Supplementary Materials for

Cyclic-GMP-AMP Is An Endogenous Second Messenger in Innate Immune Signaling by Cytosolic DNA

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Materials and Methods

Cells, Viruses, Antibodies, Nucleotides and General Methods

Cell lines including L929, HEK293T, Raw264.7, primary mouse embryonic fibroblasts (MEF) and bone marrow derived macrophages (BMDM), were cultured in DMEM (Sigma) supplemented with 10% calf serum and antibiotics. THP-1 cells were cultured in RPMI containing 10% fetal bovine serum, antibiotics, nonessential amino acids, and 50 μM of β-mercaptoethanol. Sendai virus (Cantell strain, Charles River Laboratories) was used at a final concentration of 50 hemagglutinating units/ml. Other viruses, including VSV(ΔM51)-GFP, HSV-1ΔICP34.5, and heat-inactivated Vaccinia virus (VACV, WR strain) were used at an MOI of 10.

The rabbit polyclonal antibodies against STING were generated and affinity purified as described previously(*1*). Antibodies against human and murine IRF3 were obtained from Santa Cruz Biotechnology and Invitrogen, respectively. Poly(I:C), herring testis (HT) DNA, Anti-Flag(M2)-agarose and all chemicals were from Sigma unless otherwise indicated. ISD was prepared from equimolar amounts of the sense and antisense DNA oligonucleotide (sense strand sequence: 5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTACA-3'). The oligonucleotides were heated at 95°C for 5 min and cooled to room temperature. All chromatography columns were from GE Healthcare. [α-^{32}P]-ATP and [^{35}S]-methionine were from Perkin Elmer. siRNA against indicated genes were from Sigma and transfected into L929 cells using lipofectamine 2000 according to manufacturer's instruction (Invitrogen). Lentiviral vector for STING shRNA was described previously(*1*); the targeting sequence for STING was: 5'-CCTCATCAGTGGAATGGAA (only sense strand sequence is shown). Digitonin permeabilization was used to deliver cGAMP, c-di-GMP, and c-di-AMP into cultured cells as previously described(*2*).

The procedures for native gel electrophoresis to detect IRF3 dimerization, SDS–polyacrylamide gel electrophoresis (SDS-PAGE), and Western blotting have been

described previously(*3*). Reverse transcription and real-time PCR reactions were carried out as described before(*4*). Basically, iScript cDNA synthesis kit and iQ SYBR Green Supermix (Bio-Rad) were used according to manufacturer's instructions. Quantitative real time PCR was performed on an Applied Biosystem Vii7 with the following primers: Mouse IFN-β, sense: TCCGAGCAGAGATCT TCAGGAA, anti-sense: TGCAACCACCACTCATTCTGAG;
Mouse HPRT, sense: CAGTCCCAGCGTCGTGATTAG, anti-sense: AAACACTTTTTCC-AAATCCTCGG; Human IFN-β, sense: AGGACAGGATGAACTTTGAC,
anti-sense: TGATAGACATTAGCCAGGAG; Human TNFα, sense: TGCTTGTTCCTCAGCCTCTT, anti-sense: GGTTTGCTACAACATGGGCT; Human GAPDH, sense: GAGTCAACGGATTT GGTCGT, anti-sense: TTGATTTTGGAGGGATCTCG.

Recombinant Proteins

cDNA encoding amino acids 139-379 of human STING was inserted into a modified pET28a vector in which the His_6-SUMO tag was fused to the N-terminus of the truncated STING protein. The *E. coli* strain BL21/pLys harboring this plasmid was induced with 0.5mM IPTG at 20°C overnight. *E coli* was suspended in buffer A [50mM Tris-Cl, pH8.0, 300mM NaCl, 20mM Imidazole, 5mm β-mercaptoethanol, 0.2mM PMSF] and centrifuged at 100,000 x g for 20min. The supernatant was incubated with Ni-NTA beads. After wash in buffer A, bound protein was eluted with buffer B [20mM Tris-Cl, pH7.4, 150mM NaCl, 300mM Imidazole]. The His_6-SUMO tag was removed by SUMO protease (Ulp1) cleavage followed by size exclusion chromatography on a Superdex-75 column (24ml bed volume, GE Healthcare) in buffer C [20mM Tris-Cl, pH8.0, 150mM NaCl, 5mM DTT]. The plasmid containing the coding sequence of perfringolysin O (PFO) from the bacterium *Clostridium perfringens* was a gift from Dr. Russell DeBose-Boyd (UT Southwestern Medical Center). DNA sequence encoding VC0179 was amplified from the genomic DNA of *V. cholera* strain C6709 (a gift from Dr. Vanessa Sperandio, UT Southwestern Medical Center) and was inserted into pET-21a vector in which the His_6 tag was fused to the C-terminus of VC0179 protein. BL21/pLys harboring

the PFO or VC0179 plasmid was induced with 0.5 mM IPTG at 37°C for 4hr, and the recombinant proteins were purified using Ni-NTA column as described above.

In vitro Assay for STING Activator

To isolate the cellular factor that could activate the STING pathway, L929 cells were transfected with DNA for 4-6 hr and then homogenized by douncing in the hypotonic buffer [10mM Tris-HCl, pH7.4, 10mM KCl, 1.5mM $MgCl_2$]. The homogenate was centrifuged at 100,000 rpm for 20 min, then the supernatant (S100) was heated at 95°C for 5 min and centrifuged again at 12,000 rpm for 5 min to remove denatured proteins. The heat-resistant supernatant was mixed with 10^6 THP-1 or Raw264.7 cells in an 8μl reaction containing 2 mM ATP, 1 U/μl Benzonase and 1.5 ng/μl PFO. The mixture was incubated at 30°C for 1.5 hr. Cells in the reaction were lysed by adding 0.2% NP40 and subjected to native gel electrophoresis. IRF3 dimerization was detected by immunoblotting with an IRF3 antibody. In some experiments [^{35}S]-IRF3 was added to the mixture for detection of IRF3 dimerization by autoradiography.

To synthesize the STING activator in a cell-free system, S100 from non-stimulated L929 or other cell lines as indicated was mixed with 0.1 mg/ml HT-DNA or ISD, 20 mM Hepes-OH, pH7.2, 5 mM $MgCl_2$ and 2 mM ATP. After incubation at 37°C for 45 min, the mixture was heated at 95°C for 5 min, and the heat-resistant supernatant was mixed with PFO permeabilized THP1 or Raw264.7 cells to measure IRF3 activation as described above.

Purification of the small molecule STING Activator

150 dishes (150mm) of L929 stably expressing STING-Flag were lysed in buffer D [50mM Tris-Cl, pH7.4, 100mM NaCl, 10% Glycerol, 0.5% NP40, 1mM EDTA, 1mM EGTA, 2% protease inhibitor cocktail (Roche), 0.5mM DTT] and centrifuged at 1,000 x g for 5 min. The supernatant was mixed with 1 ml of Flag (M2)-agarose and rotated at 4°C for 2 hr. The beads were washed in buffer D and used as immobilized STING affinity resin. To generate the small molecule STING activator, 50 ml reaction containing

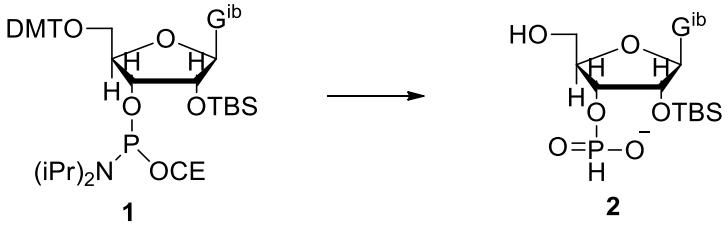
250 mg L929 S100 and 5 mg DNA was carried out at 37°C for 45 min. The heat-resistant supernatant from the reaction was mixed with the STING affinity resin and incubated at 4°C for 1 hr by constant rotation. The beads were washed in buffer E [20mM Hepes, pH7.2, 50mM NaCl, 0.1mM EGTA] and the STING activator was eluted by boiling in water for 5 min. After a brief centrifugation, the supernatant was loaded on a mono Q column (0.1ml bed volume, GE Healthcare) in 20 mM Tris-HCl, pH 7.4, and eluted with a 0-0.5M NaCl linear gradient. Active fractions (0.12-0.15M NaCl) were pooled and loaded onto a Superdex-peptide column (2.4 ml bed volume, GE Healthcare) and eluted in 20 mM Tris-HCl, pH 7.4. Active fractions were applied to a C-18 column (Eclipse Plus 4.6x30 mm, 3.5μm, Agilent Technologies) equilibrated with 0.1% formic acid and eluted with a linear gradient of 0-100% methanol. Fractions were dried at 60°C under vacuum and dissolved in water for activity assay and mass spectrometry.

cGAMP-STING Binding Assay

^{32}P-labeled cGAMP was synthesized using [α-^{32}P]-ATP, GTP, and the bacterial cyclase VC0179 as reported(*5*). cGAMP binding to STING was performed as described for the binding between c-di-GMP and STING(*6*), except that cross-linking was performed in a UV-crosslinker (Stratagene) at 1.0 Joule/cm^2.

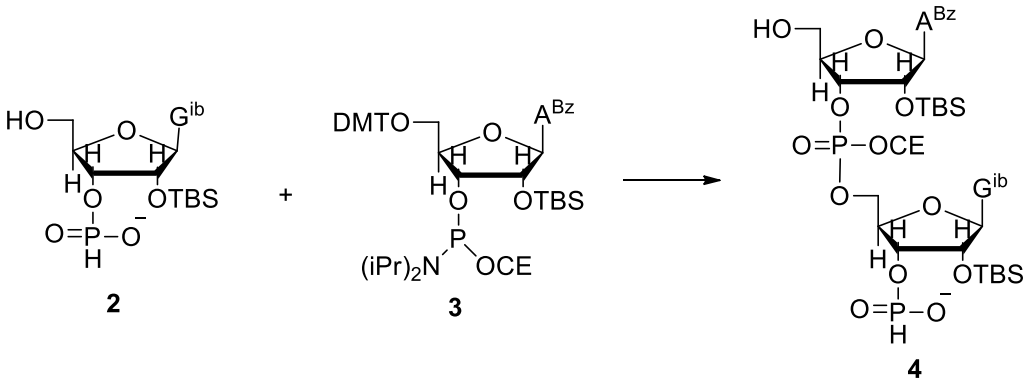
Chemical Synthesis of cGAMP

All reactions were performed in glassware under a positive pressure of argon. The synthesis scheme was modified from reference (*7*). ^{1}H NMR spectra were recorded on an Inova-400 NMR instrument. Mass spectra were acquired on an Agilent 6120 Single Quadrupole LC/MS instrument. Preparative HPLC was performed using a Waters Atlantis dC18 OBD 5 μm column (19×150 mm) or an Eclipse XDB-C18 5 μm column (9.4×250 mm).



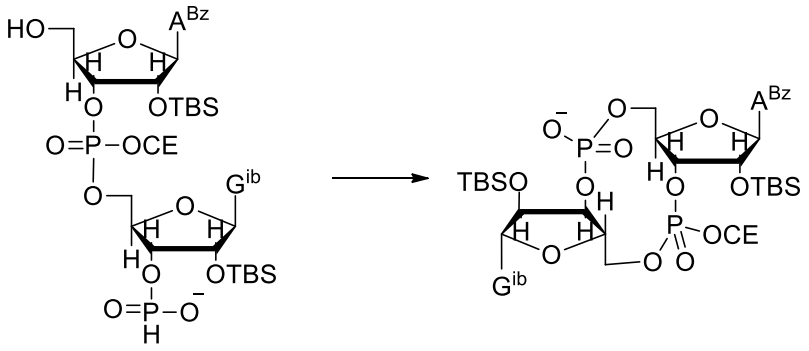
1 **2**

To a solution of **1** (0.097 g, 0.1 mmol) in CH_3CN (0.5 mL) and H_2O (0.0036 mL, 0.2 mmol, 2 equiv) was added pyridinium trifluoroacetate (0.023 g, 0.12 mmol, 1.2 equiv). After 1 min, *t*-$BuNH_2$ (0.5 mL) was added. After stirring at room temperature for 10 min, the solvent was removed and the residue was dissolved in CH_2Cl_2 (1.2 mL) and H_2O (0.018 mL, 1.0 mmol, 10 equiv), and a solution of 6% dichloroacetic acid in CH_2Cl_2 (1.2 mL, 0.88 mmol) was then added. After 10 min, the reaction was quenched by pyridine (0.14 mL, 1.74 mmol). The solution was concentrated and the crude mixture was used directly for next step.



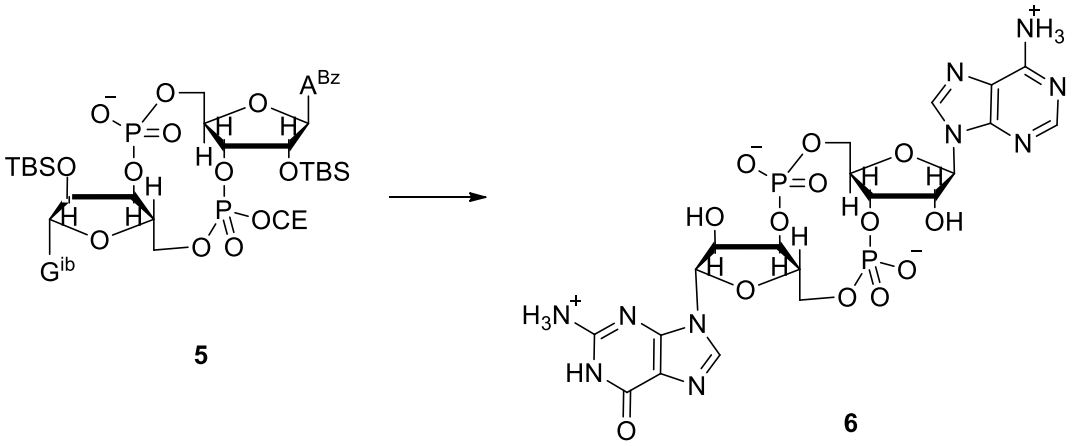
2 **3** **4**

To a solution of crude **2** obtained above in CH_3CN (0.24 mL) was added a solution of **3** (0.128 g, 0.13 mmol, 1.3 equiv) in CH_3CN (0.40 mL) under argon. After 2 min, anhydrous *t*-butyl hydroperoxide (5.5 M in decane, 0.055 mL, 0.3 mmol, 3.0 equiv) was added. After 30 min, the reaction was quenched by 33% $NaHSO_3$ aqueous solution (0.05 mL) at 0 °C. The ice bath was removed and the mixture was concentrated to an yellow oil. The residual yellow oil was dissolved in CH_2Cl_2 (1.6 mL) and H_2O (0.018 mL, 1.0 mmol, 10 equiv), and a solution of 6% dichloroacetic acid in CH_2Cl_2 (1.6 mL, 1.2 mmol) was then added. After 10 min the reaction was quenched by pyridine (1.0 mL). The solution was concentrated to an oil and the crude mixture was used directly for the next step.



4 **5**

To a solution of crude **4** obtained above in pyridine (2.0 mL) was added 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane-2-oxide (DMOCP, 95%, 0.068 g , 0.35 mmol, 3.5 equiv). After 10 min, the reaction was quenched by H_2O (0.064 mL, 3.5 mmol), and I_2 (0.033 g, 0.13 mmol, 1.3 equiv) was added immediately. After 5 min the mixture was poured into 0.14 % $NaHSO_3$ aqueous solution (14 mL). After 5 min, $NaHCO_3$ (0.40 g) was added slowly. The mixture was extracted by EtOAc/Et_2O (1:1, 4.0 mL) and the organic layer was concentrated to an oil. The crude mixture was used directly for the next step.



5 **6**

To a solution of crude **5** obtained above in CH_3CN (0.50 mL) was added *t*-$BuNH_2$ (0.5 mL). After 10 min, the yellow solution was concentrated to a yellow foam. The residue was washed with CH_2Cl_2 (0.60 mL) and the resulting white solid was reacted with a solution of CH_3NH_2 in anhydrous ethanol (33%, 3.58 mL, 29 mmol) at room temperature. After 90 min, the mixture was concentrated to an oil and pyridine:Et_3N (2.5:1.0, 0.14 mL) were added. The mixture was immediately concentrated to an oil, and this process was repeated for two more times. The resulting oil was then dissolved with

pyridine (0.080 mL) in a plastic vial and was placed in an oil bath at 50°C. Et_3N (0.50 mL, 3.6 mmol) and Et_3N•3HF (0.30 mL) were added simultaneously through syringes. After 1 h, the vial was removed from the oil bath and quenched by TMSOMe (0.5 mL). The mixture was concentrated to a yellow solid and purified by HPLC (eluent A: water with 0.1% trifluoroacetic acid, eluent B: acetonitrile with 0.1% trifluoroacetic acid, gradient: T = 0 min: 0% B, T = 7 min: 0 % B, T = 20 min: 7 % B, 4.8 mL/min). Compound **6** (cGAMP) was lyophilized and stored as a white powder (5.5 mg, 8.2% from **1**). HPLC-purified cGAMP was analyzed by ^{1}H NMR (see Supplementary Figure 3) and electrospray ionization mass spectrometry. Calculated $[M+H]^+$ for $C_{20}H_{24}N_{10}O_{13}P_2$: 675.1; measured value: 675.1. HPLC condition for LC-MS: eluent A: water with 0.1% trifluoroacetic acid, eluent B: acetonitrile with 0.1% trifluoroacetic acid, gradient: T = 0 min: 0% B, T = 5 min: 0 % B, T = 17 min: 13 % B, 0.8 mL/min. Retention time: 14.0 min.

LC-MS and LC-MS/MS

High resolution LCMS analysis was performed using a Dionex Ultimate 3000 nanoLC system (Thermofisher Scientific) coupled to a Quadrupole-Orbitrap Hybrid mass spectrometer (Q-Exactive, ThermoFisher Scientific) equipped with a nano-electrospray ion source. Ionization source parameters were set to: positive mode; capillary temperature, 250 ° C; spray voltage, 2.3 kV. Analytes were separated on a homemade analytical column (75 μm ID, 100 mm length) packed with C-18 resin (100 Å, 5 μm, MICHROM Bioresources). HPLC eluent A: 0.1% formic acid; eluent B: 0.1% formic acid + 100% acetonitrile; HPLC gradient: 2–30% B in 12 min, 30–70% B in 2 min; 100 nl/min. Full scan MS spectra were acquired in a mass range from m/z 300 to 800 with a resolution of 140,000 in the Orbitrap mass analyser.

Tandem MS/MS analysis was performed on a LTQ-XL mass spectrometer (ThermoFisher Scientific) equipped with a HPLC system (Eksigent NanoLC 2D). Analytes were eluted using a segmented gradient of 2–70% of eluent B with a constant flow of 200 nl/min in 56 min. The mass spectrometer was operated in data -dependent

mode with dynamic exclusion of 30s. Full scan MS spectra were acquired in a mass range from m/z 100 to 1500. The 3 most intense ions were sequentially isolated for CID fragmentation in the linear ion trap to generate MS/MS Spectra.

For targeted quantification of cGAMP, a selective reaction monitoring (SRM) assay was developed on the LTQ XL mass spectrometer. In the assay, three transitions of each target ion were monitored (see Table 1). The parent ion was isolated with a mass window of 1.0 m/z units, fragmented (collision energy=35, activation time=30 ms at Q=0.25), while the resulting daughter ion was scanned with a mass window of 1.6 m/z unit. The maximum ion accumulation time was 100ms, and the number of microscans was set to 1.

Precursor	Charge	Product_1	Product_2	Product_3	CE
675.11	1+	312.05	524.06	540.05	35
338.06	2+	136.06	152.06	524.06	35

Table 1. Transition List and collision energies used to monitor cGAMP by SRM. CE: collision energy.

Figure S1

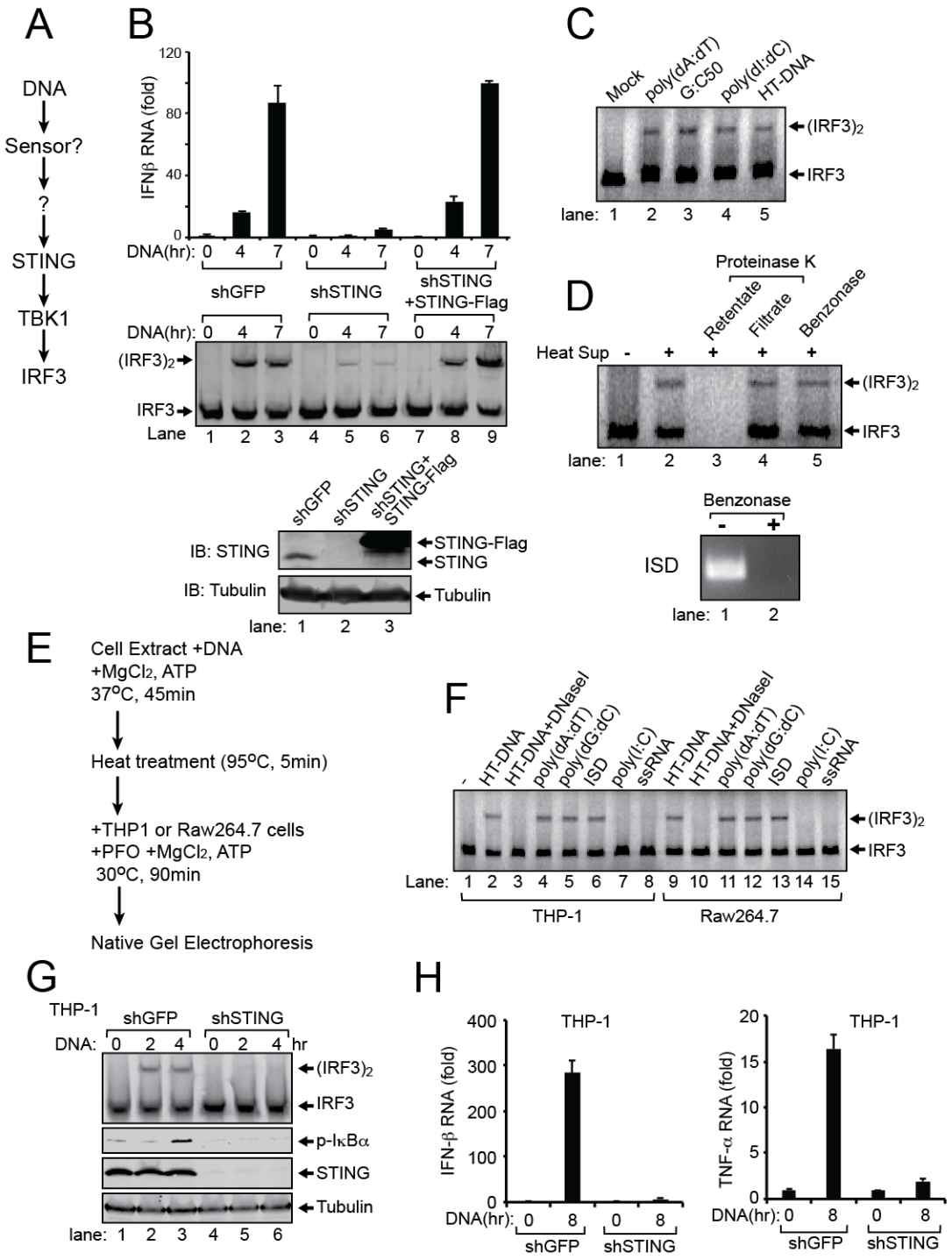

A
DNA
Sensor?
?
STING
TBK1
IRF3
B
IFNβ RNA (fold)
DNA(hr): 0 4 7 0 4 7 0 4 7
shGFP
shSTING
shSTING +STING-Flag
(IRF3)2
IRF3
Lane 1 2 3 4 5 6 7 8 9
shGFP
shSTING
shSTING+ STING-Flag
IB: STING
STING-Flag
STING
IB: Tubulin
Tubulin
lane: 1 2 3
C
Mock
poly(dA:dT)
G:C50
poly(dI:dC)
HT-DNA
(IRF3)2
IRF3
lane: 1 2 3 4 5
D
Proteinase K
Retentate
Filtrate
Benzonase
Heat Sup - + + + +
(IRF3)2
IRF3
lane: 1 2 3 4 5
Benzonase
- +
ISD
lane: 1 2
E
Cell Extract +DNA
+MgCl2, ATP
37°C, 45min
Heat treatment (95°C, 5min)
+THP1 or Raw264.7 cells
+PFO +MgCl2, ATP
30°C, 90min
Native Gel Electrophoresis
F
-
HT-DNA
HT-DNA+DNaseI
poly(dA:dT)
poly(dG:dC)
ISD
poly(I:C)
ssRNA
(IRF3)2
IRF3
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
THP-1
Raw264.7
G
THP-1
shGFP
shSTING
DNA: 0 2 4 0 2 4 hr
(IRF3)2
IRF3
p-IκBα
STING
Tubulin
lane: 1 2 3 4 5 6
H
THP-1
IFN-β RNA (fold)
DNA(hr): 0 8 0 8
shGFP
shSTING
THP-1
TNF-α RNA (fold)
DNA(hr): 0 8 0 8
shGFP
shSTING

Figure S1. Development of an in vitro complementation assay to study the STING pathway. A) A proposed DNA sensing and signaling pathway. (**B**) L929 cells stably expressing a shRNA against GFP or STING were transfected with HT-DNA for indicated times. Also transfected were L929-shSTING cells stably expressing STING-Flag that replaced endogenous STING. IFNβ RNA and IRF3 dimerization in these cells were assayed by q-RT-PCR (top) and native gel electrophoresis (middle), respectively. Cell extracts were immunoblotted with an antibody against STING or β-tubulin (bottom). (**C**) L929-shSTING cells were transfected with the indicated DNA and then cytosolic extracts from the transfected cells were incubated with PFO permeabilized THP1 cells together with [^{35}S]-IRF3. IRF3 dimerization was analyzed by native gel electrophoresis followed by autoradiography. (**D**) Similar to (C), except that cytoplasmic extracts from HT-DNA transfected L929 cells were incubated with proteinase K (lanes 3 and 4), Benzonase (lane 5) or untreated (lane 2). The proteinase K treated samples were separated by a filter with a molecular weight cut-off of 3 kDa. The retentate (large molecules; lane 3) and filtrate (small molecules, lane 4) as well as other samples were incubated with PFO permeabilized THP1 cells and then IRF3 dimerization was examined. The effectiveness of Benzonase treatment was verified by its ability to digest DNA (bottom). (**E**) Illustration of a cell-free system that synthesizes and detects the heat-resistant STING activator using DNA-supplemented L929 cytosolic extracts. (**F**) L929-shSTING cytosolic extracts were incubated with the indicated nucleic acids in the presence of ATP and then the heat-resistant supernatant was assayed for its ability to stimulate IRF3 dimerization in permeabilized THP1 cells or Raw264.7 cells. (**G and H**) THP1 cells stably expressing a shRNA against GFP or STING were transfected with HT-DNA for the indicated time, then immunoblotted with the indicated antibodies following native PAGE or SDS-PAGE (G). Total RNA was isolated for measurement of IFNβ and TNFα RNA by q-RT PCR (H).

Figure S2

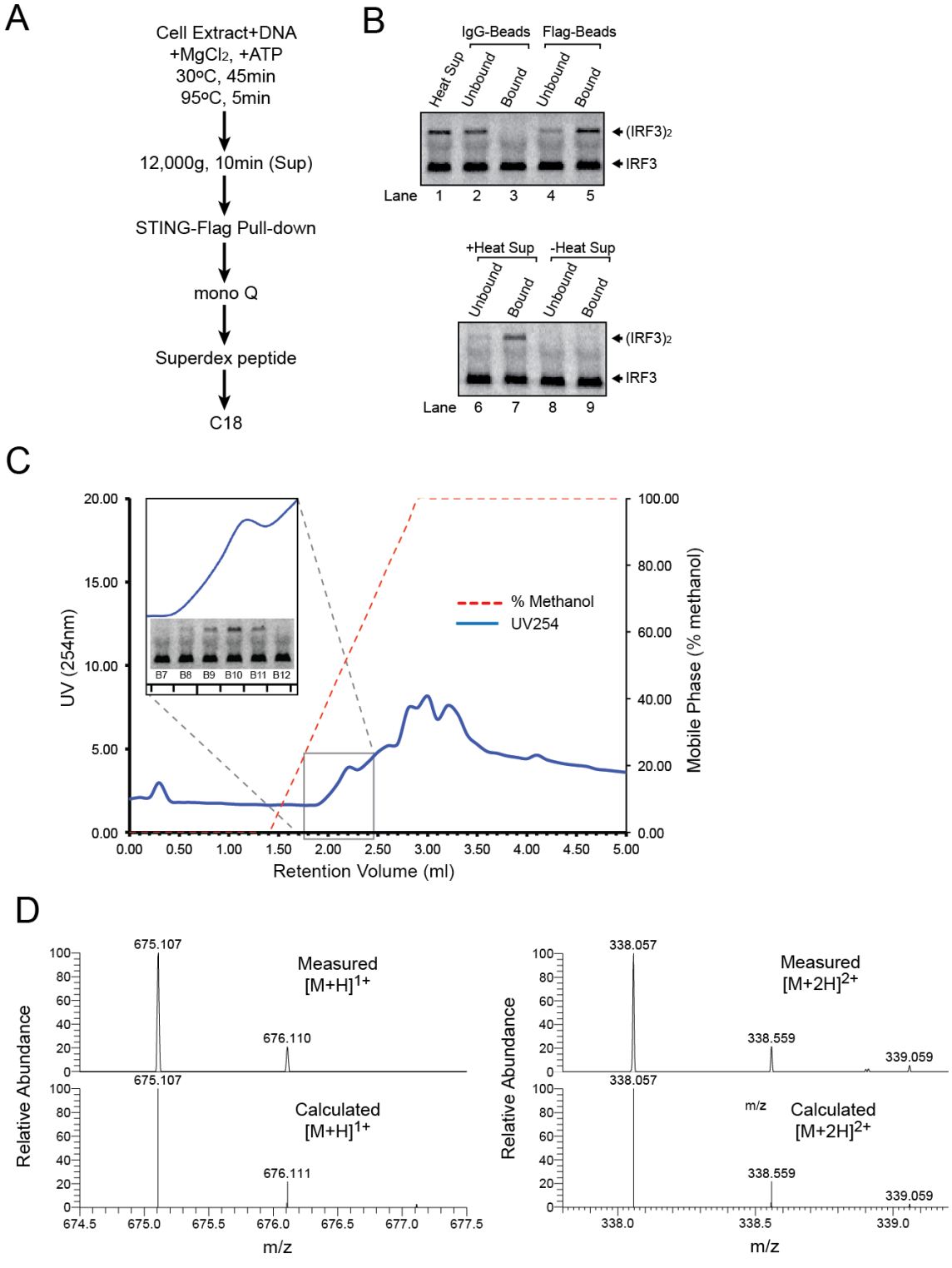
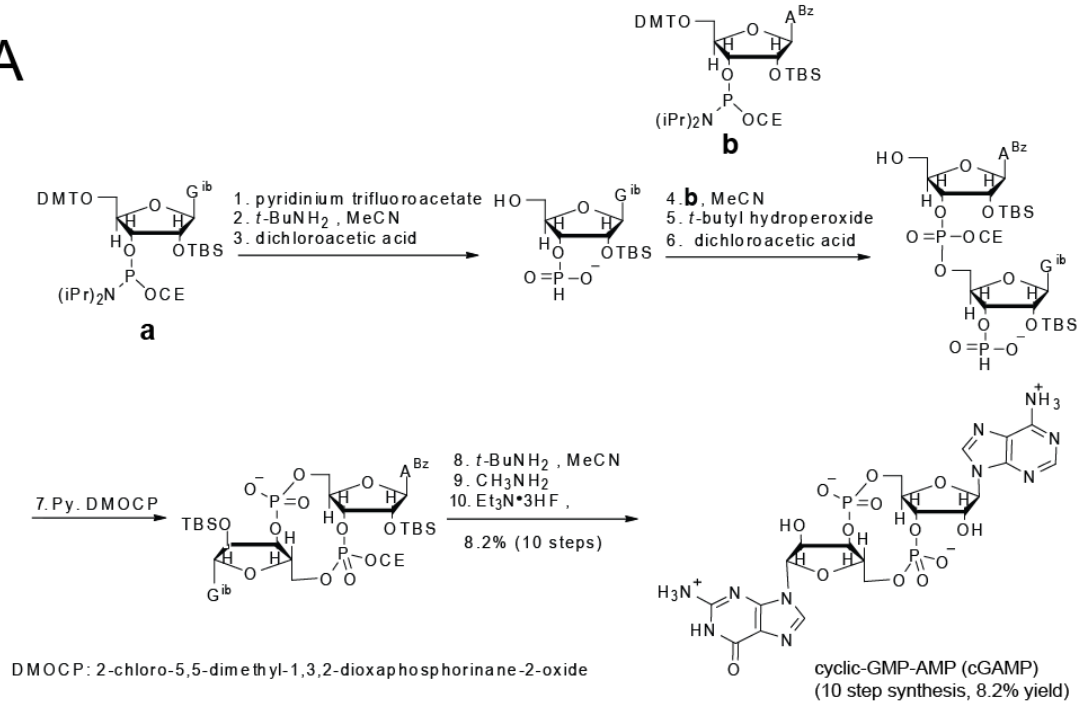

A
Cell Extract+DNA
+MgCl2, +ATP
30ºC, 45min
95ºC, 5min
12,000g, 10min (Sup)
STING-Flag Pull-down
mono Q
Superdex peptide
C18
B
IgG-Beads
Flag-Beads
Heat Sup
Unbound
Bound
Unbound
Bound
(IRF3)2
IRF3
Lane 1 2 3 4 5
+Heat Sup
-Heat Sup
Unbound
Bound
Unbound
Bound
(IRF3)2
IRF3
Lane 6 7 8 9
C
UV (254nm)
Mobile Phase (% methanol)
% Methanol
UV254
B7 B8 B9 B10 B11 B12
Retention Volume (ml)
D
Relative Abundance
675.107
Measured
[M+H]1+
676.110
675.107
Calculated
[M+H]1+
676.111
m/z
338.057
Measured
[M+2H]2+
338.559
339.059
338.057
Calculated
[M+2H]2+
338.559
339.059
m/z

Figure S2. Purification of the STING activator and its identification as cGAMP. (**A**) Illustration of the synthesis and purification of the small molecule STING activator from L929 cytosolic extracts. (**B**) Cell lysates from L929 cells stably expressing STING-Flag were incubated with Flag antibody (M2) agarose. The beads were then incubated with heat-resistant supernatant prepared from an in vitro reaction containing L929 cytosolic extracts and HT-DNA (lanes 4-7). IgG beads were used as a negative control (lanes 2 and 3). Both beads were washed and then boiled in water to elute the heat-resistant small molecule STING activator. The eluted ('bound') and unbound fractions were tested for their ability to stimulate IRF3 in permeabilized THP1 cells. In lanes 8 and 9, the beads containing STING-Flag were not incubated with heat-resistant supernatant and served as a negative control. (**C**) Purification of the small molecule STING activator by HPLC using a C18 column. The starting materials for this column had been partially purified through three steps as described in A. The inset indicates IRF3 dimerization assays for selected fractions from the C18 column. (**D**) High resolution high accuracy measurement of m/z values of the ions present in the purified fractions containing the STING activator. The measurement was performed using Orbitrap in Q Exactive. The calculated m/z values of cGAMP are shown for comparison. The lower intensity peaks represent isotopes of the major ions.

Figure S3

A


DMTO
A^Bz
OTBS
(iPr)2N
OCE
b
G^ib
a
1. pyridinium trifluoroacetate
2. t-BuNH2 , MeCN
3. dichloroacetic acid
HO
4. b, MeCN
5. t-butyl hydroperoxide
6. dichloroacetic acid
7. Py. DMOCP
TBSO
8. t-BuNH2 , MeCN
9. CH3NH2
10. Et3N•3HF ,
8.2% (10 steps)
DMOCP: 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane-2-oxide
cyclic-GMP-AMP (cGAMP)
(10 step synthesis, 8.2% yield)

B

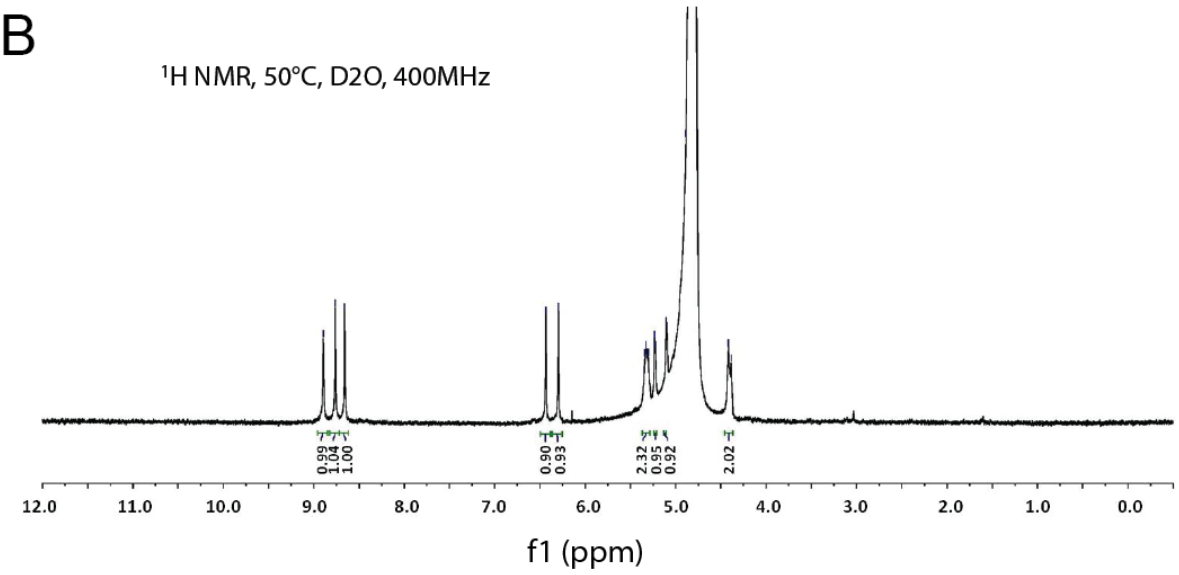

1H NMR, 50°C, D2O, 400MHz
0.99
1.04
1.00
0.90
0.93
2.32
0.95
0.92
2.02
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

Figure S3. Chemical synthesis of cGAMP. (**A**) A ten-step one-flask scheme to synthesize cGAMP from commercially available 5'-*O*-DMT-*N*-2-Isobutyryl-Guanosine-2'-*O*-TBDMS-Phosphoramidite (a) and 5'-*O*-DMT-Adenosine(Bz)-2'-*O*-TBDMS-Phosphoramidite (b). The detailed protocol is described in Materials and Methods. (**B**) ^{1}H-NMR spectrum of chemically synthesized cGAMP. Chemical shifts are reported in ppm (δ) referenced to the residual signal of the solvent (D_2O: δ 4.79 ppm). Coupling constants (*J*) are expressed in Hertz. The multiplicities are presented as follows: s = singlet, d = doublet, m = multiplet. ^{1}H NMR data: =8.90 (s, 1H), 8.76 (s,1H), 8.66 (s,1H), 6.43 (s,1H), 6.30 (s,1H), 5.36-5.28 (m, 2H), 5.23 (d, 1H, J = 4.4 Hz), 5.10 (d, 1H, J = 4.4 Hz), 4.44-4.36 (m, 2H).

Figure S4

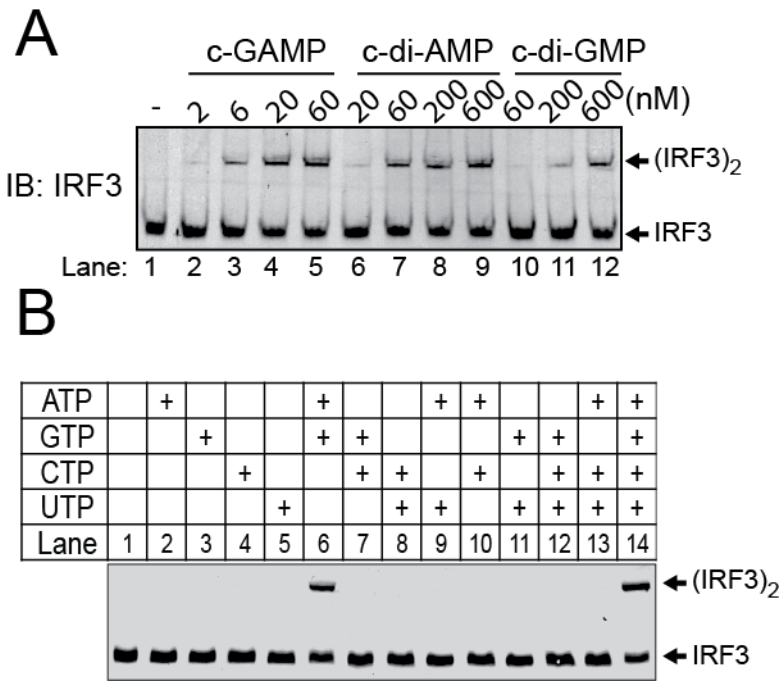


Figure S4. **cGAMP is a potent IRF3 activator and is synthesized from ATP and GTP**. (**A**) Different concentrations of cGAMP, c-di-GMP and c-di-AMP were delivered into L929 cells followed by IRF3 dimerization assays. (**B**) A partially purified fraction containing cGAMP synthesizing activity from L929 cells was incubated with the ribonucleotides as indicated. The reaction mixtures were heated to denature proteins and the supernatants were assayed for their ability to stimulate IRF3 dimerization in permeabilized Raw264.7 cells.

Figure S5

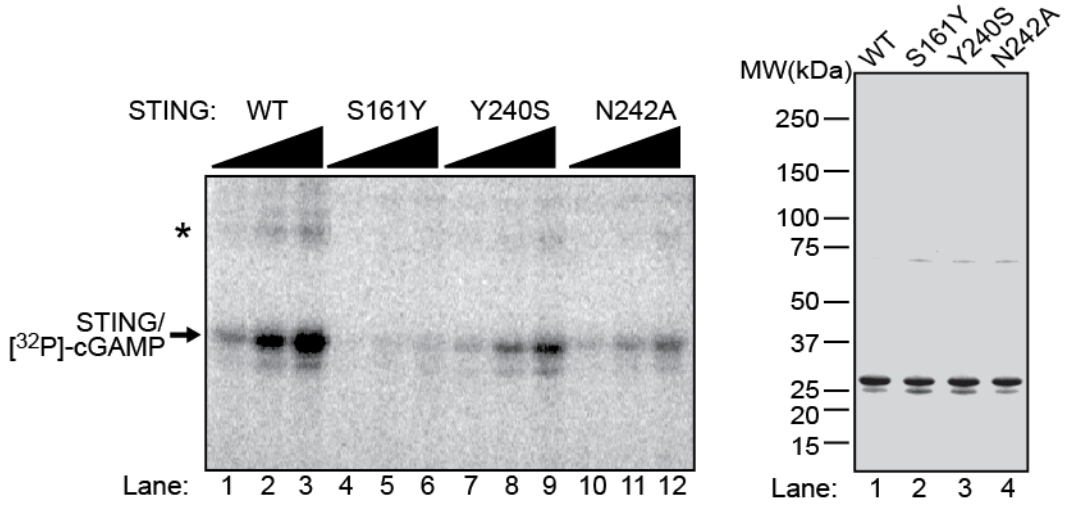


Figure S5. Characterization of STING mutants with defective binding to cGAMP. Increasing amounts of recombinant STING mutant proteins were incubated with [^{32}P]-cGAMP, followed by UV crosslinking, SDS-PAGE and autoradiography (left). The right panel shows Coomassie blue staining of the STING proteins used in the binding assays.

References

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