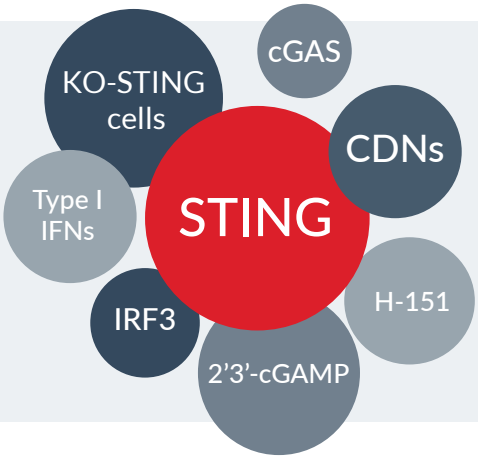
INNATE IMMUNITY

STING & cGAS

Discover the finest array of STING products

- Largest collection of STING ligands
- Functionally tested STING reporter cells
- Expression-ready STING variant genes
- STING-specific inhibitor



A rising star in innate immunity research and immunotherapy is stimulator of interferon genes (STING), whose activation by small nucleic acids called cyclic dinucleotides (CDNs) induces Type I interferons and pro-inflammatory cytokines. STING ligands include microbial CDNs released during infection and the endogenous CDN 2'3'-cGAMP, which is produced by the sensor cGAS in response to cytosolic DNA. Many questions remain on STING's functions, its interplay with other nucleic acid sensors and adaptors, and its role in areas such as infectious diseases, cancer and autoimmunity.

InvivoGen offers a broad selection of products to help you explore STING, its signaling partners, its cytokine induction activity and its therapeutic potential.

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STING: Ripe with therapeutic potential

Stimulator of interferon genes (STING; also known as TMEM173, ERIS, MITA, MPYS or NET23) is an endoplasmic adaptor and sensor protein that is paramount in innate immunity, especially for maintaining homeostasis, defending against infection, and preventing cancer and autoimmunity. Upon activation, STING induces Type I interferons (IFNs; e.g. IFN-β), via the TANK-binding kinase 1 (TBK1)/ interferon regulatory factor 3 (IRF3) pathway[1], and pro-inflammatory cytokines (e.g. TNF-α and IL-6), via the NF-κB pathway[1,2]. These cytokines orchestrate powerful cascades involving various immune cells, other cytokines and chemokines. However, the molecular and cellular mechanisms through which STING balances regulatory and inflammatory responses remain to be elucidated.

STING AND CYCLIC DINUCLEOTIDES

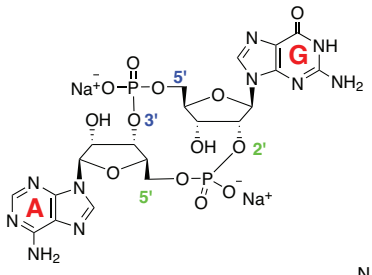
STING is activated by small nucleic acids called cyclic dinucleotides (CDNs), including cyclic diguanylate monophosphate (c-di-GMP)[3], cyclic diadenylate monophosphate (c-di-AMP)[4] and cyclic guanosine monophosphate-adenosine monophosphate (cGAMP)[5,6]. Interestingly, before STING was discovered, some of these CDNs were already known to be important second messengers in microbes. Nearly a decade ago, c-di-GMP was found to provoke a strong immune response in mammalian cells; however, it and other CDNs were only recently discovered to be STING agonists. Upon microbial infection, host STING is activated by CDNs that are released by the invading microbes (see schematic).

CYTOSOLIC DNA, cGAS AND STING

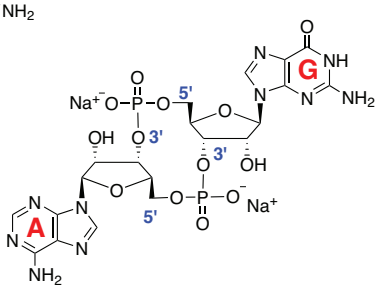
An important breakthrough in innate immunity was the recent back-to-back discovery of metazoan cGAMP and the cytosolic DNA sensor, cyclic cGAMP synthase (cGAS)[5,7]. Following detection of viral, bacterial or self DNA in the cytosol, cGAS generates cGAMP, which then binds to STING, leading to production of Type I IFNs (see schematic).

MICROBIAL vs. METAZOAN CDNs

Intriguingly, it was later discovered that metazoans synthesize their own CDNs to activate STING. Unlike microbial CDNs, in which the two nucleotides are connected by a [G(3',5') pA(3',5')p]' (or canonical) phosphodiester linkage, metazoan CDNs contain a [G(2',5')pA(3'5')] (or non-canonical) linkage[6,8].



2'3'-cGAMP (non-canonical)

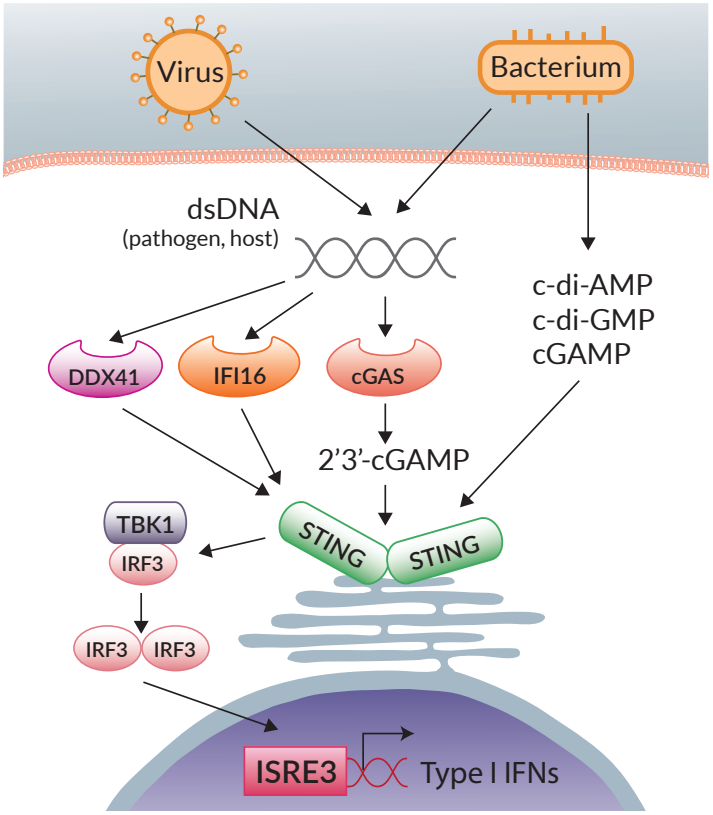


3'3'-cGAMP (canonical)

Thus arose the nomenclature 3'3'-cGAMP for the bacterial CDN, and 2'3'-cGAMP for the mammalian cGAS-produced cGAMP. Interestingly, bacterial 3'3'-cGAMP and metazoan 2'3'-cGAMP bind to the same active site in STING[9].

STING AND OTHER NUCLEIC ACID SENSORS

Increasing evidence now places cGAS as the critical cytosolic DNA sensor that leads to activation of STING. Several other sensors upstream of STING have been proposed to interact with it, including IFI16[10], DDX41[11], MRE11[12] and IFIX[13] (see schematic).



STING-induced IFN pathway

In addition to its role in the response to intracellular DNA, STING is involved in the signaling following detection of viral RNA by RIG-I, including the RNA of Japanese encephalitis virus, Newcastle disease virus and vesicular stomatitis virus[14]. The interplay among STING, cGAS and these other sensors remains opaque.

STING AND HUMAN HEALTH

As a regulator of cytokine signaling, STING has been implicated in various pathologies and in diverse clinical contexts: above all, in infectious diseases, cancer and autoimmunity (see table on next page). Generally, normal STING activity ensures immune homeostasis, deficient activity leads to immunodeficiency, and excessive activity leads to hyperimmunity. Specifically, normal signaling by the cGAS/STING complex triggers spontaneous immune responses to tumor cells[15,16] and enhances cancer radiation therapy.

STING ACTIVITY	TYPE I IFN PRODUCTION	IMMUNE STATUS	CONSEQUENCES	POSSIBLE TREATMENT
Overactive	Excessive	Hyperimmunity	• Inflammatory tumorigenesis • Autoimmunity	STING inhibitor
Normal	Normal	Homeostasis	• Protection against viral infections • Protection against bacterial infections • Tumor surveillance and suppression • Protection against autoimmunity	-
Deficient/Absent	Insufficient	Immunodeficiency	• Viral infections • Bacterial infections • Tumorigenesis and tumor growth	STING agonist

The DNA released by dying tumor cells is sensed by cGAS, which activates STING to induce Type I IFNs in dendritic cells, thereby triggering a potent anti-tumor immune response[17]. A lack of STING activity has been imputed in tumorigenesis[18,19] and in certain viral infections. For example, the Dengue virus protease NS2B3 apparently cleaves STING to block induction of IFN-α/β[20]. Thus, STING-activating CDNs have been extensively pursued as vaccine adjuvants and immunostimulatory agents[21]. Conversely, excessive STING activity has been linked to various autoimmune diseases, including lupus[22] and STING- associated vasculopathy with onset in infancy (SAV1)[23]. Therefore, STING agonists are now being sought for clinical applications in which a potent cytokine response would be beneficial, whereas STING inhibitors are being pursued for indications in which constitutive cytokine induction must be halted.

IN VIVO STABILITY OF CDNs

CDNs are hydrolyzed by various nucleases and phosphodiesterases, some of which are highly specific. In particular, the enzyme ecto-nucleotide pyrophosphatase/phospho-diesterase (ENPP1) was shown to hydrolyze 2'3'-cGAMP but not its bisphosphorothioate synthetic analog, 2'3'-cGAM(PS)$_2$ or canonical 3'3'-cGAMP[24]. Some of these enzymes are critical for microbial infection: for example, successful infection by *Mycobacterium tuberculosis* has been partially ascribed to the bacterium's ability, using one of its phosphodiesterases, to hydrolyze its own c-di-AMP in order to prevent activation of the host STING[25]. Thus, one strategy for therapeutic targeting of STING is to design synthetic CDNs that do not undergo enzymatic hydrolysis.

GENETIC VARIATIONS IN STING

Human STING is encoded by the gene *Tmem173* and appears in several variants within the human population, the most common of which is the R232 variant, considered as wild-type. Recent work has revealed that subtle differences among STING variants can have major functional consequences: for instance, the mutant R232Q is drastically less sensitive to microbial CDNs than is the wild-type. Another variant, V155M, results in a gain-of-function mutation with constitutive activation of STING and upregulation of Type I IFN production, resulting in autoinflammatory diseases[22,23]. Understanding the clinical implications of STING variants will prove crucial to development of STING-based therapies and diagnostics.

INVIVOGEN AND STING

InvivoGen offers an expanding range of products to help you study STING *in vitro* and *in vivo* (see back cover). These include STING agonists, STING variant plasmids, STING reporter cells (including STING knockout and variant knockin cells), an Anti-hSTING monoclonal antibody (mAb), and a potent human and murine STING inhibitor. Our growing selection of validated STING ligands comprises naturally occurring CDNs and analogs thereof. We also sell the potent anti-cancer agent DMXAA, which is a specific agonist of murine STING. Additionally, we offer knockout RAW cells, for major players in STING-induced IFN signaling, such as TBK1 and IRF3. Furthermore, InvivoGen offers a extensive collection of reporter cell lines with a knockin of human STING variants associated with chronic autoinflammatory disease.

1. Ishikawa H. & Barber GN., 2008. STING is an endoplasmicreticulum adaptor that facilitates innate immune signalling. Nature. 455(7213):674-8. **2. Abe T. & Barber GN., 2014.** Cytosolic-DNA-mediated, STING-dependent proinflammatory gene induction necessitates canonical NF-κB activation through TBK1. J Virol. 88(10):5328-41. **3. Burdette DL. *et al.*, 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8. **4. Barker JR. *et al.*, 2013.** STING-dependent recognition of cyclic di-AMP mediates type I interferon responses during Chlamydia trachomatis infection. MBio. 4(3):e00018-13. **5. Wu J. *et al.*, 2013.** Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science. 339(6121):826-30. **6. Ablasser A. *et al.*, 2013.** cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. Nature. 498(7454):380-4. **7. Sun L. *et al.*, 2013.** Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science. 339(6121):786-91. **8. Zhang X. *et al.*, 2013.** Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. Mol Cell. 51(2):226-35. **9. Gao P. *et al.*, 2013.** Structure-function analysis of STING activation by c[G(2',5')pA(3',5')p] and targeting by antiviral DMXAA. Cell. 154(4):748-62. **10. Unterholzner L. *et al.*, 2010.** IFI16 is an innate immune sensor for intracellular DNA. Nat Immunol. 11(11):997-1004. **11. Zhang Z., *et al.*, 2011.** The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nature Immunology. 12: 959–965. **12. Kondo T. *et al.*, 2013.** DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. Proc Natl Acad Sci U S A. 110(8):2969-74. **13. Diner BA. *et al.*, 2015.** The functional interactome of PYHIN immune regulators reveals IFIX is a sensor of viral DNA. Mol Syst Biol. 11(2):787. **14. Ran Y. *et al.*, 2014.** MITA/STING: a central and multifaceted mediator in innate immune response. Cytokine Growth Factor Rev. 25(6):631-9. **15. Woo SR. *et al.*, 2014.** STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 41(5):830-42. **16. Klarquist J. *et al.*, 2014.** STING-mediated DNA sensing promotes antitumor and autoimmune responses to dying cells. J Immunol. 193(12):6124-34. **17. Deng L. *et al.*, 2014.** STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors. Immunity. 41(5):843-52. **18. Zhu Q. *et al.*, 2014.** Cutting edge: STING mediates protection against colorectal tumorigenesis by governing the magnitude of intestinal inflammation. J Immunol. 193(10):4779-82. **19. Ahn J. *et al.*, 2015.** Diverse roles of STING-dependent signaling on the development of cancer. Oncogene. [Ahead of print]. **20. Aguirre S. *et al.*, 2012.** DENV inhibits type I IFN production in infected cells by cleaving human STING. PLoS Pathog. 8(10):e1002934. **21. Chandra D. *et al.*, 2014.** STING ligand c-di-GMP improves cancer vaccination against metastatic breast cancer. Cancer Immunol Res. 2(9):901-10. **22. Jeremiah N. *et al.*, 2014.** Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. J Clin Invest. 124(12):5516-20. **23. Liu Y. *et al.*, 2014.** Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 371(6):507-18. **24. Li L. *et al.*, 2014.** Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs. Nat Chem Biol. 10(12):1043-8. **25. Yang J. *et al.*, 2014.** Deletion of the cyclic di-AMP phosphodiesterase gene (cnpB) in Mycobacterium tuberculosis leads to reduced virulence in a mouse model of infection. Mol Microbiol. 93(1):65-79.

STING Products

• STING Ligands

Cyclic dinucleotides (CDNs) and xanthenone derivatives, such as DMXAA, were recently found to bind to and activate STING, leading to a potent Type I IFN response. CDNs are important messengers in bacteria, affecting numerous responses in prokaryotic cells as well as in mammalian cells, acting as agonists of the innate immune response. CDNs represent a promising new class of vaccine adjuvants. For your research needs, InvivoGen provides a large collection of high quality CDNs. They are chemically synthesized and are characterized by U-HPLC, NMR and MS. For each lot, the biological activity is validated and the absence of bacterial contamination (e.g. endotoxins) is verified using cell-based assays. InvivoGen's CDNs are available in two grades:

- **InvivoGen Standard Grade**: purity >95%
- **VacciGrade™**: purity >95%, sterility guaranteed, endotoxin level <5 EU/mg

PRODUCT	QTY	CAT. CODE
2'3'-cGAMP	500 µg	tlrl-nacga23
2'3'-cGAMP VacciGrade™	2 x 500 µg	vac-nacga23
2'3'-cGAM(PS)$_2$ (Rp/Sp)	250 µg	tlrl-nacga2srs
2'2'-cGAMP	500 µg	tlrl-nacga22
3'3'-cGAMP	500 µg	tlrl-nacga
3'3'-cGAMP VacciGrade™	2 x 500 µg	vac-nacga
c-di-AMP	1 mg	tlrl-nacda
c-di-AMP VacciGrade™	1 mg	vac-nacda
c-di-GMP	1 mg	tlrl-nacdg
c-di-GMP VacciGrade™	1 mg	vac-nacdg
DMXAA	5 mg	tlrl-dmx
H-151 (STING Inhibitor)	10 mg	inh-h151

Please see the website for our full range of STING ligands

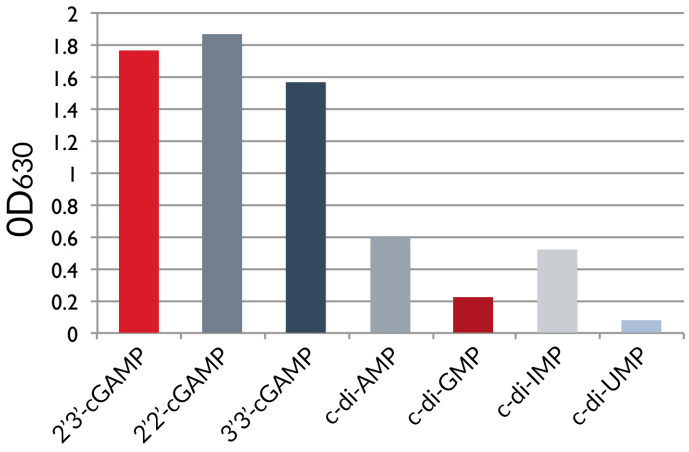
• STING Reporter Cells

STING activation induces interferon stimulated genes (ISG) through interferon regulatory factors (IRFs). To facilitate the analysis of STING ligands, InvivoGen has developed stable reporter cells in which STING has been either knocked out or knocked down. These cells feature IRF-inducible secreted reporter proteins, SEAP (secreted embryonic alkaline phosphatase) or Lucia luciferase, as convenient read-outs.

- **B16-Blue™ ISG-KO-STING and B16-Blue™ ISG cells** were derived from the mouse B16 F1 melanoma cell line and express the SEAP gene.
- **RAW-Lucia™ ISG-KO-STING and RAW-Lucia™ ISG cells** were derived from the mouse RAW 264.7 macrophage cell line and express the Lucia luciferase gene. RAW-Lucia™ ISG cells knocked out for cGAS, IFI16 or IRF3 are also available (see table below).
- **THP1-Blue™ ISG cells** were derived from the human THP-1 monocyte cell line and express the SEAP gene.
- **THP1-Dual™ KO-STING, THP1-Dual™ KI-hSTING, and THP1-Dual™** were derived from the human THP-1 monocyte cell line and express the SEAP gene and the Lucia luciferase gene.

PRODUCT	CAT. CODE
B16-Blue™ ISG	bb-ifnabg
B16-Blue™ ISG-KO-STING	bb-kostg
RAW-Lucia™ ISG	rawl-isg
RAW-Lucia™ ISG-KO-cGAS	rawl-kocgas
RAW-Lucia™ ISG-KO-IFI16	rawl-koif16
RAW-Lucia™ ISG-KO-IRF3	rawl-koirf3
RAW-Lucia™ ISG-KO-STING	rawl-kostg
THP1-Blue™ ISG	thp-isg
THP1-Dual™	thpd-nfis
THP1-Dual™ KO-STING	thpd-kostg
THP1-Dual™ KI-hSTING-S154	thpd-s154
THP1-Dual™ KI-hSTING-M155	thpd-m155
THP1-Dual™ KI-hSTING-A162	thpd-a162
THP1-Dual™ KI-hSTING-H232	thpd-h232
THP1-Dual™ KI-hSTING-R232	thpd-r232

**All cells are provided frozen in a cryotube containing 3-7 x 10^6 cells.*
Please see the website for our full range of STING reporter cell lines



THP1-Blue™ ISG cells were stimulated with 10 µg/ml of various CDNs. Cells were not permeabilized. After 24 h of incubation, the levels of IRF-induced SEAP were determined using QUANTI-Blue™, a SEAP detection reagent.

Also Available

STING Variants

Several non-synonymous variants of STING have been described in the human population, as well as various induced mutants of the human and mouse STING genes. Studies have revealed that STING variation can affect CDN recognition and signal transduction. InvivoGen provides the most relevant STING variants cloned into an expression plasmid.

STING Inhibitor

InvivoGen offers H-151, a potent, irreversible, and selective small molecule inhibitor of both human and murine STING.

Anti-hSTING mAb

InvivoGen offers Anti-hSTING-IgG, a monoclonal mouse IgG1 antibody (mAb) against human STING (hSTING). It recognizes the most prevalent isoforms, "wild-type" R232 and HAQ hSTING variants.

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