

Supplementary figure legends

Figure S1. Dose-dependent antiviral activity of CMA

A, Antiviral activity of CMA in macrophages was studied using a FFLuc encoding VSV replicon. B, Macrophages were stimulated with decreasing concentrations of CMA (500 μ g/ml – 31,25 μ g/ml in two-fold dilutions) and subsequently infected with the VSV replicon particles. 18h after stimulation luciferase activity was assessed (left panel) and cell viability was determined (right panel). C, pIFN β -firefly-luciferase macrophages were treated with decreasing concentrations of CMA (500 μ g/ml – 31,25 μ g/ml in two-fold dilutions). After 18h luciferase activity was assessed (left panel) and cell viability was determined (right panel). Representative results out of 3 independent experiments are depicted.

Figure S2. CMA-triggered type I IFN response is TLR- and MAVS-independent and mediated via TBK1/IKK ϵ

A, Wild type and TBK1/IKK ϵ double-deficient MEFs were transfected with poly(I:C), pppRNA, plasmid DNA or stimulated with CMA (500 μ g/ml) for 2h. Cells were collected and assessed for phospho-IRF3. Bone marrow-derived wild type (B), MyD88^{-/-} (C) and Trif^{-/-} (D) macrophages (M Φ) were stimulated as described above. After 18h supernatants were collected and IP-10 production was determined by ELISA. In addition phospho-IRF3 was analyzed 2h after stimulation. (E) Bone marrow-derived wild type or (F) MAVS^{-/-} macrophages were stimulated with pppRNA or CMA. ELISA and western blot were performed as described for A-D. Representative results out of 2 independent experiments are depicted.

Figure S3. Antiviral activity of CMA is STING-dependent

A, Supernatants of pretreated macrophages were assessed for antiviral properties using the VSV-FFLuc replicon in supernatant transfer experiments. B, bone marrow-derived wild type or STING-deficient macrophages were left untreated, transfected with pppRNA or stimulated with CMA (500 μ g/ml). After 18h the supernatants were harvested and diluted 1:20. The diluted supernatants (grey bars) were then transferred onto macrophages that were subsequently infected with VSV replicon particles. As a control infected macrophages were left untreated or stimulated with the equivalent concentration of CMA (25 μ g/ml; 1:20 dilution of 500 μ g/ml) in the diluted supernatants (hatched bars). Representative results out of 2 independent experiments are depicted.

Figure S4. HEK293T stably expressing murine STING respond to CMA

A-B, HEK293T cells stably expressing murine STING were transiently transfected with a total amount of 200ng DNA per 96-well, whereas 12.5ng of pIFN β -GLuc (A) or pELAM-GLuc (B)

reporter plasmid were included. An empty pCI vector served as a stuffer. After 24h cells were transfected with poly(I:C), pppRNA, ISD and c-diGMP or stimulated with LPS or decreasing concentrations of CMA (from 500 μ g/ml to 15,62 μ g/ml). Luciferase activity was measured after an additional period of 24h in the supernatant (upper panel). Cell viability was determined by CellTiter-Blue assay (lower panel). Representative results out of 3 independent experiments are depicted.

Figure S5. R231A dissociates c-diGMP- from CMA-mediated STING activation

A-C, 293T cells were transiently transfected with the indicated STING constructs (25, 12.5, 6.25 and 0ng), whereas 12.5ng of pIFN β -GLuc reporter plasmid were included. For titrations, an empty pCI vector served as a stuffer to obtain 200ng total plasmid DNA. After 24h cells were stimulated with c-diGMP or CMA (500 μ g/ml and 125 μ g/ml). Luciferase activity was measured after an additional period of 24h in the supernatant and data were normalized to the condition without STING overexpression. Plasmids coding for full-length murine STING (A), murine STING-I199N (B) and murine STING-R231A (C) were tested. D, Expression of the above-described constructs was studied in 293T cells 24h after transfection (200ng per 96-well) using western blot, whereas β -Actin served as a loading control. Representative results out of 3 independent experiments are depicted.

Figure S6. The CTT is not required for CMA binding

The interaction of mSTING-LBD- Δ CTT with c-diGMP, c-diAMP and CMA detected by thermal shift assay. Purified mSTING LBD lacking the CTT (138-341) (i) was analyzed with different concentrations of c-diGMP/c-diAMP/CMA. (ii) Thermal shifts of (iii) fluorescence intensity vs. temperature are shown. Representative results out of 2 experiments are depicted.

Figure S7. Comparison of mouse STING:CMA and human STING:c-diGMP

Superposition of mouse STING, bound to two CMA molecules (brown ribbon model and with magenta CMA stick models), with human STING, bound to c-diGMP (PDB accession code 4F5D, cyan ribbon model with green c-diGMP stick model). The superposition shows a similar dimer structure, shared ligand binding site and overall similar folding of the lid region, suggesting that CMA activates murine STING by a similar structural mechanism than c-diGMP.

Table S1. Data Collection and Refinement Statistics

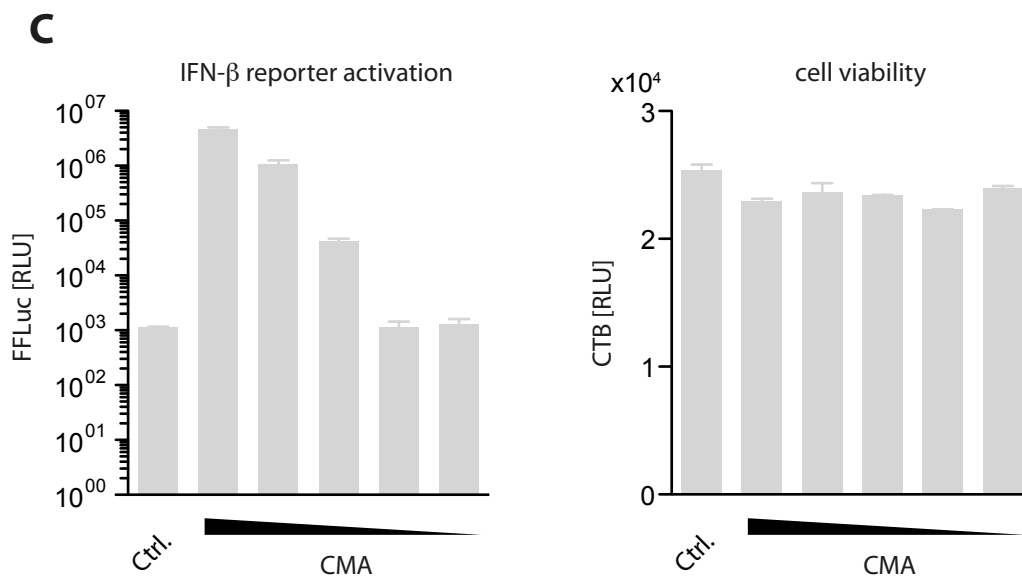
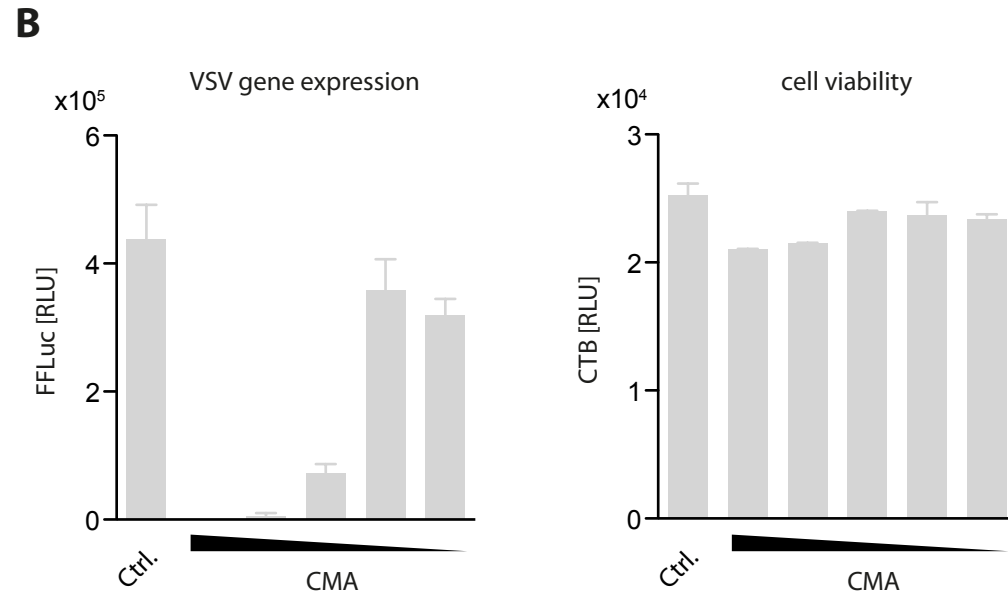
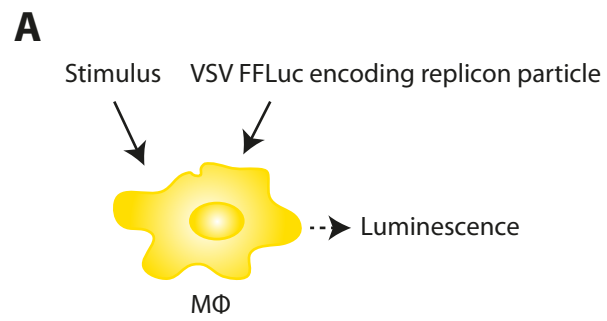
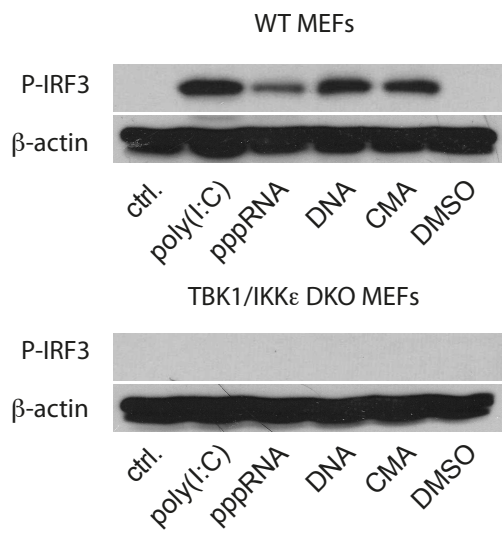
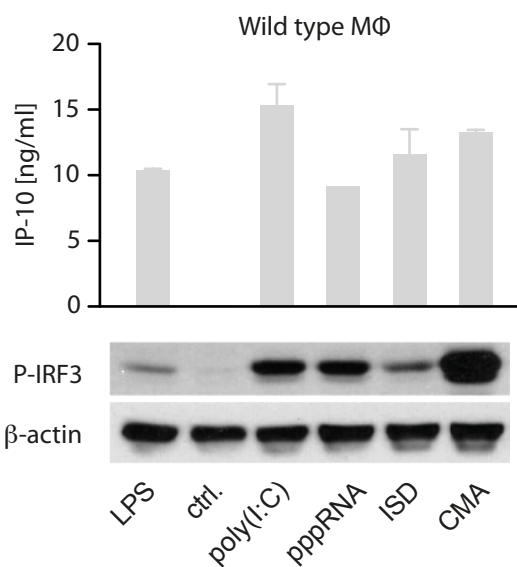
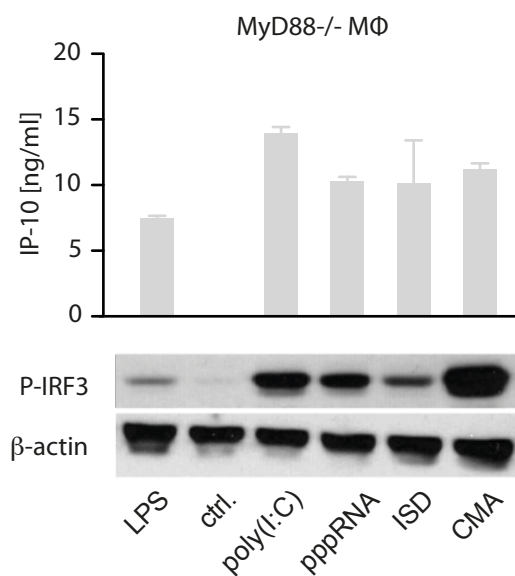
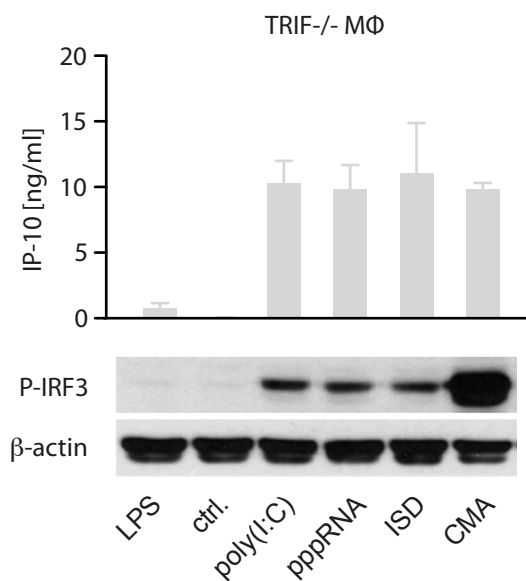
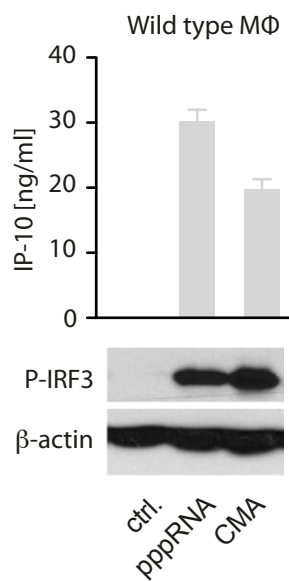
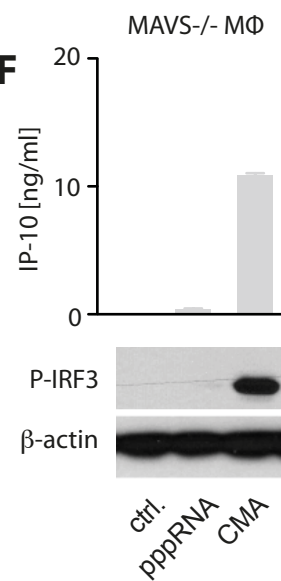
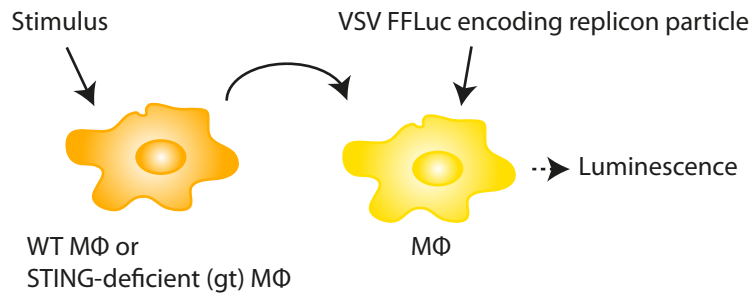
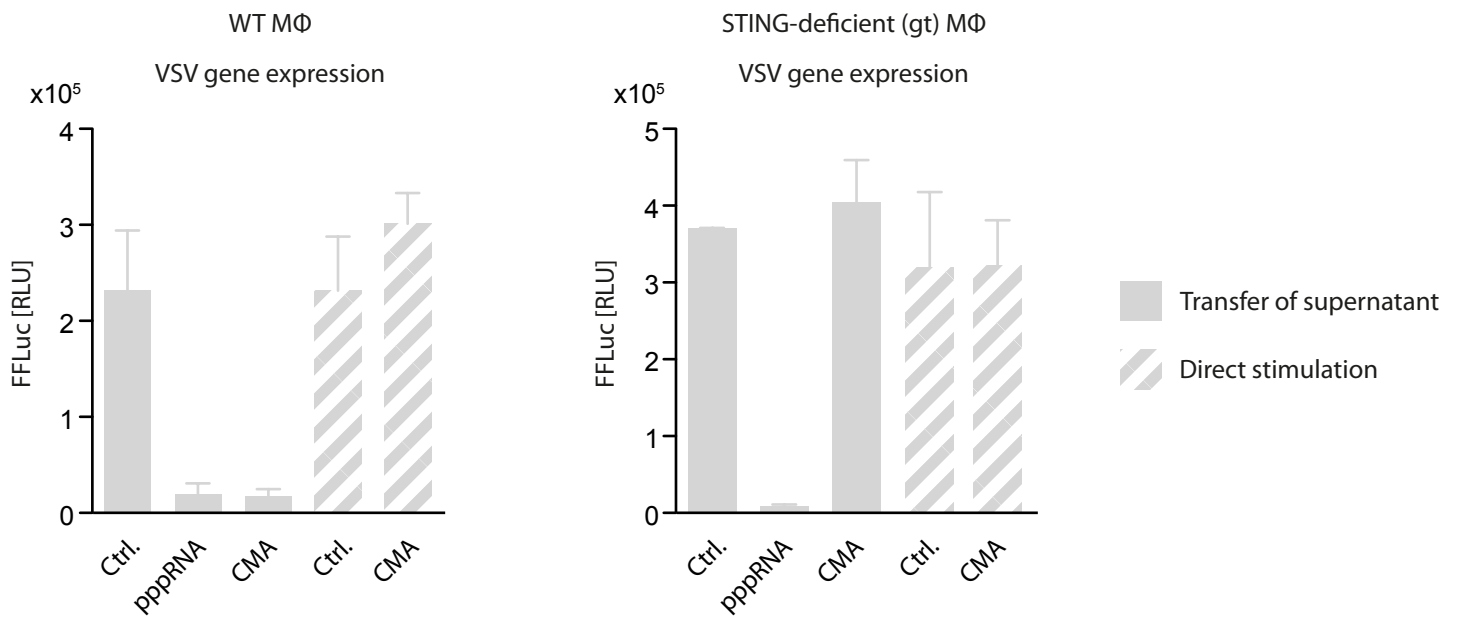
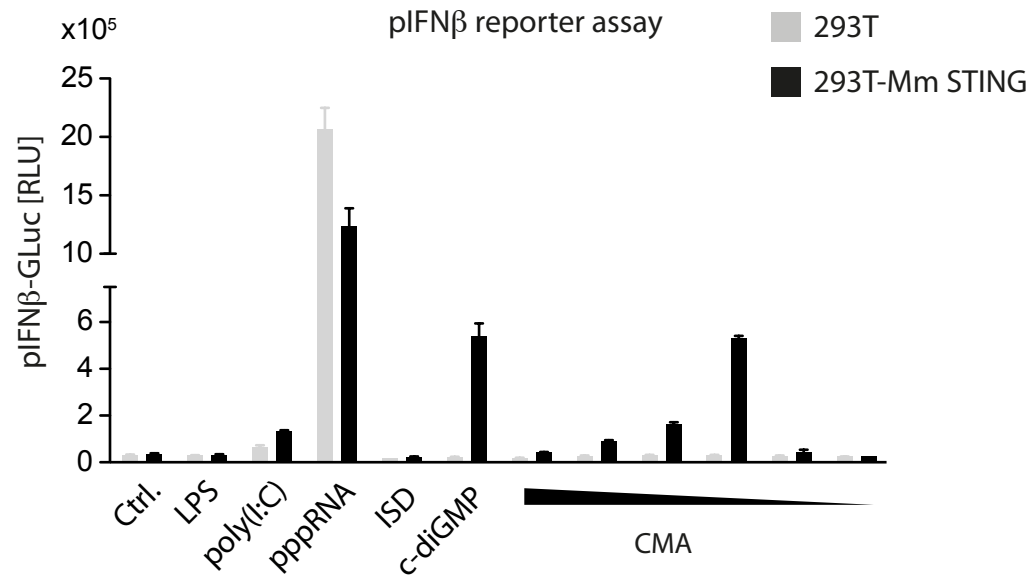
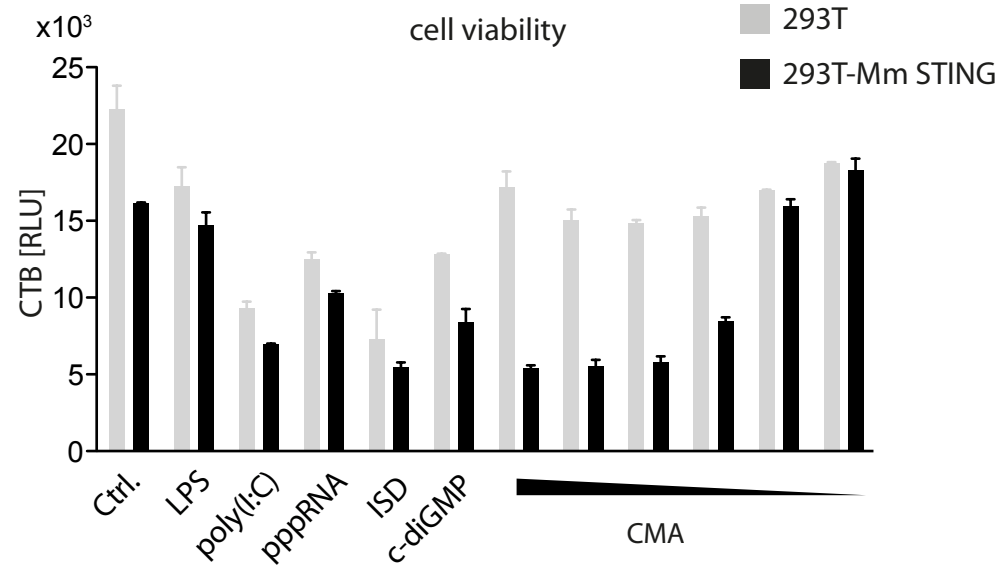
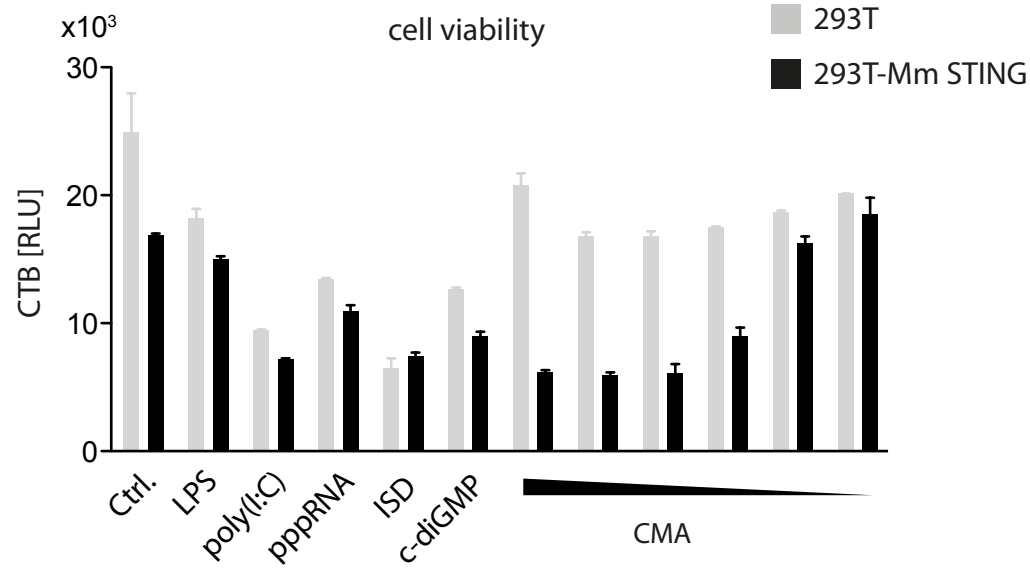
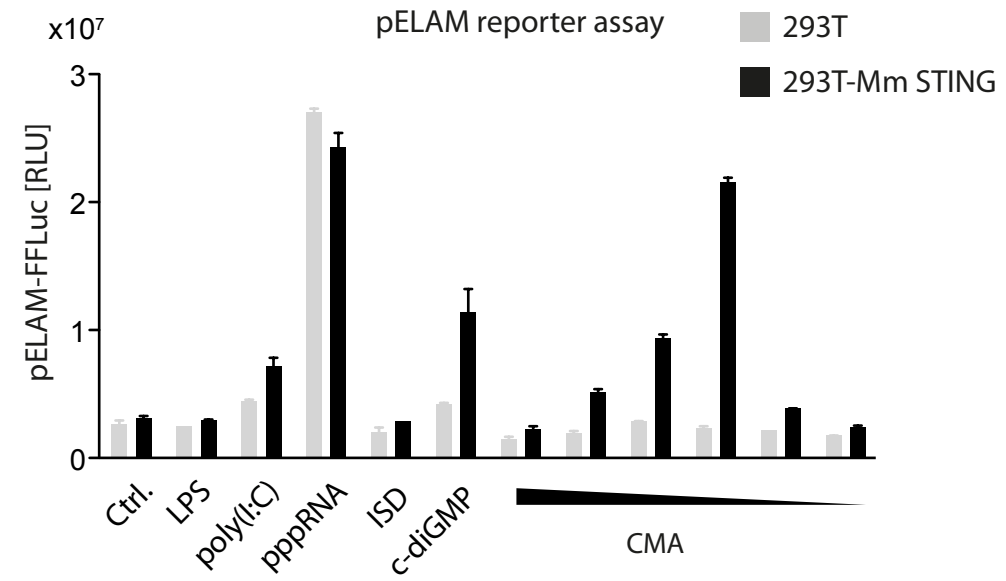


Figure S1

A**B****C****D****E****F****Figure S2**

A**B****Figure S3**

A**B****Figure S4**

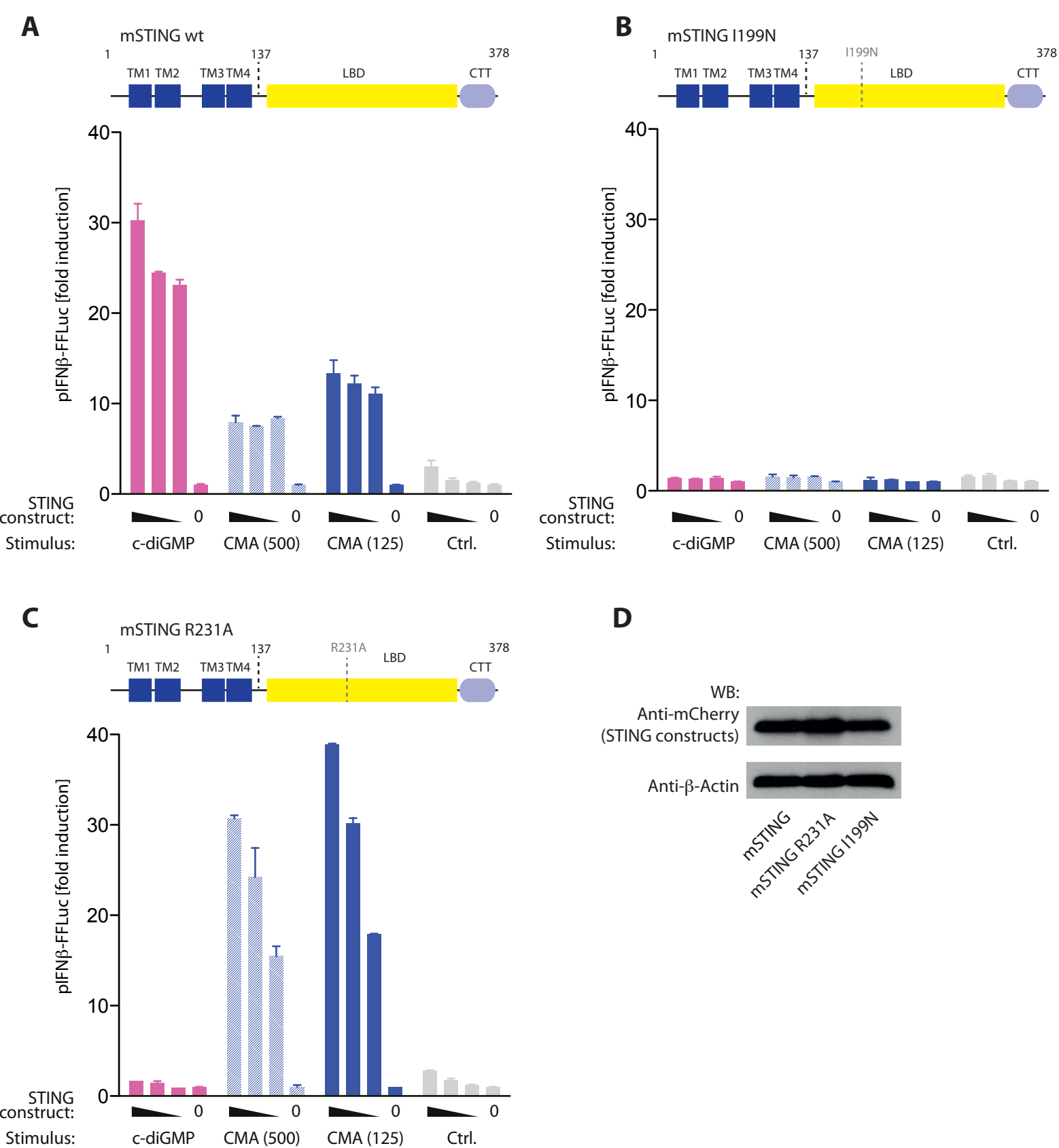


Figure S5

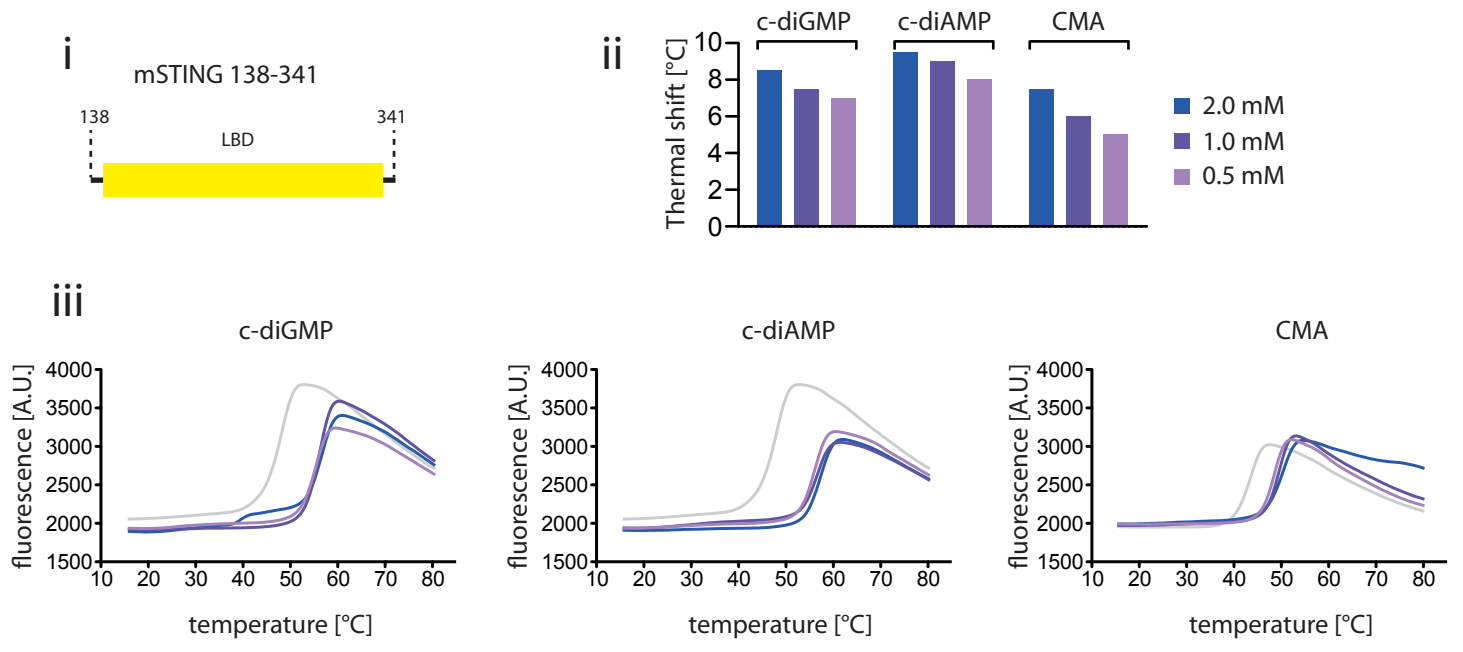


Figure S6

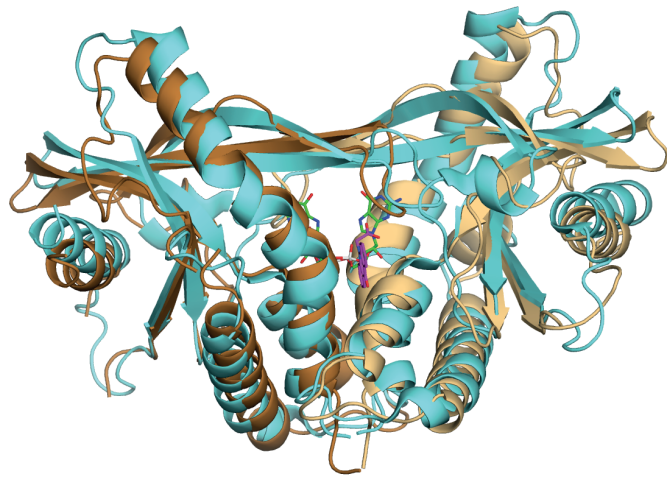


Figure S7

Data collection

Space group	R3
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	108,54 108.54, 101.83
<i>a</i> , <i>b</i> , <i>g</i> (°)	90.0, 90.0, 120.0
Resolution (Å)	50.0 – 2.75 (2.91 – 2.75) *
<i>R</i> _{meas} (%)	5.1 (77.2)
<i>I</i> / <i>σ</i> <i>I</i>	19.17 (1.97)
Completeness (%)	99.3 (98.3)
Redundancy	4.0 (3.9)

Refinement

Resolution (Å)	34.38 – 2.75
No. reflections	11444
<i>R</i> _{work} / <i>R</i> _{free} (%)	21.0 / 23.7
No. atoms	
Protein	2989
Ligand/ion	38
Water	32
<i>B</i> -factors	
Protein	73,5
Ligand/ion	39,2
Water	62,5
R.m.s deviations	
Bond lengths (Å)	0,007
Bond angles (°)	1,159
Ramachandran values	
Favored	343
Allowed	19
Outliers	0
PDB Accession code	4JC5

* Values in parentheses are for highest resolution shell

Table S1. Data Collection and Refinement Statistics