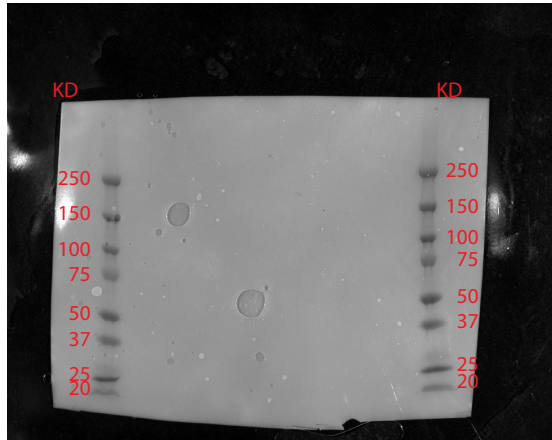

Supplementary information

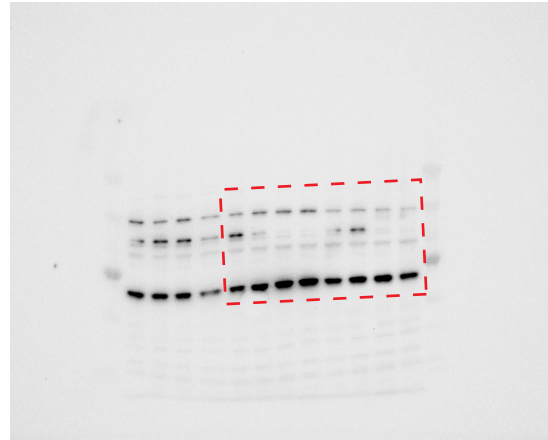
Ubiquitin-like conjugation by bacterial cGAS enhances anti-phage defence

In the format provided by the authors and unedited

Used in Fig. 1b

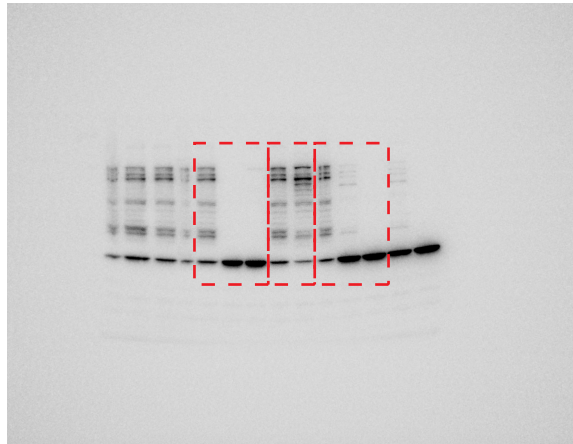
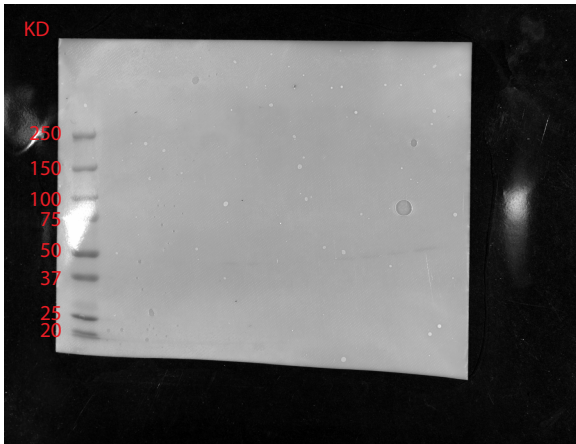


DTT (mM): - 1 10 100 - - - -
H₂O (mM): - - - - - 1 10 100



anti-Flag
Flag-Cap2

Used in Fig. 1e

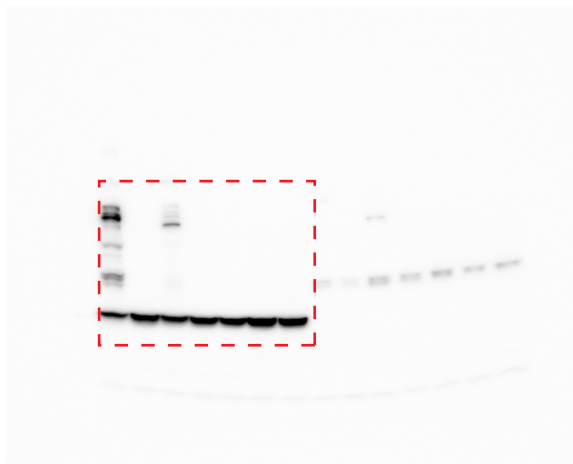
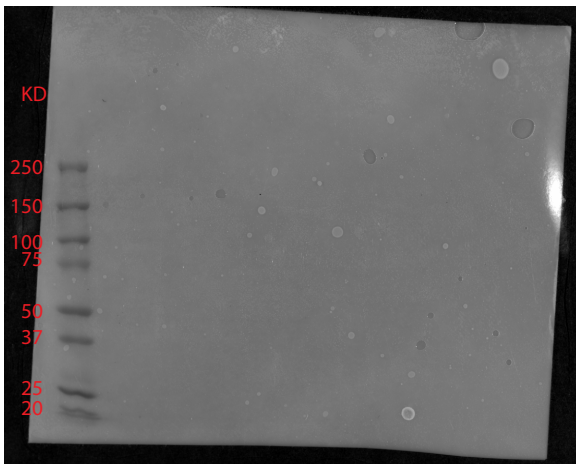


anti-Flag
Flag-cGAS

WT Cap2 C493A/C496A
Cap2 C91A WT Cap3 E38A
WT cGAS G432A
cGAS ΔG432

left right middle
panel panel panel

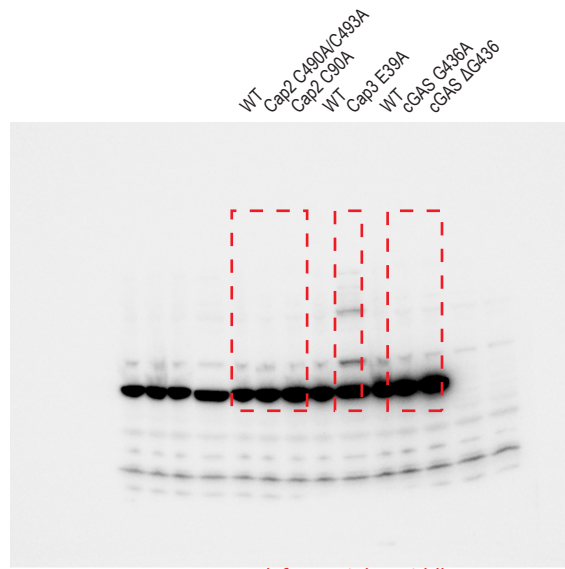
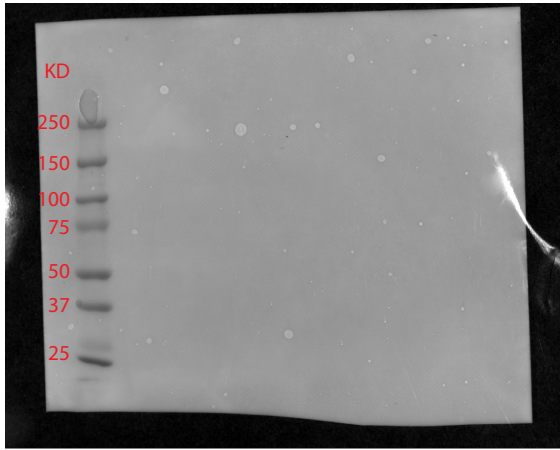
Used in Fig. 1f



anti-Flag
Flag-cGAS

WT cGAS ΔG432
cGAS G432A
cGAS G432S
cGAS G432V
cGAS G432R
cGAS G432W

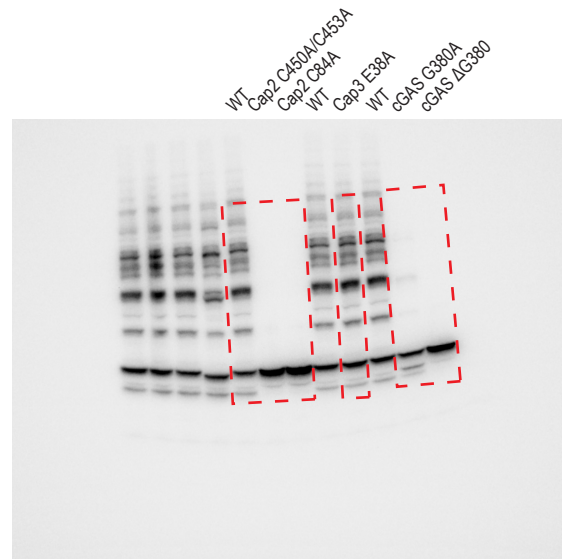
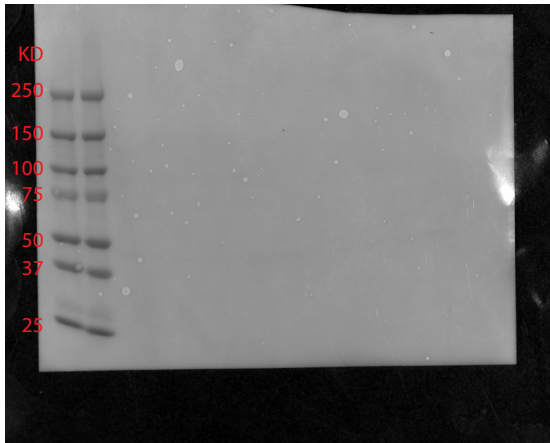
Used in Fig. 1g



anti-Flag
Flag-cGAS

left panel right middle panel panel

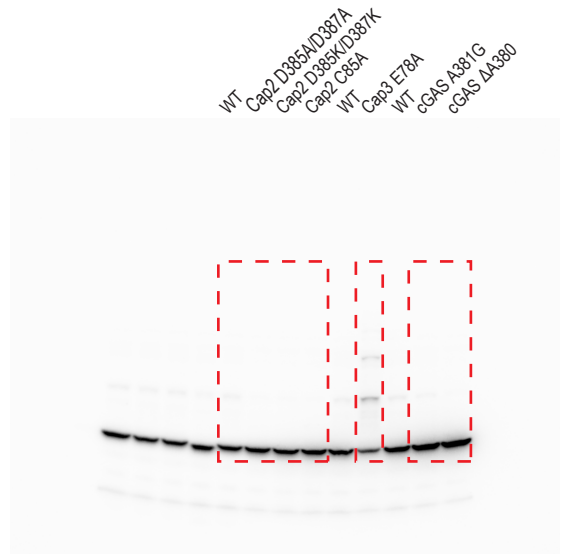
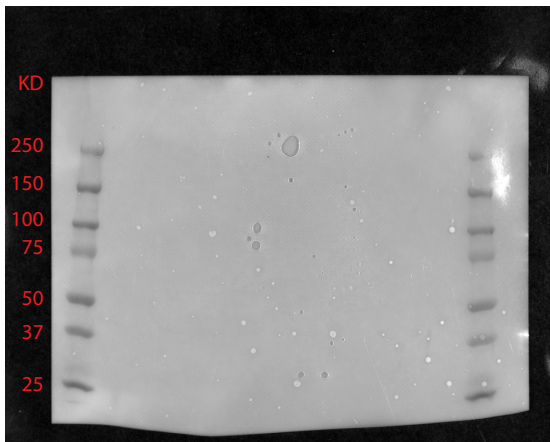
Used in Fig. 1h



anti-Flag
Flag-cGAS

left panel right middle panel panel

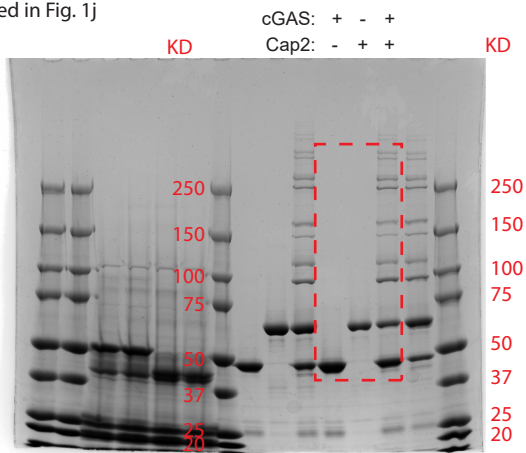
Used in Fig. 1i



anti-Flag
Flag-cGAS

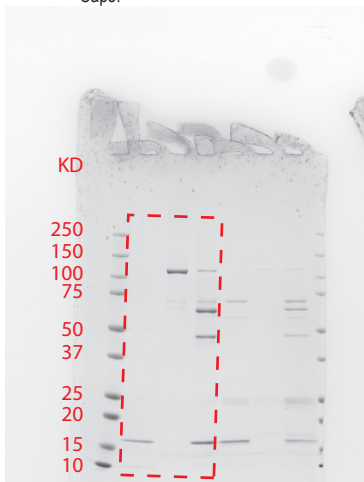
left panel right middle panel panel

Used in Fig. 1j



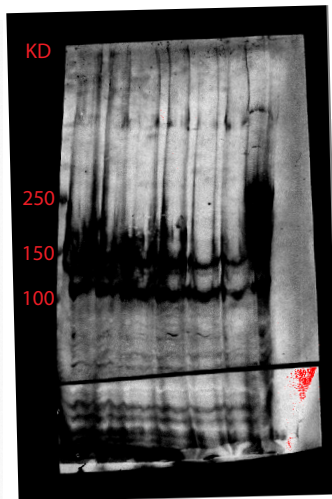
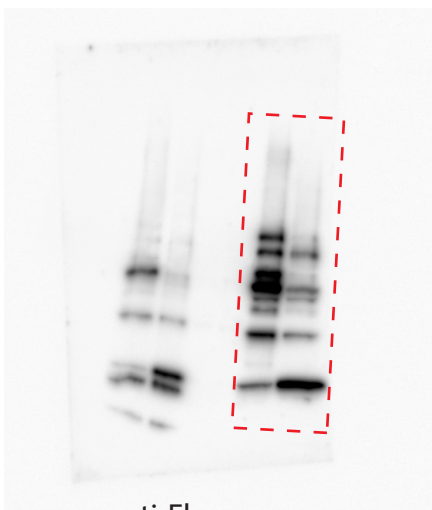
Used in Extended Data Fig. 1a

Sumo-cGAS-cGAS:	-	+	+
Cap3:	+	-	+



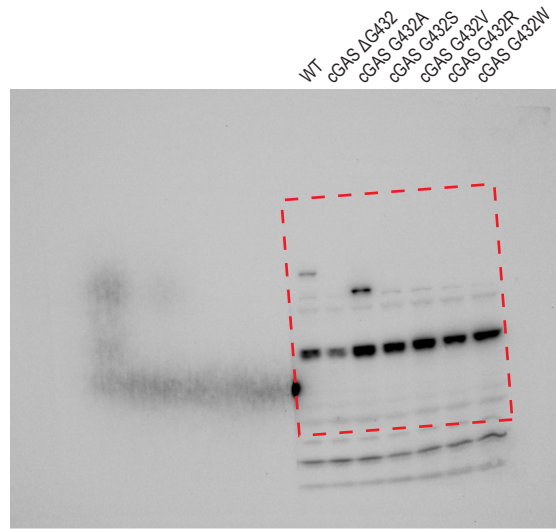
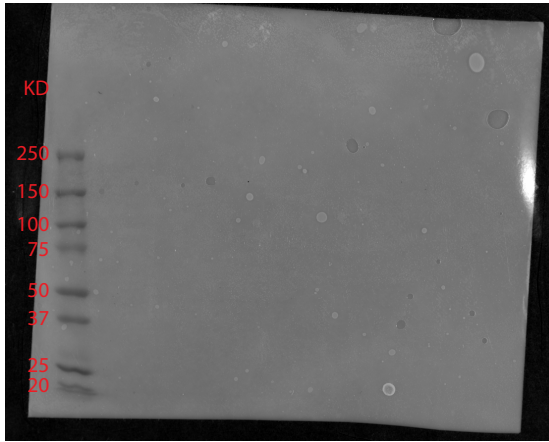
Used in Extended Data Fig. 1d

flag-IP:	+	+
Cap3:	-	+



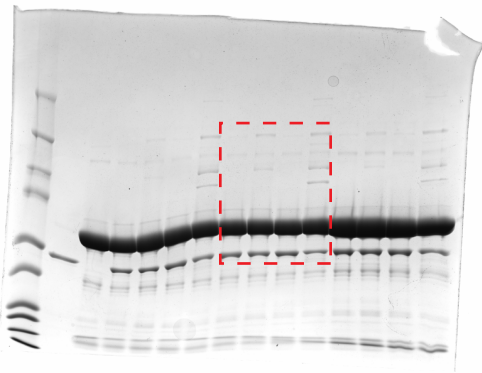
other half of membrane
with visible mw markers

Used in Extended Data Fig. 1f

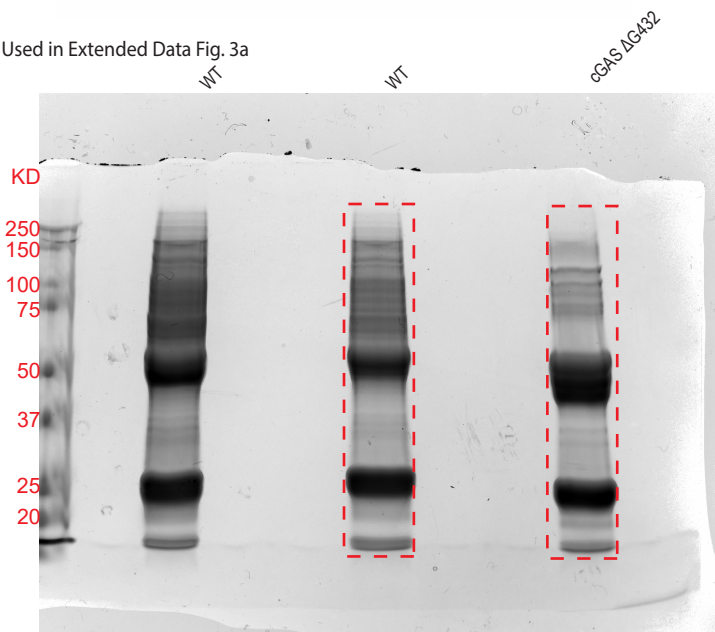


Used in Extended Data Fig. 1g

ATP (10 mM): - - + +
MgCl₂ (20 mM): - + - +



Used in Extended Data Fig. 3a



Supplementary Fig. 1 uncropped and unedited versions of all gels used in this study.

Supplementary Table 1| Top 20 cGAS conjugated proteins.

Accession	Description	Gene	Coverage [%]	# Peptides	MW [kDa]	Score Sequest HT	Abundance		TMVSG sites
							Δ G432	WT	
P06959	Dihydrolipoyllysine-residue acetyltransferase	aceF	91	74	66	6613	181	86	1
P09373	Formate acetyltransferase 1	pflB	93	86	85	5254	10	114	2
E1E2	ThiF family adenylyltransferase	E1E2	90	75	67	3889	6	135	5
P0A6F5	60 kDa chaperonin	groL	92	50	57	2781	2	169	2
P07395	Phenylalanine--tRNA ligase beta subunit	pheT	85	73	87	2696	3	152	1
P36683	Aconitate hydratase 2	acnB	89	75	93	2528	5	141	1
P0A8M3	Threonine--tRNA ligase	thrS	85	64	74	1719	6	131	2
P09152	Respiratory nitrate reductase 1 alpha chain	narG	83	88	140	1642	67	101	1
P0A853	Tryptophanase	tnaA	89	42	53	1596	28	119	4
P0A6M8	Elongation factor G	fusA	81	49	78	1444	52	106	2
P0CE48	Elongation factor Tu 2	tufB	86	29	43	1149	6	110	1
P0A9B2	Glyceraldehyde-3-phosphate dehydrogenase A	gapA	92	39	36	1037	29	125	4
P05055	Polyribonucleotide nucleotidyltransferase	pnp	69	48	77	960	124	94	1
P08839	Phosphoenolpyruvate-protein phosphotransferase	ptsI	86	48	64	752	15	94	1
P0A799	Phosphoglycerate kinase	pgk	94	37	41	613	4	126	2
P0A9C5	Glutamine synthetase	glnA	85	34	52	612	2	148	1
P00961	Glycine--tRNA ligase beta subunit	glyS	85	58	77	605	51	113	1
P0A6P9	Enolase	eno	86	36	46	597	1	144	1
P0A6T1	Glucose-6-phosphate isomerase	pgi	80	44	62	576	1	128	2
P00957	Alanine--tRNA ligase	alaS	74	57	96	546.36	31	138	1

Supplementary Table 2| 20 most enriched cGAS conjugated proteins in cells infected with phage T4.

Description	Sum PEP Score	Coverage [%]	Abundance Ratio: (T4 infected) / (Uninfected)	Found in Sample: Uninfected	Found in Sample: T4 infected	Modifications
Uncharacterized 9.5 kDa protein in dexA-dda intergenic region OS=Enterobacteria phage T4 OX=10665 GN=y00H PE=4 SV=1	22.617	70	100	Not Found	High	TMVSG [K5]
Fibrin OS=Enterobacteria phage T4 OX=10665 GN=wac PE=1 SV=5	111.34	69	21.028	High	High	TMVSG [K100]
50S ribosomal protein L4 OS=Escherichia coli (strain K12) GN=rpL4 PE=1 SV=1	64.668	59	18.773	High	High	TMVSG [K6]
50S ribosomal protein L9 OS=Escherichia coli (strain K12) GN=rpL9 PE=1 SV=1	48.507	78	12.396	High	High	TMVSG [K22]
Protein-export protein SecB OS=Escherichia coli (strain K12) GN=secB PE=1 SV=1	27.533	54	4.542	High	High	TMVSG [K19]
Glycerol kinase OS=Escherichia coli (strain K12) GN=glpK PE=1 SV=2	220.956	72	2.741	High	High	TMVSG [K143]
Citrate synthase OS=Escherichia coli (strain K12) GN=gltA PE=1 SV=1	181.654	85	2.078	High	High	TMVSG [K38]
60 kDa chaperonin OS=Escherichia coli (strain K12) GN=groL PE=1 SV=2	527.371	90	1.983	High	High	TMVSG [K7]
Lipid A export ATP-binding/permease protein MsbA OS=Escherichia coli (strain K12) GN=msbA PE=1 SV=1	5.504	5	1.943	Peak Found	High	TMVSG [K528]
3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Escherichia coli (strain K12) GN=fabF PE=1 SV=2	62.461	65	1.832	High	High	TMVSG [K49]
Ribosomal protein S12 methyltransferase RimO OS=Escherichia coli (strain K12) GN=rpmO PE=1 SV=1	46.233	37	1.752	High	High	TMVSG [K19]
Alkyl hydroperoxide reductase subunit C OS=Escherichia coli (strain K12) GN=ahpC PE=1 SV=2	171.942	90	1.603	High	High	TMVSG [K17]
Glycine cleavage system H protein OS=Escherichia coli (strain K12) GN=gcvH PE=1 SV=2	10.867	27	1.542	High	High	TMVSG [K12]
Galactitol-specific phosphotransferase enzyme IIA component OS=Escherichia coli (strain K12) GN=gatA PE=4 SV=1	48.243	90	1.41	High	High	TMVSG [K29]
Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Escherichia coli (strain K12) GN=accA PE=1 SV=2	57.406	56	1.303	High	High	TMVSG [K53]
Elongation factor Tu 1 OS=Escherichia coli (strain K12) GN=tufA PE=1 SV=1	216.33	90	1.294	High	High	TMVSG [K38; K177]
Elongation factor Tu 2 OS=Escherichia coli (strain K12) GN=tufB PE=1 SV=1	216.33	90	1.294	High	High	TMVSG [K38; K177]
Lactaldehyde dehydrogenase OS=Escherichia coli (strain K12) GN=aldA PE=1 SV=2	138.001	76	1.288	High	High	TMVSG [K356]
Lysine-tRNA ligase OS=Escherichia coli (strain K12) GN=lysS PE=1 SV=2	219.952	77	1.116	High	High	TMVSG [K82]
Bifunctional purine biosynthesis protein PurH OS=Escherichia coli (strain K12) GN=purH PE=1 SV=1	72.007	45	1.069	High	High	TMVSG [K17]