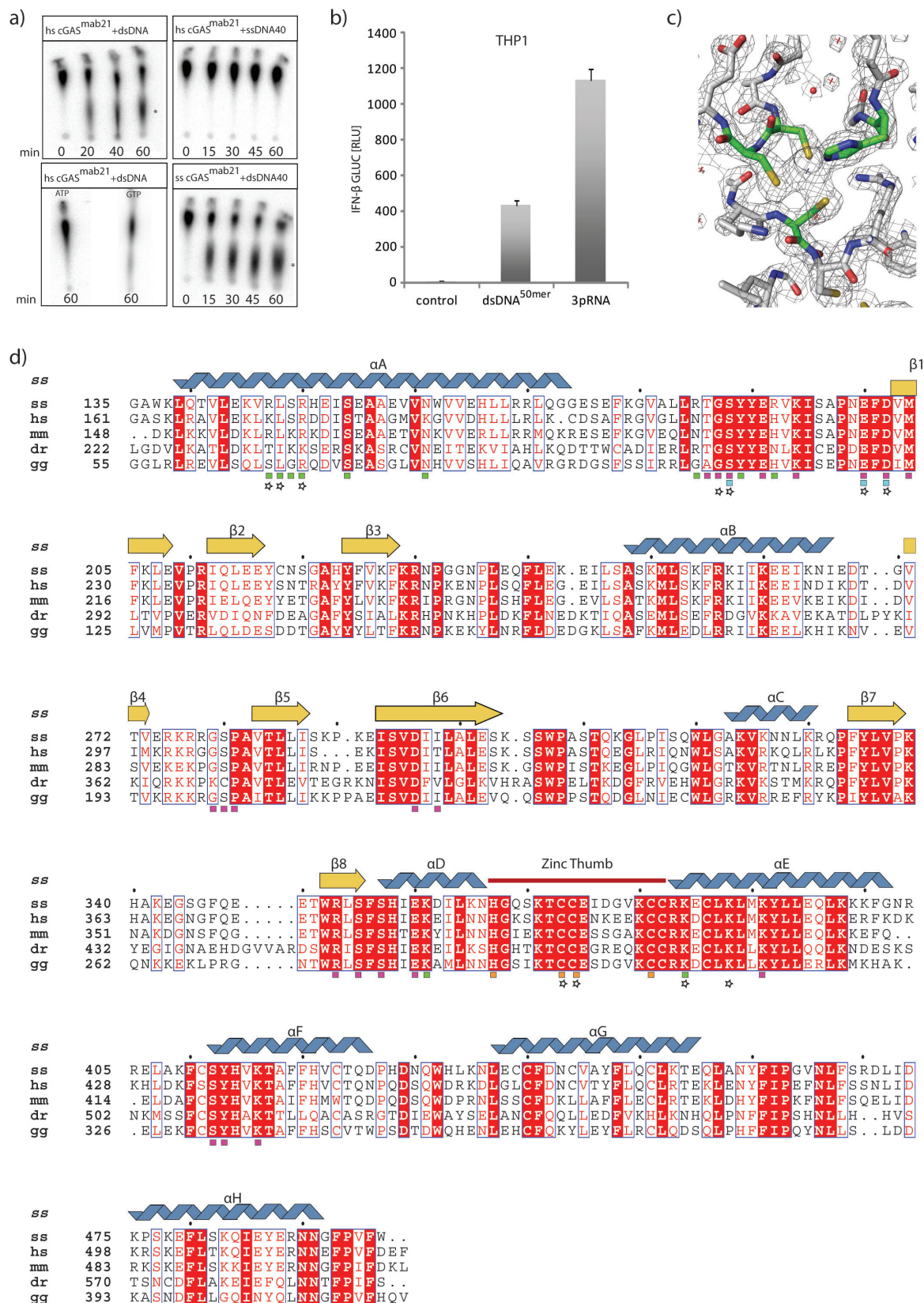


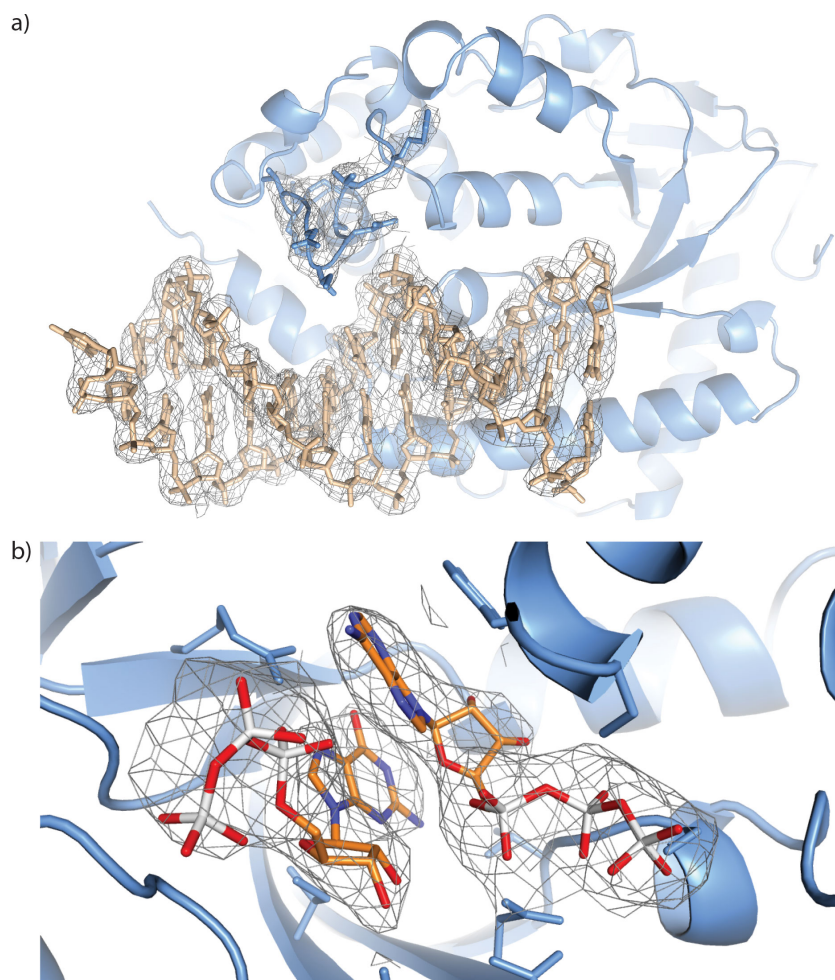
SUPPLEMENTARY INFORMATION

doi:10.1038/nature12305



Supplemental Figure S1: Activity assays, electron density and cGAS sequence alignment

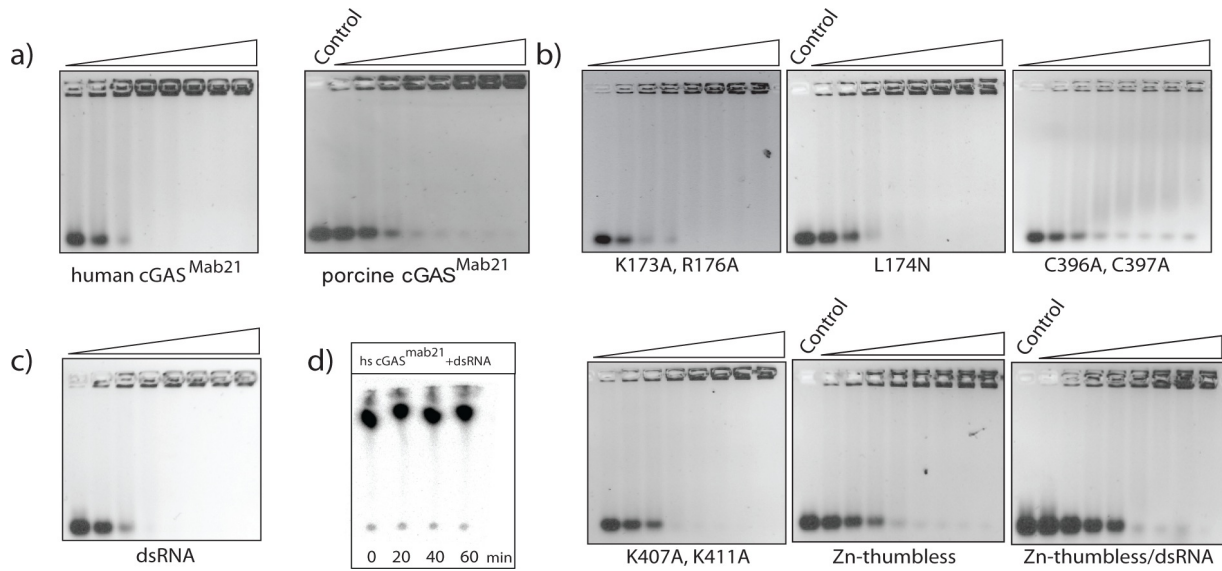
- a) Activity assays with human and porcine cGAS^{Mab21} in the presence of ATP, GTP and ATP $\alpha^{32}\text{P}$. Upper panel left: 2 μM human cGAS^{Mab21} + dsDNA (50mer), right: 1 μM human cGAS^{Mab21} + 0.5 μM ssDNA (40mer). Lower panel left: 2 μM human cGAS^{Mab21} + 3 μM dsDNA (50mer) using only ATP/ATP $\alpha^{32}\text{P}$ and only GTP/GTP $\alpha^{32}\text{P}$, respectively; right: 1 μM porcine cGAS^{Mab21} + 0.5 μM dsDNA (40mer). The reactions were stopped at indicated time points by plotting on TLC plates. Both human and porcine cGAS^{Mab21} are activated by dsDNA while ssDNA and single nucleotides fail to induce activity.
- b) IFN- β stimulation in THP1 cells by dsDNA^{50mer} or with 5'triphosphate dsRNA (3pRNA). 200ng of indicated ligand was transfected to THP1 cells along with IFN- β promoter reporter plasmid pIFN- β -GLUC. Luciferase activity is plotted: mean \pm sd (n=3). The dsDNA^{50mer} used in *in vitro* assays induces interferon production in THP1 cells. 3pRNA, which induces the RIG-I pathway, is used as positive control. The negative control is without ligand.
- c) 2F_o-F_c electron density overlaid with the final model around the thumb (carbons green). The 2F_o-F_c map is contoured at 2 σ .
- d) Structure based alignment of selected cGAS^{Mab21} sequences (abbreviations: *Sus scrofa*: **ss**, *Homo sapiens*: **hs**, *Mus musculus*: **mm**, *Danio rerio*: **dr**, *Gallus gallus*: **gg**) with highlighted conserved residues and annotated motifs. The secondary structural elements are shown on top of the alignment for the porcine homolog, color coding is analogous to Fig. 1. The squares mark residue contacts: green => DNA, magenta => GTP/ATP, cyan => Mg²⁺, orange => Zn²⁺. Stars denote residues that are mutated in this study.



Supplemental Figure S2: Electron densities

a) Ribbon model of cGAS^{Mab21(td)} (blue) with bound dsDNA (beige stick representation). 2F_o-F_c electron density for the DNA and the Zn-thumb residues are shown at a contour level of 1σ.

b) 2F_o-F_c electron density around the two nucleotides in the active site (contour level of 1σ).



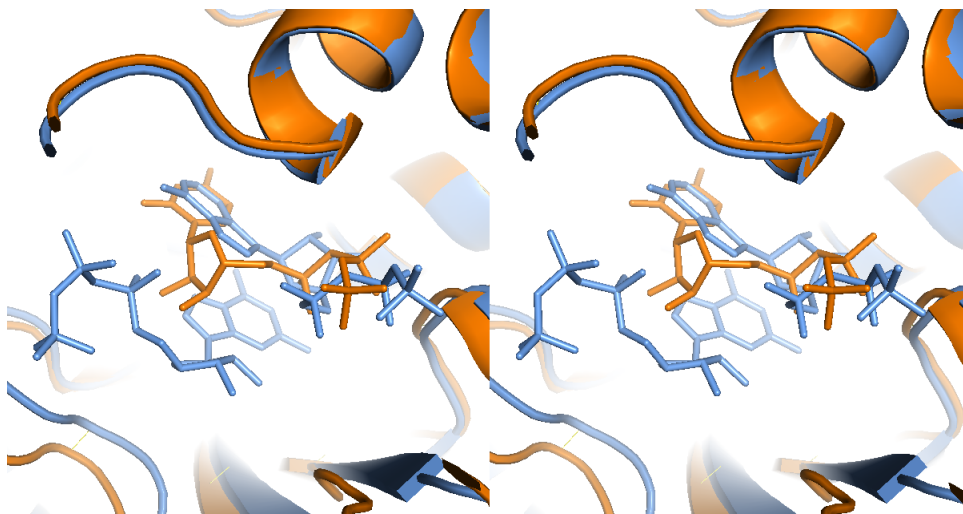
Supplementary Figure S3: Electrophoretic mobility shift and activity assays

a) Electrophoretic mobility shift analysis of dsDNA^{50mer} (0.2 μM) with human or porcine cGAS^{Mab21} (protein concentrations are 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50 μM (triangle)). Control: without protein.

b) Like a), but with human cGAS^{Mab21} mutants.

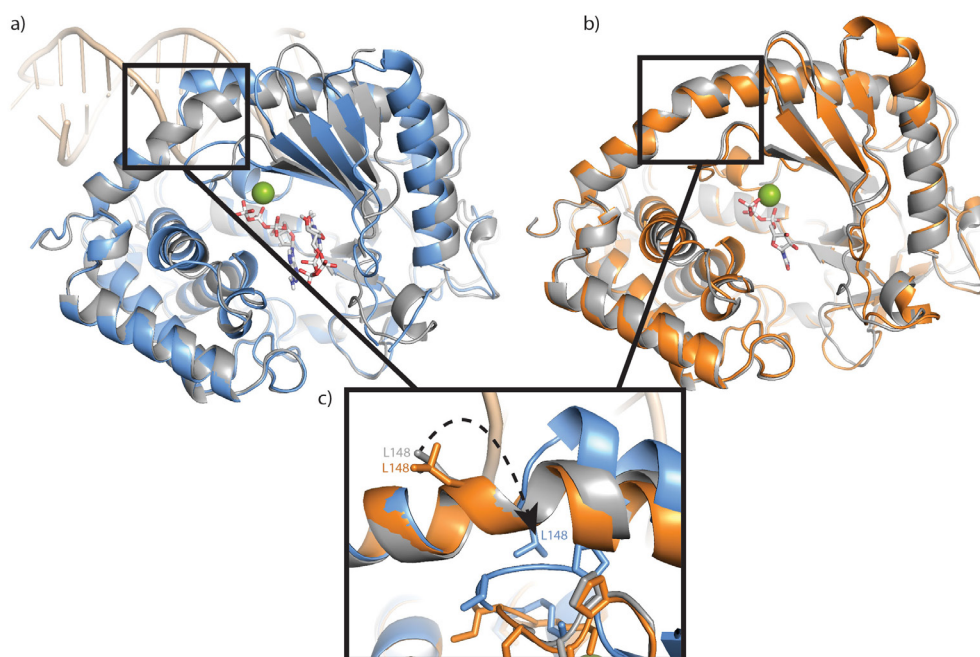
c) Like a) but binding of human cGAS^{Mab21} to dsRNA^{50mer}.

d) Activity assay with 2 μM human cGAS^{Mab21} + 3 μM dsRNA^{50mer}.



Supplementary Figure S4: Comparison of UTP- and ATP/GTP-bound structure of cGAS^{Mab21}

The stereo figure shows the active site of the superimposed structures of the UTP-bound state of cGAS^{Mab21} (orange) and the cGAS^{Mab21(td)}:DNA:GTP:ATP complex (blue), respectively. The ribose moiety is flipped in UTP as compared to ATP.

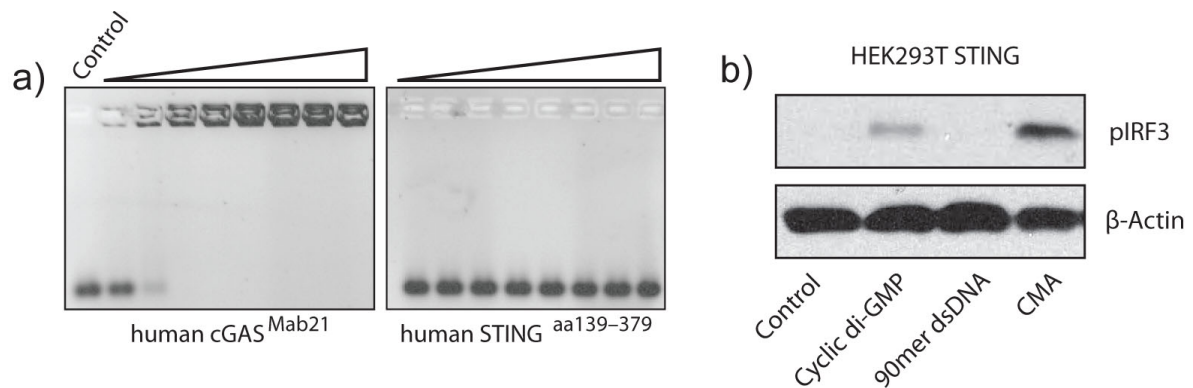


Supplementary Figure S5: Comparison of cGAS^{Mab21} structures

a) Superposition of the DNA-bound (blue) and apo (grey) cGAS^{Mab21} structures.

b) Superposition of apo (grey) cGAS^{Mab21} and UTP-bound (orange) cGAS^{Mab21}.

c) The Leu148 flip upon DNA binding. In the absence of DNA (orange UTP-bound and grey apo structures) Leu148 is solvent exposed. In the DNA binding conformation (blue), Leu148 flips and helps stabilize the nucleotide-binding loop.



Supplementary Figure S6: Comparison of STING and cGAS DNA binding and STING in vivo activity.

a) Electrophoretic mobility shift analysis of dsDNA (0.2 μ M) with human cGAS^{Mab21} (left) and human STING^{aa139-379} (right) (protein concentrations are 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, 10, 15 μ M, control: without protein). While human cGAS^{Mab21} readily binds dsDNA at a concentration as low as 0.5 μ M, human STING^{aa139-379} fails binding even at concentration as high as 15 μ M.

b) Western blot analysis of IRF3 phosphorylation which indicates interferon stimulation. The HEK293T cells stably expressing STING were induced with cyclic-di-GMP, dsDNA^{90mer} and 10-carboxymethyl-9-acridanone (CMA). The proven STING ligands cyclic-di-GMP and CMA induce IRF3 phosphorylation while DNA^{90mer} fails, suggesting requirement of a sensor that detects DNA upstream of STING. β -actin was used as loading control.

Supplemental Table S1 Data collection and refinement statistics

	SeMet	Apo	+UTP	DNA-GTP-ATP complex
Data collection				
Space group	C222 ₁	C222 ₁	P2 ₁ 2 ₁ 2 ₁	C222 ₁
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	47.5, 119.9, 140.9	47.4, 118.0, 142.6	80.6, 97.7, 107.0	86.2, 111.7, 117.6
α , β , γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	47.1 (2.5)*	47.5 (2.0)*	46.9 (2.28)*	999.0 (3.1)*
<i>R</i> _{merge}	9.0 (44.7)*	4.7 (57.5)*	5.8 (59.1)*	9.0 (90.7)*
<i>I</i> / σ <i>I</i>	13.8 (3.3)*	20.9 (2.6)*	12.0 (2.0)*	17.0 (1.9)*
Completeness (%)	98.7 (92.0)*	99.6 (98.4)*	95.9 (93.3)*	98.6 (91.7)*
Redundancy	6.8 (6.4)*	6.4 (5.9)*	2.3 (2.3)*	6.9 (6.6)*
Wavelength (Å)	0.97961	1.00665	0.97934	0.97626
Refinement				
Resolution (Å)		45.5 - 2.0	44.6 - 2.27	68.3 - 3.08
No. reflections		51611	72224	10739
<i>R</i> _{work} / <i>R</i> _{free} (%)		18.6 / 21.3	17.0 / 20.4	25.5 / 25.8
No. atoms				
Protein		2878	5969	2899
DNA		-	-	555
Ligand/ion		54	62	73
Water		132	306	2
<i>B</i> -factors				
Protein		52.2	38.0	101.4
DNA		-	-	113.6
Ligand/ion		65.6	41.5	129.5
Water		45.1	38.2	95.59
R.m.s. deviations				
Bond lengths (Å)		0.008	0.009	0.004
Bond angles (°)		1.07	1.19	0.888
Ramachandran (%)				
Favored		97.4	98.6	96.0
Allowed		2.3	1.4	4.0
Outliers		0.3	0	0
PDB Accession Code		4JLX	4JLZ	4KB6

*Values in parentheses are for the highest-resolution shell.