Supplementary Figure 1

STING and cGAS mutations in human tumors. (a) Alteration frequency for *STING* or *cGAS* gene in human tumors. The data were obtained from cBioPortal. (b) STING or cGAS mutations found in human tumors. The data were obtained from cBioPortal. The mutants tested in this study are colored in red. SNP data were obtained from dbSNP, 1000 Genomes Project, and ExAC databases. (c) STING or cGAS mutations found in human tumors. The data were obtained from cBioPortal, COSMIC, and ICGC databases. (d) Allele frequency of STING or cGAS mutants. The data obtained from ExAC. (e) STING/cGAS expression profiles in a variety of cancer cell lines compared to normal counterparts.

Supplementary Figure 2

STING crystal structure. Human STING dimer in complex with cGAMP (PDB ID: 4LOH). R169 and P203 are highlighted in red.

Supplementary Figure 3

STING mutants are dominant-negative mutants. (a) As described in Figure 2a, the luciferase activity in the cell lysates was measured after the transfection of the indicated plasmids. The numbers under the graph indicate the amount of transfected plasmids (μg). (b) As described in Figure 4b, the reconstituted MEF cells were treated with dsDNA and then western blots were performed with the indicated antibodies.

Supplementary Figure 4

Pair-wise correlation matrix for methylation probes. (a and b) The correlations for the 16 cGAS probes are shown in a and the 11 probes for STING in b. The Pearson correlation was calculated across 9173 samples.

Supplementary Figure 5

Tumor Purity vs. methylation levels for cGAS and STING. The tumor purity was used to establish that the methylation signal for cGAS and STING comes from tumor cells and not from infiltrating immune cells. The figures show that the methylation is muted due to infiltrating cells. Only pure tumor samples (CPE TP = 1) show a full range in methylation levels.

Supplementary Figure 6

cGAS and STING promoters are highly methylated in a variety of human tumors. The methylation data were obtained from MethHC database that collects methylation data focusing on human tumors from TCGA. MethHC database provides dataset of methylation for 18 different human tumors and uses β-value to measure the methylation level. The β-value for *cGAS (MB21D1)*, *STING (TMEM173)*, *RIG-I (DDX58)*, *MDA5 (IFIH1)*, *MAVS*, or *TBK1* gene in human tumors (T) was statistically analyzed compared to normal tissues (N) and is shown in box plots that were made by using BoxPlotR. The β-value used in this figure indicates the methylation level for promoter region including TSS1500, TSS200, and 5'UTR. The statistical analysis was performed by Student's *t*-test. * and ** indicate $p < 0.05$ and $p < 0.005$, respectively. Abbreviation for each tumor type is shown in Supplementary Information.

Supplementary Figure 1

a

	STING	cGAS
ACbC (MSKCC/Breast 2015)	0%	0%
ACC (TCGA)	0%	0%
ACyC (FMI 2014)	0%	0%
ACyC (MDA 2015)	0%	0%
ACyC (MSKCC, 2013)	0%	0%
ACyC (Sanger 2013)	0%	0%
ALL (St. Jude)	0%	0%
AML (TCGA)	0%	0%
Bladder (BGI 2013)	1%	1%
Bladder (DFCI/MSKCC 2014)	2%	2%
Bladder (MSKCC 2012)	0%	0%
Bladder (MSKCC 2014)	0%	0%
Bladder (TCGA)	0%	1.50%
Bladder PV (MSKCC)	0%	0%
Breast (BCCRC 2012)	0%	0%
Breast (BCCRC Xenograft)	0%	0%
Breast (Broad 2012)	0%	0%
Breast (METABRIC)	0%	0%
Breast (Sanger)	0%	0%
Breast (TCGA)	0.10%	0.10%
CCLE (Novartis/Broad 2012)	0%	0%
ccRCC (BGI 2012)	0%	0%
ccRCC (IRC)	0%	0%
ccRCC (TCGA)	0%	0%
ccRCC (U Tokyo)	0%	0%
Cervical (TCGA)	0%	1%
Cholangiocarcinoma (JHU)	0%	0%
Cholangiocarcinoma (NCCS)	0%	0%
Cholangiocarcinoma (NUS)	0%	0%
Cholangiocarcinoma (TCGA)	0%	0%
chRCC (TCGA)	0%	0%
CLL (Broad 2013)	0%	0%
CLL (IUOPA 2015)	0%	0%
Colorectal (DFCI 2016)	1.10%	1.10%
Colorectal (Genentech)	1.40%	4.20%
Colorectal (MSKCC)	0%	0%
Colorectal (TCGA)	0%	1.80%
CSCC (DFCI 2015)	0%	0%
CTCL (Columbia 2015)	0%	0%
DESM (Broad 2015)	0%	0%
DLBC (TCGA)	0%	0%
DLBCL (Broad 2012)	0%	1.70%
ESCC (UCLA 2014)	0%	0%
Esophagus (Broad)	0%	0.70%
Esophagus (TCGA)	0.50%	0%
Esophagus sq (ICGC)	0%	1.10%
Ewing Sarcoma (DFCI)	0%	0%
Ewing Sarcoma (Institut Curie)	0%	0%
GBC (Shanghai)	0%	3.10%
GBM (TCGA)	0%	0%
Glioma (TCGA)	0%	0.30%
Glioma (UCSF)	1.60%	0%
Head & neck (Broad)	0%	0%
Head & neck (JHU)	3.10%	0%
Head & neck (MDA)	2.50%	0%
Head & neck (TCGA)	0.40%	0.40%
hnc_mskcc_2016	0%	0%
LGG-GBM (TCGA 2016)	0%	0.10%
Liad (Inserm 2014)	0%	0%
Liver (AMC)	0.40%	0.40%
Liver (RIKEN)	0%	0%
Liver (TCGA)	1.10%	0.80%
Lung adeno (Broad)	0%	0%
Lung adeno (MSKCC)	0%	0%
Lung adeno (TCGA)	0.90%	0.40%
Lung adeno (TSP)	0%	0%
Lung squ (TCGA)	1.10%	0%

	STING	cGAS
MBL (Broad)	0%	0%
MBL (ICGC)	0%	0%
MBL (PCGP)	0%	0%
MCL (IDIBIPS 2013)	0%	0%
MDS (Tokyo)	0%	0%
Melanoma (Broad)	1.70%	1.70%
Melanoma (Broad/DFCI)	0%	0%
Melanoma (TCGA)	0.80%	1.40%
Melanoma (Yale)	0%	1.10%
MM (Broad)	0%	0.50%
MPNST (MSKCC)	0%	0%
NBL (AMC)	0%	0%
NBL (Cologne 2015)	0%	0%
nccRCC (Genentech 2014)	0%	0%
NCI-60	1.70%	1.70%
NEPC (Trento/Cornell/Broad 2016)	0%	0%
NPC (Singapore)	0%	0%
NSCLC (TCGA 2016)	0.70%	0.30%
Ovarian (TCGA)	0%	0%
Ovary SC (MSKCC)	0%	0%
PAAC (JHU)	0%	0%
Pancreas (ICGC)	0%	0%
Pancreas (QCMG 2016)	0%	0%
Pancreas (TCGA)	0.70%	0%
Pancreas (UTSW)	0%	0%
Panet (Shanghai 2013)	0%	0%
PANET (Johns Hopkins 2011)	0%	0%
PCNSL (Mayo Clinic)	10%	10%
PCPG (TCGA)	0%	0%
PLMESO (NYU 2015)	0%	0%
PRAD Hallmarks (CPCG-GENE, 2017)	0%	0%
pRCC (TCGA)	0.70%	0.40%
Prostate (Broad/Cornell 2012)	0%	0%
Prostate (Broad/Cornell 2013)	0%	0%
Prostate (FHCRC, 2016)	0%	0%
Prostate (MICH)	0%	0%
Prostate (MSKCC 2010)	0%	0%
Prostate (SU2C)	0%	0%
Prostate (TCGA)	0.40%	0%
Prostate Organoids	0%	0%
Renal unclass (MSKCC)	0%	0%
RMS (NIH)	0%	0%
Sarcoma (MSKCC)	0%	0%
Sarcoma (TCGA)	0%	0%
Small Cell Lung (CLCGP)	0%	0%
Small Cell Lung (JHU)	0%	2.40%
Small Cell Lung (UCOLOGNE)	0%	0.90%
Stomach (Pfizer UHK)	1%	0%
Stomach (TCGA)	0.50%	2%
Stomach (TMUCIH 2015)	1.30%	1.30%
Stomach (UHK)	0%	0%
Stomach (UTokyo)	0%	0%
Testicular germ cell (TCGA)	0%	0%
TET (NCI)	0%	0%
Thymoma (TCGA)	0%	0%
Thyroid (MSKCC 2016)	0%	0%
Thyroid (TCGA)	0%	0%
ucs (Johns Hopkins 2014)	0%	0%
Uterine (TCGA)	1.60%	0.80%
Uterine CS (TCGA)	1.80%	0%
Uveal melanoma (TCGA)	0%	1.30%

Supplementary Figure 1

b

STING (TMEM173)

Sample ID	Cancer Study	AA change	Type	Copy # *1	Allele Freq (T)	Count	Total Count	dbSNP *2	1000 Genomes *3	ExAC *4
TCGA-73-4675-01	Lung adeno (TCGA pub)	S4C	Missense	Gain	0.26	1	1	N		N
TCGA-73-4675-01	Lung adeno (TCGA)	S4C	Missense	Gain	0.26					
TCGA-73-4675-01	NSCLC (TCGA 2016)	S4C	Missense	Gain	0.26					
TCGA-YJ-A8SW-01	Prostate (TCGA)	L49I	Missense	Gain	0.56	1	1	N		N
B105-0	Bladder (BGI 2013)	H50Q	Missense	NA	NA	1	1	N		N
TCGA-BH-A0BF-01	Breast (TCGA pub)	R71H	Missense	ShallowDel	NA	1	1	Y	R: 0.7969/H: 0.2031	Y (0.1834)
TCGA-CZ-5464-01	ccRCC (TCGA pub)	H74Y	Missense	AMP	NA	1	1	Y (H: 0.99998355/Y: 0.00001647) ss1687970267		N
coadread_dfci_2016_4500	Colorectal (DFCI 2016)	R78W	Missense	NA	NA	1	1	Y (R: 0.99999177/W: 0.00000825) ss1687970252		Y (0.000009113)
TCGA-D3-A51K-06	Melanoma (TCGA)	W82R	Missense	Gain	0.69	1	1	N		N
PGM52	Stomach (TMUCIH 2015)	T84S	Missense	NA	NA	1	1	N		N
coadread_dfci_2016_3152	Colorectal (DFCI 2016)	V85Cfs*46	FS del	NA	NA	1	1	N		N
TCGA-L9-A8F4-01	NSCLC (TCGA 2016)	G90C	Missense	Diploid	0.39	1	1	N		N
coadread_dfci_2016_229	Colorectal (DFCI 2016)	R94C	Missense	NA	NA	1	1	Y (R: 0.99999177/C: 0.00000824) ss1687970244		Y (0.000008590)
TCGA-BR-8486-01	Stomach (TCGA)	G96E	Missense	Diploid	NA	1	1	N		N
TCGA-63-A5MB-01	NSCLC (TCGA 2016)	V113L	Missense	ShallowDel	0.6	1	1	N		N
TCGA-N7-A4Y8-01	Uterine CS (TCGA)	S127T	Missense	ShallowDel	0.46	1	1	N		N
TCGA-18-3409-01	Lung squ (TCGA pub)	L133F	Missense	Diploid	0.26	1	1	N		N
TCGA-18-3409-01	Lung squ (TCGA)	L133F	Missense	ShallowDel	0.26					
TCGA-18-3409-01	NSCLC (TCGA 2016)	L133F	Missense	ShallowDel	0.26					
325	Head & neck (JHU)	L136P	Missense	NA	NA	1	2	N		N
OSCJM-PT10-325-T	Head & neck (MDA)	L136P	Missense	NA	NA	1				
TCGA-IB-7651-01	Pancreas (TCGA)	W161*	Nonsense	Diploid	0.19	1	1	N		N
TCGA-58-8387-01	NSCLC (TCGA 2016)	R169W	Missense	ShallowDel	0.11	1	3	Y (No frequency data)		N
TCGA-IB-7651-01	Pancreas (TCGA)	R169W	Missense	Diploid	0.2	1				
PCNSL_4	PCNSL (Mayo Clinic)	R169W	Missense	NA	NA	1				
coadread_dfci_2016_593	Colorectal (DFCI 2016)	R180Q	Missense	NA	NA	1	3	N		N
coadread_dfci_2016_3235	Colorectal (DFCI 2016)	R180Q	Missense	NA	NA	1				
TCGA-D1-A16X-01	Uterine (TCGA pub)	R180Q	Missense	Diploid	0.19	1				
TCGA-D1-A16X-01	Uterine (TCGA)	R180Q	Missense	Diploid	0.19					
ME017	Melanoma (Broad)	R191L	Missense	NA	NA	1	1	N		N
TCGA-L5-A43J-01	Esophagus (TCGA)	R191Q	Missense	Diploid	NA	1	1	Y (R: 0.99998355/Q: 0.00001647) ss1687970161		Y (0.00001647)
coadread_dfci_2016_3209	Colorectal (DFCI 2016)	R191W	Missense	NA	NA	1	1	Y (R: 0.99961287/W: 0.00038711) ss1687970162		Y (0.0003871)
TCGA-B5-A11E-01	Uterine (TCGA pub)	R197Q	Missense	Diploid	0.38	1	1	Y (R: 0.99996704/Q: 0.00003295) ss1687970156		Y (0.00003295)
TCGA-B5-A11E-01	Uterine (TCGA)	R197Q	Missense	Diploid	0.38					
TCGA-EE-A3AA-06	Melanoma (TCGA)	L202F	Missense	Gain	0.09	1	1	N		N
TCGA-D6-6516-01	Head & neck (TCGA pub)	P203S	Missense	Diploid	0.14	1	2	N		N
TCGA-D6-6516-01	Head & neck (TCGA)	P203S	Missense	Diploid	0.14					
MEL-Ma-Mel-65	Melanoma (Broad)	P203S	Missense	NA	NA	1				
TCGA-AN-A0AK-01	Breast (TCGA 2015)	D210N	Missense	ShallowDel	0.53	1	1	N		N
TCGA-AN-A0AK-01	Breast (TCGA)	D210N	Missense	ShallowDel	NA					
TCGA-DD-A3A0-01	Liver (TCGA)	N218Tfs*129	FS del	Diploid	0.28	1	1	N		N
HCT_15	NCI-60	A233S	Missense	ShallowDel	NA	1	1	N		N
MSKCC-0620_R	Bladder (DFCI/MSKCC 2014)	R238W	Missense	NA	NA	1	1	N		N
TCGA-MG-AAMC-01	Prostate (TCGA)	R253W	Missense	Diploid	0.44	1	1	Y	R: 0.9998/W: 0.002	Y (0.0001006)
TCGA-18-3406-01	Lung squ (TCGA pub)	A254A	splice_region	Diploid	0.09	1	1	Y		N
P01_Rec	Glioma (UCSF)	T263I	Missense	NA	0.11	1	1	N		N
coadread_dfci_2016_390	Colorectal (DFCI 2016)	L265Cfs*82	FS del	NA	NA	1	2	N		N
TCGA-BR-8487-01	Stomach (TCGA pub)	L265Cfs*82	FS del	Diploid	0.43	1				
TCGA-BR-8487-01	Stomach (TCGA)	L265Cfs*82	FS del	Diploid	NA					
H071591	Liver (AMC)	M271V	Missense	Diploid	0.18	1	1	N		N
TCGA-DD-AAD8-01	Liver (TCGA)	Y274C	Missense	ShallowDel	0.25	1	1	N		N
TCGA-AP-A0LF-01	Uterine (TCGA pub)	Q276P	Missense	Diploid	0.18	1	1	N		N
TCGA-AP-A0LF-01	Uterine (TCGA)	Q276P	Missense	Diploid	0.18					
TCGA-46-3768-01	Lung squ (TCGA pub)	R281L	Missense	ShallowDel	0.07	1	1	N		N
TCGA-37-4141-01	Lung squ (TCGA pub)	E282*	Nonsense	Diploid	0.68	1	1	N		N
TCGA-37-4141-01	Lung squ (TCGA)	E282*	Nonsense	Gain	0.68					
TCGA-37-4141-01	NSCLC (TCGA 2016)	E282*	Nonsense	Gain	0.68					
TCGA-55-7815-01	Lung adeno (TCGA pub)	R284M	Missense	Diploid	0.22	1	1	N		N
TCGA-55-7815-01	Lung adeno (TCGA)	R284M	Missense	Diploid	0.22					
TCGA-55-7815-01	NSCLC (TCGA 2016)	R284M	Missense	Diploid	0.22					
TCGA-60-2725-01	NSCLC (TCGA 2016)	P303Lfs*44	FS del	ShallowDel	0.08	1	1	N		N
587376	Colorectal (Genentech)	R310H	Missense	NA	NA	1	1	Y (R: 0.99993414/H: 0.00006589) ss1687970089		Y (0.00006740)
TCGA-CV-7410-01	Head & neck (TCGA)	A318T	Missense	Diploid	NA	1	1	N		N
TCGA-Y8-A8RY-01	pRCC (TCGA)	D319E	Missense	Diploid	0.2	1	1	N		N
TCGA-FV-A3R2-01	Liver (TCGA)	V329F	Missense	Gain	0.19	1	1	Y (V: 0.99999177/F: 0.00000824) ss1687970061		Y (0.000008304)
pfg127T	Stomach (Pfizer UHK)	T356M	Missense	NA	0.34	1	1	Y (T: 0.99999177/M: 0.00000824) ss1687970048		Y (0.000009704)
TCGA-FW-A3R5-06	Melanoma (TCGA)	P371L	Missense	Diploid	0.33	1	1	N		N
TCGA-D1-A16F-01	Uterine (TCGA pub)	R375C	Missense	NA	0.24	1	1	Y (R: 0.99994224/C: 0.00005775) ss1687970037		Y (0.0001269)
TCGA-D1-A16F-01	Uterine (TCGA)	R375C	Missense	NA	0.24					
TCGA-B1-A656-01	pRCC (TCGA)	R375H	Missense	Gain	0.42	1	1	Y	R: 0.9970/H: 0.0030	Y (0.0001064)
TCGA-BC-A10R-01	Liver (TCGA)	T376K	Missense	ShallowDel	0.46	1	1	Y (No frequency data)		N

*1
ShallowDel: possibly heterozygous deletion
Gain: Amplification
NA: Not available

*2
Y: Yes (Data exists in dbSNP)
N: No (No data exists in dbSNP)
Number means allele frequency.
ss means submitted SNP number.

*3
Number means allele frequency.

*4
Y: Yes (Data exists in ExAC)
N: No (No data exists in ExAC)
Number means allele frequency.

Supplementary Figure 1

b (Continued)

cGAS (MB21D1)

Sample ID	Cancer Study	AA change	Type	Copy #	Allele Freq (T)	Count	Total Count	dbSNP	1000 Genomes	ExAC
TCGA-DD-A113-01	Liver (TCGA)	G6R	Missense	Diploid	0.23	1	1	N		N
XHDG38	GBC (Shanghai)	G16R	Missense	NA	NA	1	1	N		N
587350	Colorectal (Genentech)	M32I	Missense	NA	NA	1	1	N		N
TCGA-DD-A3A0-01	Liver (TCGA)	A54Pfs*119	FS del	Diploid	0.07	1	1	N		N
PCNSL_5	PCNSL (Mayo Clinic)	K82T	Missense	NA	NA	1	1	Y (K: 0.99998903/T: 0.00001097) ss1688394886		N
coadread_dfci_2016_2624	Colorectal (DFCI 2016)	G145R	Missense	NA	NA	1	2	N		N
TCGA-HU-A4GU-01	Stomach (TCGA pub)	G145R	Missense	Diploid	0.2	1				
TCGA-HU-A4GU-01	Stomach (TCGA)	G145R	Missense	Gain	NA					
DLBCL-MAYO_DLBCL_234	DLBCL (Broad 2012)	V154I	Missense	NA	NA	1	1	N		N
coadread_dfci_2016_2369	Colorectal (DFCI 2016)	A159V	Missense	NA	NA	1	1	N		N
coadread_dfci_2016_1208	Colorectal (DFCI 2016)	R166Q	Missense	NA	NA	1	1	Y (R: 0.99998355/Q: 0.00001648) ss1688394836		Y (0.00001717)
TCGA-DD-A3A7-01	Liver (TCGA)	E216D	Missense	Diploid	0.34	1	1	N		N
TCGA-AA-A010-01	Colorectal (TCGA pub)	V234A	Missense	Diploid	NA	1	1	N		N
TCGA-AA-A010-01	Colorectal (TCGA)	V234A	Missense	Diploid	NA					
TCGA-90-A4ED-01	NSCLC (TCGA 2016)	E241K	Missense	Diploid	0.29	1	1	N		N
TCGA-ER-A193-06	Melanoma (TCGA)	S243T	Missense	Diploid	0.29	1	1	N		N
MSKCC-0100_R	Bladder (DFCI/MSKCC 2014)	R246C	Missense	NA	NA	1	1	Y (R: 0.99994230/C: 0.00005767) ss1688394779		Y (0.00005850)
TCGA-AP-A056-01	Uterine (TCGA pub)	Y248*	Nonsense	Diploid	0.22	1	1	N		N
TCGA-AP-A056-01	Uterine (TCGA)	Y248*	Nonsense	Diploid	0.22					
TCGA-EE-A29E-06	Melanoma (TCGA)	P257S	Missense	Diploid	0.54	1	2	N		N
TCGA-D3-A3C7-06	Melanoma (TCGA)	P257S	Missense	Diploid	0.45	1				
TCGA-B5-A0JY-01	Uterine (TCGA pub)	E259*	Nonsense	Diploid	0.33	1	1	N		N
TCGA-B5-A0JY-01	Uterine (TCGA)	E259*	Nonsense	Diploid	0.33					
MEL-JWCI-WGS-12	Melanoma (Broad)	G303E	Missense	NA	NA	1	1	N		N
TCGA-95-7039-01	Lung adeno (TCGA pub)	V318L	Missense	Diploid	0.51	1	1	N		N
TCGA-95-7039-01	Lung adeno (TCGA)	V318L	Missense	Diploid	0.51					
TCGA-95-7039-01	NSCLC (TCGA 2016)	V318L	Missense	Diploid	0.51					
TCGA-FS-A1Z0-06	Melanoma (TCGA)	S326L	Missense	AMP	0.63	1	1	N		N
H080750	Liver (AMC)	W330C	Missense	Diploid	0.3	1	1	N		N
coadread_dfci_2016_1202	Colorectal (DFCI 2016)	W330R	Missense	NA	NA	1	1	N		N
ESO-007	Esophagus (Broad)	S333I	Missense	NA	0.13	1	1	N		N
B70	Bladder (BGI 2013)	R339H	Missense	NA	NA	1	5	Y	R: 0.9998/H: 0.0002	Y (0.00002472)
coadread_dfci_2016_345	Colorectal (DFCI 2016)	R339H	Missense	NA	NA	1				
PGM21	Stomach (TMUCIH 2015)	R339H	Missense	NA	NA	1				
TCGA-BR-4257-01	Stomach (TCGA pub)	R339H	Missense	Gain	0.19	1				
TCGA-BR-4257-01	Stomach (TCGA)	R339H	Missense	Gain	NA					
TCGA-B5-A0JY-01	Uterine (TCGA pub)	R339H	Missense	Diploid	0.33	1				
TCGA-B5-A0JY-01	Uterine (TCGA)	R339H	Missense	Diploid	0.33					
TCGA-AP-A056-01	Uterine (TCGA pub)	N342H	Missense	Diploid	0.34	1	1	N		N
TCGA-AP-A056-01	Uterine (TCGA)	N342H	Missense	Diploid	0.34					
TCGA-DK-A1AC-01	Bladder (TCGA 2014)	W343*	Nonsense	Gain	0.35	1	1	N		N
TCGA-DK-A1AC-01	Bladder (TCGA)	W343*	Nonsense	Gain	0.35					
MM-0509	MM (Broad)	W343C	Missense	NA	NA	1	1	N		N
TCGA-G2-A2EO-01	Bladder (TCGA 2014)	S345*	Nonsense	ShallowDel	0.28	1	1	N		N
TCGA-G2-A2EO-01	Bladder (TCGA)	S345*	Nonsense	ShallowDel	0.28					
TCGA-CV-A45W-01	Head & neck (TCGA)	S345L	Missense	Diploid	0.09	1	1	N		N
TCGA-B9-A8YI-01	pRCC (TCGA)	N368H	Missense	Diploid	0.27	1	1	N		N
TCGA-IN-A7NT-01	Stomach (TCGA)	R376L	Missense	ShallowDel	NA	1	1	N		N
coadread_dfci_2016_237641	Colorectal (DFCI 2016)	R376Q	Missense	NA	NA	1	2	Y (R: 0.99995881/Q: 0.00004119) ss1688394685		Y (0.00004133)
TCGA-AA-3864-01	Colorectal (TCGA pub)	R376Q	Missense	Diploid	NA	1				
TCGA-AA-3864-01	Colorectal (TCGA)	R376Q	Missense	Diploid	NA					
TCGA-BR-8487-01	Stomach (TCGA pub)	L377P	Missense	Diploid	0.23	1	1	N		N
TCGA-BR-8487-01	Stomach (TCGA)	L377P	Missense	Diploid	NA					
HCT_116	NCI-60	L377V	Missense	Diploid	NA	1	1	N		N
587256	Colorectal (Genentech)	F379S	Missense	NA	NA	1	1	N		N
coadread_dfci_2016_578	Colorectal (DFCI 2016)	E383K	Missense	NA	NA	1	3	Y (E: 0.99984348/K: 0.00015651) ss1688394678		Y (0.0001567)
TCGA-BR-8680-01	Stomach (TCGA pub)	E383K	Missense	Diploid	0.27	1				
TCGA-BR-8680-01	Stomach (TCGA)	E383K	Missense	Diploid	NA					
TCGA-SW-A7EA-01	Stomach (TCGA)	E383K	Missense	Diploid	NA	1				
587222	Colorectal (Genentech)	E385D	Missense	NA	NA	1	2	N		N
TCGA-AP-A056-01	Uterine (TCGA pub)	E385D	Missense	Diploid	0.33	1				
TCGA-AP-A056-01	Uterine (TCGA)	E385D	Missense	Diploid	0.33					
TCGA-AA-A00A-01	Colorectal (TCGA pub)	I386N	Missense	Diploid	NA	1	1	N		N
TCGA-AA-A00A-01	Colorectal (TCGA)	I386N	Missense	Diploid	NA					
TCGA-BR-4361-01	Stomach (TCGA pub)	X406_splice	Splice	Diploid	0.24	1	1	N		N
TCGA-BR-4361-01	Stomach (TCGA)	X406_splice	Splice	Diploid	NA					
TCGA-GN-A266-06	Melanoma (TCGA)	D408N	Missense	Gain	0.5	1	1	N		N
DLBCL-MAYO_DLBCL_234	DLBCL (Broad 2012)	Q419E	Missense	NA	NA	1	1	N		N
TCGA-AR-A0TX-01	Breast (TCGA 2015)	F433L	Missense	Diploid	0.24	1	1	Y (F: 0.99983525/L: 0.00016473) ss1688394636		Y (0.0001667)
TCGA-AR-A0TX-01	Breast (TCGA pub)	F433L	Missense	Diploid	NA					
TCGA-AR-A0TX-01	Breast (TCGA)	F433L	Missense	Diploid	NA					
TCGA-JW-A5VL-01	Cervical (TCGA)	S434C	Missense	Diploid	NA	1	1	N		N
TCGA-CN-A63V-01	Head & neck (TCGA)	S434P	Missense	Diploid	NA	1	1	N		N
TCGA-B7-A5TN-01	Stomach (TCGA)	S435P	Missense	Diploid	NA	1	1	N		N
TCGA-56-8307-01	NSCLC (TCGA 2016)	H437D	Missense	Diploid	0.28	1	1	N		N
TCGA-JW-A5VL-01	Cervical (TCGA)	H437Y	Missense	Diploid	NA	1	1	N		N
S02348	Small Cell Lung (UCOLOGNE)	T440A	Missense	NA	NA	1	1	N		N
TCGA-DU-8161-01	Glioma (TCGA)	F442V	Missense	ShallowDel	0.28	1	1	N		N
TCGA-DU-8161-01	LGG-GBM (TCGA 2016)	F442V	Missense	ShallowDel	0.28					
TCGA-IR-A3LK-01	Cervical (TCGA)	T447I	Missense	Diploid	NA	1	1	N		N
585208	Small Cell Lung (JHU)	Q473*	Nonsense	NA	NA	1	1	N		N
ESCC-210T	Esophagus sq (ICGC)	T477A	Missense	NA	0.21	1	1	N		N
MEL-Ma-Mel-16	Melanoma (Broad)	L480I	Missense	NA	NA	1	1	N		N
YUPAT	Melanoma (Yale)	N494D	Missense	NA	NA	1	1	N		N
TCGA-AG-A002-01	Colorectal (TCGA pub)	E502*	Nonsense	Diploid	NA	1	1	N		N
TCGA-AG-A002-01	Colorectal (TCGA)	E502*	Nonsense	Diploid	NA					
TCGA-V4-A9F8-01	Uveal melanoma (TCGA)	I508T	Missense	Diploid	0.41	1	1	N		N
TCGA-34-8454-01	NSCLC (TCGA 2016)	E515Q	Missense	Diploid	0.24	1	1	N		N

Supplementary Figure 1

c

STING	Count		
Mutation	cBioPortal	COSMIC	ICGC
S4C	1		
A18D		1	1
P40S		2	
L49I	1		
H50Q	1	2	1
S53F		1	
R71H	1		1
H74Y	1	1	
R76G		1	
R78W	1		
W82R	1	1	1
T84S	1		
V85Cfs*46	1	1	
G90C	1		
P92L		1	
R94C	1	1	1
G96E	1		
V113L	1		
S127T	1		
N131fs*13		1	
L133F	1	1	1
L136P	2	2	
W161*	1		
R169W	3		
R180Q	2	1	1
N183S		1	
R191L	1		
R191Q	1		
R191W	1		
G192S		1	
R197Q	1	1	1
R197W		1	
L202F	1	1	2
P203S	2		
D205N		1	
D210N	1	1	1
N218Tfs*129	1		1
G230A			1
H232Y		1	1
A233S	1		
R238W	1		1
G251E		1	
R253W	1		
R253Q		1	1
T263I	1		
P264fs			1
L265Cfs*82	2		
M271V	1		
Y274C	1		
Y274D		1	
Q276P	1	1	1
R281L	1		
E282*	1	1	1
R284M	1		
L285I		1	
L285P		1	
R293Q			1
P303Lfs*44	1		
R310H	1	1	
A318T	1		
D319E	1		
V329F	1	1	1
G344C		1	1
G344D		2	1
T356M	1	1	
LL363LF			1
P371L	1	1	1
R375C	1	3	2
R375H	1	1	1
T376K	1	1	1
T376K			
F378L			1

cGAS	Count		
Mutation	cBioPortal	COSMIC	ICGC
G6R	1	1	1
G16R	1	1	
A27T			1
M32I	1	1	
A54Pfs*119	1		
K82T	1		
P85S			1
T97S			1
R122C		1	
G145R	2	1	1
S149L		1	1
V154I	1		
A159V	1		
R166Q	1		
A202T		1	
E216D	1	1	1
H217Q		1	
E233D		1	
V234A	1	1	
E240Q			1
E241K	1		
S243T	1	1	1
R246C	1		
Y248*	1	1	1
P257S	2	2	2
E259K		1	
E259*	1	1	1
R281K		1	
D295Y		1	1
G303E	1		
I316L		1	
V318L	1		
D319Y		1	
S326L	1	1	1
W330C	1		
W330R	1		
S333I	1	1	
R339H	5	4	3
Q341*		2	1
N342H	1	1	1
W343*	1	1	1
W343C	1		
S345*	1	1	1
S345L	1		
V360E		1	
N368H	1		
R376L	1		
R376Q	2		
L377P	1	1	1
L377V	1		
F379S	1	1	
I382T		1	1
E383K	3	2	1
E385K		1	
E385D	2	2	1
I386N	1		
C396G			1
N399fs*22		1	
E401*		1	1
E402D		1	1
D408N	1	1	1
Q419E	1		
K428fs			1
F433L	1	1	1
S434C	1	1	1
S434P	2		
H437Y	1	1	1
H437D	1		
T440A	1	1	
F442V	2	1	1
H444L		1	1
V445I		1	
T447I	1	1	1
P450R		1	
Q473*	1	1	
T477A	1	1	1
L480I	1		
P486L		1	
N494D	1	1	
R499I		2	2
E502*	1	1	
I508T	1		
E515Q	1		

Supplementary Figure 1

d

STING
(TMEM173)
R191W

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Latino	3	11578	0	0.000259112
African	5	10406	0	0.000480492
European (Finnish)	16	6614	0	0.002419111
European (Non-Finnish)	22	66728	0	0.000329697
East Asian	0	8654	0	0
South Asian	0	16512	0	0
Other	1	908	0	0.001101322
Total	47	121400	0	0.00038715

STING
(TMEM173)
R253W

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Latino	5	11560	0	0.000432526
African	2	10270	0	0.000194742
European (Finnish)	0	6612	0	0
European (Non-Finnish)	5	66174	0	7.55584E-05
East Asian	0	8622	0	0
South Asian	0	15148	0	0
Other	0	882	0	0
Total	12	119268	0	0.000100614

cGAS
(MB21D1)
R339H

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Latino	2	11576	0	0.000172771
African	1	10390	0	9.62464E-05
European (Finnish)	0	6614	0	0
European (Non-Finnish)	0	66728	0	0
East Asian	0	8654	0	0
South Asian	0	16512	0	0
Other	0	908	0	0
Total	3	121382	0	2.47154E-05

cGAS
(MB21D1)
R376Q

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Latino	0	11552	0	0
African	0	10364	0	0
European (Finnish)	0	6610	0	0
European (Non-Finnish)	4	66446	0	6.01993E-05
East Asian	1	8630	0	0.000115875
South Asian	0	16464	0	0
Other	0	906	0	0
Total	5	120972	0	4.13319E-05

cGAS
(MB21D1)
E383K

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
Latino	4	11570	0	0.000345722
African	1	10394	0	9.62094E-05
European (Finnish)	0	6614	0	0
European (Non-Finnish)	14	66622	0	0.000210141
East Asian	0	8646	0	0
South Asian	0	16494	0	0
Other	0	906	0	0
Total	19	121246	0	0.000156706

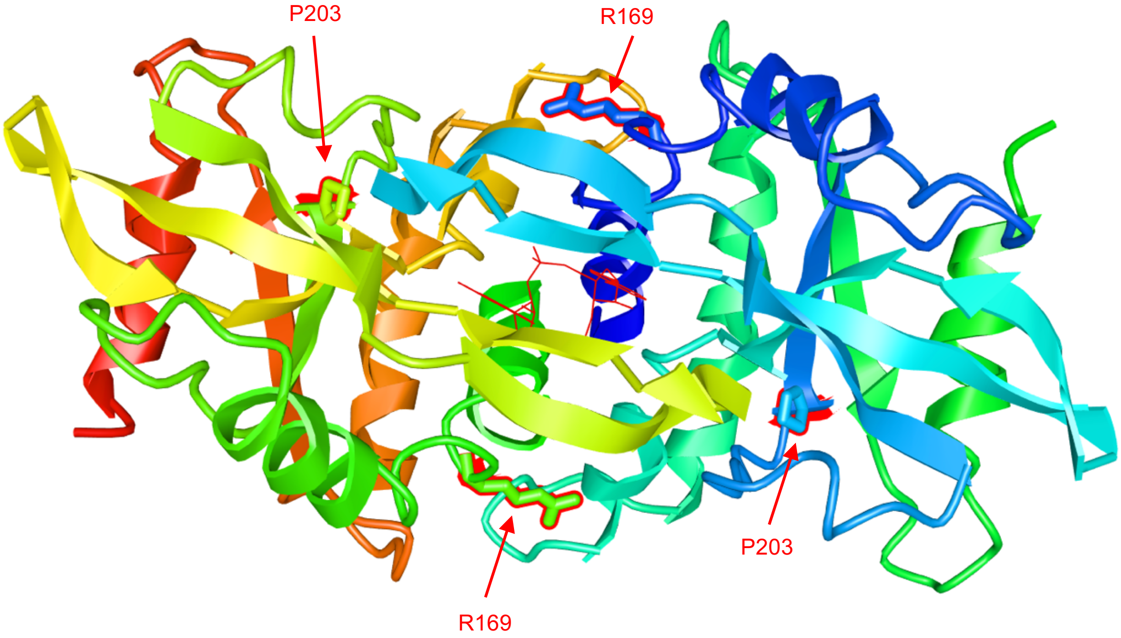
Supplementary Figure 1

e

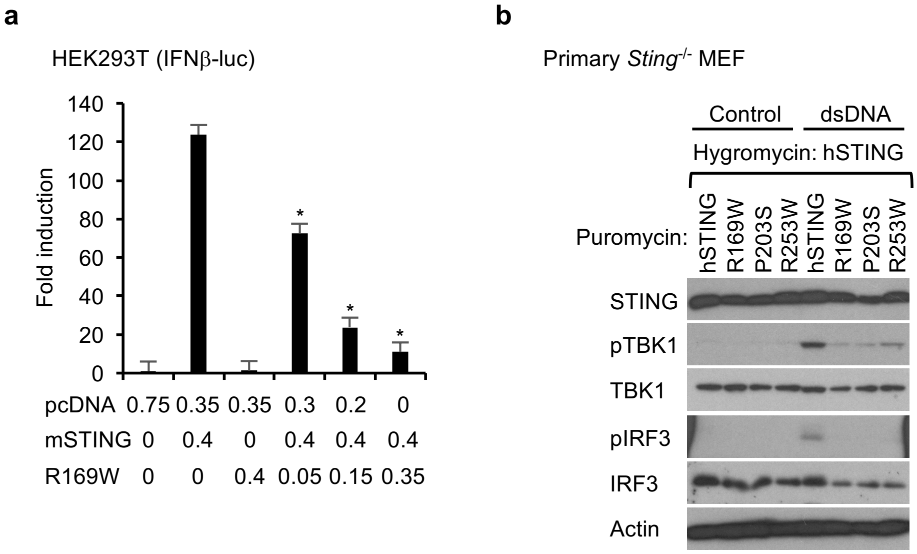
Tissue	Cell line	Expression		Response to dsDNA	Reference
		STING	cGAS		
Breast	MCF-10A (non-tumorigenic epithelial cells)	Yes	Not tested	Not tested	Bhatelia et al., Biochim. Biophys. Acta, 1842 (2014), 144-53.
	MCF-7	No	Not tested	Not tested	
	T47-D	No	Not tested	Not tested	
	HBL-100	Yes	Not tested	Not tested	
	MDA-MB-231	Yes	Not tested	Not tested	
	ZR-75-1	Yes	Not tested	Not tested	
	MDA231	Very weak	Not tested	No	Xia et al., Cell Reports, 14 (2016), 282-97.
Cervix	HeLa	Very weak	Not tested	No	Xia et al., Cell Reports, 14 (2016), 282-97.
Kidney	HEK293T	No	No	No	Sun et al., Science, 339 (2013), 786-91.
	HEK293	Yes	Not tested	Yes	Zhong et al., Immunity, 29 (2009), 538-50.
Large intestine	FHC (normal colon epithelial cells)	Yes	No (mRNA; Yes)	Yes	Xia et al., Cell Reports, 14 (2016), 282-97.
	SW1116	Yes	Yes	Yes	
	LS123	Yes	Yes	Yes	
	LS174T	Yes	No	No	
	SW480	Yes	No	No	
	SW948	Yes	Yes	No	
	SW1417	Yes	No	Yes	
	LOVO	Yes	Yes	Yes	
	SW48	No	No	No	
	HT29	Yes	Yes	Yes	
	HT116	Yes	No	No	
	COLO205	Yes	No	No	
Leukocyte	THP1	Yes	Yes	Yes	Sun et al., Science, 339 (2013), 786-91.
Liver	Huh7	No	Not tested	Not tested	Zhong et al., Immunity, 29 (2009), 538-50.
Lung	PASMC (normal primary pulmonary artery smooth muscle cells)	Not tested	Not tested	Yes	Xia et al., Cell Reports, 14 (2016), 282-97.
	H1299	Very weak	Not tested	No	
	A549	Yes	Not tested	Not tested	Zhong et al., Immunity, 29 (2009), 538-50.
Ovary	SK-OV-3	Yes	Not tested	No	Xia et al., Cell Reports, 14 (2016), 282-97.
	OVCAR3	Very weak	Not tested	No	
Skin	hTERT-BJ1 (telomerase-immortalized human foreskin fibroblasts)	Yes	Yes	Yes	Abe at al., Mol Cell, 50 (2013), 5-15/Konno at al., Cell, 155 (2013), 688-98.
	NHDF-Ad (normal human dermal fibroblasts)	Not tested	Not tested	Yes	Xia et al., Cell Reports, 14 (2016), 282-97.
	HEMa (normal human epidermal melanocytes)	Yes	Yes	Yes	Xia et al., Cancer Research, 76 (2016), 6747-59.
	A375	Yes	No	No	
	G361	No	No	No	
	MeWo	No	Yes	No	
	RPMI7951	Yes	Yes	No (WB: Yes)	
	SK-MEL-2	Yes	Yes	No	
	SK-MEL-3	Yes	Yes	No (WB: Yes)	
	SK-MEL-5	No	No	No	
	SK-MEL-24	Yes	No (mRNA; Yes)	Yes	
	SK-MEL-28	Yes	Yes	No	
	SK-MEL-31	Yes	Yes	Yes	
	WM115	Yes	Yes	No	

Supplementary Figure 2

Human STING (TMEM173) crystal structure in complex with cGAMP

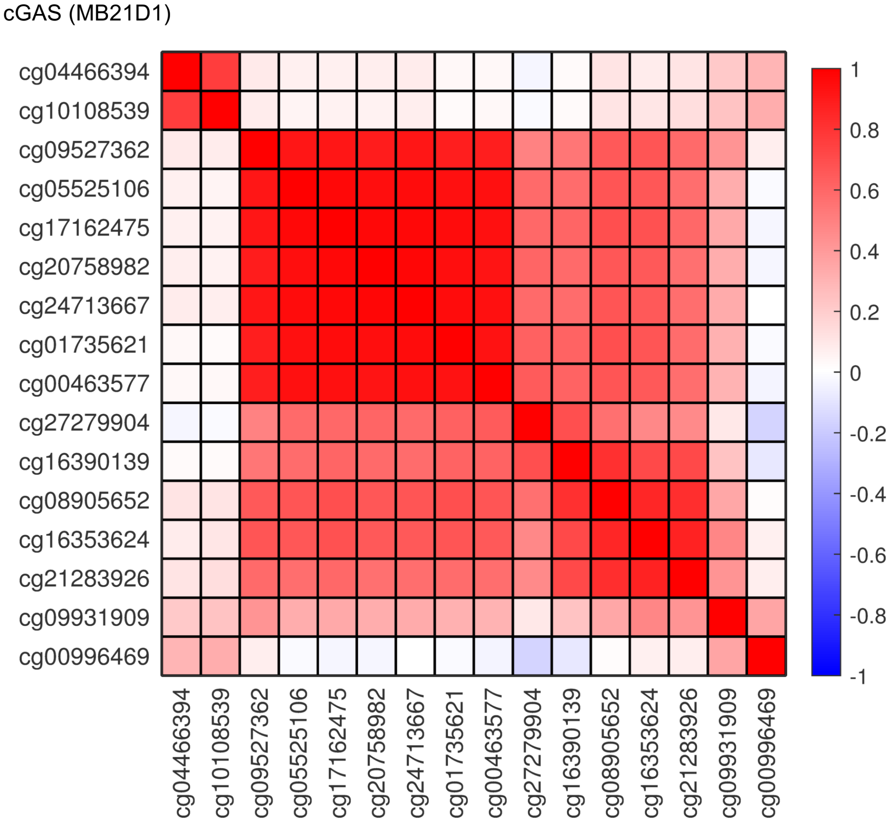


Supplementary Figure 3

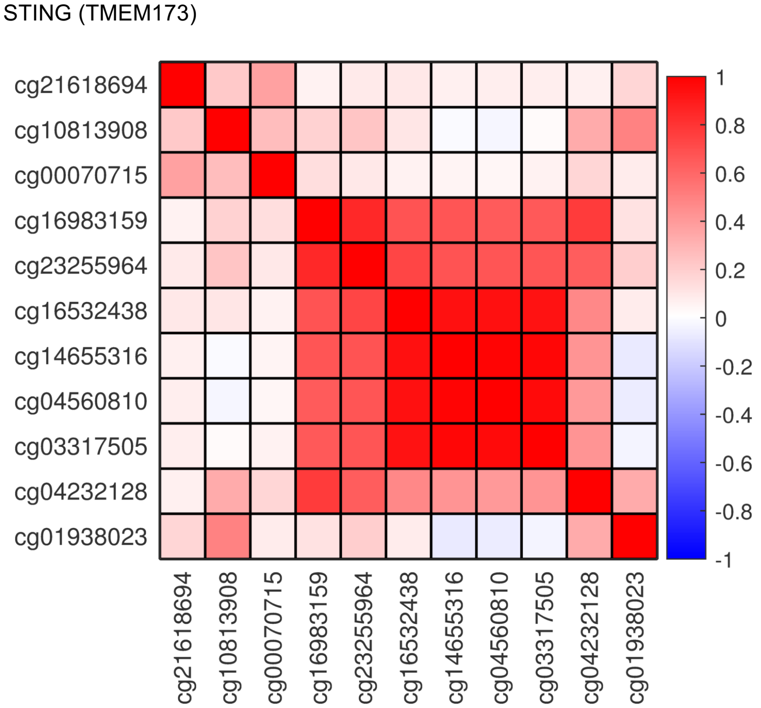


Supplementary Figure 4

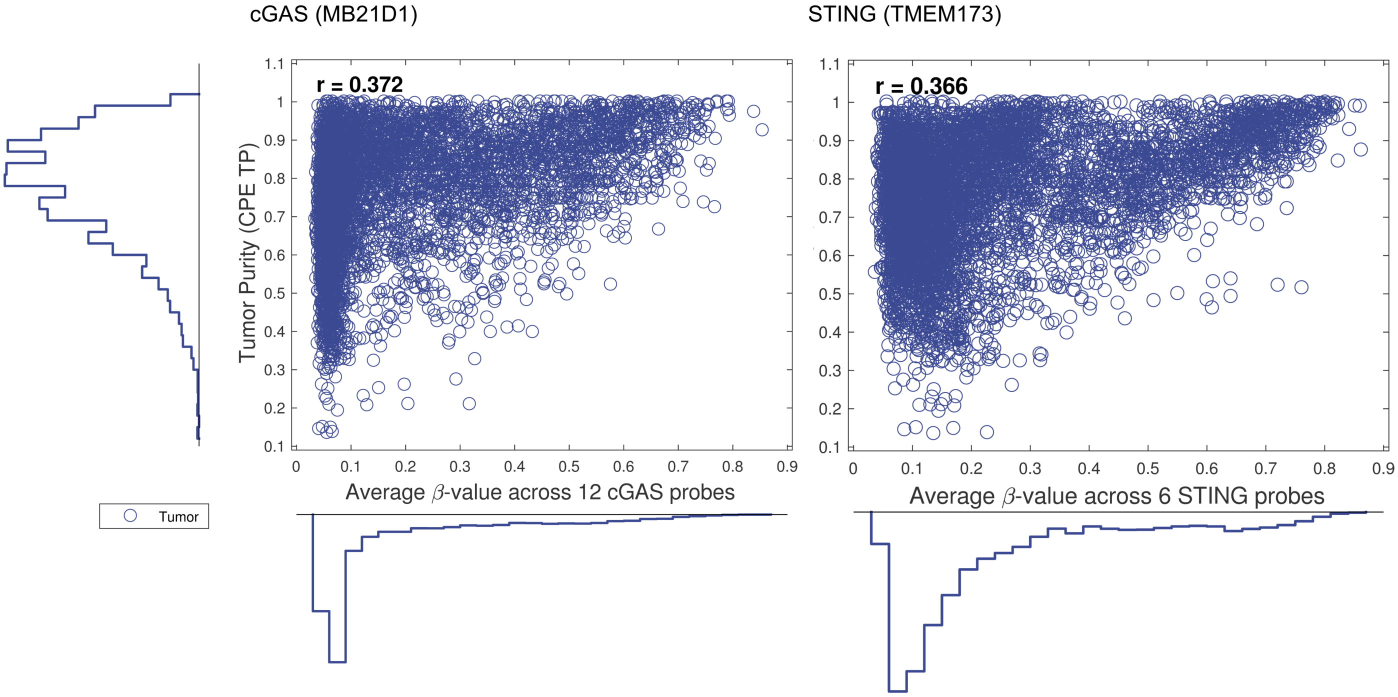
a



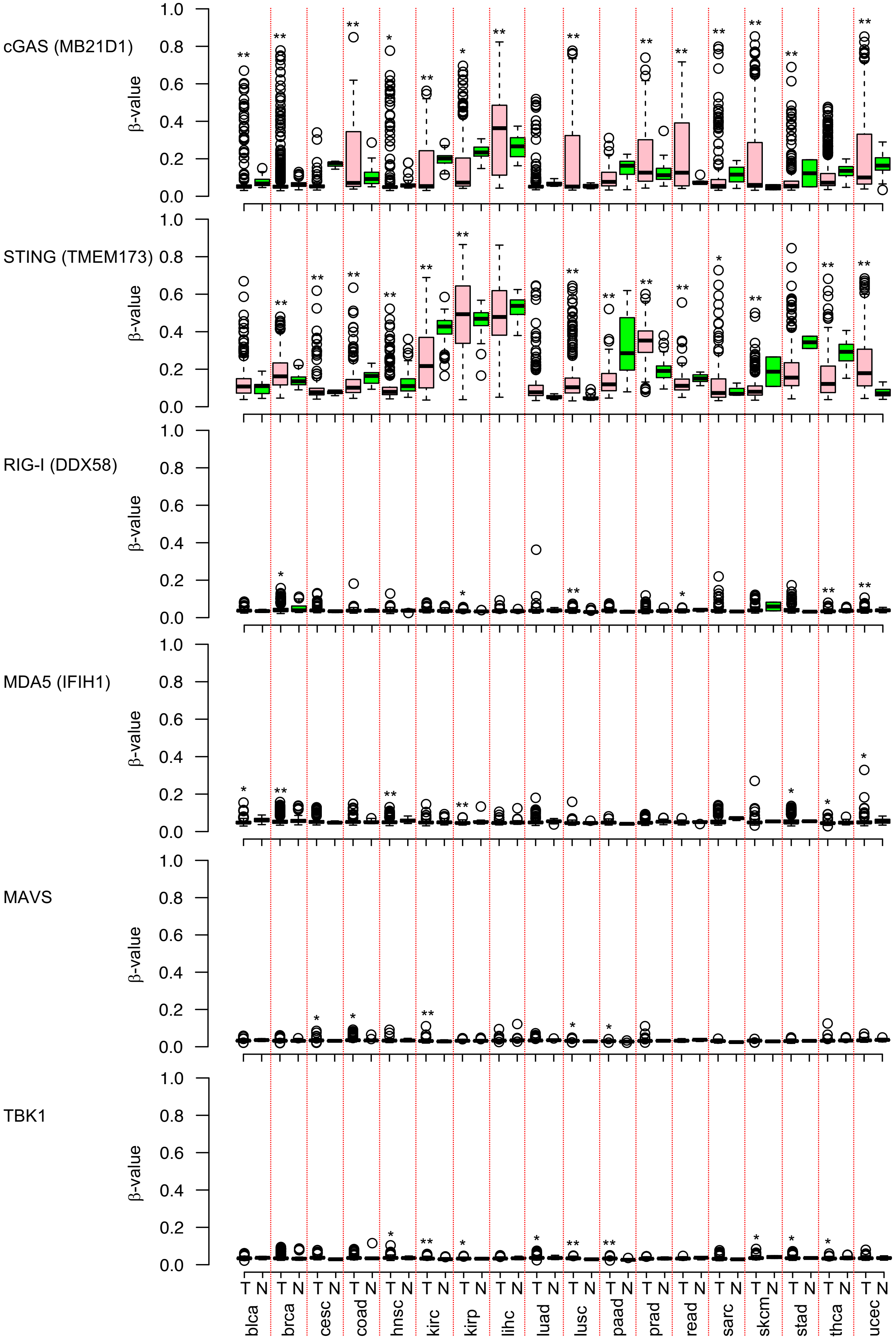
b



Supplementary Figure 5



Supplementary Figure 6



Abbreviation

ACC	Adrenocortical carcinoma
BLCA	Bladder Urothelial Carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
HNSC	Head and Neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LGG	Brain Lower Grade Glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and Paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin Cutaneous Melanoma
STAD	Stomach adenocarcinoma
TGCT	Testicular Germ Cell Tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine Corpus Endometrial Carcinoma
UCS	Uterine Carcinosarcoma
UVM	Uveal Melanoma

Primers used in this study

hSTING mutation

R169W-Fwd	TACATCGGATATCTGTGGCTGATCCTGCCAGAG
R169W-Rev	CTCTGGCAGGATCAGCCACAGATATCCGATGTA
R180Q-Fwd	CTCCAGGCCCGGATTCAAACTTACAATCAGCAT
R180Q-Rev	ATGCTGATTGTAAGTTTGAATCCGGGCCTGGAG
R191W-Fwd	TACAACAACCTGCTATGGGGTGCAGTGAGCCAG
R191W-Rev	CTGGCTCACTGCACCCCATAGCAGGTTGTTGTA
P203S-Fwd	CTGTATATTCTCCTCTCATTGGACTGTGGGGTG
P203S-Rev	CACCCCACAGTCCAATGAGAGGAGAATATACAG
R238W-Fwd	GCTGGCATCAAGGATTGGGTTTACAGCAACAGC
R238W-Rev	GCTGTTGCTGTAAACCCAATCCTTGATGCCAGC
R253W-Fwd	CTGGAGAACGGGCAGTGGGCGGGCACCTGTGTC
R253W-Rev	GACACAGGTGCCCGCCCACTGCCCGTTCTCCAG

hcGAS mutation

G145R-Fwd	GACGTGCCCAGCCCCCGCCTGCCGGTCTCGG
G145R-Rev	CCGAGACCGGCAGGCGGGGGCTGGGCACGTC
P257S-Fwd	CTTTGTGAAATTTAAAAGAAATTCGAAAGAAAATCATCTGAGTC
P257S-Rev	GACTCAGATGATTTTCTTTCGAATTTCTTTTAAATTTCACAAAG
W330R-Fwd	GAATCAAAAAGTAGCCGGCCTGCTAGCACCC
W330R-Rev	GGGTGCTAGCAGGCCGGCTACTTTTTGATTC
R339H-Fwd	CCCAAGAAGGCCTGCACATTCAAAACTGGCT
R339H-Rev	AGCCAGTTTTGAATGTGCAGGCCTTCTTGGG
R376Q-Fwd	AAGAAGAAACATGGCAGCTATCCTTCTCTCA
R376Q-Rev	TGAGAGAAGGATAGCTGCCATGTTTCTTCTT
L377P-Fwd	AAGAAACATGGCGGCCATCCTTCTCTCACAT
L377P-Rev	ATGTGAGAGAAGGATGGCCGCCATGTTTCTT
E383K-Fwd	TCCTTCTCTCACATCAAAAAGGAAATTTTGA
E383K-Rev	TCAAAATTTCCTTTTTGATGTGAGAGAAGGA
E385D-Fwd	TCACATCGAAAAGGATATTTTGAACAATCAT
E385D-Rev	ATGATTGTTCAAAATATCCTTTTCGATGTGA
S434C-Fwd	CATCTGGATAAATTCTGTTCTTATCATGTGAAAACTG
S434C-Rev	CAGTTTTCACATGATAAGAACAGAATTTATCCAGATG
H437D-Fwd	AAATTCTCTTCTTATGATGTGAAAACTGCCT
H437D-Rev	AGGCAGTTTTCACATCATAAGAAGAGAATTT
R499I-Fwd	CTAGCAACTTAATTGACAAAATAAGTAAAGAATTTCTGACAAAGC
R499I-Rev	GCTTTGTCAGAAATTCTTTACTTATTTTGTCAATTAAGTTGCTAG

hcGAS cloning

hcGAS-Fwd	GTTAGATCTATGCAGCCTTGGCACGGAAAGGCCATGC
hcGAS-Rev	GAGTCGACTTACTTGTCATCGTCGTCCTTGTAGTCAAATTCATCAAAAACTGGAAACTCATTG