SUPPLEMENTAL DATA

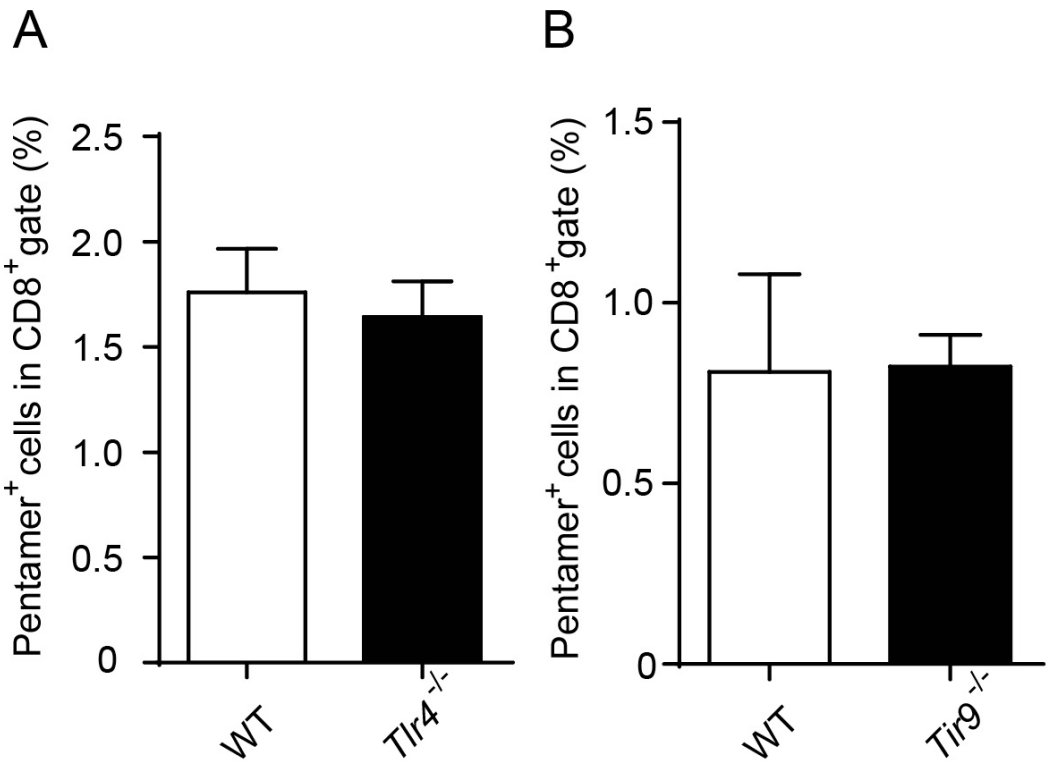

Figure S1, related to Figure 1. Antigen-specific CD8$^+$ T cell response in *Tlr*4$^{-/-}$ and *Tlr*9$^{-/-}$ mice is comparable to WT Mice. B16.SIY melanoma cells were injected into WT or *Tlr*4$^{-/-}$ (A) or *Tlr*9$^{-/-}$ (B) mice. After 1 week, spleens were isolated and SIY peptide-specific pentamer staining was performed as described in Experimental Procedures. Data represent mean ± SEM and are representative of two independent experiments.

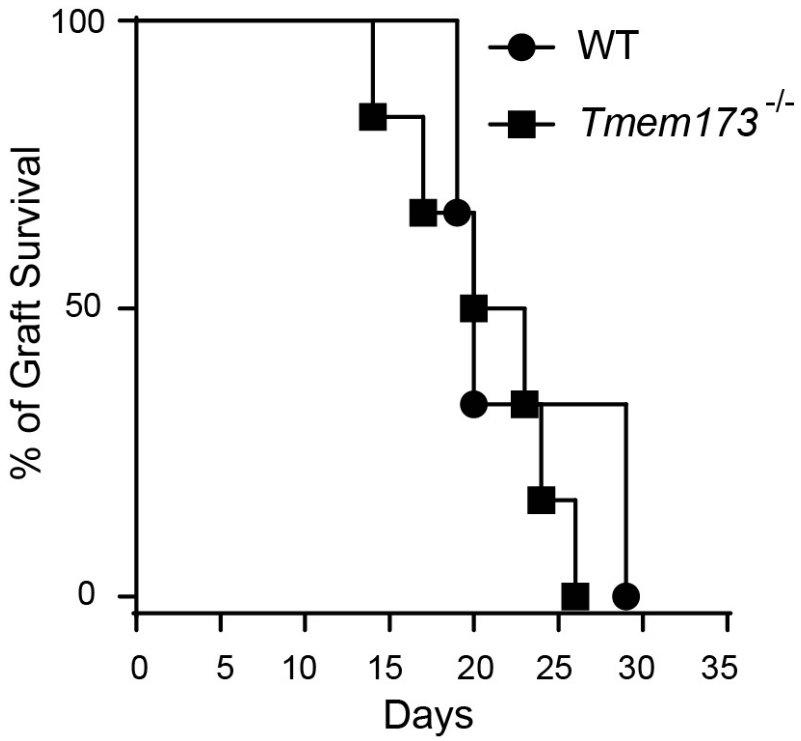

Figure S2, related to Figure 1. *Tmem173*$^{-/-}$ mice reject skin grafts with similar kinetics as wild type recipients. Skin from male mice was transplanted into female recipients using either C57BL/6 wildtype (n=3) or *Tmem173*$^{-/-}$ (n=6) donor/recipient pairs. Percent surviving grafts was assessed over time.

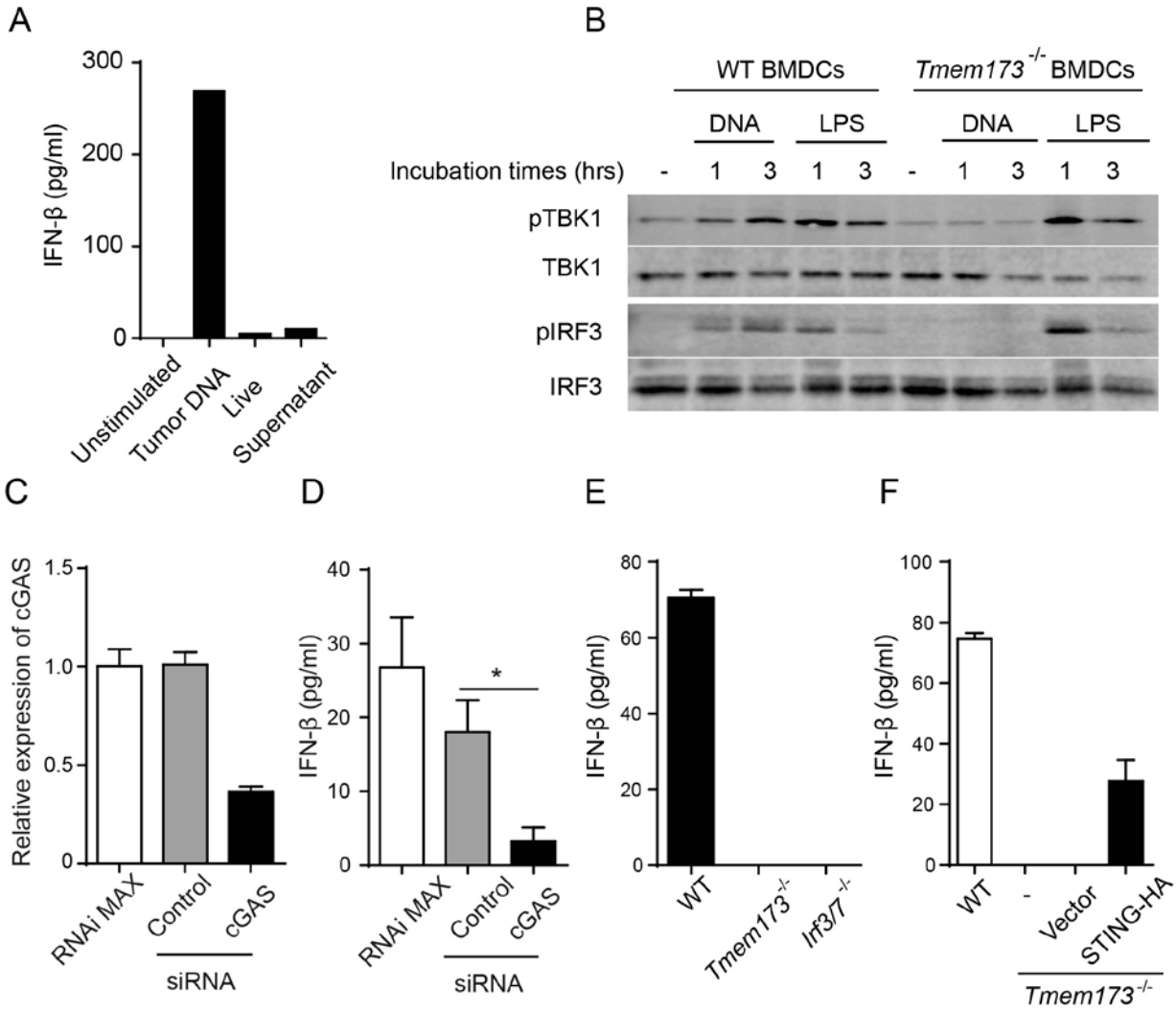

Figure S3, related Figure 3. Tumor-derived DNA induces production of IFN-β via a cGAS/STING/IRF3-dependent pathway. (A) Immortalized macrophage cell lines were stimulated with either tumor-derived DNA + Lipofectamine, live tumor cells, or culture supernatant from tumor cells, and the amount of produced IFN-β was measured by ELISA. (B) BMDCs were stimulated with either tumor-derived DNA (1 µg/ml) or LPS (20ng/ml) for the indicated times. Whole cell extracts were incubated with antibodies against pTBK1, total TBK1, pIRF3 and total IRF3. Images were acquired using Odyssey Scan (Licor) and analysed by Image Studio (Licor). (C-D) Murine macrophage cells were treated with control or cGAS siRNA. (C) Assessment of cGAS expression by RT-PCR. (D) After 48hrs, treated cells were stimulated with tumor DNA and production of IFN-β was measured from cell culture supernatants by ELISA. Data are mean ± SEM and are representative of three independent experiments. (E-F) Murine macrophage cells were stimulated with tumor-derived DNA (1µg/ml) and the amount of IFN-β was measured from cell culture supernatants by ELISA. (E) *Tmem173*$^{-/-}$ and *Irf3/7*$^{-/-}$ macrophages. (F) *Tmem173*$^{-/-}$ macrophages transduced with empty vector or a STING-HA construct to restore STING expression.

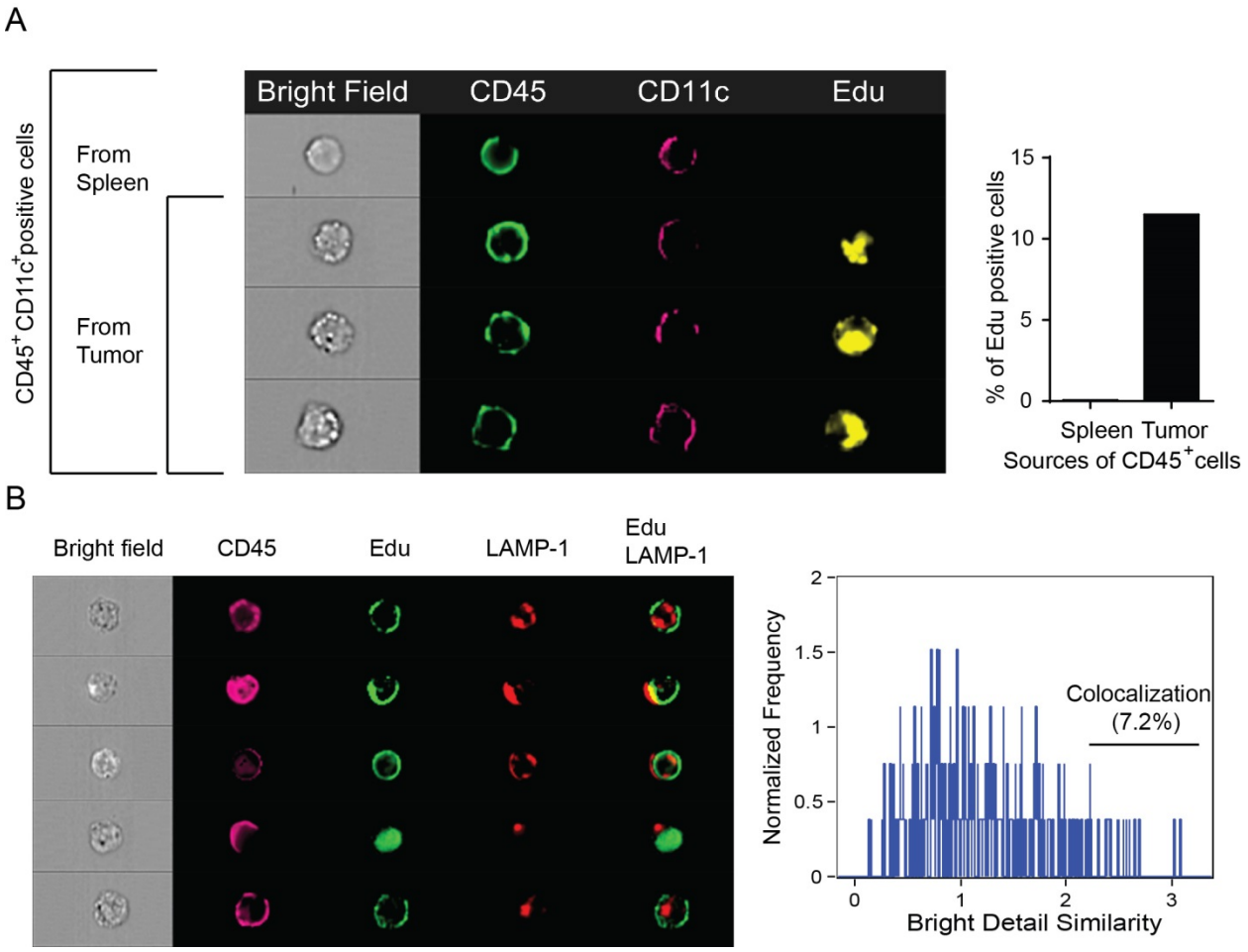

Figure S4, related Figure 4. DNA from 1969 tumor cells can be detected within host APCs in vivo and most of the tumor-derived EdU signal does not co-localize with the lysosomal marker LAMP-1 in tumor-infiltrating CD45$^+$ cells. (A) 1969 tumor cells were labeled with EdU and injected subcutaneously into mice. After 1 day, early tumors including tumor-infiltrating immune cells were isolated and stained for CD45, CD11c, and EdU as described in the Experimental Procedures. Images were acquired using the Amnis ImageStream system and data were analyzed using Amnis software (IDEAS). Data illustrate one representative of two independent experiments. (B) EdU-labeled B16 melanoma cells were injected subcutaneously into mice. After 3 days, the early tumor including tumor-infiltrating immune cells were isolated and stained for CD45, LAMP-1, and EdU as described in the Experimental Procedures. Images were acquired using the Amnis ImageStream system and data were analyzed using Amnis software (IDEAS) to quantify the degree of co-localization. Data illustrate one representative of two independent experiments.

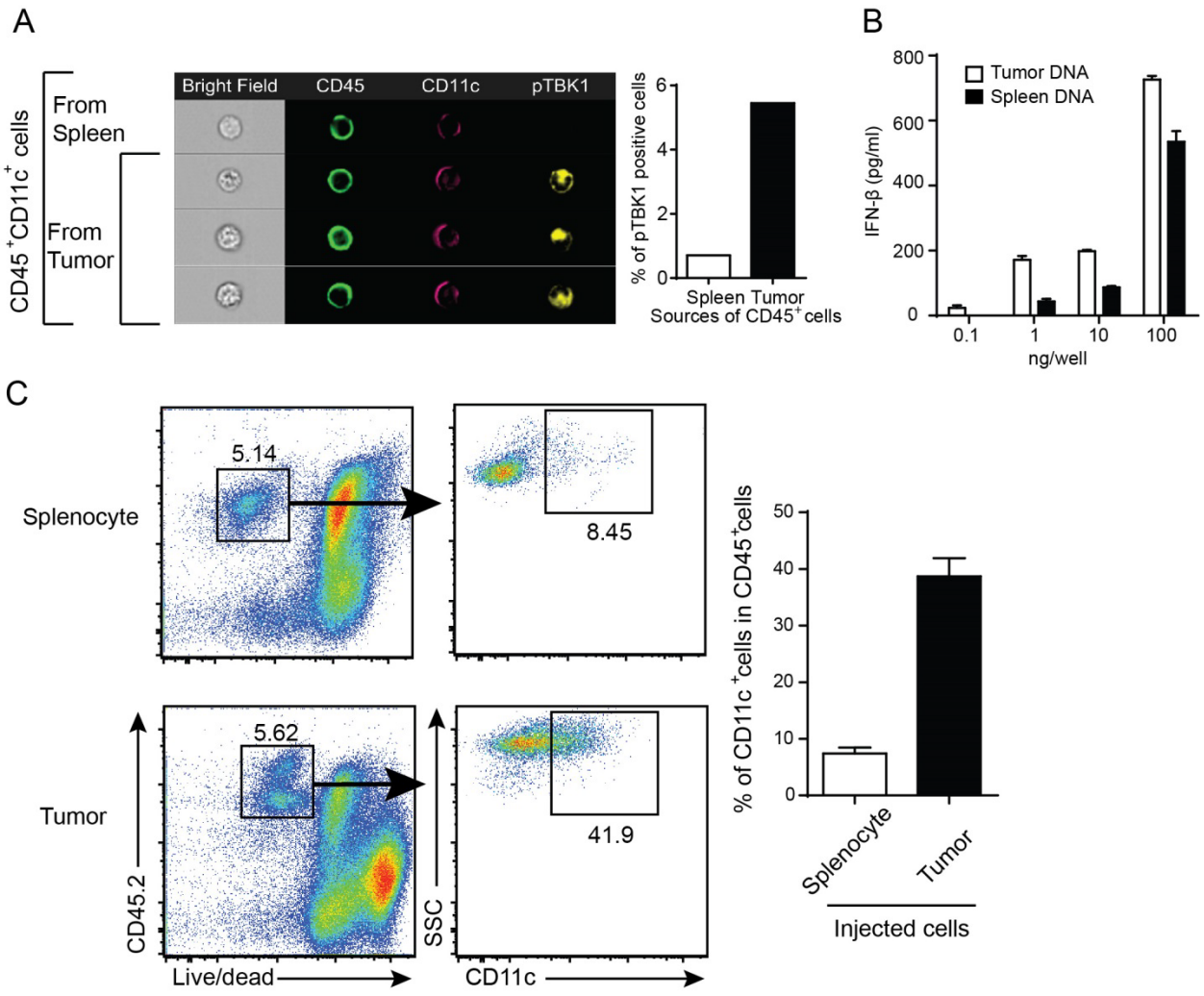

Figure S5, related Figure 5. pTBK1 staining is detected in tumor-infiltrating CD45$^+$CD11c$^+$ cells and injection of splenocytes fails to recruit the number of DCs that are recruited with tumor cell injection subcutaneously although DNA from splenocytes induce production of IFN-β comparable to tumor-derived DNA in vitro. (A) B16 melanoma cells were injected into mice. After 1 day, the early tumors including tumor-infiltrating host immune cells were isolated and stained with indicated antibodies. Images were acquired using the Amnis ImageStream system and data were analyzed using Amnis software (IDEAS). (B) DNA from spleen of WT B6 mice or B16 melanoma tumor cells was isolated using DNA isolation kit (Qiagen). BMDCs were stimulated with indicated concentrations of DNA along with Lipofectamine, and IFN-β production was measured from cell culture supernatants. Data are means ± SEM and are representative of three independent experiments. (C) Either syngeneic splenocytes or B16 tumor cells (5×10^6) were injected subcutaneously into mice. After one day, tissues from the injected sites were isolated and stained for CD45 and CD11c as described in the Experimental Procedures. Stained cells were acquired and analyzed by flow cytometry and FlowJo software. Data are representative of two independent experiments.

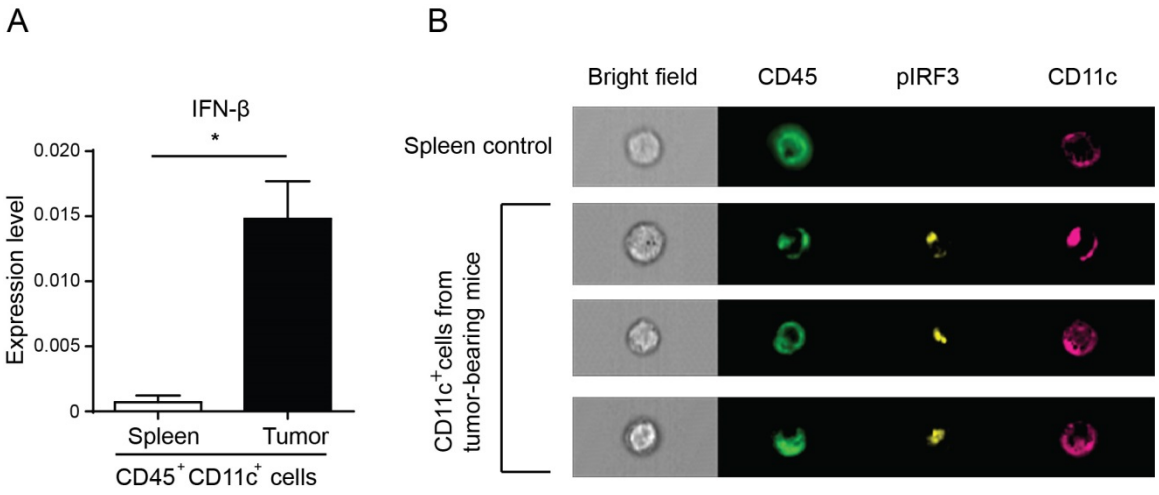

Figure S6, related to Figure 5. DCs from tumor tissues of BRaf^{V600E}/PTEN$^{-/-}$ mice show induction of IFN-β transcripts and pIRF3 staining. (A) Live CD45^{+} CD11c^{+} cells were isolated from spleen and tumors of tamoxifen-treated BrafV600E/PTEN$^{-/-}$ mice using flow cytometric sorting. Expression of IFN-β transcript was measured by qRT-PCR as described in Experimental Procedures. Expression level of IFN-β was calculated by comparison of 18s control. * P = 0.05 (one-sided Mann-Whitney U test, n=4 each samples). (B) CD45^{+} CD11c^{+} live cells were isolated from tumors obtained from tamoxifen-treated Braf^{V600E}/PTEN$^{-/-}$ mice using flow cytometric sorting. Sorted cells were stained with anti-pIRF3 antibody, and stained cells were acquired by Amnis ImageStream. Acquired images were analyzed using Amnis software (IDEAS). Data are representative of two independent experiments.

Supplemental Experimental Procedures

Western blot and siRNA-mediated interference

WT or STING$^{-/-}$ BM-DCs were stimulated with 1μg/ml of tumor-derived DNA for 1, 3 or 6 hours. Proteins were extracted with Triton-X buffer (150 mM sodium chloride, 50 mM Tris, 1% Triton-X, pH 8.0) with proteinase inhibitors (Thermo scientific) and phosphatase inhibitors (Sigma). 30 μg of protein was electrophoresed in 10% SDS-PAGE gels and transferred onto Immobilon-FL membranes (Millipore). The blots were incubated with antibodies specific for pTBK1 (Ser172), pIRF3 (Ser396), total TBK1, total IRF3 and GAPDH (all antibodies from CellSignaling, except anti-total IRF3, which was from from Invitrogen). Anti-rabbit IRDye 680RD label secondary antibody was used for visualization of bands in the Odyssey Scan (Licor) and densitometry of each band was calculated using Li-cor software. The siRNAs for STING and IRF3 were purchased from Invitrogen (Silencer® Select siRNA). IFN-β reporter cells were cultured in 96-well plates at a density of 5×10^4 cells per well and transfected with siRNA targeting mouse IRF-3 (sense strand: 5′-GGAAAGAAGUGUUGCGGUUtt-3′), mouse STING (sense strand: 5′-GGAUCCGAAUGUUCAAUCAtt-3′), in the presence of Lipofectamine. siRNA transfection was performed for 24 hours, after incubation total RNA was isolated using the RNeasy® kit (Qiagen). cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (applied biosystems™), and knock down of each gene was measured by quantitative RT-PCR using specific primer/probe mouse STING (forward 5′-AACACCGGTCTAGGAAGCAG-3′, reverse 5′-CATATTTGGAGCGGTGACCT-3′ and probe 5′-CATCCAGC-3′), mouse IRF-3 (forward 5′-CAAGAGGCTTGTGATGGTCA-3′, reverse 5′-GCAAGTCCACGGTTTTCAGT-3′ and probe 5′-AGGAGCTG-3′). The siRNA-transduced cells were stimulated with tumor-derived DNA and amount of IFN-β was measured. WT

macrophages were cultured in 96-well plates at a density of 5×10^4 cells per well and transfected with 10nM siRNA targeting mouse cGAS (sense strand: 5′-AUUUCUGCUCCUAAUGAAtt-3′; antisense strand: 3'-UUCAUUAGGAGCAGAAAUCtt-5'), or scrambled siRNA in complex with Lipofectamine RNAiMAX. siRNA transfection was performed for 48 hours, then total RNA was isolated using the RNeasy® kit (Qiagen). cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (applied biosystems™), and knock down of cGAS was measured by quantitative RT-PCR using specific primer/probe sets (mouse cGAS--forward: 5'- GAA TCT TCC GGA GCA AAA TG-3', reverse: 3'-GGC AGT TTT CAC ATG GTA GGA-5'and probe: 5′-CATCCAGC-3′). The siRNA-transfected cells were stimulated with 20 or 200 ng of tumor-derived DNA per well. After 12 hours, supernatants were collected and the amount of IFN-β was assessed by ELISA (PBL Interferon Source). For IFN-β mRNA assessment, tumor cells were injected into mice and $CD45^+$ cells were isolated by cell sorting. Q-PCR analysis was performed described above.

Dendritic cell cytokine and microarray analysis

BM-DCs were generated from WT or $STING^{-/-}$ mice as described above. After tumor-derived DNA stimulation for 7 hours, supernatants were collected and the amount of IL-6, IL-12p40, and TNF-α was measured by ELISA (eBioscience). Stimulated BMDCs with tumor-derived DNA were lysed and total RNA was isolated using RNeasy® kit (Qiagen). Isolated RNA was submitted for Affymetrix GeneChip analysis to the Functional Genomics Facility at the University of Chicago. The RNA integrity was assessed by Agilent 2100 Bioanalyzer (Agilent Technologies), and the concentration/purity of RNA was determined by NanoDrop 1000 (Thermo Scientific). All RNA samples used for microarray analysis had RNA Integrity

Number > 8.0, OD260/280 and OD260/230 ratio >1.8. The arrays (Affymetrix mouse genome 430 2.0_ were scanned by Affymetrix Gene Chip Scanner 3000 7G and CEL. Intensity files were generated by Gene Chip Operating Software v. 1.4 (MicroArray Suite 5.0). dChip software was used to analyze the microarray data. Using dChip software, the genes scored as "absent" or with signal intensity <100 were first filtered out. Microarray data can be found in Gene Expression Omnibus (GEO) under accession GSE61835.

Skin transplantation

Skin transplantation was performed as previously described (Molinero et al., 2008). Briefly, full-thickness donor flank skin pieces (0.5–1 cm^2) were positioned on a graft bed prepared on the flank of the recipient. The time point of rejection was defined as the complete necrosis of the graft.

RT-PCR analysis of IFN-β

For RT-PCR analysis of IFN-β, B16.SIY melanoma cells were inoculated into mice (5 mice per group). Single cell suspensions were prepared described above and stained with antibodies anti-mouse CD45-PE (30-F11), anti-mouse CD11b-PacBlue (M1/70) and anti-mouse CD11c-PEcy7 (N418). Stained cells were collected by cell sorting with FACSAria III (BD). Total RNA was isolated using the RNeasy® kit (Qiagen). cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (applied biosystems™). Q-PCR reactions were performed using TagMan Gene Expression Master Mix (A&B) and a 7300 Real Time PCR system (A&B).

RT-PCR analysis of IFN-β within inducible genetic melanoma mouse model

Mice harboring flox-stop-flox $Braf^{V600E}$ (endogenous Braf-locus) and floxed PTEN (exon 3) were crossed onto a tryosinase-ER-Cre mouse as described previously (Dankort et al., 2009). Cre expression was induced by application of 4-hydroxy-tamoxifen at a single location (5µl volume) on the back of mice. Following tumor development at 6 weeks, tumor-tissues (and in some experiments lymphoid organs) were harvested and single cell suspensions were prepared. As controls in some cases, back skin, LN, and spleen were harvested from littermates without tamoxifen application. Cells were stained with a Fixable Viability Dye 450 (eBioscience) and Fc-block prior to staining with CD19-PacBlue (eBio1D3), CD45-Ax488 (30-F11) and CD3-APC (145-2C11). $CD45^{+}CD19^{-}CD3^{-}$ cells were sorted (FACSAria III, BD) directly into Trizol and subsequently RNA was isolated according to manufactures instructions (Life Technologies). cDNA was synthesized and Q-PCR analysis for IFN-β was performed as above.

ImageStream analysis

Harvested tumors were incubated with collagenase (50 unit/ml; Worthington Biochemical Corporation) for 2 hrs at 37^{o}C. Single suspensions of tumor-derived cells were prepared by homogenization using a syringe plunger and cell strainer. After antibody staining, single cell images were acquired with ImageStreamxMark II (Amnis). Collected data were analyzed with IDEAS 5.0 software (Amnis). Single-stained control cells were used for compensation. Cells were gated for single cells with the area and aspect ratio and for focused cells with the Gradient RMS feature. For the DRAQ5 uptake assay, B16 melanoma cells were incubated with DRAQ5 (5µM) for 15 minutes. After extensive washing with PBS, stained tumor cells were inoculated into mice subcutaneously. The next day, the tumor bump was harvested

and tumor-derived cells were isolated and a single cell suspension was prepared as described above. Cells were stained with the LIVE/DEAD Fixable Dead cell stain kit (Invitrogen), anti-mouse-CD45-PECy5 (30-F11), and CD11c-PECy7 (N418), followed by analysis with the ImageStreamxMarkII (Amnis). For the EdU labeling experiments, B16 melanoma or 1969 sarcoma cells were incubated with EdU (10μM) for overnight in complete DMEM culture medium prior to preparation for inoculation. At the indicated times, the tumor bump was harvested, made into a single cell suspension as above, and stained with anti-mouse CD45-PECy5 and CD11c-PECy7. EdU detection (either Alexa Fluor 555 or Alexa Fluor 647) was performed using Click-iT® EdU Imaging Kits (Invitrogen). Non-labeled tumor cells were used as a negative control through the same staining procedure. For pIRF3 staining, tumor single cell suspensions were stained with LIVE/DEAD Fixable Dead cell stain, anti-mouse CD45-PECy5, CD11c-PECy7 and permeabilized the Fixation/Permeabilization kit (eBioscience). After blocking with Normal Mouse Serum, cells were stained with pIRF3 antibody (Cell Signaling, Cat # 4947) and subsequently were stained with anti-rabbit IgG-PE secondary antibody (Invitrogen). For nuclear staining, stained cells were incubated with NucBlue™Fixed Cell Stain (Invitrogen) for 5 minutes. For pTBK1 staining, the same procedure was used as above except using a pTBK1-specific antibody (cell signaling, Cat #5483). For EdU localization experiments, anti-Lamin A/C (Cell Signaling) and anti-LAMP-1 (Biolegend) antibodies were used.