Cell Reports

Supplemental Information

Deregulation of STING Signaling in Colorectal Carcinoma Constrains DNA-Damage Responses and Correlates With Tumorigenesis

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Supplemental Figures

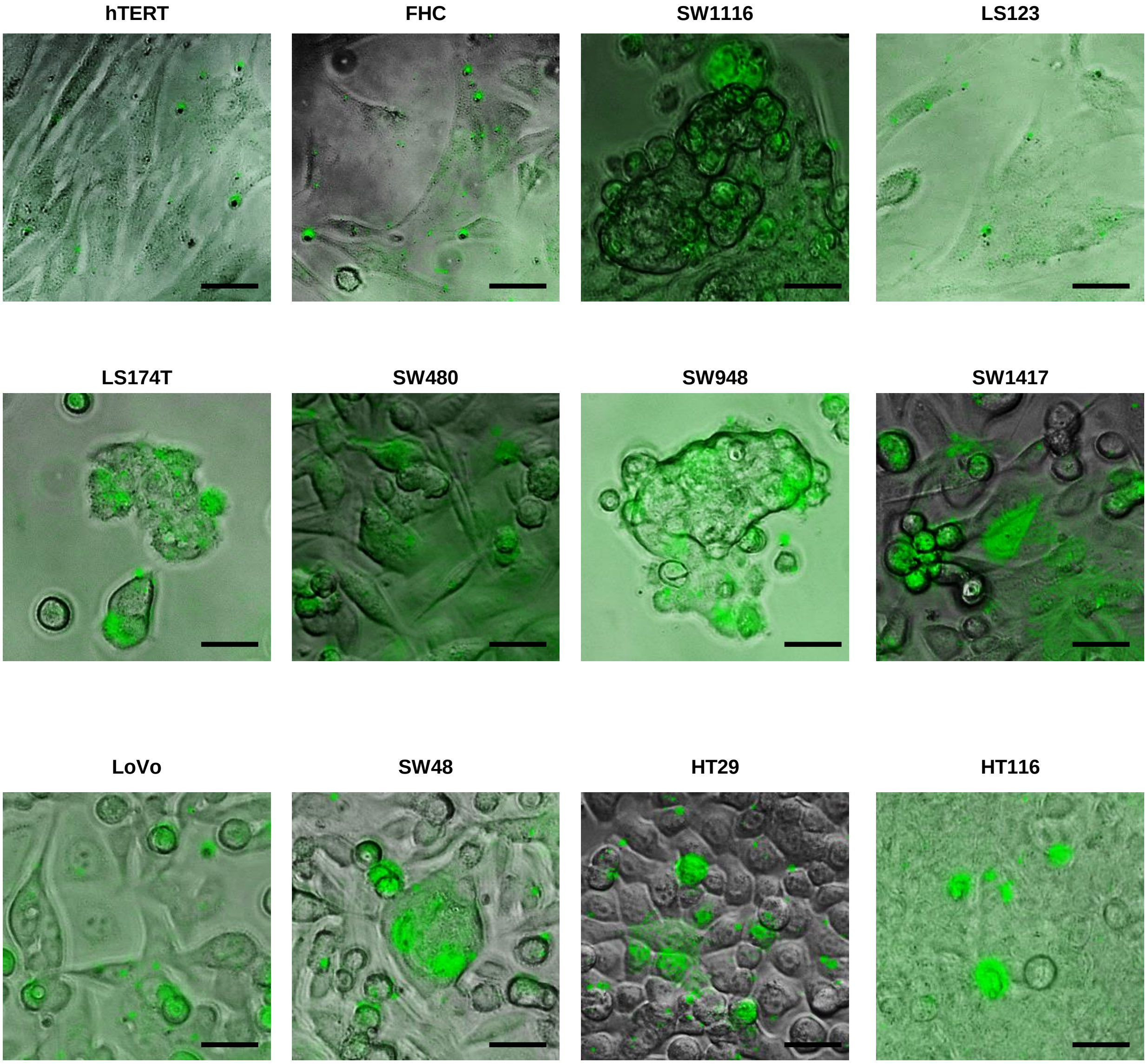


Figure S1, Related to Figure 1. dsDNA90 Transfection efficiency into Colon Cancer Cell Lines were monitor with FITC-dsDNA90 3 hours post Lipofectamine 2000 transfection under fluorescent microscopy. Images shown are at 600X. Bar size, 5µm.

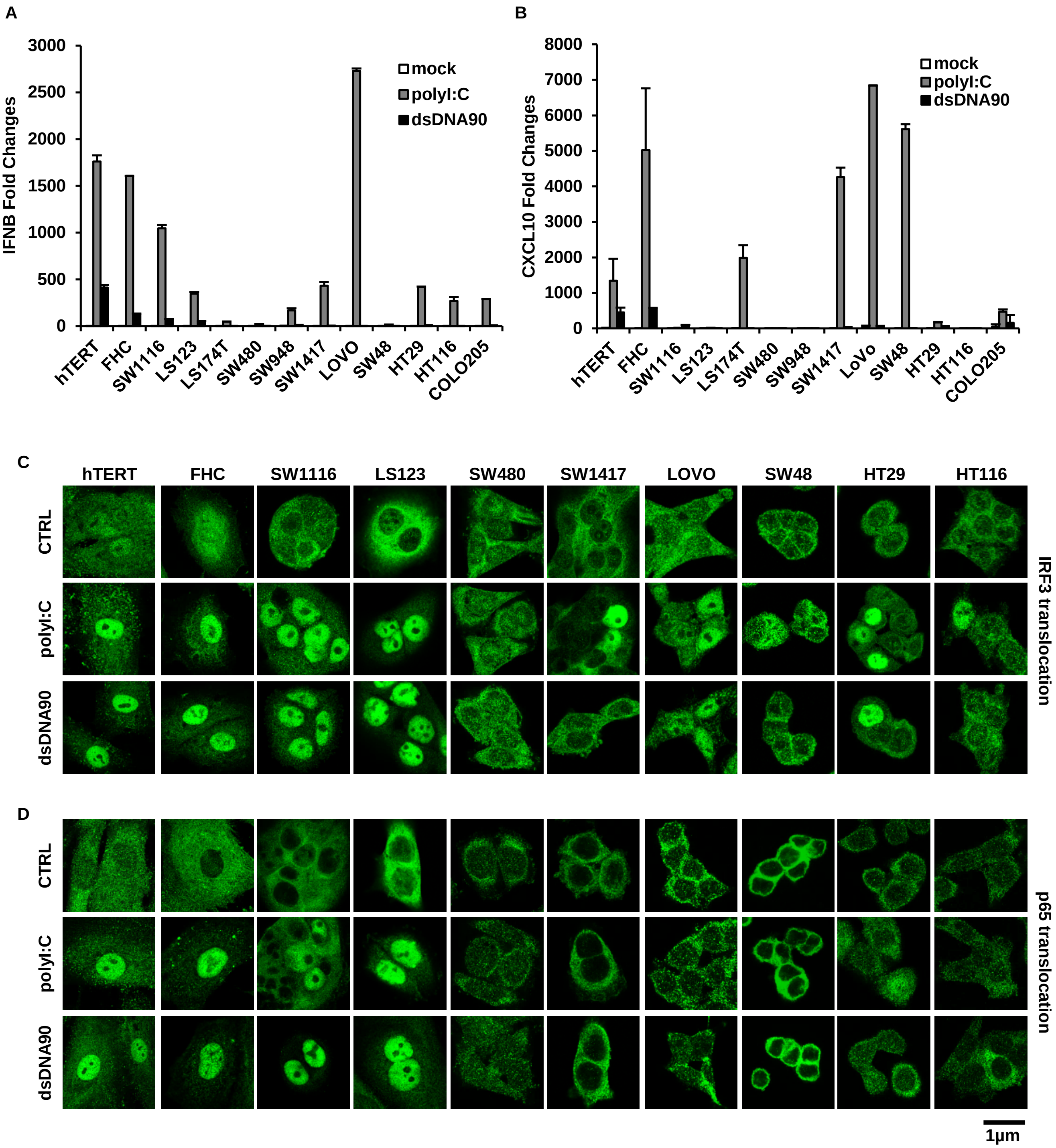


Figure S2, Related to Figure 1 and 2. Normal and colon cancer cells were transfected with 3µg/ml polyIC or dsDNA90 or mock transfected for 3 hour followed by qPCR analysis of human IFNB (A) and CXCL10 (B) expression. Data is representative of at least two independent experiments. Cells similarly treated as A were subjected to Immunofluorescence Microscopy analysis of IRF3 translocation (C) and p65 translocation (D). Representative images are shown at original magnification, 1260X. Bar size, 1µm.

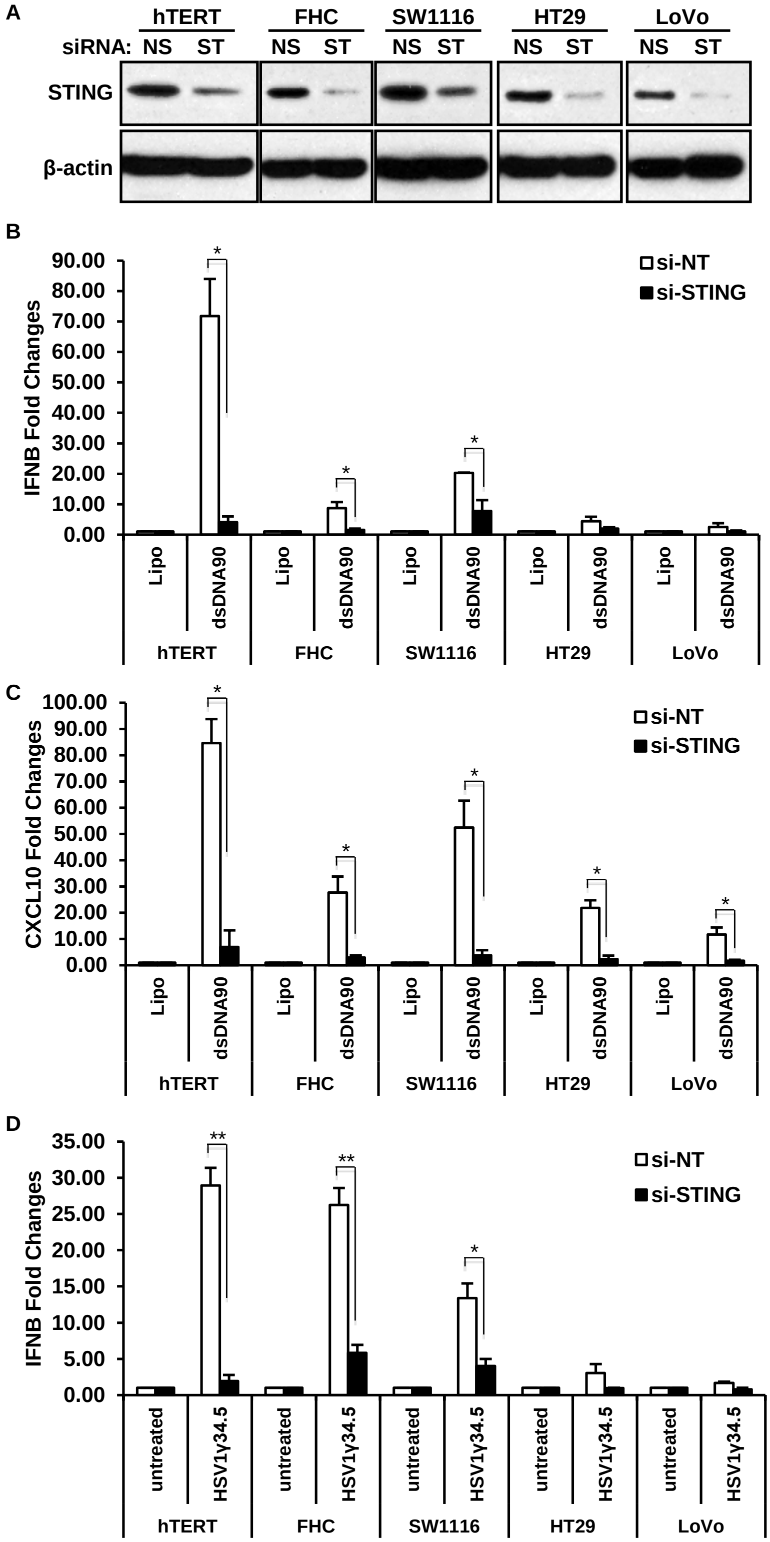


Figure S3, Related to Figure 1 and 4. (A) Normal and colon cancer cells were treated with non-specific siRNA (si-NT) or STING siRNA (si-STING) for 3 days. Cells were then analyzed for STING siRNA efficiency by immunoblot. (B) siRNA treated Cells same as A were transfected with dsDNA90 at 3µg/ml for 3 hours followed by qPCR analysis for IFNB expression. (C) qPCR analysis for CXCL10 expression in cells same as B. (D) siRNA treated Cells same as A were infected with HSVγ34.5 at M.O. I. 5 for 3 hours. Cells were then analyzed by qPCR for IFNB expression. Data is representative of at least two independent experiments. Error bars indicate s.d. *, $p<0.05$; **, $p<0.01$; Student's t-test.

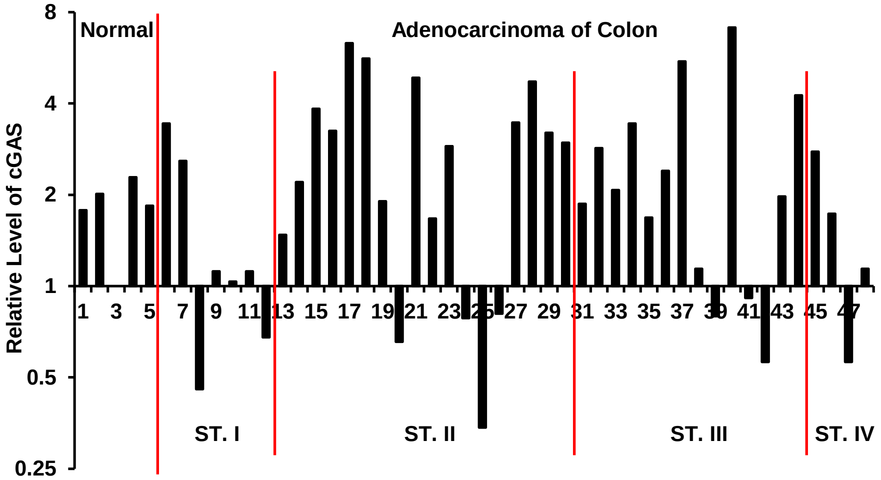
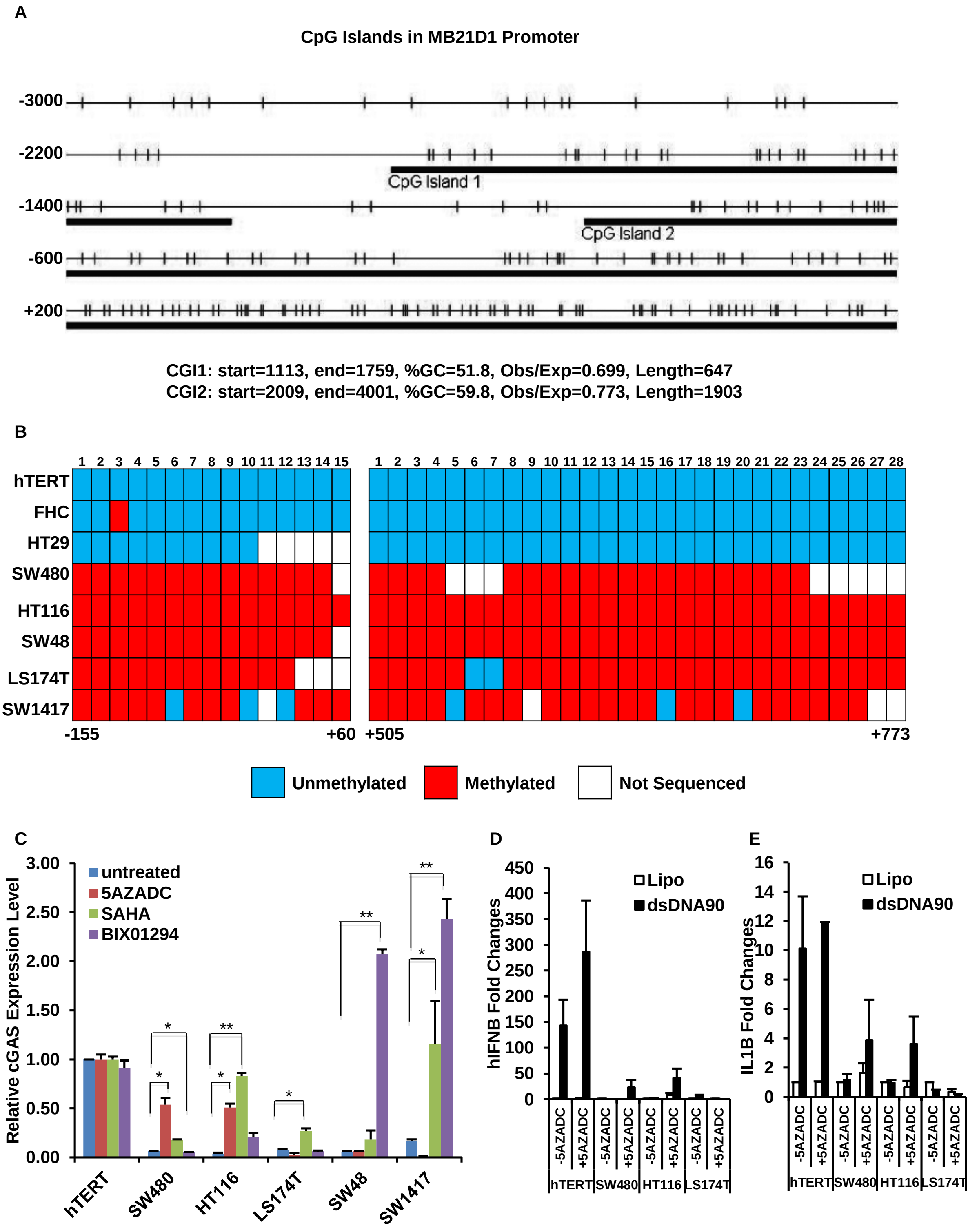


Figure S4, Related to Figure 3. cDNA from 5 normal human colon tissues and 43 human colon cancers of various stages were analyzed by qPCR for cGAS expression.



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CGI2: start=2009, end=4001, %GC=59.8, Obs/Exp=0.773, Length=1903

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hTERT	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
FHC	🟦	🟦	🟥	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
HT29	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	⬜	⬜	⬜	⬜	⬜
SW480	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	⬜
HT116	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
SW48	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	⬜
LS174T	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	⬜	⬜	⬜
SW1417	🟥	🟥	🟥	🟥	🟥	🟦	🟥	🟥	🟥	🟦	⬜	🟦	🟥	🟥	🟥

+505 to +773

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
hTERT	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
FHC	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
HT29	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
SW480	🟥	🟥	🟥	🟥	⬜	⬜	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	⬜	⬜	⬜	⬜	⬜
HT116	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
SW48	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
LS174T	🟥	🟥	🟥	🟥	🟥	🟦	🟦	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
SW1417	🟥	🟥	🟥	🟥	🟦	🟥	🟥	🟥	⬜	🟥	🟥	🟥	🟥	🟥	🟥	🟦	🟥	🟥	🟥	🟦	🟥	🟥	🟥	🟥	🟥	🟥	⬜	⬜

🟦 Unmethylated 🟥 Methylated ⬜ Not Sequenced

Figure S5, Related to Figure 3. (A) Schematic representation of CpG Islands located in the proximal promoter regions of cGAS. (B) Bisulfite sequencing analysis of cGAS promoter region. Each box represent s one CpG dinucleotide located within the promoter region indicated by the position marker at the bottom. Red represents methylated CpG; Blue represents unmethylated CpG; White, not sequenced). (C) Colon cancer cells were treated with 5AZADC (DNA methyltransferase inhibitor), SAHA (histone deacetylase inhibitor) and BIX01294 (histone-lysine methyltransferase inhibitor) at 1µM for 5 days. cGAS expression were then examined by qPCR. (D) IFNB qPCR analysis of cells (same as in Figure 3C) treated with 5AZADC followed by dsDNA transfection at 3µg/ml dsDNA90 for 3 hours. (E) IL1B qPCR analysis of cells same as D. Data is representative of at least two independent experiments. Error bars indicate s.d. *, $p<0.05$; **, $p<0.01$; Student's t-test.

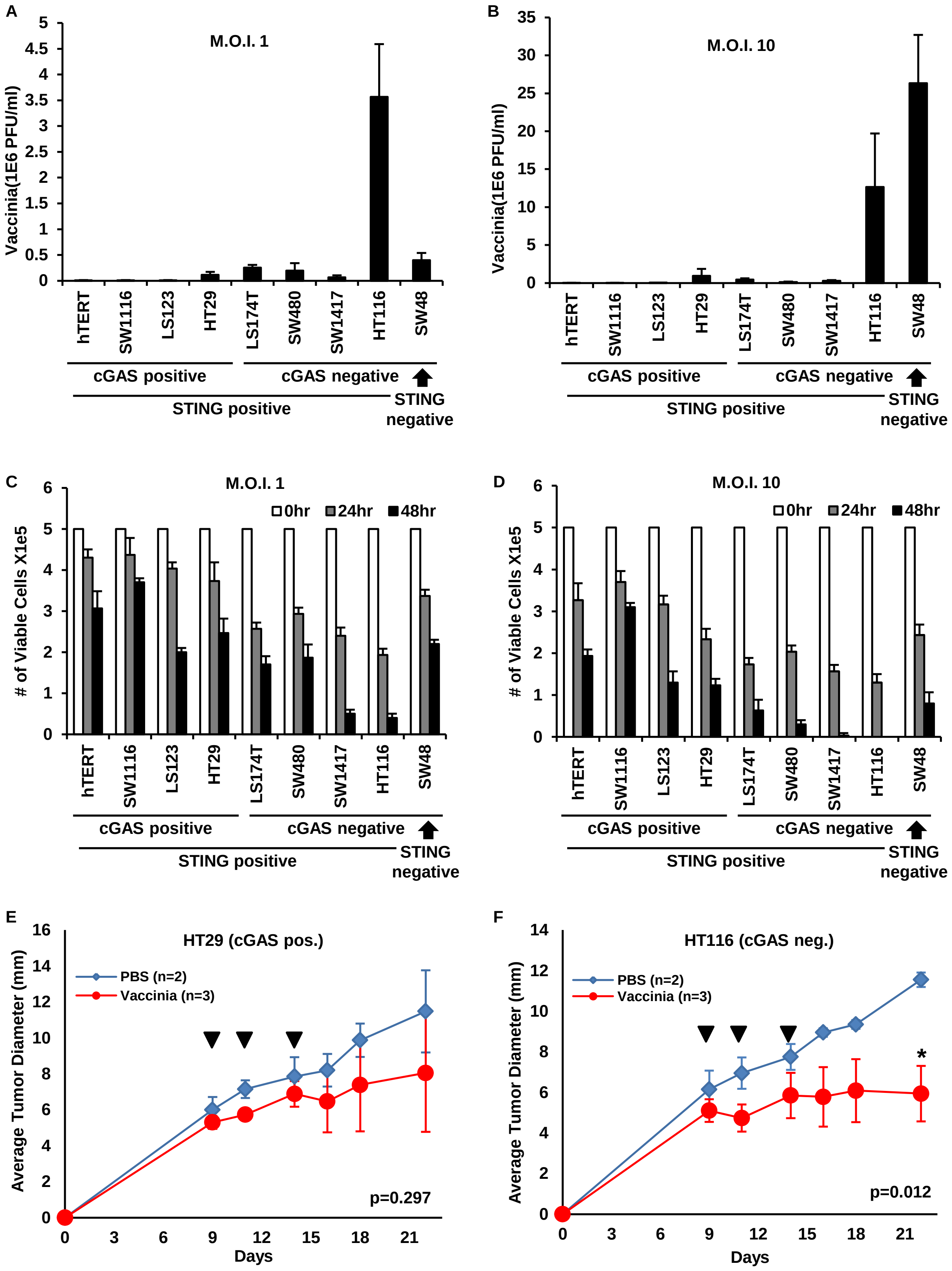

Figure S6, Related to Figure 4 and 7. Normal and Colon Cancer Cells were infected with Vaccinia Virus at M.O.I. 1 (A) or M.O.I. 10 (B) for 24 hours, and viral replication was analyzed by standard plaque assay in Vero cells. Cells were infected with Vaccinia Virus at M.O.I. 1 (C) or M.O.I. 10 (D), and cell viability was analyzed by trypan blue staining 24 hours and 48 hours later. Colon Cancer Cell HT29 (E) or HT116 (F) xenograft tumors were generated in the right flank of nude Balb/c mice. When tumors had reached approximately 0.5 cm in diameter, tumors were injected every other day a total of three times (arrows) with 1E7 PFU Vaccinia Virus in 50 µl PBS (N=3) or 50 µl PBS only (N=2) and tumor growth measured every other day. Statistical analysis was carried out comparing the two treatment groups at the last time point using the unpaired Student's t-test. P values are as indicated.

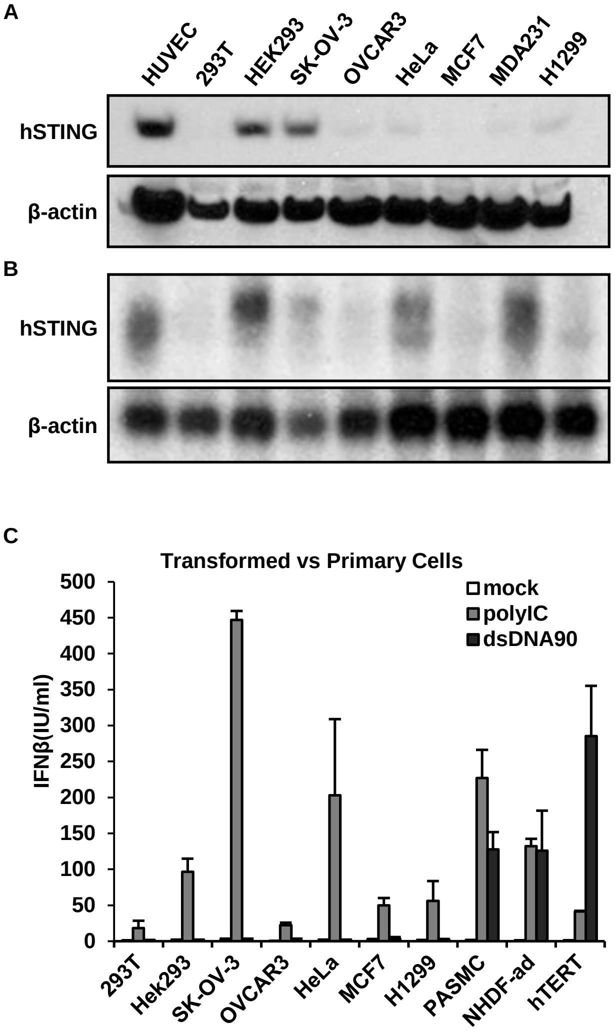

Figure S7, Related to Figure 6. STING expression and dsDNA induced Interferon β production in human cancer cell lines. (A) Immunoblot of STING in various transformed or cancer derived human cell lines (as indicated in the top row). Normal HUVEC cells was used as positive control. (B) Northern blot analysis of STING mRNA expression in cell lines same as A. HUVEC was used as positive control. (C), ELISA analysis of Interferon β production in the media of cells (same as A) transfected with 3µg/ml polyIC or dsDNA90 or mock transfected for 16 hours. Normal human cells (PASMC, NHDF-ad and hTERT) were included as positive controls. Data is representative of at least two independent experiments. Error bars indicate s.d.

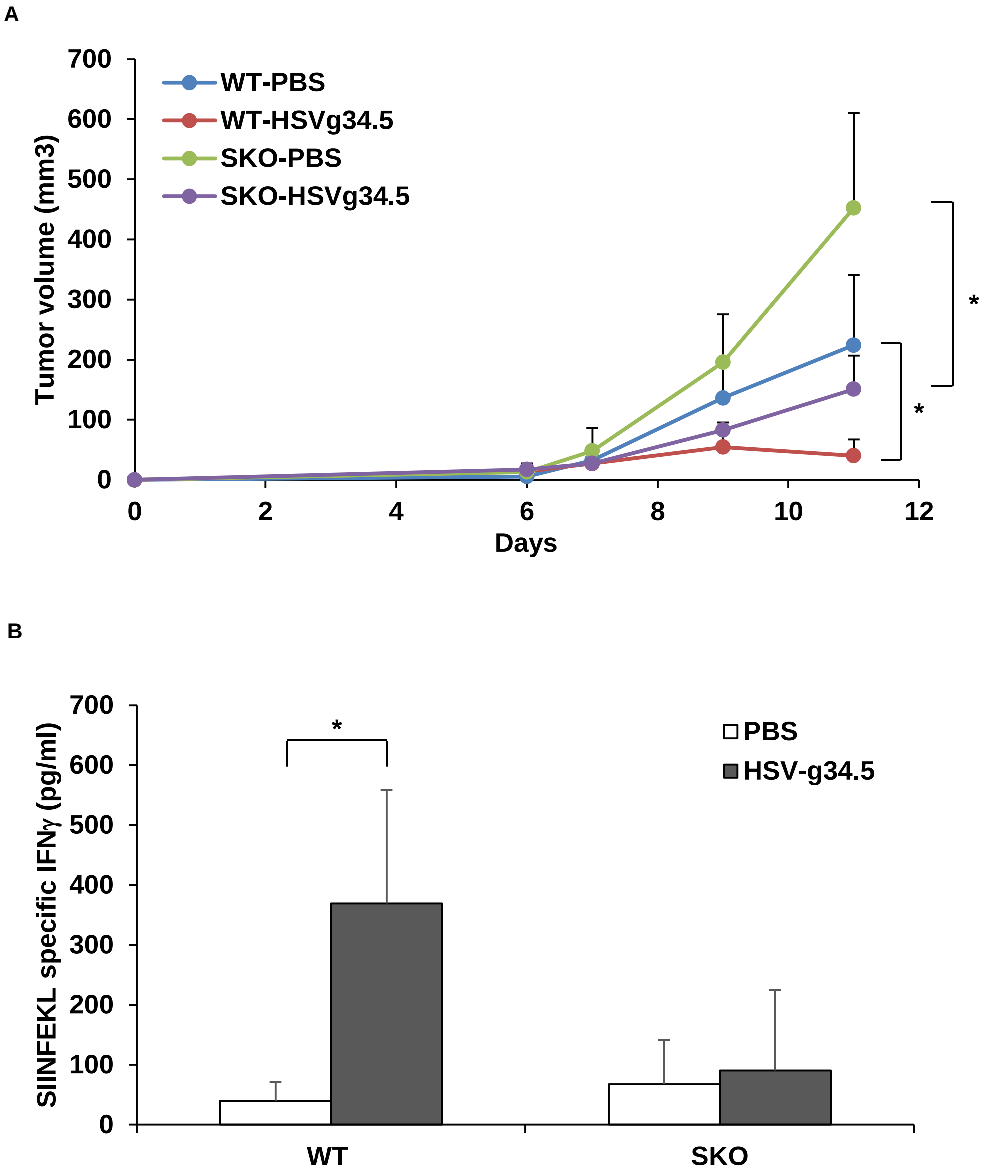

Figure S8, Related to Figure 7. (A) B16-OVA cells (5X10^5/mouse) were subcutaneously injected into C57/BL6 WT and Sting knockout (SKO) mice on day 0. On days 7, 9, 11, mice were injected intratumorally with HSV1γ34.5 (5X10^5 PFU/mouse) and tumor volume was monitored every other day. (B) ELISA of IFNγ in 1X10^6 splenocytes from mice same as A and stimulated with SIINFEKL peptides for 48 hours. Data presented is the mean SD of 4 to 6 mice. Error bars indicate s.d.; *, $p<0.05$; Student's t-test.

Table S1, Related to Figure 1. Sequencing of STING in Colon Cancer Cell Lines

Sample Name	Age	Gender	Ethnicity	Colon Cancer Grade	Target Region	Position in Target	Ref. Base	Variant Found	Variant Name	Variant Class	Variant Function	Reference Codon	Variant Codon
FHC	normal				TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
SW1116	73	male	Caucasian	Dukes' type A, grade III	TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
LS123	65	female	Caucasian	Dukes' type B	TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
LS174T	58	female	Caucasian	Dukes' type B	TMEM173_Intron1	16	C	T	rs80059114	SNP	UTR		
					TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
SW480	50	male	Caucasian	Dukes' type B	TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon3	212	G	R	rs11554776	SNP	Coding, Non-Synon.	CGC=Arg	CAC=His
					TMEM173_Intron4	140	G	R	rs7380272	SNP	Intronic		
					TMEM173_Exon6	169	G	S	rs78233829	SNP	Coding, Non-Synon.	GGT=Gly	GCT=Ala
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
					TMEM173_Intron6_3	3	A	R	rs75746446	SNP	Intronic		
					TMEM173_Exon7	119	G	R	rs7380824	SNP	Coding, Non-Synon.	CGG=Arg	CAG=Gln
					TMEM173_Intron7_2	52	G	S	rs73257329	SNP	Intronic		
SW948	81	female	Caucasian	Dukes' type C, grade III	TMEM173_Intron1	16	C	Y	rs80059114	SNP	UTR		
					TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	R	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
SW1417	53	female	Caucasian	Dukes' type C, grade III	TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	R	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
LOVO	56	male		Dukes' type C, grade IV	TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon3	212	G	R	rs11554776	SNP	Coding, Non-Synon.	CGC=Arg	CAC=His
					TMEM173_Intron4	140	G	R	rs7380272	SNP	Intronic		
					TMEM173_Exon6	169	G	S	rs78233829	SNP	Coding, Non-Synon.	GGT=Gly	GCT=Ala
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
					TMEM173_Intron6_3	3	A	R	rs75746446	SNP	Intronic		
					TMEM173_Exon7	119	G	R	rs7380824	SNP	Coding, Non-Synon.	CGG=Arg	CAG=Gln
					TMEM173_Intron7_2	52	G	S	rs73257329	SNP	Intronic		
SW48	82	female	Caucasian	Dukes' type C, grade IV	TMEM173_Intron1	16	C	Y	rs80059114	SNP	UTR		
					TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon3	226	A	R	Novel	SNP	Coding, Non-Synon.	AG=Arg	GG=His
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
HT29	44	female	Caucasian	colorectal adenocarcinoma	TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
HT116	adult	male		colorectal carcinoma	TMEM173_Exon3	144	G	C	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	G	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg
Colo205	70	male	Caucasian	Dukes' type D	TMEM173_Exon3	144	G	S	rs7447927	SNP	Coding, Synon.	GTG=Val	GTC=Val
					TMEM173_Exon6	175	A	R	rs1131769	SNP	Coding, Non-Synon.	CAT=His	CGT=Arg

Table S2, Related to Figure 3. Sequencing of cGAS in Colon Cancer Cell Lines

Sample Name	Age	Gender	Ethnicity	Colon Cancer Grade	Target Region	Position in Target	Ref. Base	Variant Found	Variant Name	Variant Class	Variant Function	Reference Codon	Variant Codon
FHC	normal				MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
					MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	M	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon4	32	C	Y	rs73754628	SNP	Coding, Synon.	ATC=Ile	ATT=Ile
SW1116	73	male	Caucasian	Dukes' type A, grade III	MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	A	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
LS123	65	female	Caucasian	Dukes' type B	MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_2	11	C	M	rs35629782	SNP	Coding, Non-Synon.	GCG=Ala	GAG=Glu
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	A	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	R	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
LS174T	58	female	Caucasian	Dukes' type B	MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	A	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
SW480	50	male	Caucasian	Dukes' type B	MB21D1_Exon1_1	33	T	-	rs34413328	Deletion	UTR		
					MB21D1_Exon1_1	243	C	M	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	Y	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon5_2	70	G	R	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
SW948	81	female	Caucasian	Dukes' type C, grade III	MB21D1_Exon1_1	243	C	M	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	Y	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	M	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
SW1417	53	female	Caucasian	Dukes' type C, grade III	MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	A	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
LOVO	56	male		Dukes' type C, grade IV	MB21D1_Exon1_1	33	T	HD	rs34413328	Deletion	UTR		
					MB21D1_Exon1_1	243	C	M	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	Y	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon5_2	70	G	R	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
SW48	82	female	Caucasian	Dukes' type C, grade IV	MB21D1_Exon1_1	33	T	HD	rs34413328	Deletion	UTR		
					MB21D1_Exon1_1	243	C	M	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon5_2	70	G	R	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
HT29	44	female	Caucasian	colorectal adenocarcinoma	MB21D1_Exon1_1	243	C	M	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
HT116	adult	male		colorectal carcinoma	MB21D1_Exon1_1	33	T	HD	rs34413328	Deletion	UTR		
					MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	M	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon4	15	C	S	Novel	SNP	Coding, Non-Synon.	CTA=Leu	GTA=Val
					MB21D1_Exon5_2	70	G	R	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys
Colo205	70	male	Caucasian	Dukes' type D	MB21D1_Exon1_1	243	C	A	rs9352000	SNP	Coding, Non-Synon.	ACC=Thr	AAC=Asn
					MB21D1_Exon1_3	11	T	C	rs9446904	SNP	Coding, Synon.	CCT=Pro	CCC=Pro
					MB21D1_Exon2	125	C	A	rs610913	SNP	Coding, Non-Synon.	CCT=Pro	CAT=His
					MB21D1_Exon5_2	70	G	A	rs311678	SNP	Coding, Synon.	AAG=Lys	AAA=Lys

Table S3, related to Figure 6. IHC analysis of STING and cGAS in Colon Cancer Tissue Microarray

Type	Stage	Position	Age	Sex	Diagnose	STING				cGAS			
						Area	Intensity	H-Score	Status	Area	Intensity	H-Score	Status
Tumor	I	E5	59	Female	Adenocarcinoma of colon	3	3	9	+	3	3	9	+
	IIA	B2	68	Male	Adenocarcinoma of colon	3	2	6	+	3	2	6	+
	IIA	C2	69	Female	Adenocarcinoma of colon, mucinous	3	2	6	+	0	0	0	-
	IIA	C4	66	Male	Adenocarcinoma of colon	3	3	9	+	0	0	0	-
	IIA	D1	75	Male	Adenocarcinoma of rectum	3	3	9	+	0	0	0	-
	IIA	D5	83	Female	Adenocarcinoma of colon	3	2	6	+	3	1	3	+
	IIA	F4	57	Female	Adenocarcinoma of colon	2	3	6	+	1	2	2	-
	IIA	H3	70	Female	Adenocarcinoma of colon	3	3	9	+	3	2	6	+
	IIB	G5	58	Female	Adenocarcinoma of colon	3	2	6	+	3	3	9	+
	IIB	H5	75	Male	Adenocarcinoma of colon	3	2	6	+	0	0	0	-
	IIIA	H1	71	Female	Adenocarcinoma of colon, metastatic	3	2	6	+	3	2	6	+
	IIIB	B1	79	Male	Adenocarcinoma of colon	1	1	1	-	3	2	6	+
	IIIB	C5	42	Female	Adenocarcinoma of colon	3	2	6	+	3	3	9	+
	IIIB	E2	50	Male	Adenocarcinoma of colon	3	3	9	+	0	0	0	-
	IIIB	F1	60	Female	Adenocarcinoma of colon	3	3	9	+	0	0	0	-
	IIIB	F5	78	Male	Adenocarcinoma of colon, mucinous	1	2	2	-	1	2	2	-
	IIIB	G4	89	Female	Adenocarcinoma of colon	3	3	9	+	3	3	9	+
	IIIC	C1	55	Female	Adenocarcinoma of colon	2	1	2	-	3	1	3	+
	IIIC	D3	38	Male	Adenocarcinoma of colon	3	3	9	+	0	0	0	-
	IIIC	F2	45	Male	Adenocarcinoma of colon	3	3	9	+	3	3	9	+
	IIIC	G1	64	Male	Adenocarcinoma of colon	3	3	9	+	3	3	9	+
	IIIC	G2	76	Female	Adenocarcinoma of colon	3	2	6	+	1	1	1	-
	IIIC	G3	56	Male	Adenocarcinoma of colon, mucinous	3	3	9	+	2	1	2	-
	IIIC	H4	60	Female	Adenocarcinoma of colon	1	2	2	-	2	3	6	+
	IV	A1	72	Male	Adenocarcinoma of colon, metastatic	1	1	1	-	0	0	0	-
	IV	A3	36	Male	Adenocarcinoma of colon	3	2	6	+	2	1	2	-
	IV	A4	57	Female	Adenocarcinoma of colon, metastatic	2	3	6	+	0	0	0	-
	IV	A5	66	Female	Adenocarcinoma of colon, metastatic	2	3	6	+	3	2	6	+
	IV	B3	77	Male	Adenocarcinoma of colon, metastatic	3	2	6	+	0	0	0	-
	IV	B4	70	Male	Adenocarcinoma of colon, metastatic	3	2	6	+	3	2	6	+
	IV	B5	49	Female	Adenocarcinoma of colon, metastatic	3	2	6	+	3	3	9	+
	IV	D2	72	Male	Adenocarcinoma of colon	3	3	9	+	3	3	9	+
	IV	D4	64	Male	Adenocarcinoma, metastatic	2	3	6	+	0	0	0	-
	Not Reported	A2			Adenocarcinoma of colon	2	1	2	-	3	3	9	+
	Not Reported	E1			Adenocarcinoma of colon	3	3	9	+	3	2	6	+
	Not Reported	E3			Adenocarcinoma of colon	2	3	6	+	3	2	6	+
	Not Reported	E4			Adenocarcinoma of colon	2	3	6	+	3	2	6	+
	Not Reported	F3			Adenocarcinoma of colon	3	3	9	+	2	2	4	+
	Not Reported	H2			Adenocarcinoma of colon	3	3	9	+	0	0	0	-
Normal		I1	71	Female	Within normal limits	3	3	9	+	3	2	6	+
		I3	70	Female	Within normal limits	3	3	9	+	3	3	9	+
		I5	75	Male	Within normal limits	3	2	6	+	3	2	6	+
		I4	60	Female	Within normal limits	3	2	6	+	3	2	6	+
		J1	43	Female	Within normal limits	3	3	9	+	3	3	9	+
		J2	71	Female	Within normal limits	3	3	9	+	3	2	6	+
		J4	47	Male	Within normal limits	3	3	9	+	3	3	9	+
		J5	31	Female	Within normal limits	3	3	9	+	3	2	6	+

Statistical analysis of STING/cGAS expression in TMA-IHC

STAGE	STING expression		cGAS expression	
	H-Score	p-value	H-Score	p-value
Normal	8.25±1.4 (n=8)	N/A	7.1±1.6 (n=8)	N/A
I	9 (n=1)	N/A	9 (n=1)	N/A
II	7±1.5 (n=9)	0.096	2.9±3.4 (n=9)	0.005***
III	6.3±3.2 (n=14)	0.119	4.4±3.7 (n=14)	0.064
IV	5.8±2.0 (n=9)	0.012*	3.6±3.9 (n=9)	0.030*
Unkown Stage	6.8±2.8 (n=6)	0.233	5.2±3 (n=6)	0.136