**Figure 2-1. Establishment of 293T cell lines and condition tests for optimal stimulation.**

(A) 293T\_STING cells respond to cGAS transfection and activate downstream signaling. 293T cells stably expressing STING (293T\_STING) and 293T parental cells were transfected with 0.5ug/mL empty pcDNA vector (EV), pcDNA-cGAS (cGAS\_wt), pcDNA-cGAS with G198A and S199A mutations (cGAS\_mt), or pcDNA-MAVS (MAVS) for 24 hr. Activation of IRF3 was analyzed by native PAGE and immunoblotting.

(B) 293T\_STING cells produce IFN-b with cGAS transfection. Similar to (A), the cells were transfected with the indicated DNA vector for 24 hours before total RNA was isolated. IFN-b mRNA level was analyzed by q-RT-PCR. Unless indicated otherwise, error bars represent standard deviations of triplicate assays.

(C-E) Titration test of DNA concentration for optimal activation. 293T cells were transfected with the indicated amount of DNA vectors for 24 hours. Total RNA was isolated and IFN-b, CXCL-10 and TNF-a mRNA levels were analyzed by q-RT-PCR.

(F) cGAS catalytic dead mutation is null functional. 293T\_STING cells were transfected with 0.1ug/mL pcDNA\_EV or pcDNA\_cGAS\_mt for 24 hours. Total RNA was isolated and further processed for mRNA-sequencing. Sequencing data was mapped to 33615 genes and the result was plotted. X and Y axis represent the log base 2 transformed RPKM (Reads per kilobase per million mapped reads) values of the indicated experiment samples.

**Figure 2-2. cGAS induced immune response is STING dependent in 293T cells.**

(A) Wild type cGAS plasmid transfection induces gene expression changes in 293T\_STING cells. Scatter plot representation of mRNA-sequencing expression data. Each data point represents a single RefSeq gene plotted according to the fold change between cGAS\_wt and cGAS\_mt transfected samples (x-axis) and the expression values (RPKM) in cGAS\_wt transfected sample (y-axis). The identity of several data points is indicated for reference. Vertical red lines indicate the 4-fold differential expression cutoff to define the cGAMP activated genes. Genes were further sorted according to their mean cGAMP activated expression into high, intermediate, and low expression categories. (We used a mean cGAMP activated RPKM cutoff of 0.1 to ensure genes in lists were sufficiently expressed.)

(B) In 293T cells, transient expression of cGAS\_wt does not alter gene expression changes. Similar scatter plot as (A), but 293T parental cells were used.

**Figure 2-3. cGAS induces broader gene expression changes than MAVS.**

(A) MAVS expression in 293T\_STING cells induces gene expression changes. Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between MAVS and cGAS\_mt transfected samples. Y-axis represents the RPKM values in MAVS transfected sample.

(B) Venn diagram comparing genes that are induced by cGAS and MAVS. With the sequencing data represented in figure 2-2A and 2-3A, a cut-off of induction fold greater than 4.0 and induced expression RPKM greater than 1.0 was applied.

(C) Heatmap of RPKM data showing the gene expressions in 293T\_STING cells transfected with pcDNA-cGAS\_mt, pcDNA-cGAS\_wt, or pcDNA-MAVS.

**Figure 2-4. Representing lists of genes that are commonly or specifically induced by cGAS and MAVS.**

(A-B) List of representing genes and their RPKM values that were only induced by pcDNA-cGAS\_wt transfection. (A) Genes with induced RPKM value less than 25; (B) Genes with induced RPKM value larger than 25.

(C-D) List of representing genes and their RPKM values that were induced in both pcDNA-cGAS\_wt and pcDNA-MAVS transfected samples. (C) Genes with induced RPKM value less than 25; (D) Genes with induced RPKM value larger than 25.

(E) List of representing genes and their RPKM values that were only induced by pcDNA-MAVS transfection.

**Figure 2-5. Confirmation of genes induced by cGAS and MAVS.**

(A) 293T\_STING cells were transfected with 0.1ug/ml pcDNA-cGAS\_wt, pcDNA-cGAS\_mt, or pcDNA-MAVS for 24 hours. Total RNA was isolated and mRNA levels of indicated genes were analyzed by q-RT-PCR.

(B-E) The expression of GADD family genes was induced specifically by cGAS expression. 293T\_STING cells were either treated with recombinant TNF-a for the indicated time or transfected with indicated DNA vector for the indicated time. Total RNA was isolated and mRNA levels of GADD34, GADD45-a, GADD45-b, and GADD45-g were analyzed by q-RT-PCR.

**Figure 2-6. Condition tests for optimal cGAMP stimulation in BMDM and LF.**

(A-B) Delivery methods test for cGAMP treatment. BMDM cells were plated 7 days after differentiation. The cells were treated by following methods for indicated time: Lipofectamine 2000 alone, Lipofectamine 2000 along with 1.5uM cGAMP, 1.5uM cGAMP alone added to culture medium, Digitonin permeabilization buffer alone, Digitonin permeabilization buffer with 0.1uM or 0.2uM cGAMP. Total RNA was isolated and mRNA levels of IFN-b (A) and CXCL-10 (B) were analyzed by q-RT-PCR.

(C-D) cGAMP dosage test for optimal cGAMP induced activation. Lung fibroblast cells were treated with Digitonin permeabilization buffer alone, or Digitonin permeabilization buffer with 0.1, 0.2, or 0.4uM cGAMP, for the indicated time. Total RNA was isolated and mRNA levels of IFN-b (A) and CXCL-10 (B) were analyzed by q-RT-PCR.

(E-F) Sample test for subsequent sequencing data collection. BMDM (E) and lung fibroblast (F) cells were treated with Digitonin permeabilization buffer (Ctl) or Digitonin permeabilization buffer with 0.1uM cGAMP (cGAMP). Total RNA was isolated and mRNA levels of IFN-b and CXCL-10 were analyzed by q-RT-PCR.

**Figure 2-7. cGAMP induced immune response is STING dependent in lung fibroblast cells.**

(A) cGAMP induces gene expression changes in wild type lung fibroblast cells. Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between cGAMP and Digitonin alone treated samples. Y-axis represents the RPKM values in cGAMP treated sample.

(B) In lung fibroblast from STING Goldenticket mice, cGAMP does not alter gene expression changes. Similar scatter plot as (A), but lung fibroblast from STING Goldenticket mice were used.

**Figure 2-8. cGAMP induced immune response is STING dependent in BMDM cells.**

(A) cGAMP induces gene expression changes in wild type BMDM cells. Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between cGAMP and Digitonin alone treated samples. Y-axis represents the RPKM values in cGAMP treated sample.

(B) In BMDM from STING Goldenticket mice, cGAMP does not alter gene expression changes. Similar scatter plot as (A), but BMDM from STING Goldenticket mice were used.

**Figure 2-9. cGAMP induced immune response is STING dependent in conventional dendritic cells.**

(A-B) cGAMP induces gene expression changes in wild type cDC cells. Conventional dendritic cells were treated for 6 hours (A) or 12 hours(B). Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between cGAMP and Digitonin alone treated samples. Y-axis represents the RPKM values in cGAMP treated sample.

(C-D) Similar to A and B, but cDC cells from STING Goldenticket mice were used.

(E-F) Similar to A and B, but cDC cells from interferon alpha receptor knockout mice were used.

**Figure 2-10. Liposome and ISD induce IFN-b and ISG expression in conventional dendritic cells.**

(A-C) Liposome, ISD, and HT-DNA induce the expression of IFN-b, TNF-a and CXCL-10 in cDCs. cDC cells were plated at day 8 post differentiation and were treated with indicated amount of liposome alone (Lipo2K), liposome with ISD, or liposome with HT-DNA for 6 hours. Total RNA was isolated and mRNA levels of IFN-b (A), TNF-a (B), and CXCL-10 (C) were analyzed by q-RT-PCR.

(D) Liposome or DNA stimulation is toxic to cells and lower total RNA yield. cDC cells were treated as in A-C, the total amount of RNA (in ug) isolated from per million cells was measured and plotted.

(E) The mRNA level of IFN-b decreases after 6 hours of DNA stimulation. cDC cells from wild type mice were treated with either 6ul/ml Lipofectamine 2000 alone or along with 2ug/ml ISD for the indicated time before total RNA was isolated. mRNA level of IFN-b was analyzed by q-RT-PCR.

(F) Sample test for subsequent sequencing data collection. cDC cells from wild type, cGAS knockout, and STING Goldenticket mice were treated with either 6ul/ml Lipofectamine 2000 alone or along with 2ug/ml ISD for the indicated time before total RNA was isolated. mRNA level of IFN-b was analyzed by q-RT-PCR.

**Figure 2-11. ISD induced immune response is largely cGAS and STING dependent in conventional dendritic cells.**

(A-B) ISD induces gene expression changes in wild type cDC cells. Conventional dendritic cells were treated for 3 hours (A) or 6 hours(B). Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between ISD treated and non-treated (Ctl) samples. Y-axis represents the RPKM values in ISD treated sample.

(C-D) Similar to A and B, but cDC cells from STING Goldenticket mice were used.

(E-F) Similar to A and B, but cDC cells from cGAS knockout mice were used.

**Figure 2-12. Liposome induced immune response is largely cGAS and STING dependent in conventional dendritic cells.**

(A-B) Liposome induces gene expression changes in wild type cDC cells. Conventional dendritic cells were treated for 3 hours (A) or 6 hours(B). Scatter plot representation of mRNA- sequencing expression data. X- axis represents the fold change between liposome treated and non-treated (Ctl) samples. Y-axis represents the RPKM values in liposome treated sample.

(C-D) Similar to A and B, but cDC cells from STING Goldenticket mice were used.

(E-F) Similar to A and B, but cDC cells from cGAS knockout mice were used.

(G) Venn diagram comparing genes that are induced by 6 hours treatment of ISD and liposome alone. With the sequencing data represented in figure 2-11B and 2-12B, a cut-off of induction fold greater than 2.0 and induced expression RPKM greater than 1.0 was applied.

**Figure 2-13. Representing genes that are induced by ISD independent of cGAS and STING**

(A-B) List of representing genes and their RPKM values that were induced by 6 hours of ISD treatment in cGAS knockout and STING Goldenticket cDC cells. (A) Genes with induced RPKM value less than 100; (B) Genes with induced RPKM value larger than 100.

(C) List of representing genes and their RPKM values whose basal expression level is 1.5 folds lower in cGAS knockout and STING Goldenticket cDC cells than in wild type cDC cells.

(D) Venn diagram comparing genes that are induced by 6 hours treatment of ISD independent of cGAS/STING and genes with lower basal expression level in cGAS knockout and STING Goldenticket cDC cells.

**Figure 2-14. IL-12b gene expression is induced by ISD, but not cGAMP in cDCs.**

(A) RPKM values of IL-12b gene in RNA-sequencing dataset (as shown in Figure 2-11 and 12).

(B-C) The induction of IFN-b and IL-6, but not IL-12b by ISD was dependent on cGAS and STING. cDC cells from wild type, cGAS knockout, and STING Goldenticket mice were plated and treated with liposome alone, with ISD, cGAMP, or infected with herpes simplex virus (HSV) or Sendai virus (SeV) for the indicated time. Total RNA was isolated and mRNA levels of IFN-b (B), IL-6 (C), and IL-12b (D) were analyzed by q-RT-PCR.

**Figure 2-15. Gene expression by ISD and CpG-DNAs in pDC and cDC cells.**

Plasmacytoid dendritic cells (pDCs, B, D and F) and Conventional dendritic cells (cDCs, A, C and E) from wild type, cGAS knockout, and TLR9 knockout mice were treated by liposome, ISD, CpG-DNAs, or in combination for 6 hours. Total RNA was isolated and mRNA levels of IFN-b (A-B), IL-6 (C-D), and IL-12b (E-F) were analyzed by q-RT-PCR.