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Unravel the Mysteries of STING:

A Hub for Innate Immune Responses to Nucleic Acids

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InvivoGen sas Europe (Toulouse, France)

Founded in 1977 by Pr. Gerard Tiraby

InvivoGen Inc. America (San Diego, U.S.)

US subsidiary created in 1997

InvivoGen Ltd. Asia (Hong Kong, China)

Asian subsidiary incorporated in 2014

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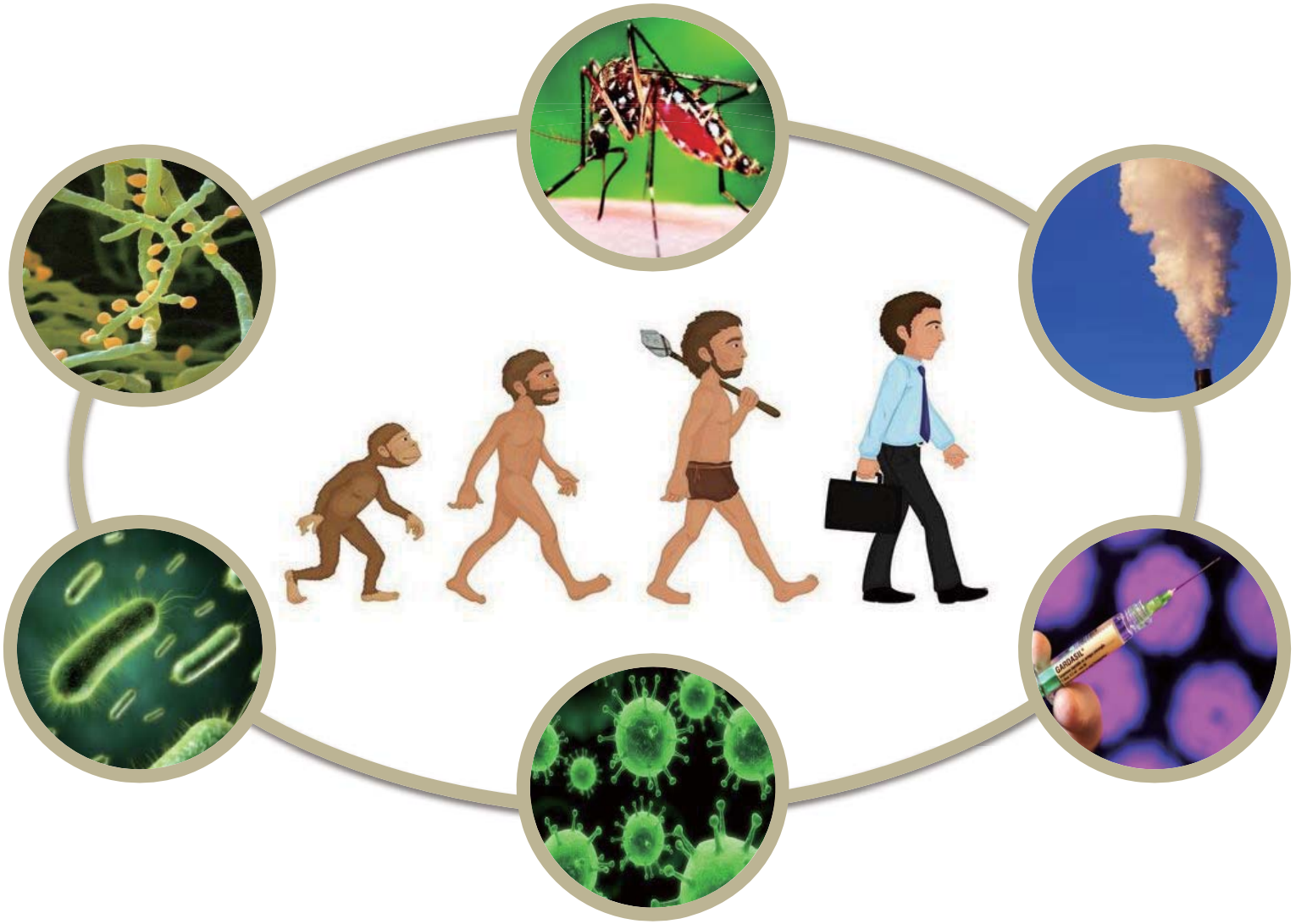
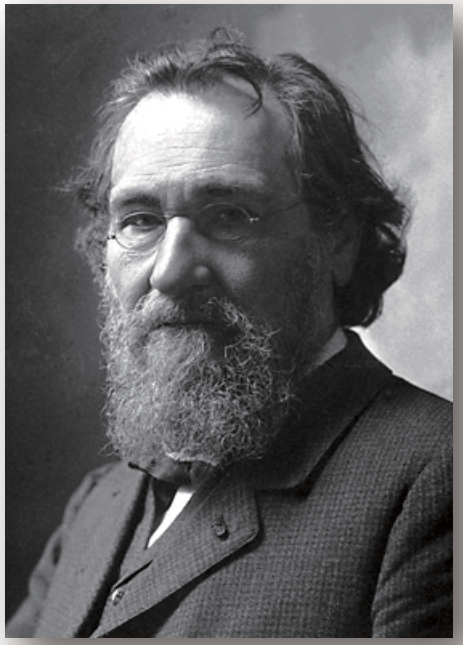
Our bodies are constantly being exposed to infectious agents



Research in defense of infectious agents is lasting for centuries

Chinese began practicing a form of immunization by drying and inhaling powders derived from the crusts of smallpox lesions in 1000 A.D.

Edward Jenner developed the first vaccine(Small pox vaccine) in the world in 1798.



Élie Metchnikoff discovered phygocytes in 1882, won the 1908 Nobel Prize in Physiology or Medicine

Approaching the Asymptote? Evolution and Revolution in Immunology

C.A. JANEWAY, JR.
Section of Immunology, Howard Hughes Medical Institute at Yale University School of Medicine New Haven, Connecticut 06510

In 1989

Cited by 3442 articles

Table 1. Effector Mechanisms in Immunity, Nonspecific and Specific

Nonspecific effector mechanism	Specific effector mechanism
Alternative complement cascade	antibody + classic complement cascade
Phagocytosis	antibody + Fc-receptor-induced phagocytosis
C-reactive protein	specific antibody to phosphocholine
Macrophage sequestration	T-dependent macrophage activation
Natural killer cells	cytolytic T cells

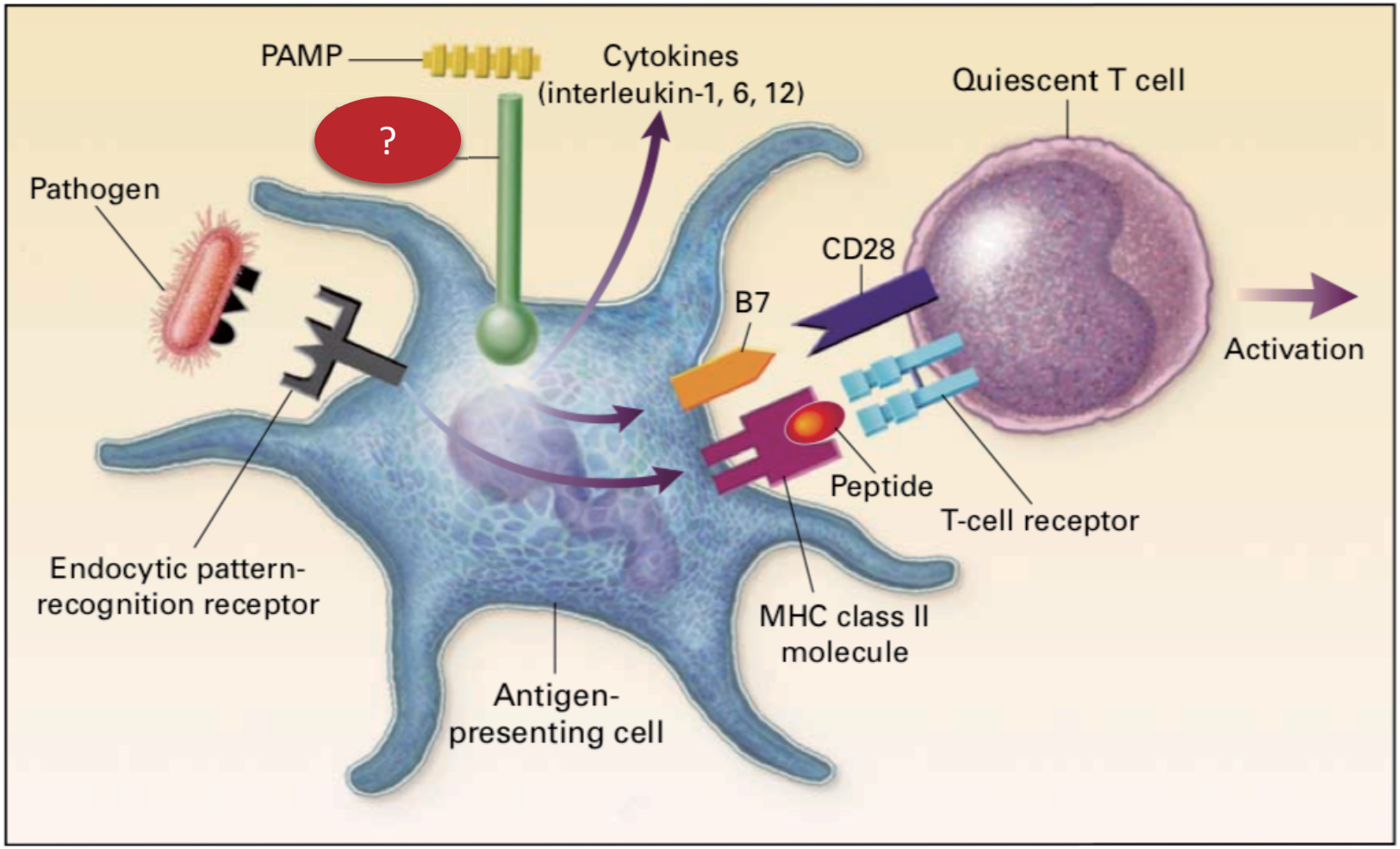
Innate Immune System

- non specific
- first line of defense
- immediate response
- memory is non-specific

Adaptive Immune System

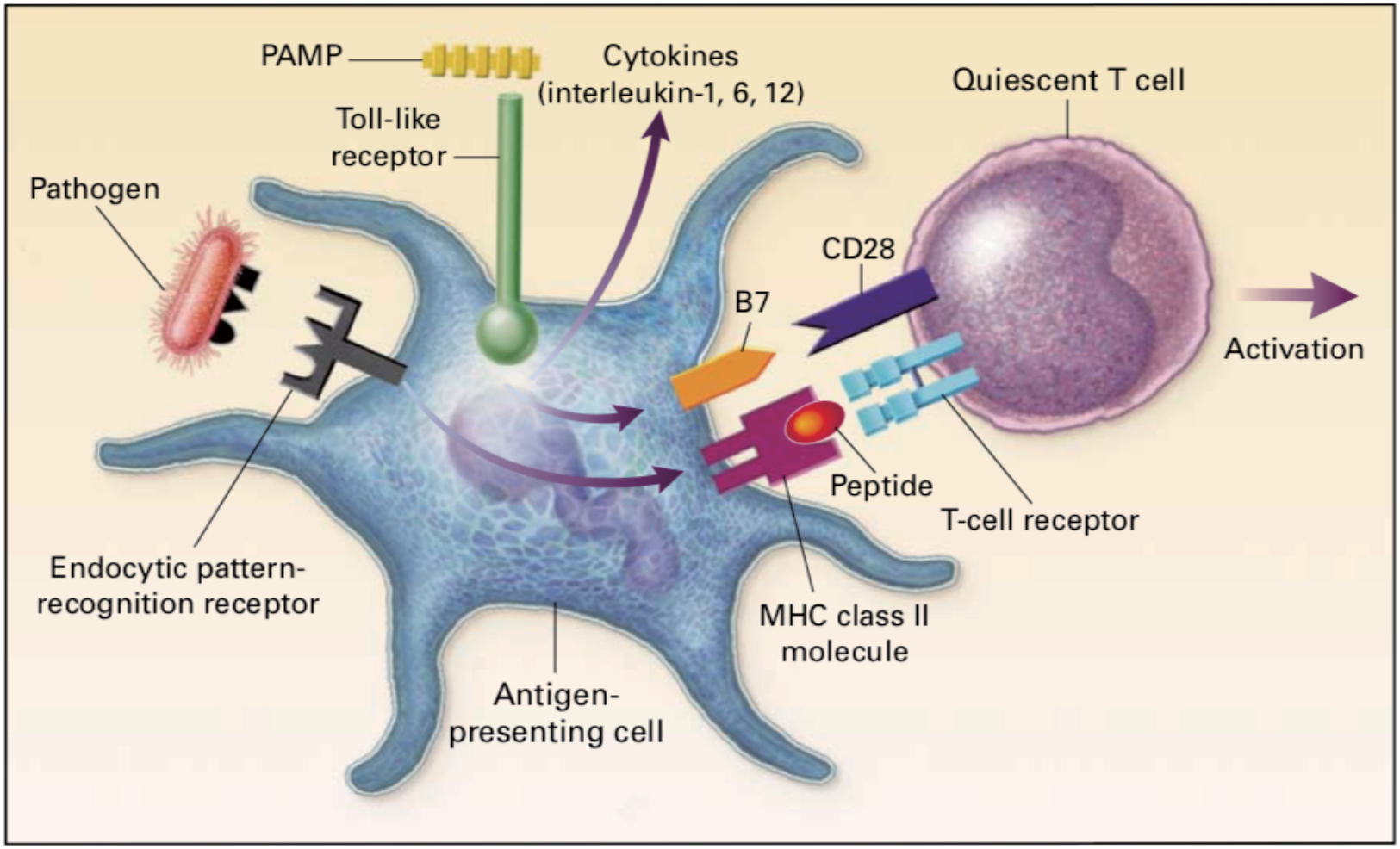
- specific
- second line of defense
- slow response
- long term memory

Innate immune system active Adaptive Immune system: pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) , in turn trigger B7 (CD80/86) signal and cytokines



Janeway, C.A. Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54, 1–13 (1989).

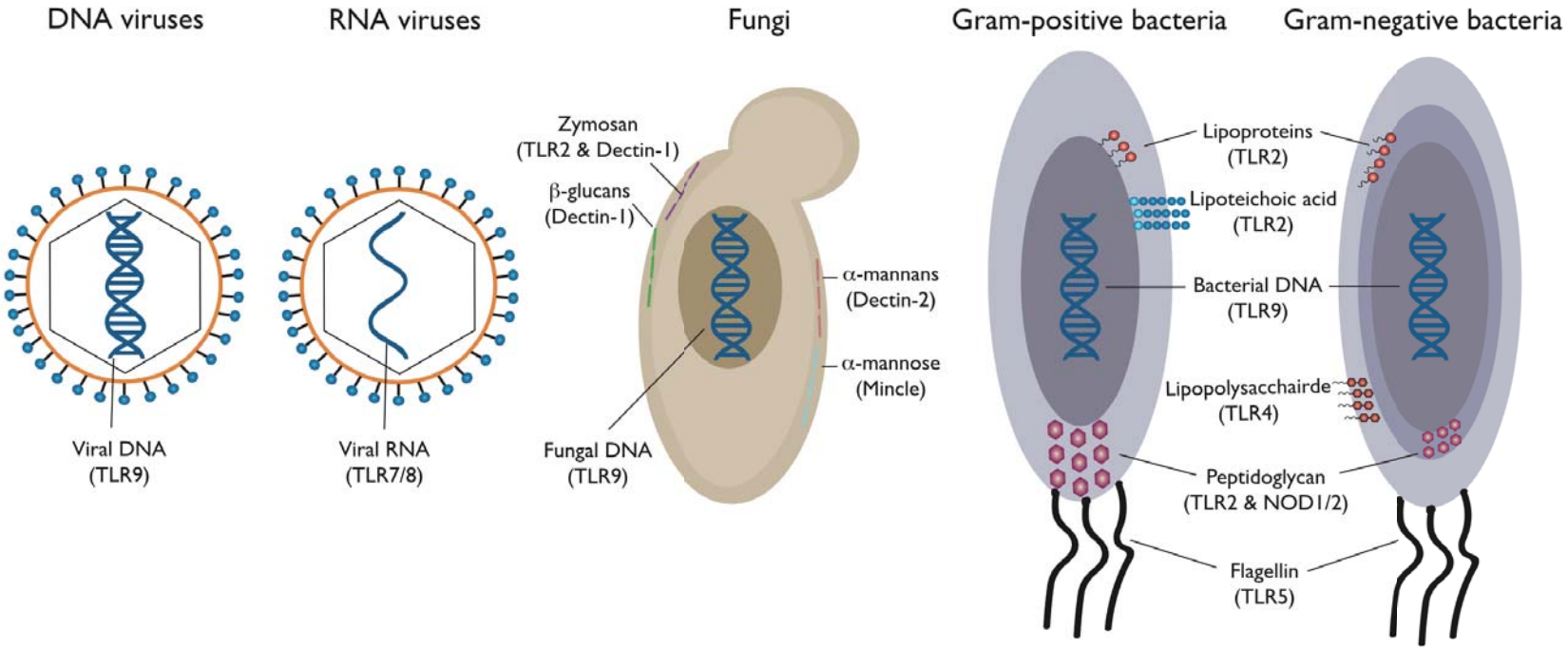
Innate immune system active Adaptive Immune system: pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) , in turn trigger B7 (CD80/86) signal and cytokines



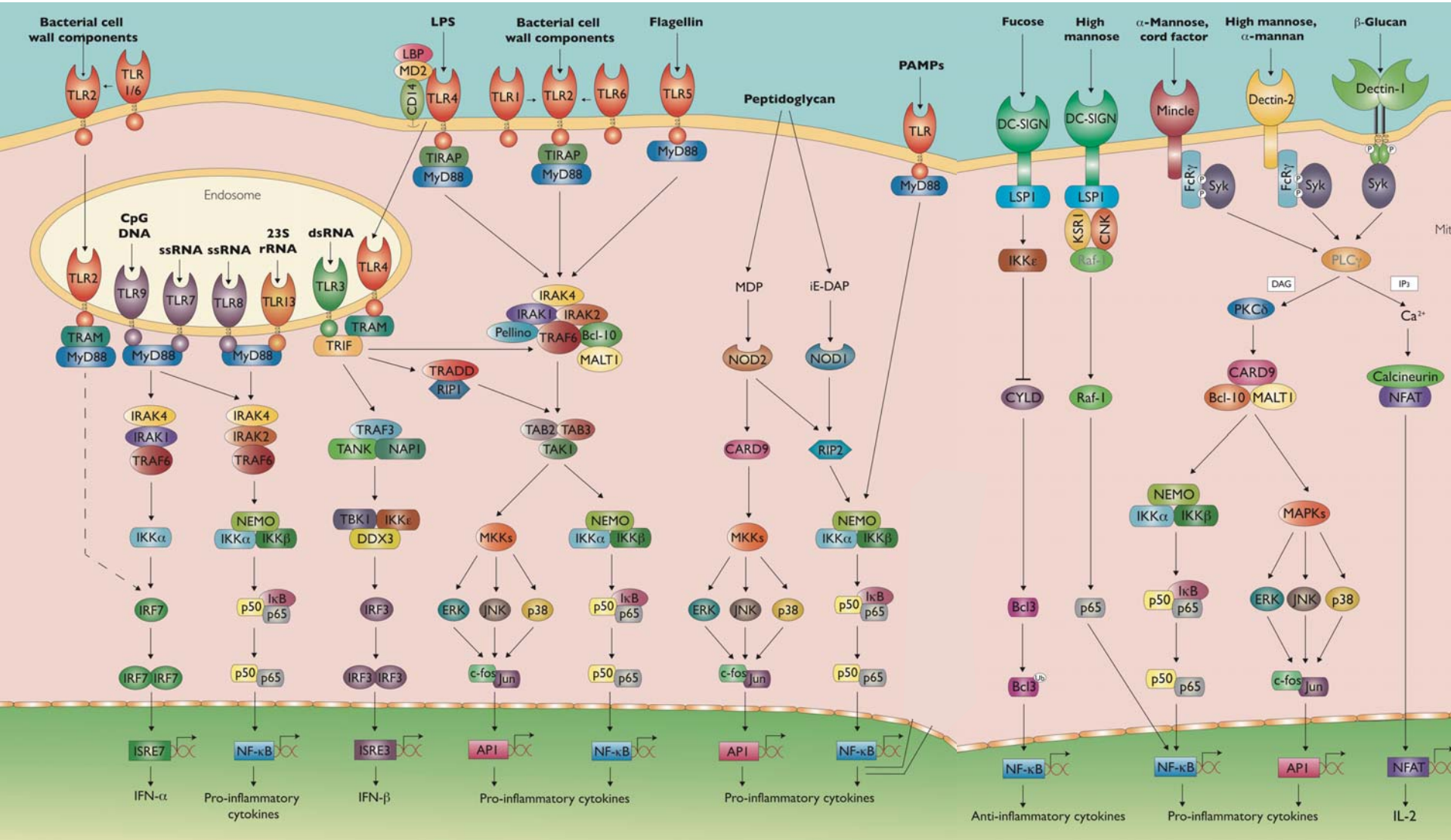
Janeway, C.A. Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. 54, 1–13 (1989).

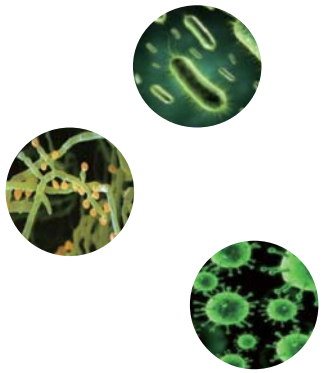
Ruslan Medzhitov. et al. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997

Recognition of microbial pathogens: Microbial pathogens expressed highly conserved structural motifs named pathogen-associated molecular patterns (PAMPs) which could be specifically recognized by pattern recognition receptors (PRRs).

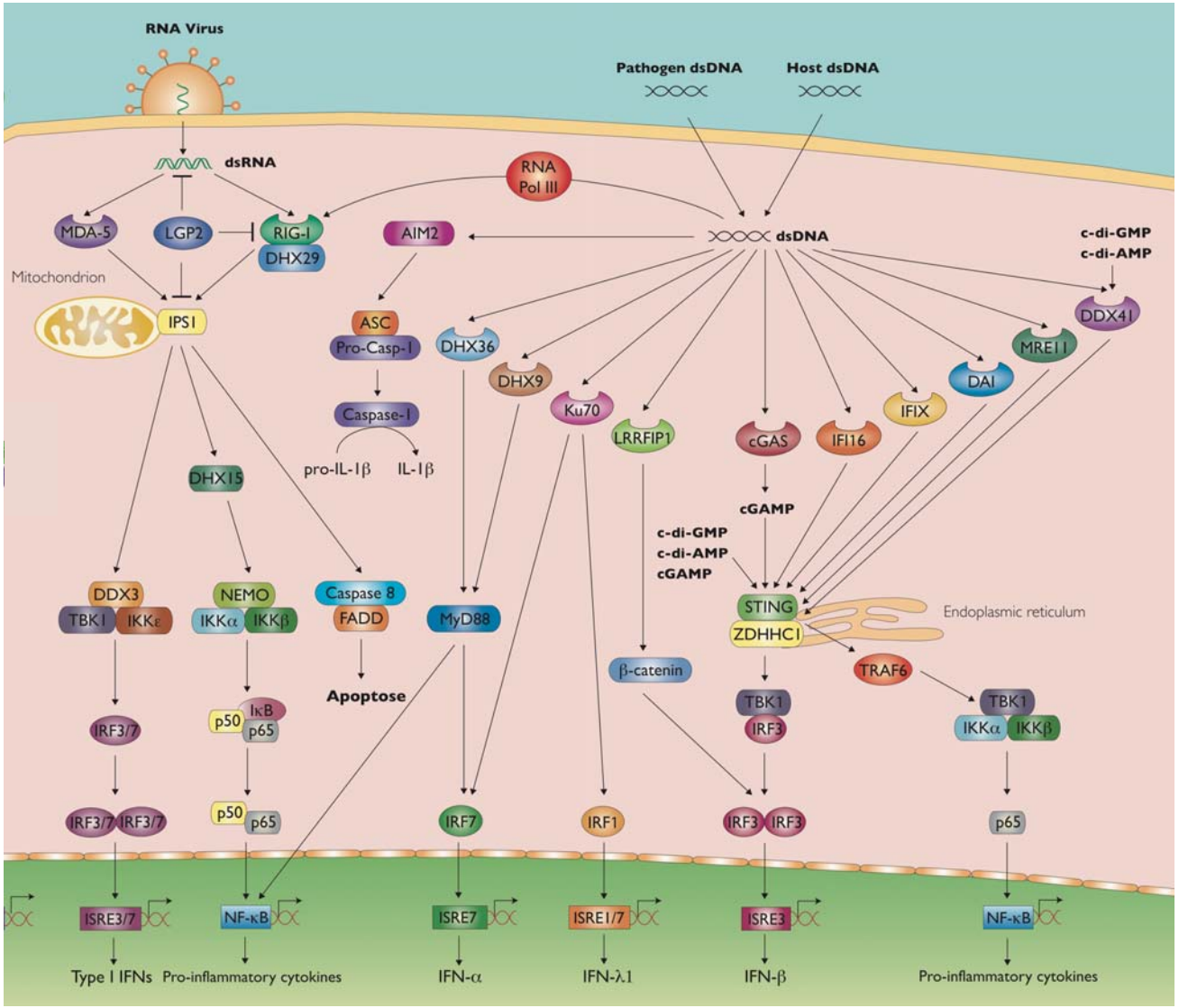


PRRs responses to PAMPs on cell surface and in endosome





PRRs responses to replicating microbes in cytoplasm



STING research

Publication 711

Publication 849

Publication 1439

2008~April of 2020

Interferon Stimulatory DNA (ISD)

STING

TBK1

IRF3

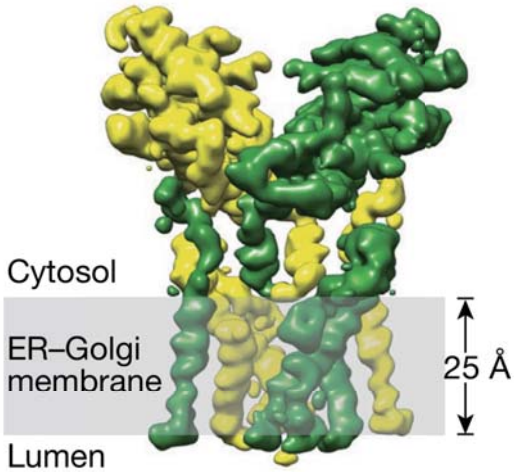
IFNs

Ishikawa H. et al., Nature. 2008
Ishikawa H. et al., Nature. 2009

Product Box

Product	Cat. code
ISD Naked	tlrl-isdn

STING – Stimulator of interferon genes (also know as TMEM173, ERIS, MITA and MPYS) is essential for DNA sensing in cytosol, where in turn induce type I interferon. However, cytosolic DNA does not directly bind with STING.



Guijun Shang, et al. Nature. 2019

Sendai Virus

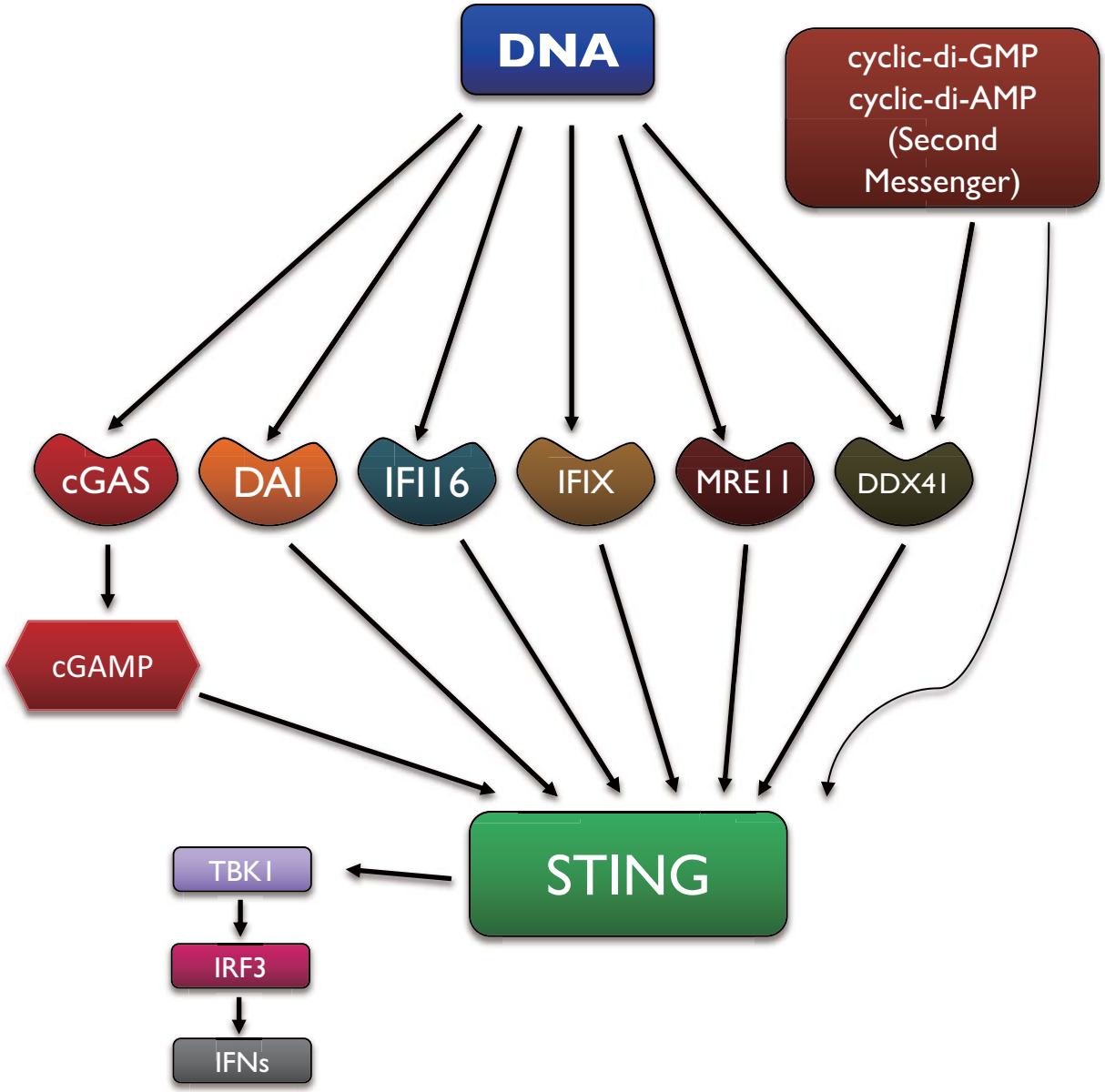
MITA

TBK1

IRF3

IFNs

Zhong B. et al., Immunity. 2008
Zhong B. et al., Immunity. 2009



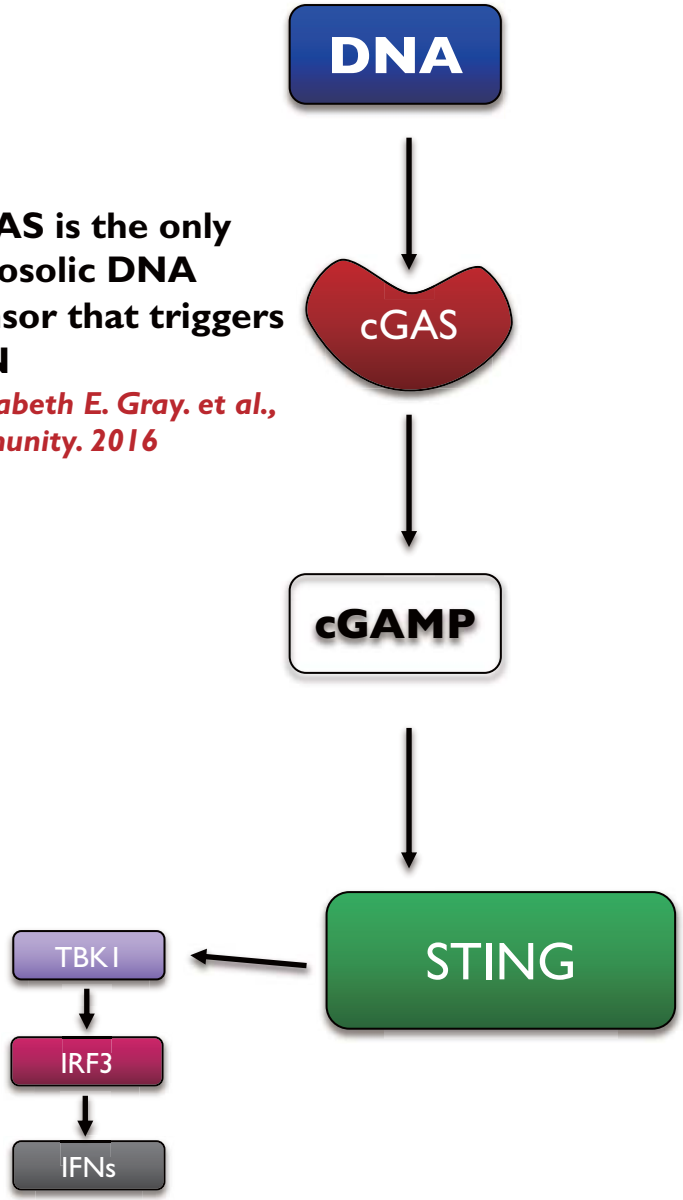
Product Box

Product	Cat. code	Sensor
Poly(dA:dT)	tlrl-patn	DAI
Poly(dG:dC)	tlrl-pgcn	DAI
HSV-60	tlrl-hsv60n	IFI16
VACV-70	tlrl-vav70n	IFI16
ISD Naked	tlrl-isdn	controversial
G3-YSD **[NEW]**	tlrl-ydna	cGAS
c-di-GMP	tlrl-cdg	DDX41, STING
c-di-AMP	tlrl-cda	DDX41, STING
c-di-IMP	tlrl-cdi	DDX41, STING

Ishikawa H. et al., Nature. 2008
Takaoka,A. et al.,Nature. 2007
Upton. et al., Cell Host Microbe. 2012
Unterholzner,l., et al., Nat Immnol. 2010
Diner B.A., et al., Mol syst Biol. 2015
Horan, K.A., et al., J. Immunol. 2013
Kondo,T., et al., PNAS. 2013
Zhang, Z.Q., et al., Nat Immnol. 2011
Burdette, D.L., et al., Nature. 2011
Sun L. et al., Science. 2013
Wu J. et al., Science. 2013

cGAS is the only cytosolic DNA sensor that triggers IFN

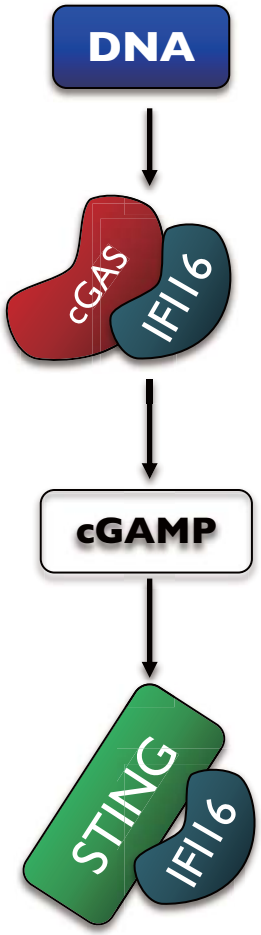
Elizabeth E. Gray. et al., Immunity. 2016

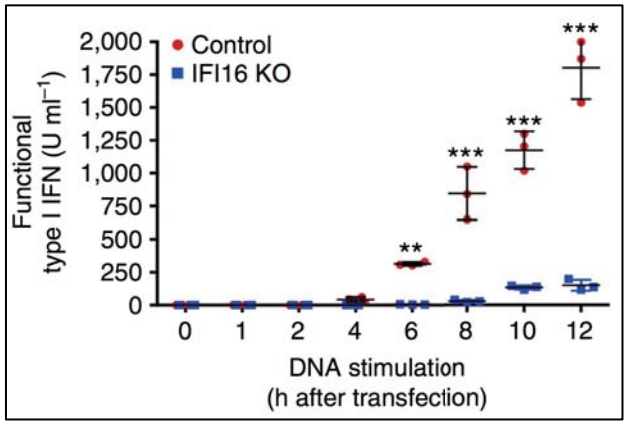


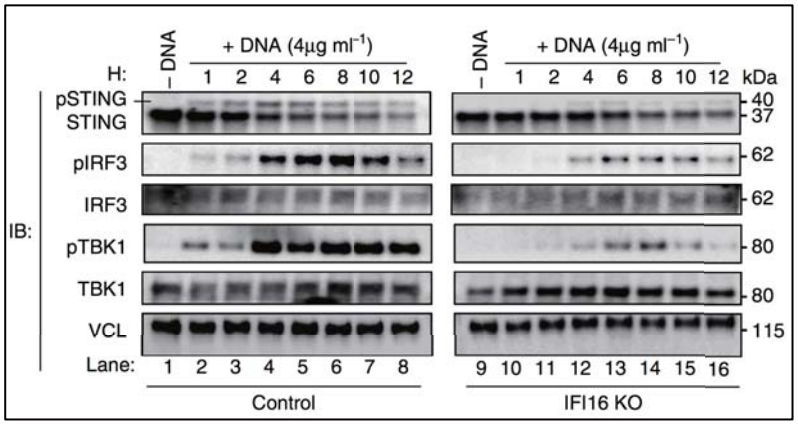
A Narrowing Field of Cytosolic DNA Sensors Triggering IFN Production

Genetic data now point to cyclic-GMP-AMP synthase (cGAS) as the only viable IFN-inducing DNA sensor, depicted in this illustration as the last man standing. It remains unclear whether other putative cytosolic DNA sensors, previously proposed to induce type I IFN expression in response to dsDNA, are truly dead and buried or whether these proteins will have roles in response to specific stimuli in certain cellular contexts.

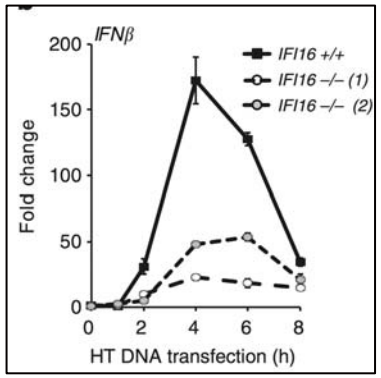
Russell E. Vance. Immunity. 2016



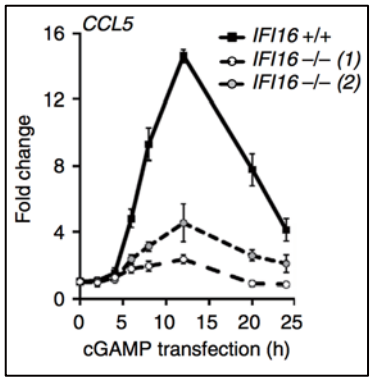




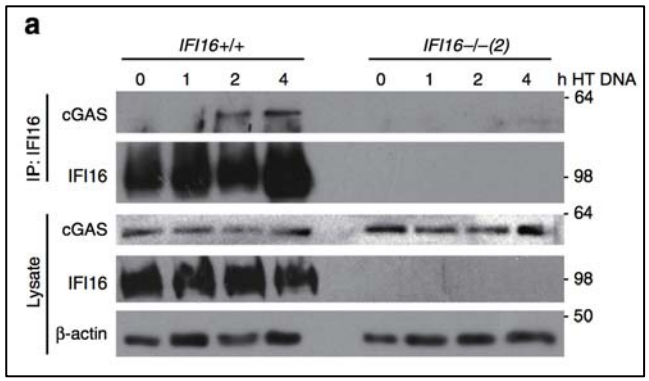
K.L. Jønsson et al. IFI16 is required for DNA sensing in human macrophages by promoting production and function of cGAMP. Nature Communication.2017



IFI16 is required for DNA in HaCaT keratinocytes.

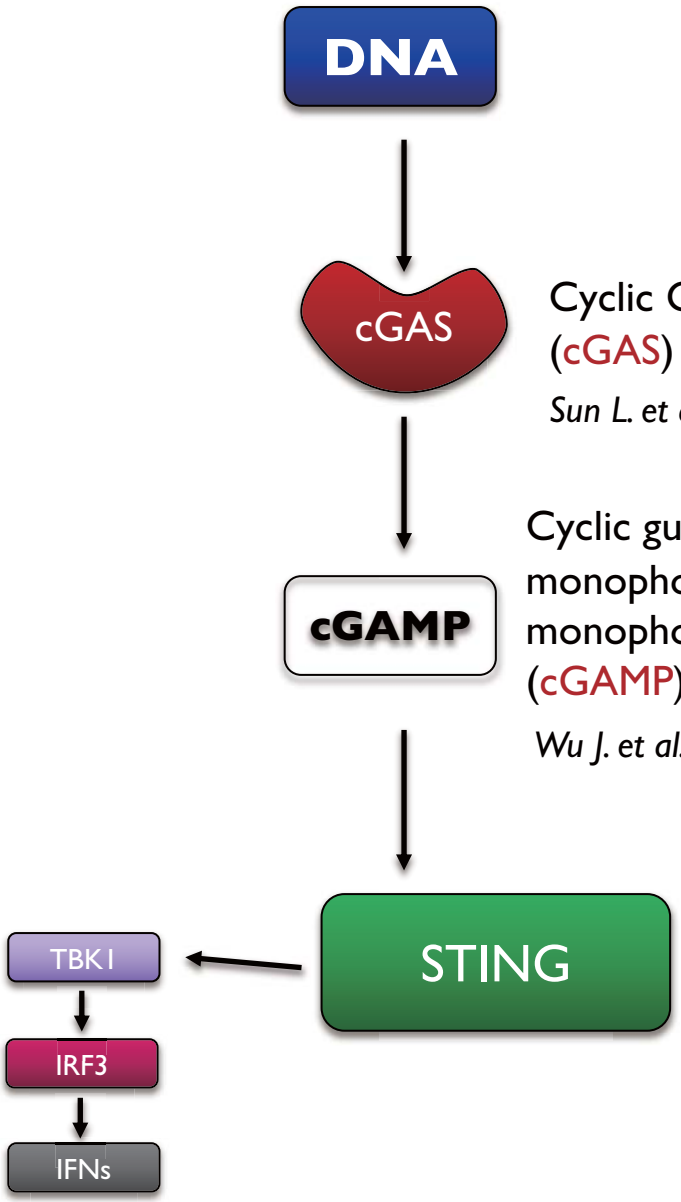


IFI16 is required for STING response to cGAMP .



Interaction between endogenous IFI16 and cGAS that was enhanced by stimulation with DNA

Jessica F. Almine et al. IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. Nature Communication.2017



Cyclic GMP-AMP synthase (cGAS)

Sun L. et al., Science. 2013

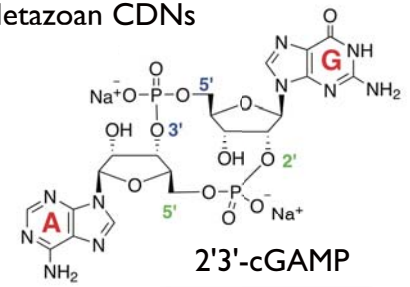
Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP)

Wu J. et al., Science. 2013

Product Box

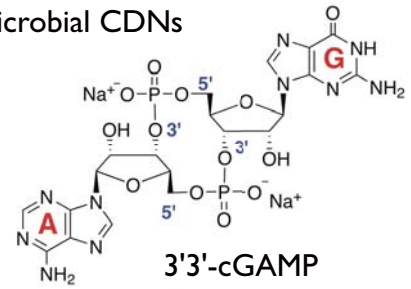
Product	Cat. code	Sensor
3'3'-cGAMP	tlrl-nacga	STING
2'3'-cGAMP	tlrl-nacga23	STING
2'2'-cGAMP	tlrl-nacga22	STING

Metazoan CDNs



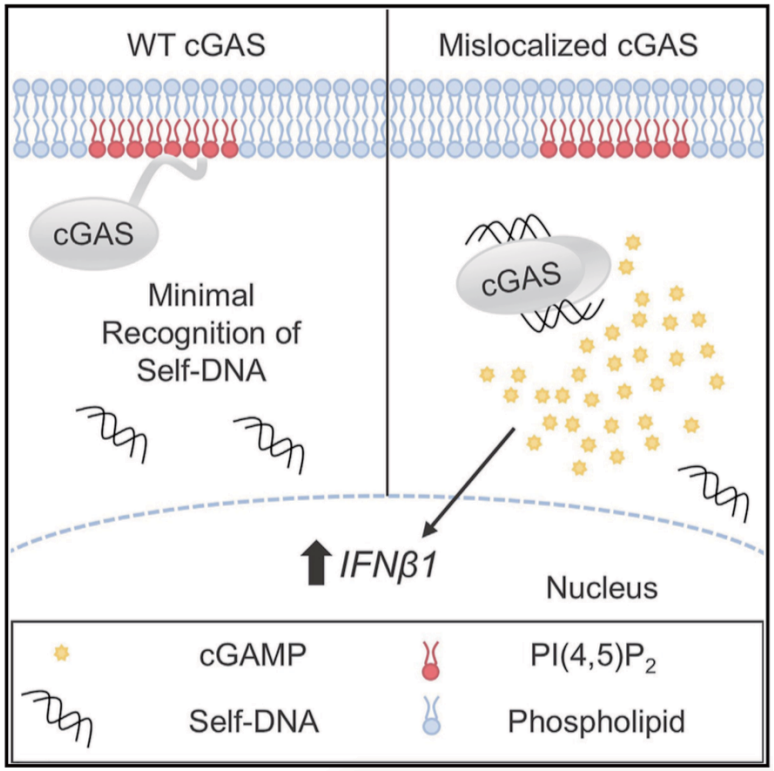
2'3'-cGAMP

Microbial CDNs

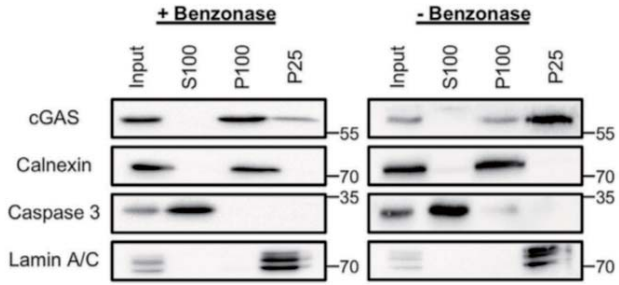


3'3'-cGAMP

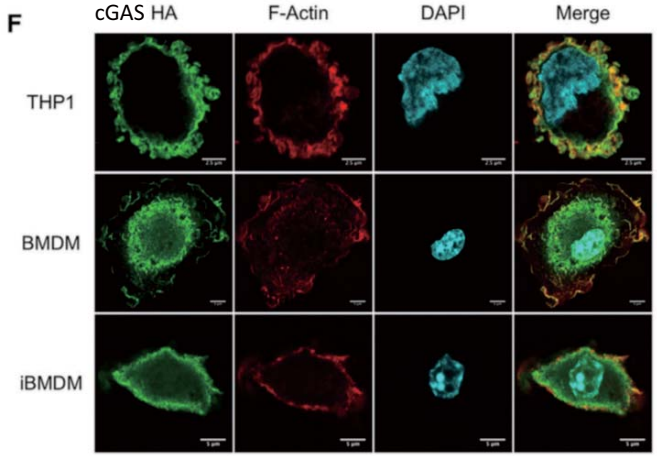
cGAS is not cytosolic sensor



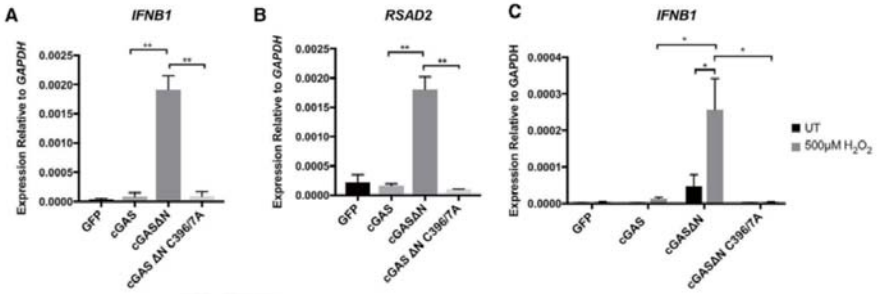
Phosphoinositide Interactions Position cGAS at the Plasma Membrane to Ensure Efficient Distinction between Self- and Viral DNA. Katherine C. Barnett, Julia M. Coronas-Serna, Wen Zhou, Michael J. Ernandes, Anh Cao,1 Philip J. Kranzusch and Jonathan C. Kagan. Cell. 2019



Plasma membrane cGAS cannot be detected in the absence of benzonase



cGAS Associates with the Plasma Membrane



Mislocalized cGAS mutants drive interferon responses to genotoxic stress

Molecular Mechanisms

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2020

REVIEWS

Molecular mechanisms and cellular functions of cGAS–STING signalling

Karl-Peter Hopfner[1,2]✉ *and Veit Hornung*[1,2]✉

Abstract | The cGAS–STING signalling axis, comprising the synthase for the second messenger cyclic GMP–AMP (cGAS) and the cyclic GMP–AMP receptor stimulator of interferon genes (STING), detects pathogenic DNA to trigger an innate immune reaction involving a strong type I interferon response against microbial infections. Notably however, besides sensing microbial DNA, the DNA sensor cGAS can also be activated by endogenous DNA, including extranuclear chromatin resulting from genotoxic stress and DNA released from mitochondria, placing cGAS–STING as an important axis in autoimmunity, sterile inflammatory responses and cellular senescence. Initial models assumed that co-localization of cGAS and DNA in the cytosol defines the specificity of the pathway for non-self, but recent work revealed that cGAS is also present in the nucleus and at the plasma membrane, and such subcellular compartmentalization was linked to signalling specificity of cGAS. Further confounding the simple view of cGAS–STING signalling as a response mechanism to infectious agents, both cGAS and STING were shown to have additional functions, independent of interferon response. These involve non-catalytic roles of cGAS in regulating DNA repair and signalling via STING to NF-κB and MAPK as well as STING-mediated induction of autophagy and lysosome-dependent cell death. We have also learnt that cGAS dimers can multimerize and undergo liquid–liquid phase separation to form biomolecular condensates that could importantly regulate cGAS activation. Here, we review the molecular mechanisms and cellular functions underlying cGAS–STING activation and signalling, particularly highlighting the newly emerging diversity of this signalling pathway and discussing how the specificity towards normal, damage-induced and infection-associated DNA could be achieved.

Innate immune system
A heterogeneous system of molecules, signal transducers and cells that has evolved to detect invading microbes, and elicits a first line of antimicrobial defence and activates the adaptive immune system.

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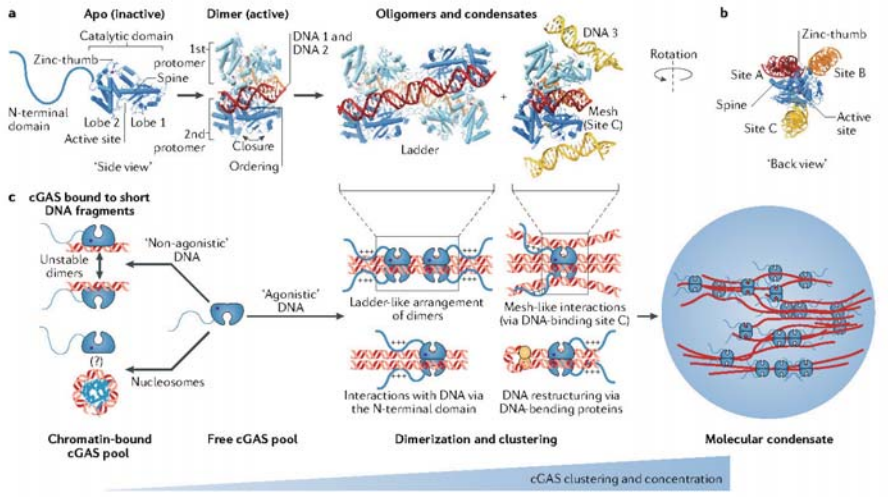
https://doi.org/10.1038/s41580-020-0244-x

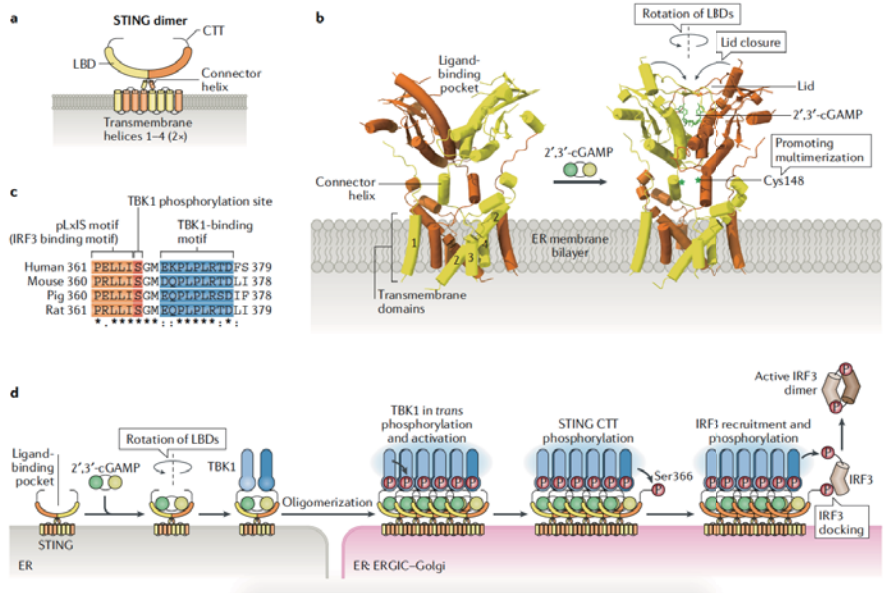
Cell intrinsic recognition and defence systems against foreign genetic material encompass an ancient and fundamental feature of living systems. A first line of defence in mammals is orchestrated by the innate immune system. Germline-encoded pattern recognition receptors (PRRs) detect various pathogen and damage-associated molecular patterns (PAMPs and DAMPs). Their activation elicits signalling cascades that lead to the initiation of cell autonomous defence mechanisms, as well as the production of soluble mediators, such as type I interferons and pro-inflammatory cytokines (BOX 1). Type I interferons play a central role in impeding viral propagation, hence their production is typically governed by PRRs that have evolved to sense viral infection. By inducing the expression of interferon-stimulated genes, type I interferons boost cell autonomous defence mechanisms in an autocrine manner, and furthermore can spread antiviral immunity and activate the adaptive immune system.

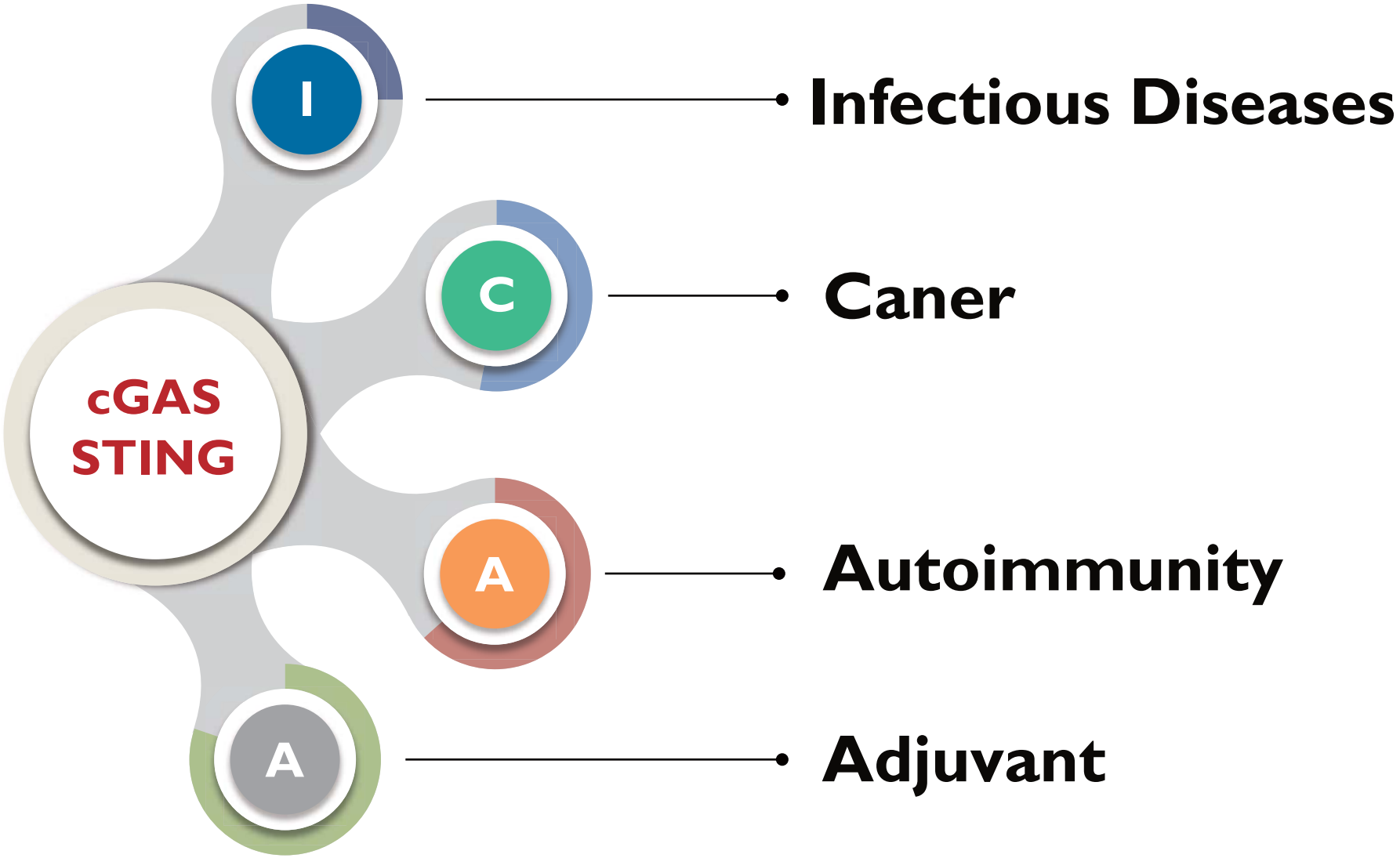
Cytosolic DNA is a potent activator of a type I interferon response[1,3] (FIG. 1). Under normal conditions, DNA is confined to the nucleus and mitochondria, and is rapidly degraded by nucleases in the cytosol and endolysosomal compartments. Following infections, for example, increased amounts of intracellular DNA are detected in a pathway that involves cyclic GMP–AMP synthase (cGAS; also known as MB21D1)[3,4], a member of the nucleotidyl transferase (NTase) enzyme family[5] that functions upstream of stimulator of interferon genes (STING). cGAS normally resides as inactive protein in the cell. Upon binding to DNA, cGAS undergoes a conformational change to an active state and produces the second messenger cyclic GMP–AMP (cGAMP) from ATP and GTP[6–8], which is subsequently detected by the cyclic-dinucleotide sensor STING[3,4,9], an ~40-kDa dimeric transmembrane protein at the endoplasmic reticulum (ER)[10]. Binding of cGAMP activates STING, which then translocates to the Golgi and activates TANK-binding kinase 1 (TBK1). TBK1 then phosphorylates itself, STING and, subsequently, the interferon regulatory factor 3 (IRF3) transcription factor.

cGAS



STING



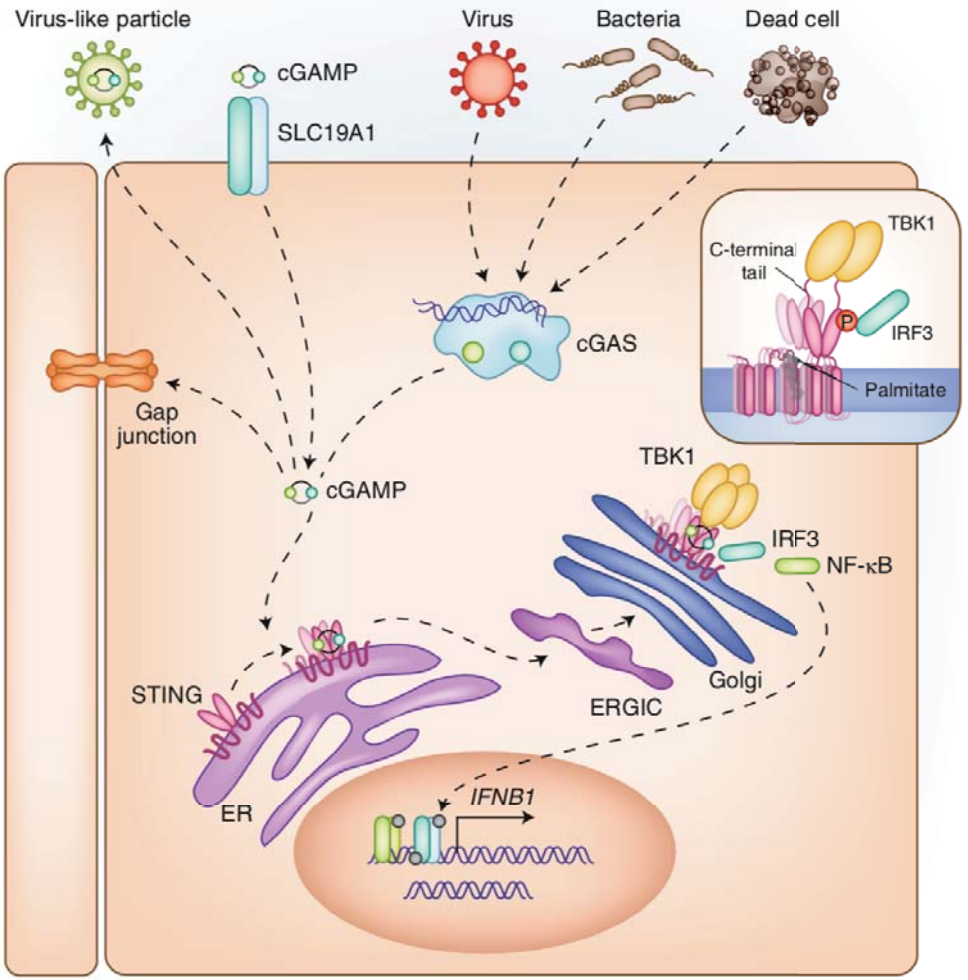

cGAS
STING
I
C
A
A
Infectious Diseases
Caner
Autoimmunity
Adjuvant

Infectious Diseases

I

cGAS STING

- Invasion
- Evasion
- Interplay



Andrea Ablasser and Sun Hur. Nature Immunology. 2019

STING and Infectious Diseases - Invasion

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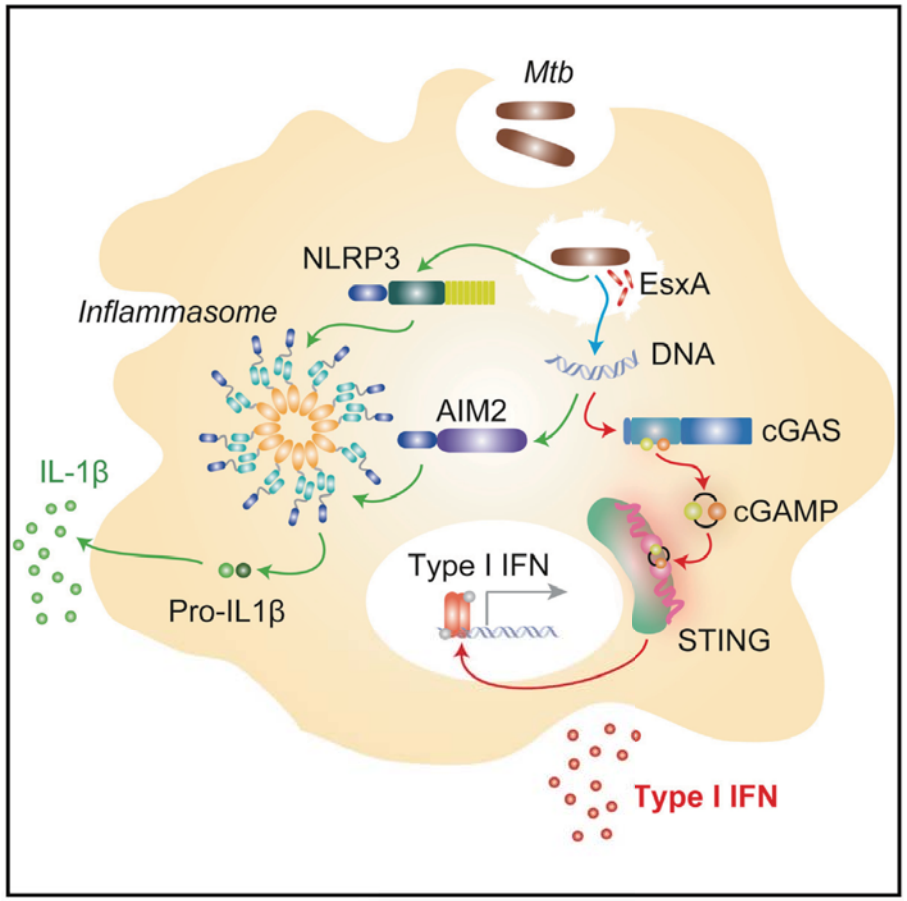
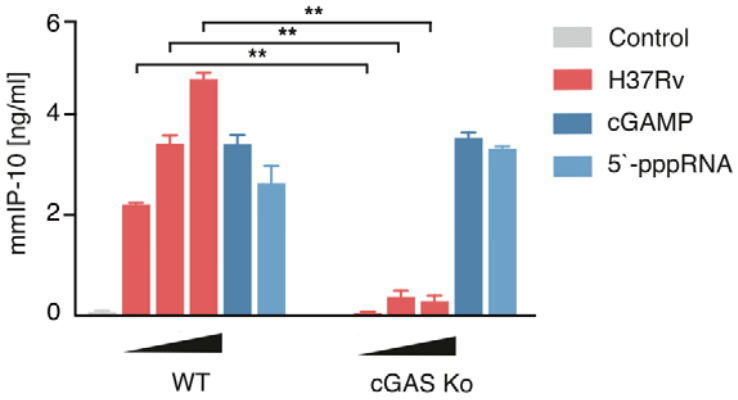
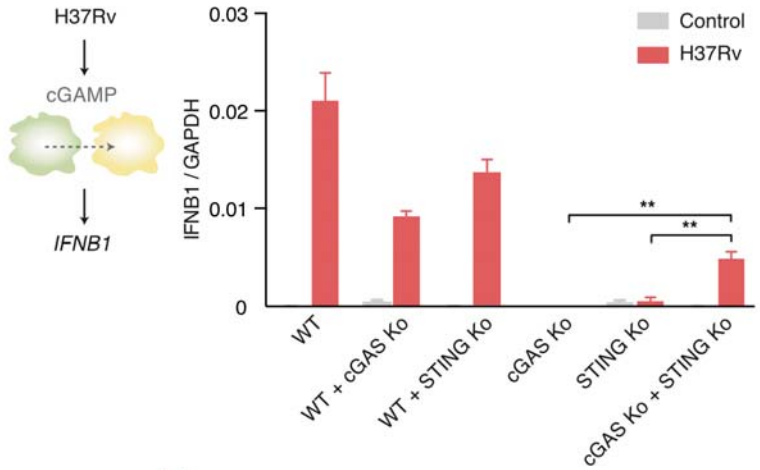
cGAS/STING & *Mycobacterium tuberculosis* –





cGAS Is Essential for Type I IFN Responses Triggered by Mtb



cGAMP Shuttling Restores Type I IFN Responses

Product	Cat. code
5'ppp-dsRNA	tlrl-3prna-100
THP1-Dual™ KO-STING Cells	thpd-kocgas
THP1-Dual™ KO-cGAS Cells	thpd-kostg

Ruth Wassermann, et. al. Mycobacterium tuberculosis Differentially Activates cGAS- and Inflammasome-Dependent Intracellular Immune Responses through ESX-1. Cell Host & Microbe. 2015

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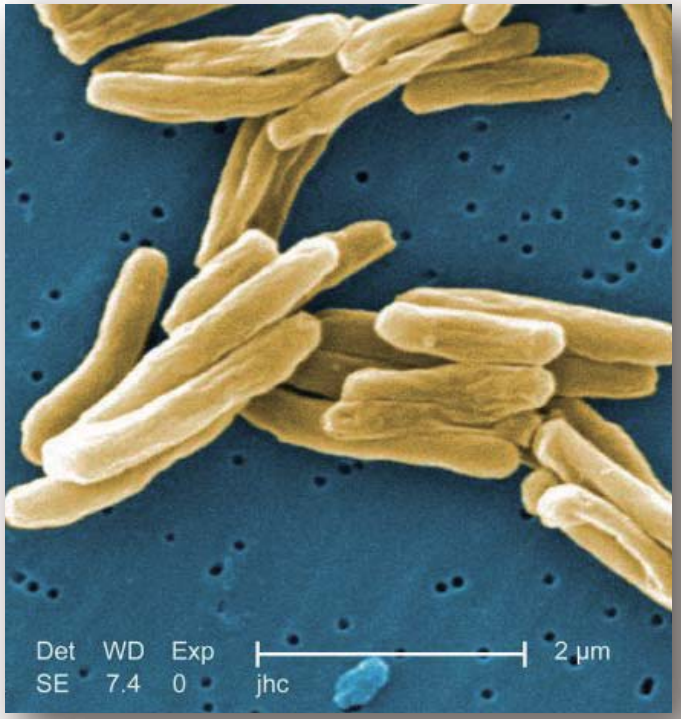
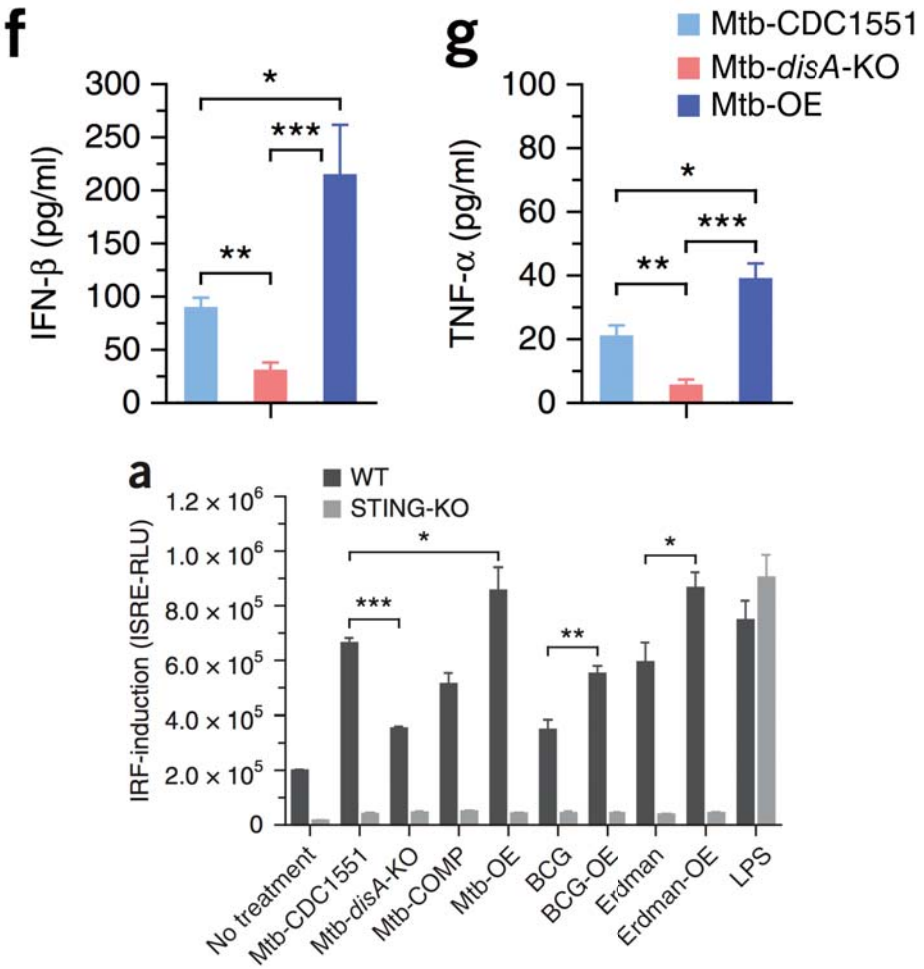
STING & *Mycobacterium tuberculosis*

M. tuberculosis genome encodes a di-adenylate cyclase enzyme (disA) that synthesizes c-di-AMP from ATP or ADP.



Bappaditya Dey,et al. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. Nature Medicine. 2015



Product	Cat. code
RAW-Lucia™ ISG Cells	rawl-isg
RAW-Lucia™ ISG KO-STING Cells	rawl-kostg

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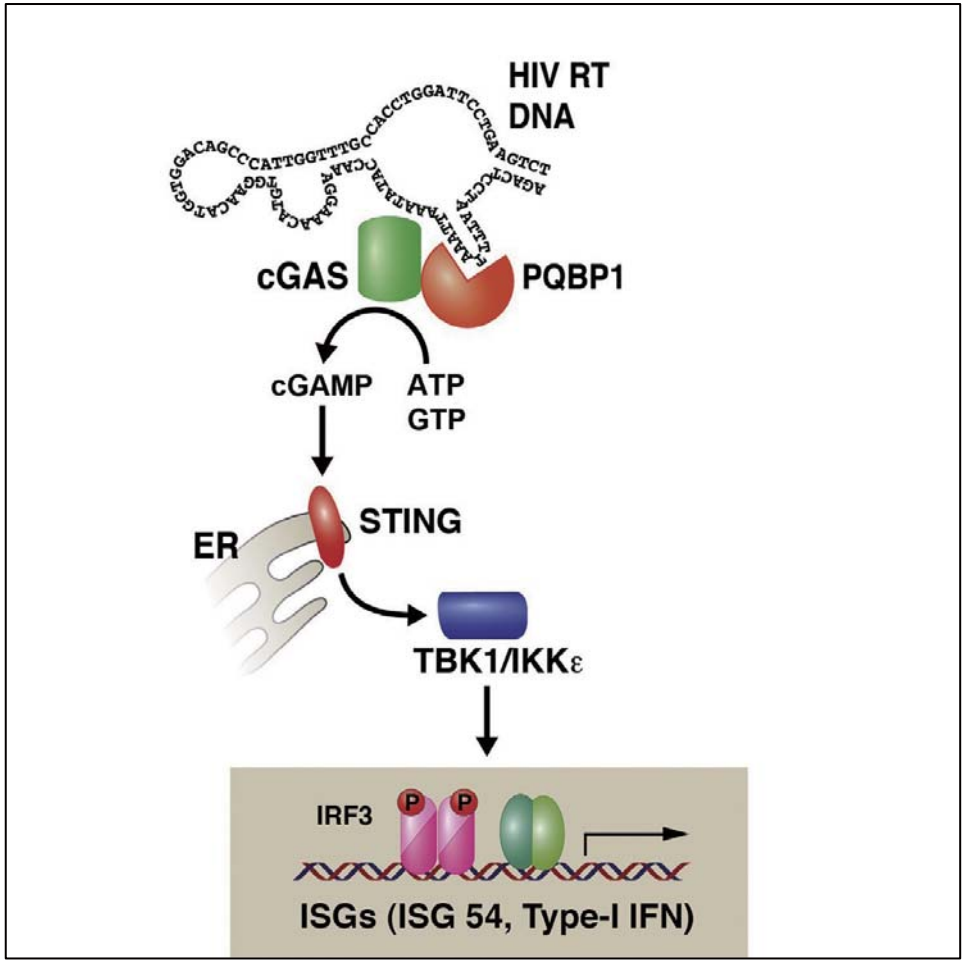
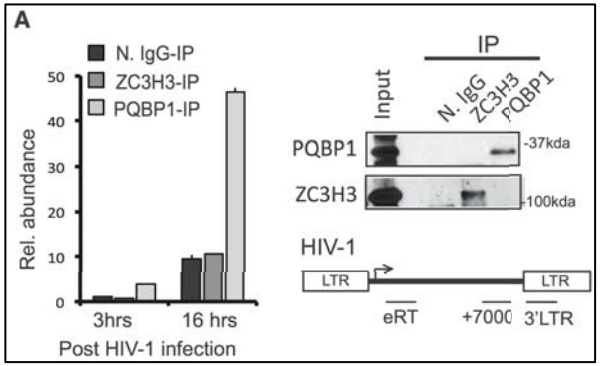
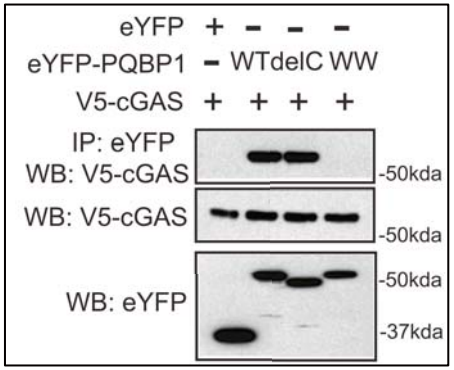
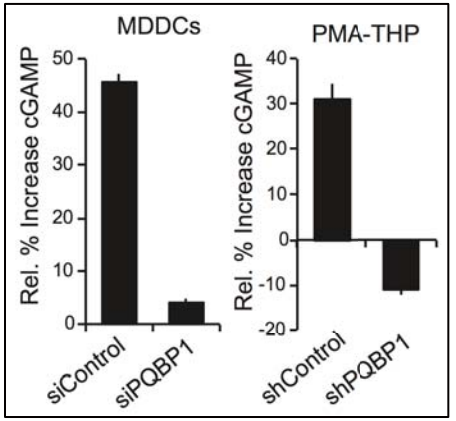
STING & HIV









Sunnie M.Yoh, , et al. PQBP1 Is a Proximal Sensor of the cGAS-Dependent Innate Response to HIV-1 . Cell .2016

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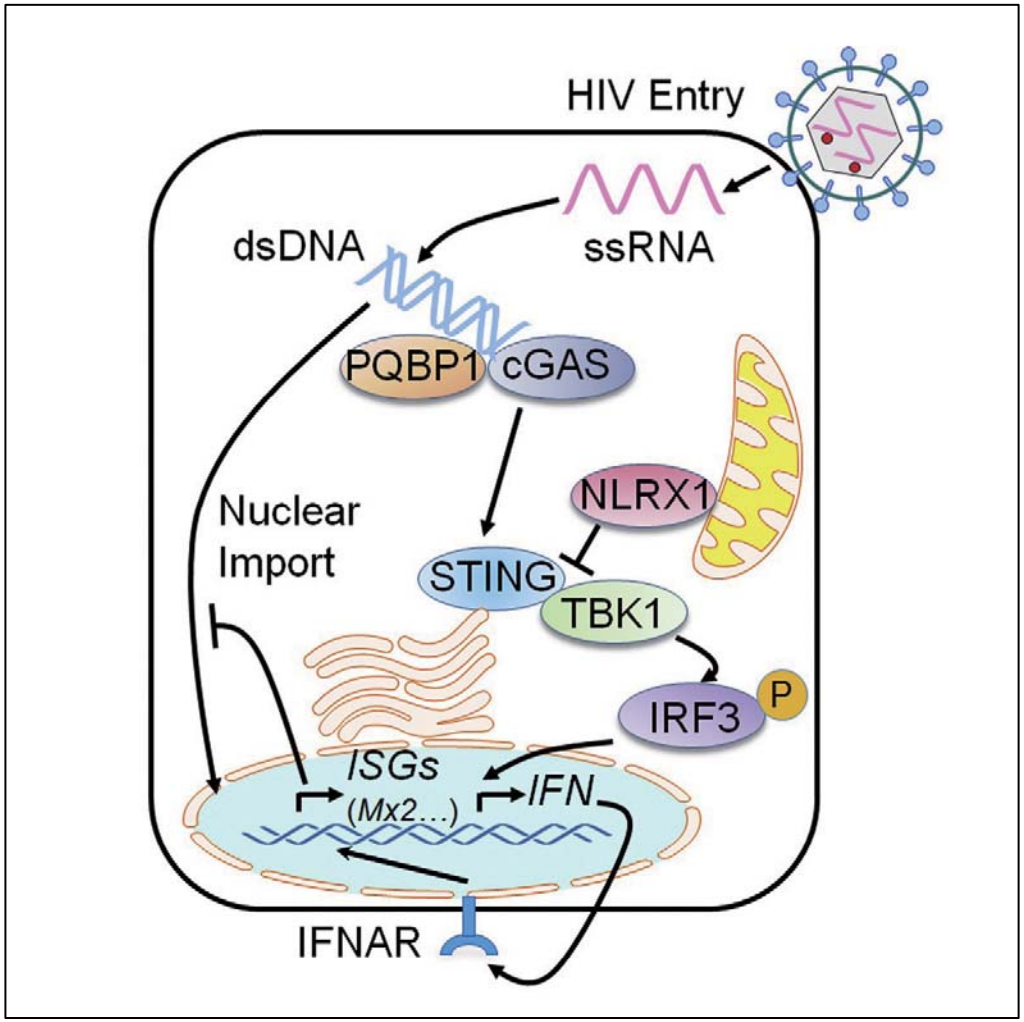
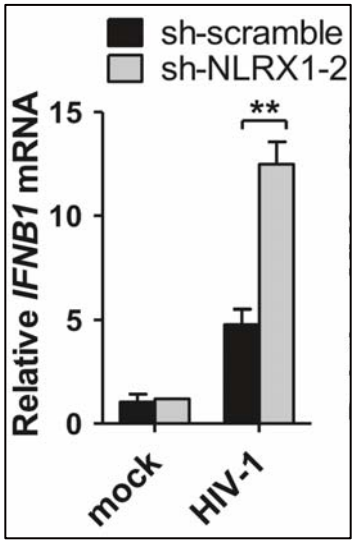
INNOVATION WITHIN REACH

STING & HIV

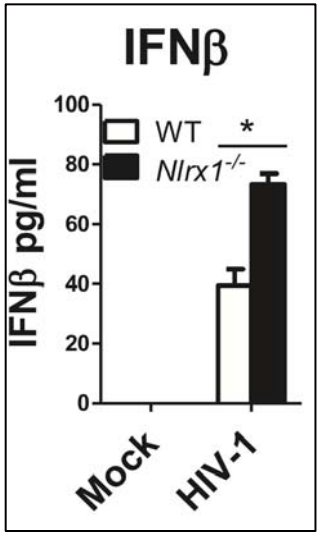




THP-1

Mitochondrial-localized NLR, NLRX1

BMDM



Guo et al., NLRX1 Sequesters STING to Negatively Regulate the Interferon Response, Thereby Facilitating the Replication of HIV-1 and DNA Viruses. Cell Host & Microbe, 2016

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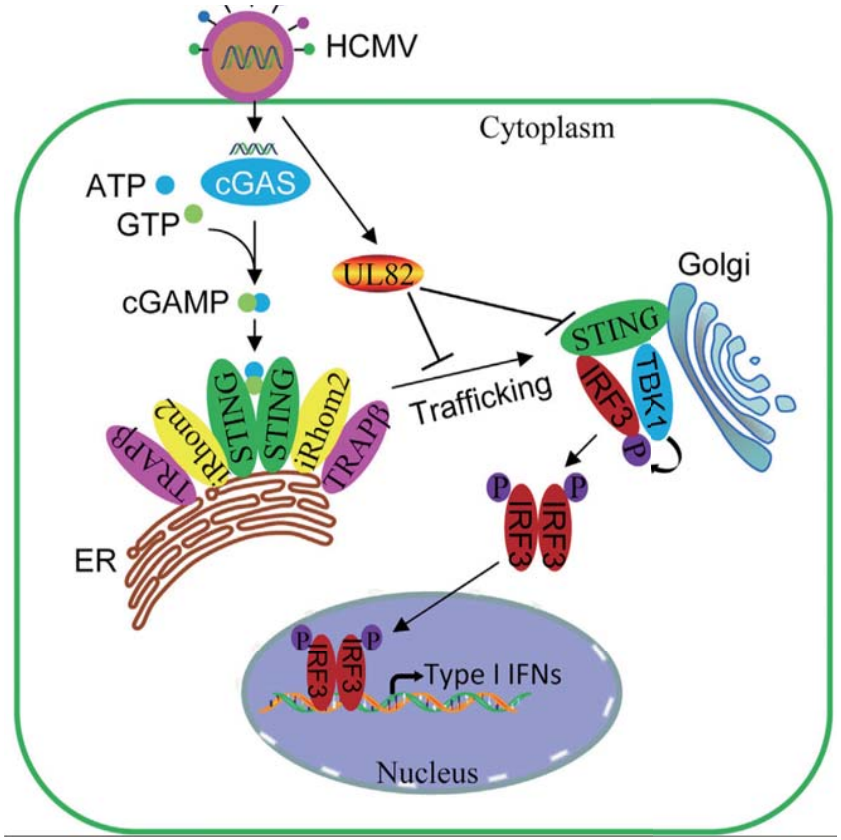
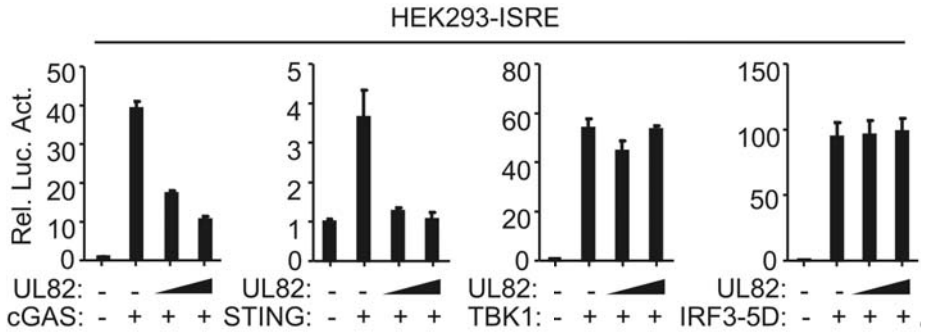
INNOVATION WITHIN REACH

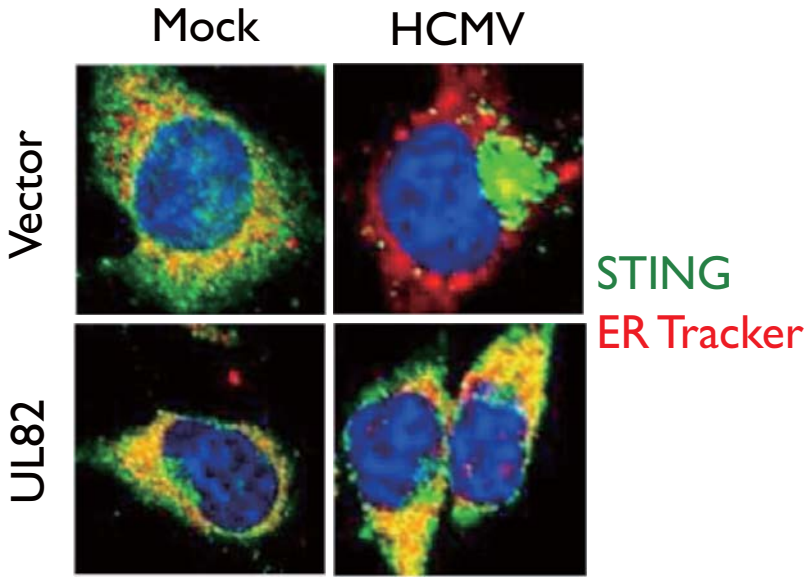
STING & HCMV



Yu-Zhi Fu, et al. Human Cytomegalovirus Tegument Protein UL82 Inhibits STING-Mediated Signaling to Evade Antiviral Immunity. Cell Host & Microbe. 2017



-UL82 Inhibits STING-Mediated Signaling



-UL82 Impairs the Trafficking of STING

STING and Infectious Diseases – Evasion

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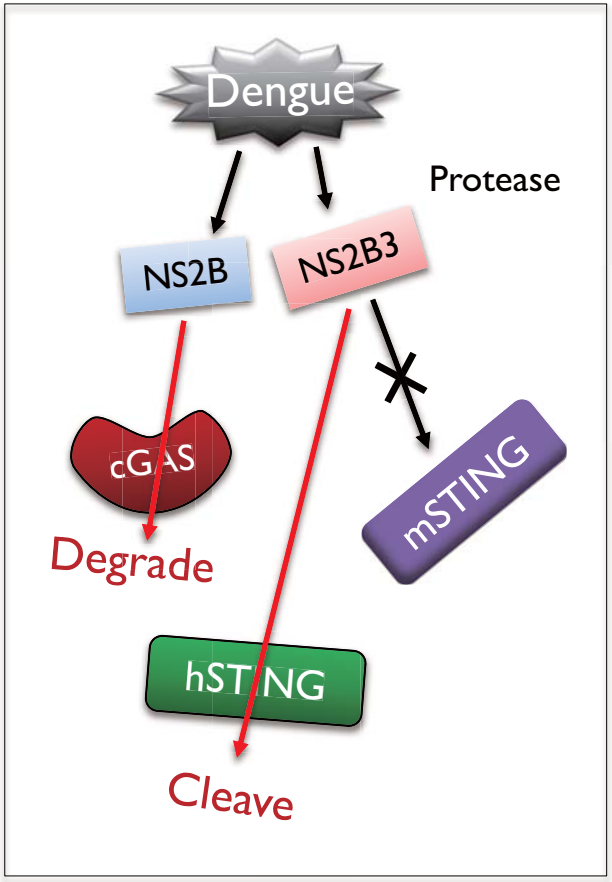
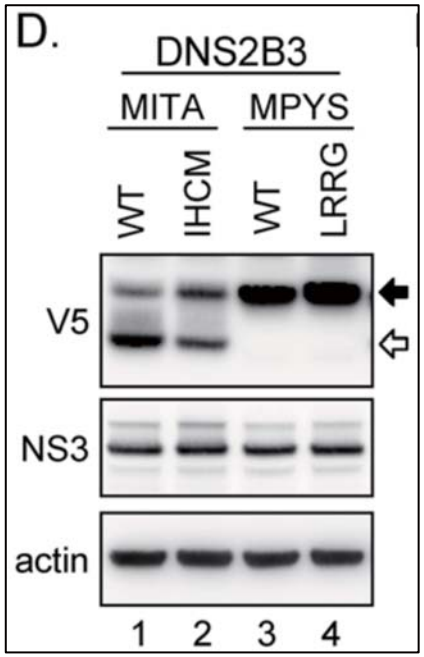
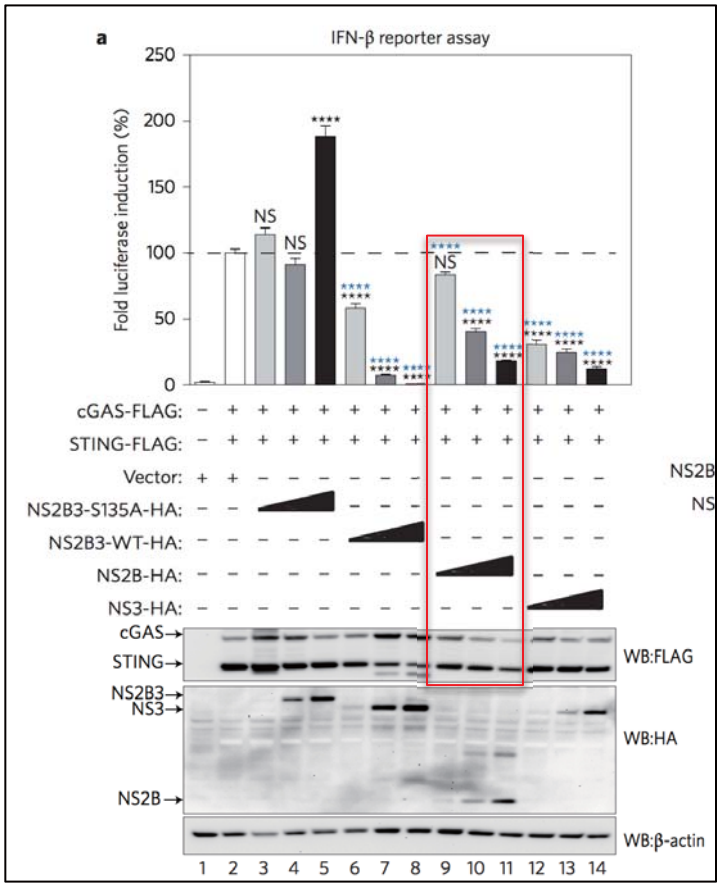
INNOVATION WITHIN REACH

STING & Dengue





[1] DNS2B3 Cleave hSTING but not mSTING



[2] NS2B degrades cGAS

[1] *Yu, C.Y. et al. Dengue virus targets the adaptor protein MITA to subvert host innate immunity. PLoS Pathog. 2012.*

[2] *Sebastian Aguirre. et al. Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection NATURE MICROBIOLOGY 2017.*

Product	Cat. code
Poly(dA:dT)	tlrl-patn-1
VACV-70	tlrl-vav70n

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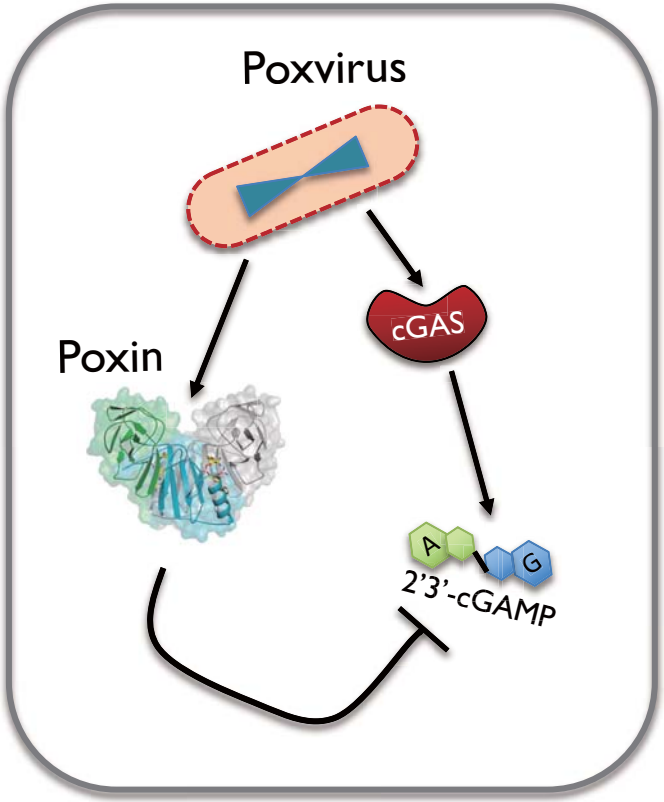
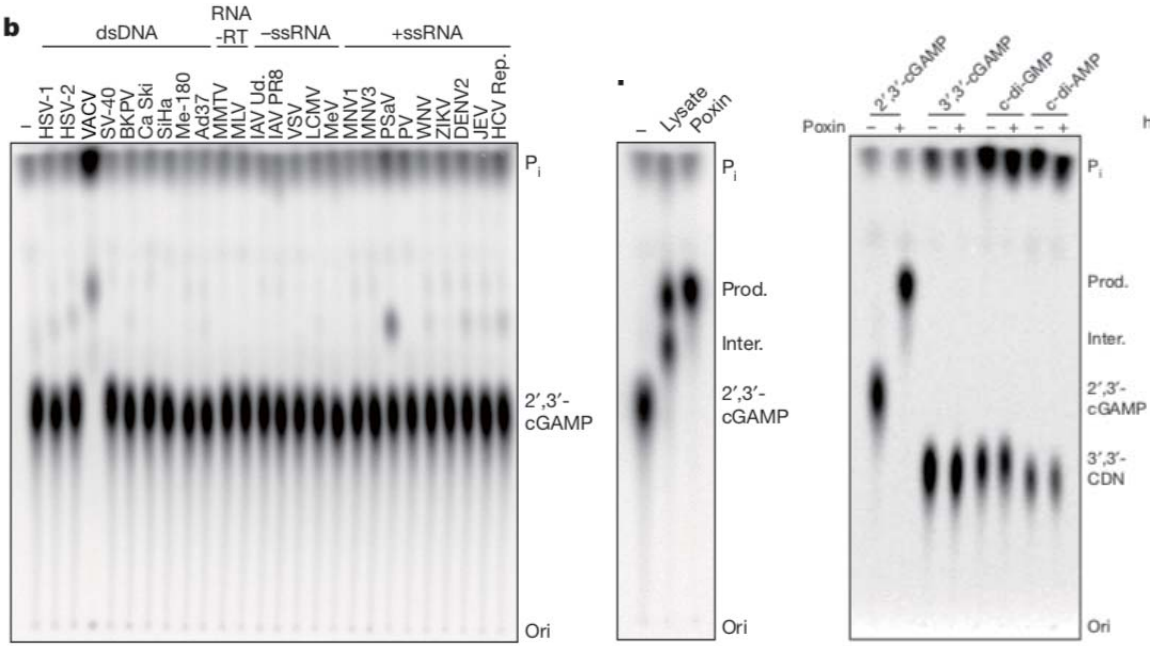
INNOVATION WITHIN REACH

STING & Poxvirus





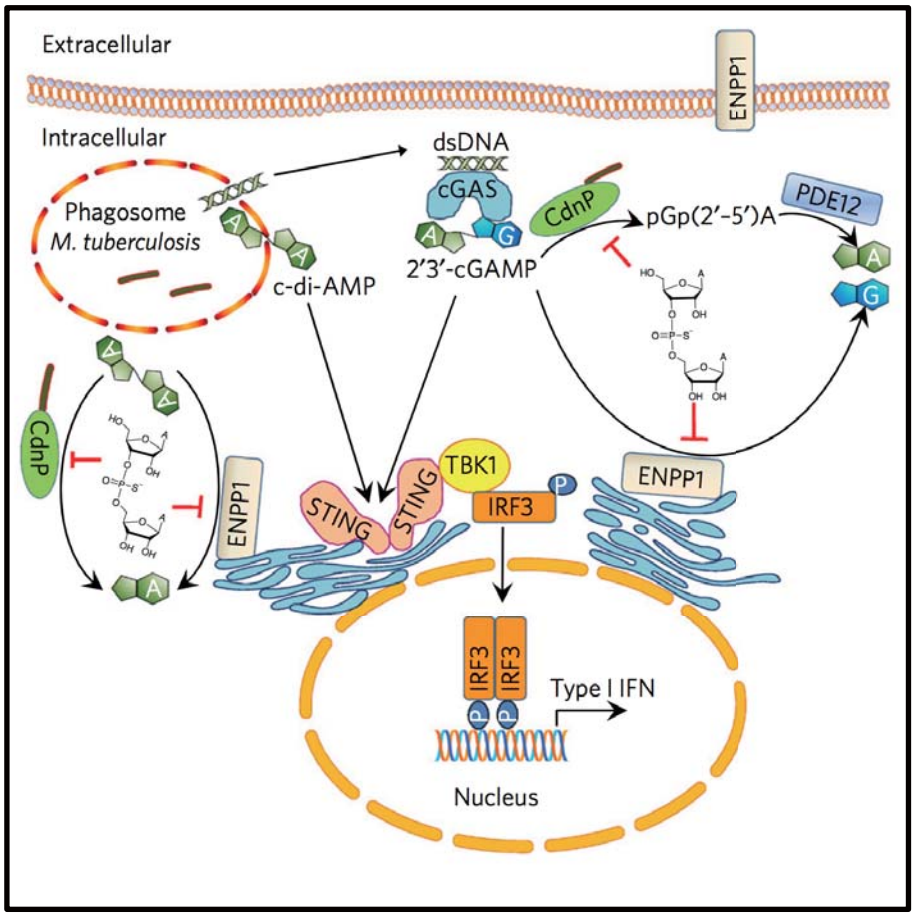
Product	Cat. code
3'3'-cGAMP	tlrl-nacga
2'3'-cGAMP	tlrl-nacga23
c-di-GMP	tlrl-cdg
c-di-AMP	tlrl-cda

- Vaccinia virus(VACV) degrades 2’3’-cGAMP
- Poxin, a virus nuclease is identified
- Poxin specifically degrades 2’3’-cGAMP

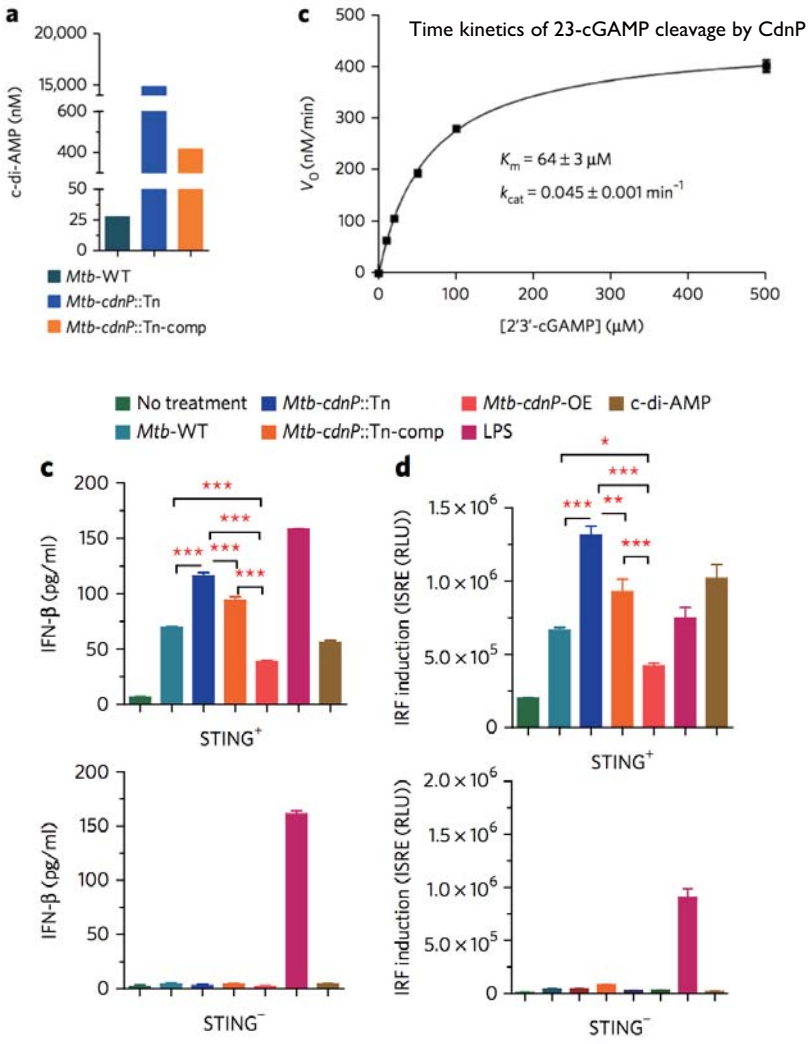
James B. eaglesham. et al.Viral and metazoan poxins are cGAMP-specific nucleases that restrict cGAS–STING signalling. Nature. 2019.

STING and Infectious Diseases – Evasion

STING & *Mycobacterium tuberculosis*

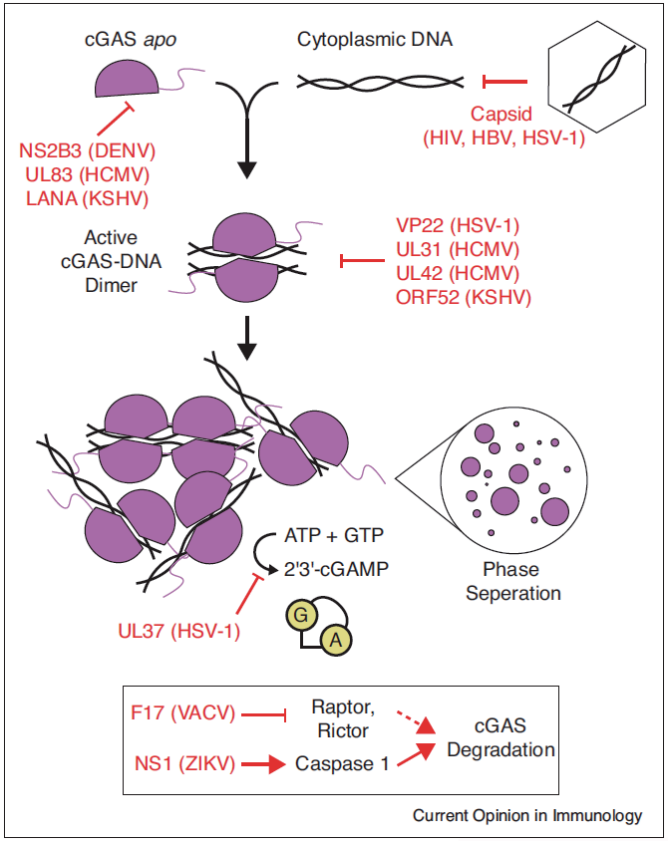


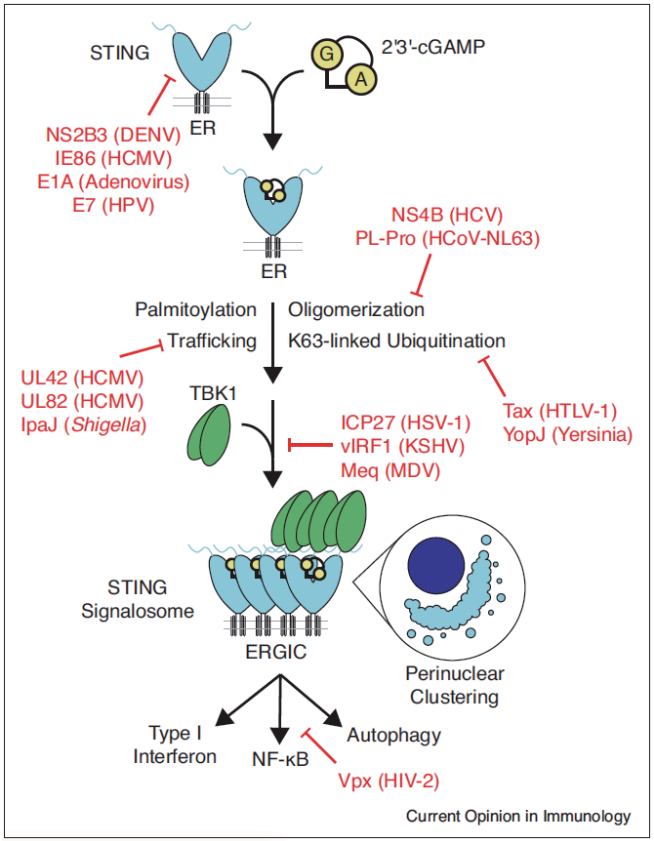
Product	Cat. code
3'3'-cGAMP	tlrl-nacga
2'3'-cGAMP	tlrl-nacga23
c-di-GMP	tlrl-nacdg
RAW-Blue ISG Cells	raw-isg
RAW-Blue ISG KO-STING Cells	rawl-kostg
THP-1-Dual Cells	thpd-nfis

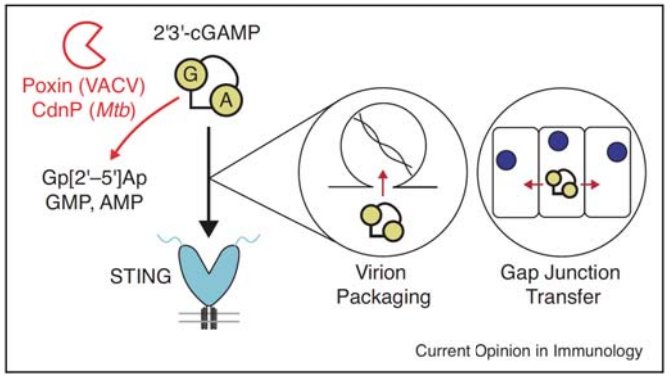


Ruchi Jain dey, et. al. Inhibition of innate immune cytosolic surveillance by an M. tuberculosis phosphodiesterase. Nature Chemical Biology. 2017

STING and Infectious Diseases – Evasion

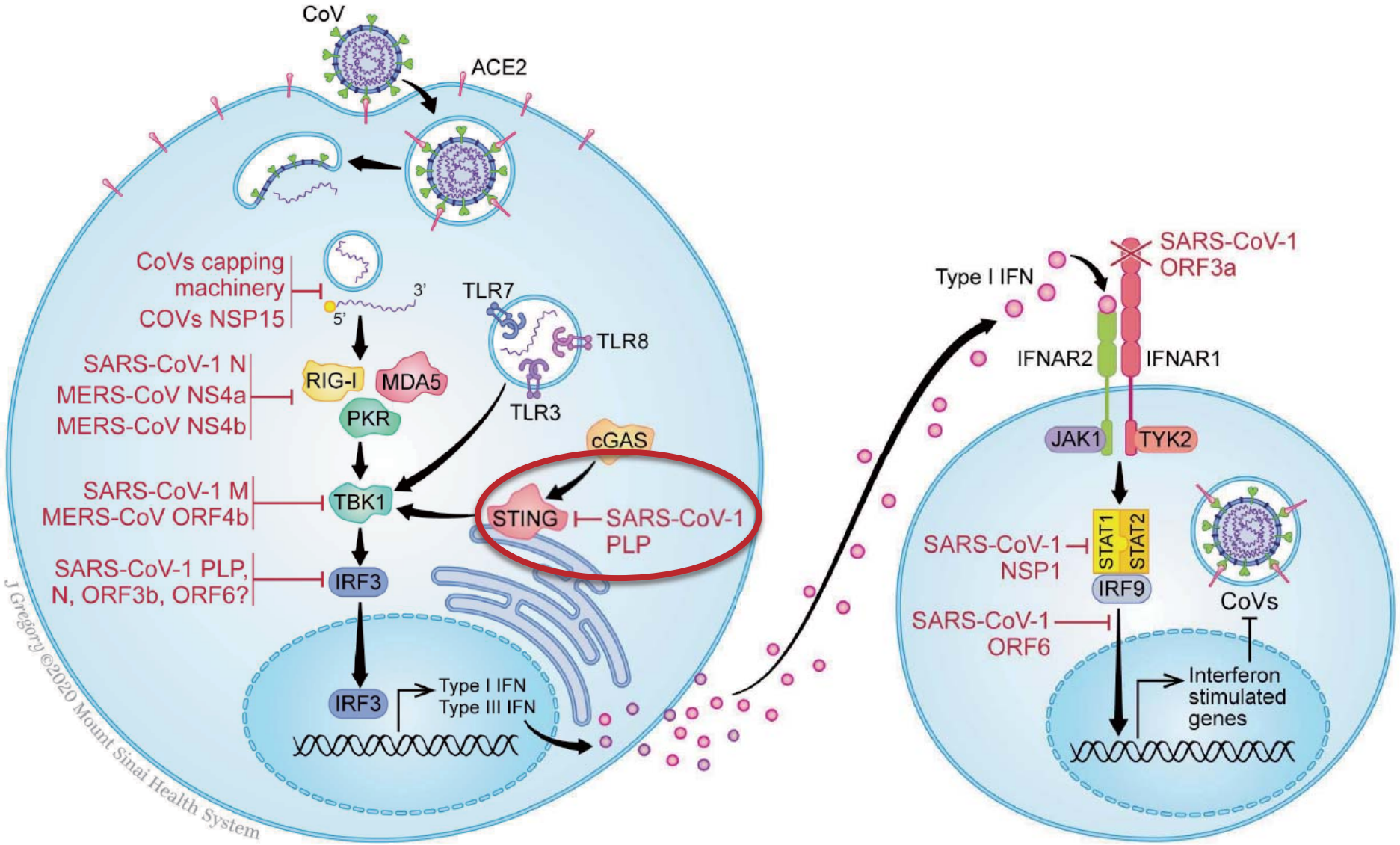






James B Eaglesham and Philip J Kranzusch. Conserved strategies for pathogen evasion of cGAS–STING immunity. Current Opinion in Immunology. 2020

STING and Infectious Diseases – Evasion



Nicolas Vabret, et. al. Immunology of COVID-19: current state of the science. Immunity. 2020

STING and Infectious Diseases – Interplay

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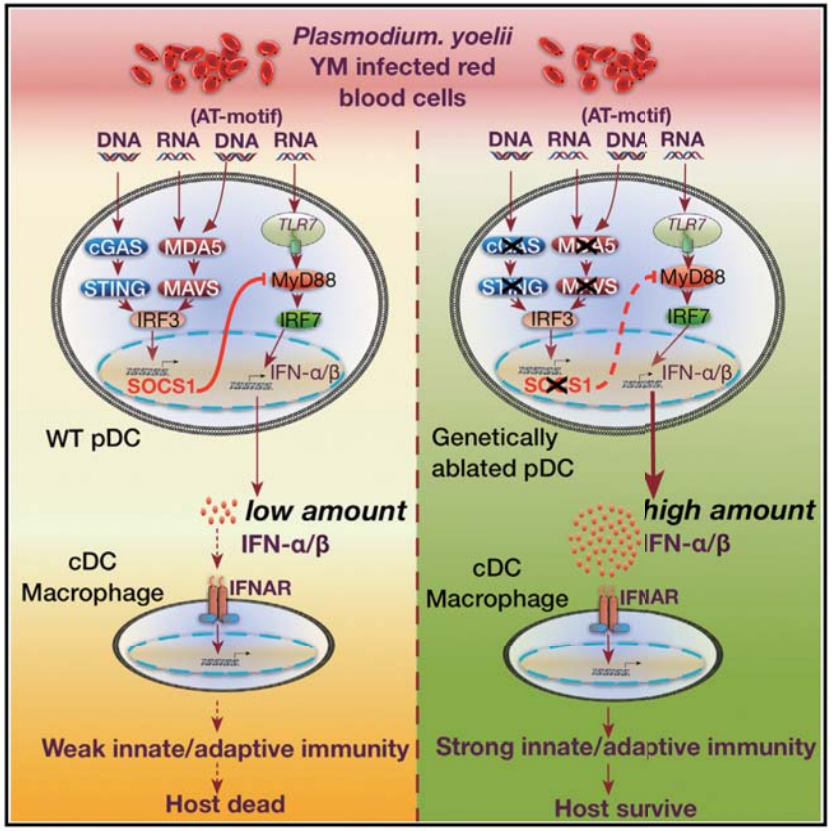
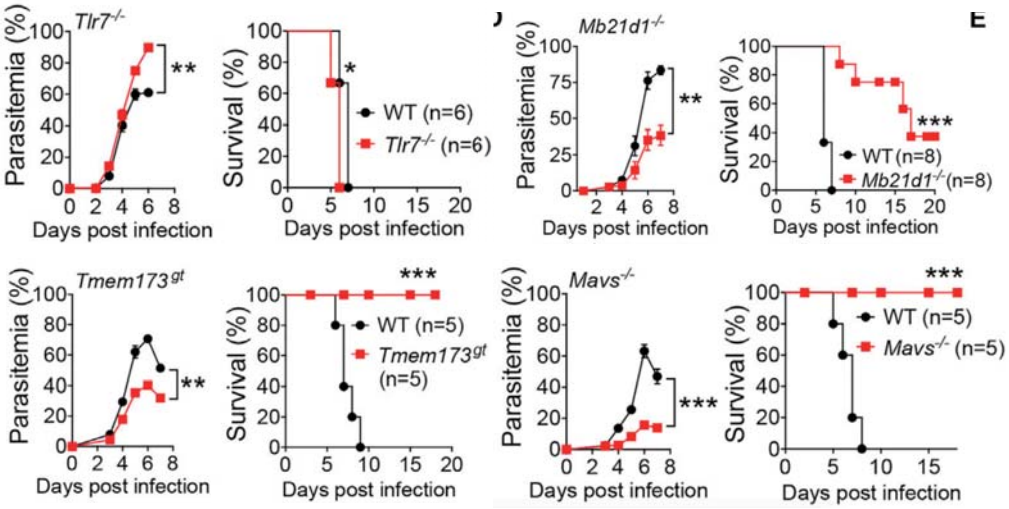
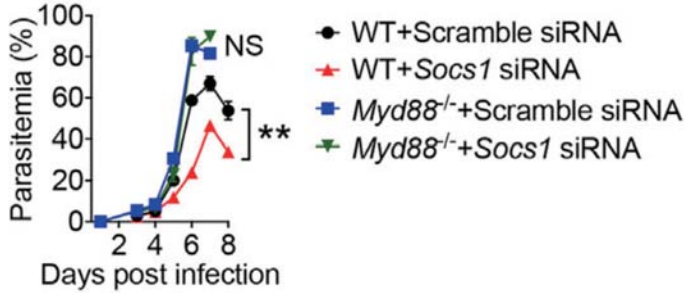
STING & TLR7 - Malaria



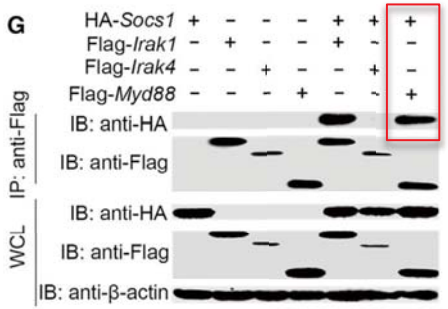
Xiao Yu, et al. Cross-Regulation of Two Type I Interferon Signaling Pathways in Plasmacytoid Dendritic Cells Controls Anti-malaria Immunity and Host Mortality. Immunity. 2016



-Mice deficient in cGAS, STING, MDA5, MAVS, but not in TLR7 signaling molecules, are protected from YM Infection



-SOCS1 inhibit Myd88-dependent anti-malaria



-SOCS1 associate with Myd88

STING and Infectious Diseases – Interplay

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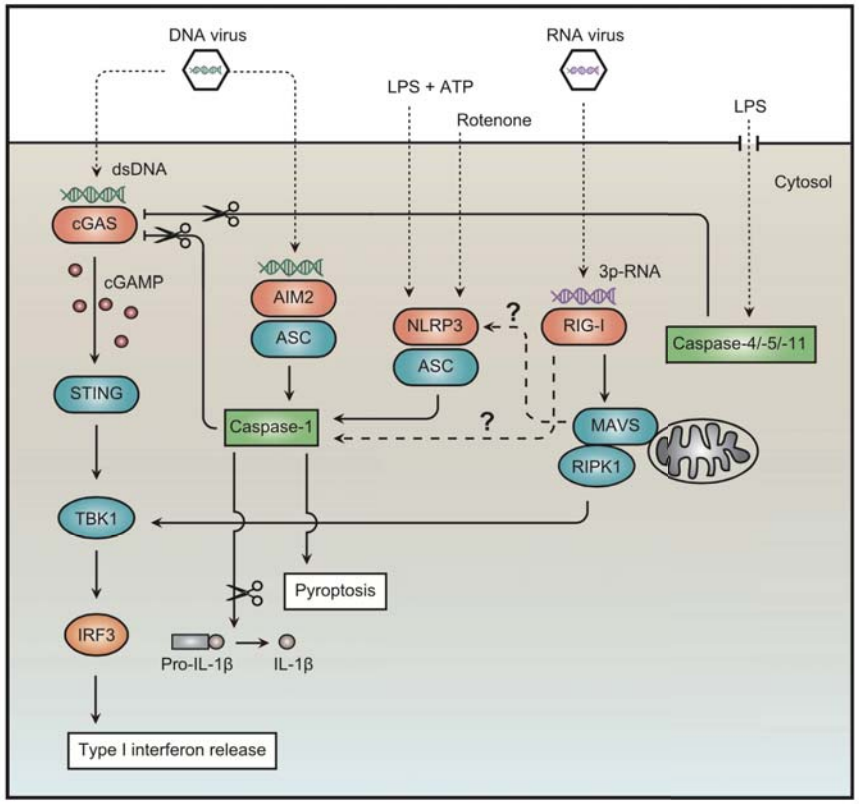
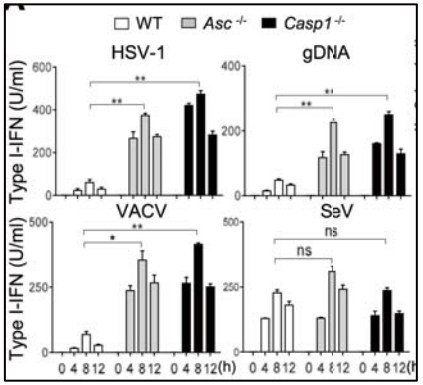
STING & Inflammasome – DNA Viurs

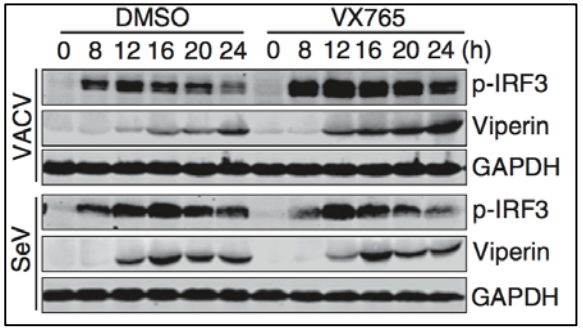


Simon Heidegger, et al. Cutting Edge in IFN Regulation: Inflammatory Caspases Cleave cGAS. Immunity. 2017

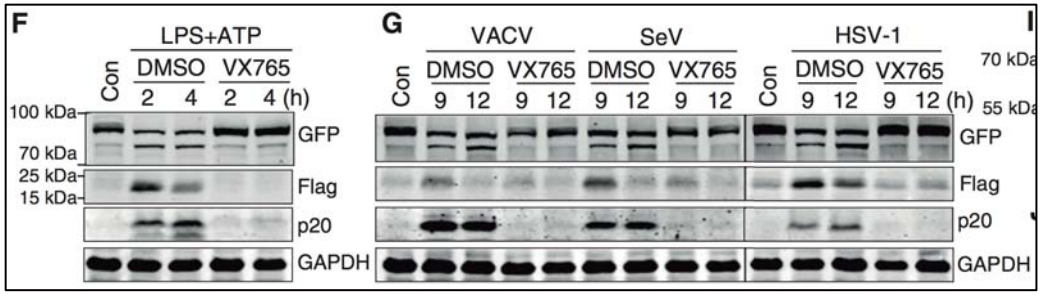
Yutao Wang, et al. Inflammasome Activation Triggers Caspase-1-Mediated Cleavage of cGAS to Regulate Responses to DNA Virus Infection Immunity. 2017



-Inflammasome Defect Augments Cytokine Production



-Inflammasome Inhibition Potentiates DNA Virus-Triggered Signaling



-cGAS Is Recruited and Cleaved by Caspase-11and Caspase-1during Inflammasome Activation

Product Box

Products	Cat.
HSV-60 Naked	tlrl-hsv60n
VACV-70	tlrl-vav70n
VX-765	Inh-vx765i-1

STING and Infectious Diseases – Interplay

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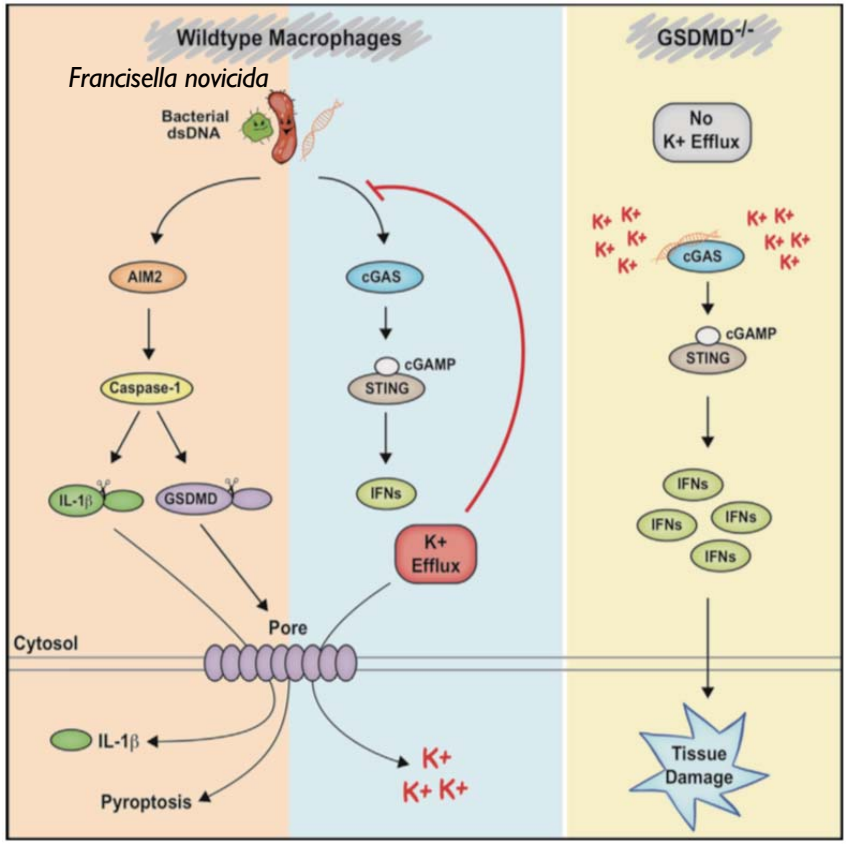
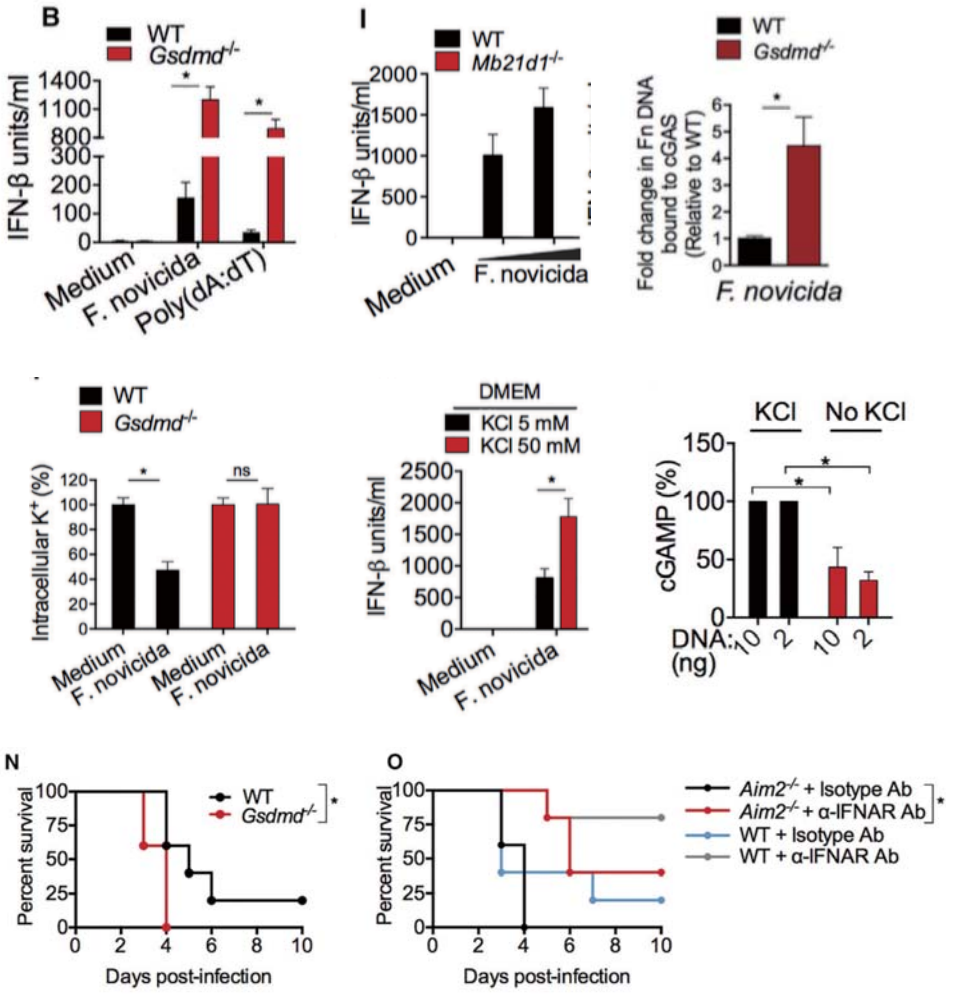
STING & Gasdermin D - Bacteria



Products	Cat.
Ultrapure LPS E.coli O111:B4	tlrl-3pelps
2'3'-cGAMP	tlrl-nacga23-1
Poly I:C	tlrl-pic
Pam3CSK4	tlrl-pms
Nigericin	tlrl-nig
Ionomycin	Inh-ion



Ishita Banerjee, et al. Gasdermin D Restrains Type I Interferon Response to Cytosolic DNA by Disrupting Ionic Homeostasis. Immunity. 2018

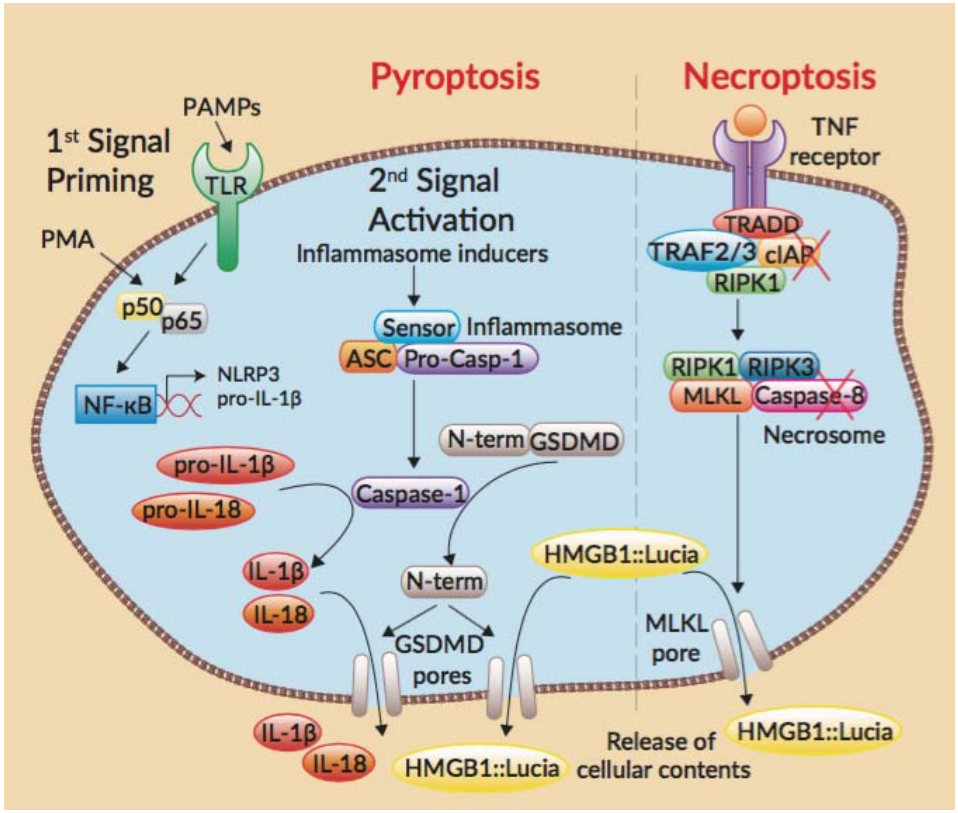
Rebecca Feltham and James E. Vince. Ion Man: GSDMD Punches Pores to Knock Out cGAS. Immunity. 2018

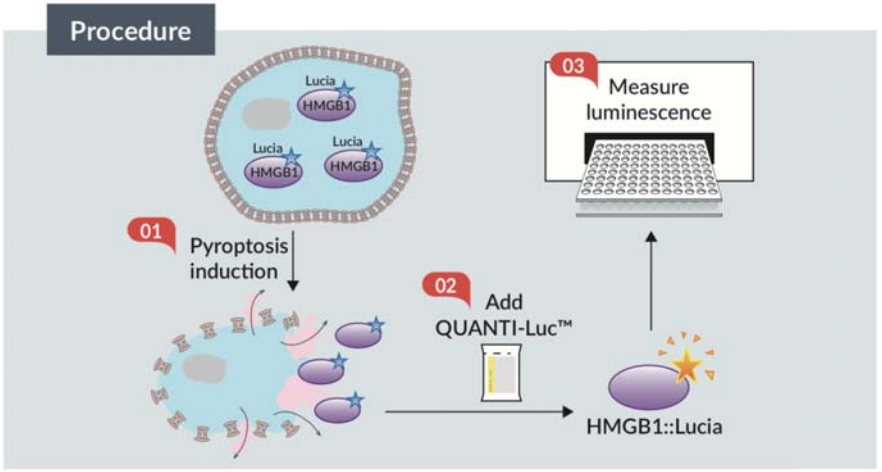
Pyroptosis and Necroptosis Reporter Cell Lines

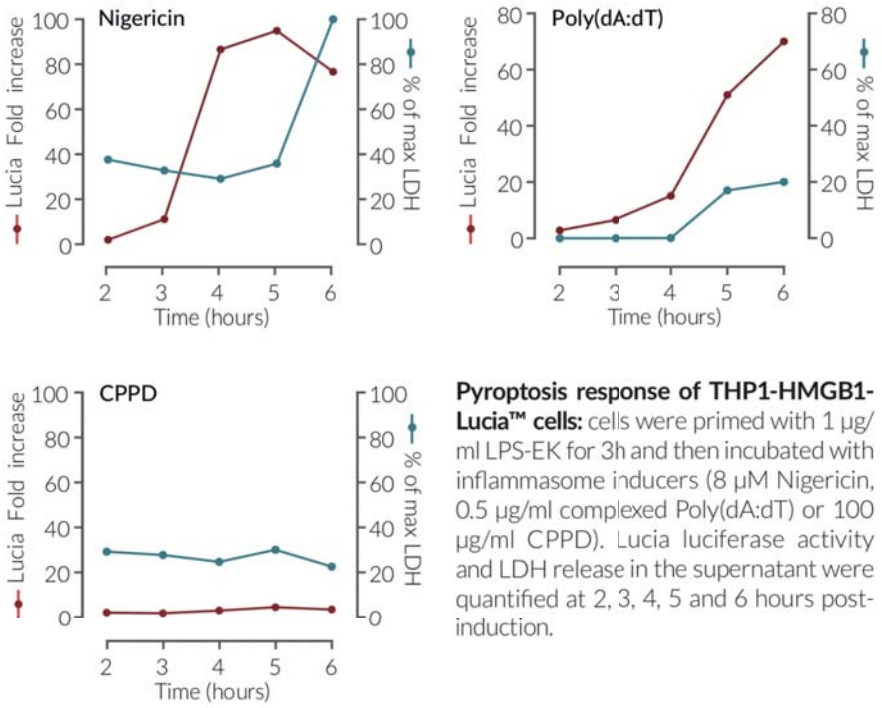
InvivoGen
INNOVATION WITHIN REACH

THP1-HMGB1-Lucia™ Cells

Cat. Code: thp-hmgluc







Pyroptosis response of THP1-HMGB1-Lucia™ cells: cells were primed with 1 µg/ml LPS-EK for 3h and then incubated with inflammasome inducers (8 µM Nigericin, 0.5 µg/ml complexed Poly(dA:dT) or 100 µg/ml CPPD). Lucia luciferase activity and LDH release in the supernatant were quantified at 2, 3, 4, 5 and 6 hours post-induction.

STING and Infectious Diseases - Bacteria

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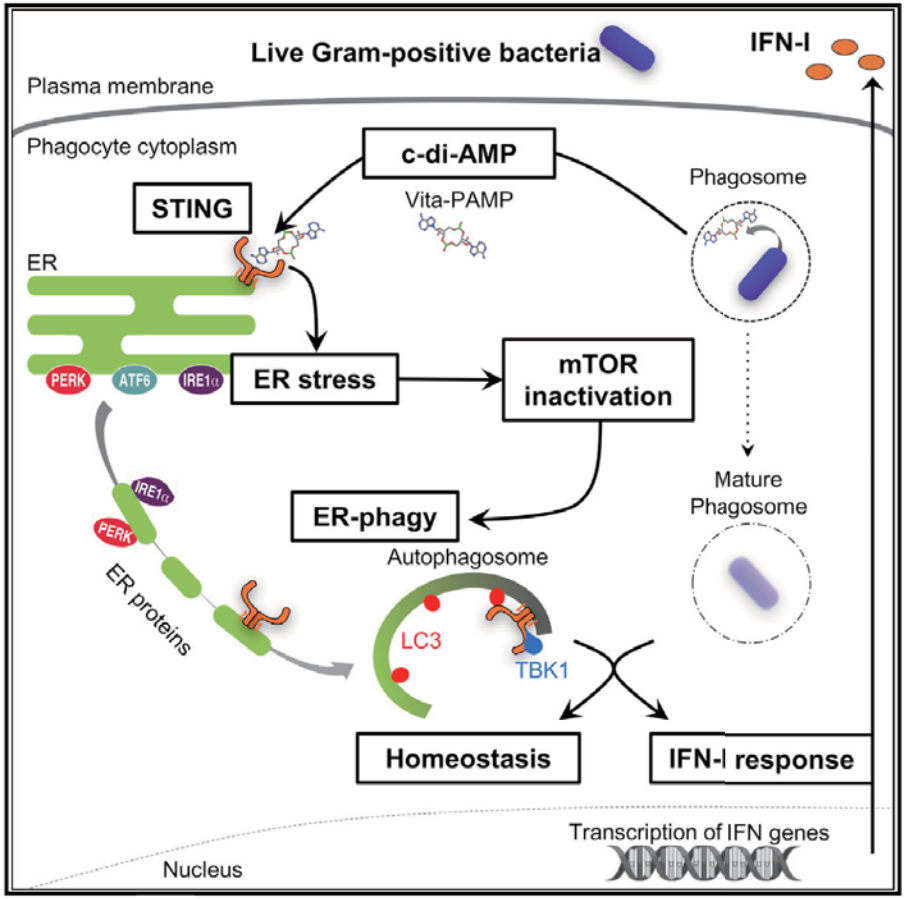
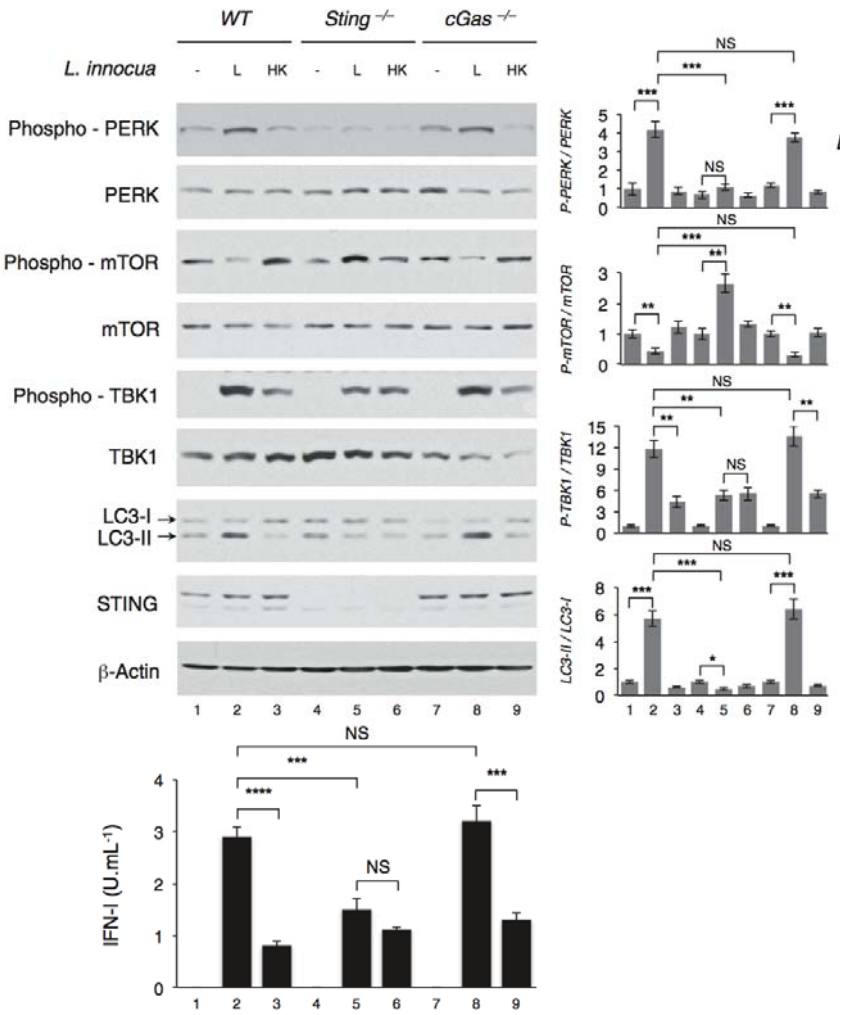
STING mediates Autophagy



Product	Cat. code
c-di-AMP	tlrl-nacda
Rapamycin	tlrl-rap
Bafilomycin AI	tlrl-baf1
Wortmannin	tlrl-wtm
HeLa-Difluo™ hLC3 Cells	heldf-hlc3



Julien Moretti, et. al. STING Senses Microbial Viability to Orchestrate Stress-Mediated Autophagy of the Endoplasmic Reticulum.Cell. 2017

STING and Autophagy

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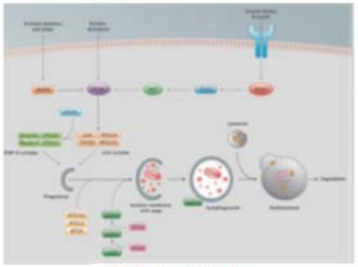
Autophagy

Autophagy is a pathway by which cytoplasmic constituents, including organelles and intracellular pathogens, are sequestred in a double-membrane-bound autophagosome and delivered to the lysosome for degradation.
The role of autophagy is to eliminate unwanted constituents from the cell and recycle cytoplasmic material allowing the cell to maintain macromolecular synthesis and energy homeostatis during starvation and other stressful conditions.



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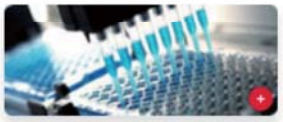
NEW AUTOPHAGY & INNATE IMMUNITY REVIEW READ MORE
http://www.invivogen.com/review-autophagy

Autophagy Inducers



Autophagy Inhibitors

Autophagy Genes

Autophagy Reporter Cells

http://www.invivogen.com/autophagy

http://www.invivogen.com/newsletters.php

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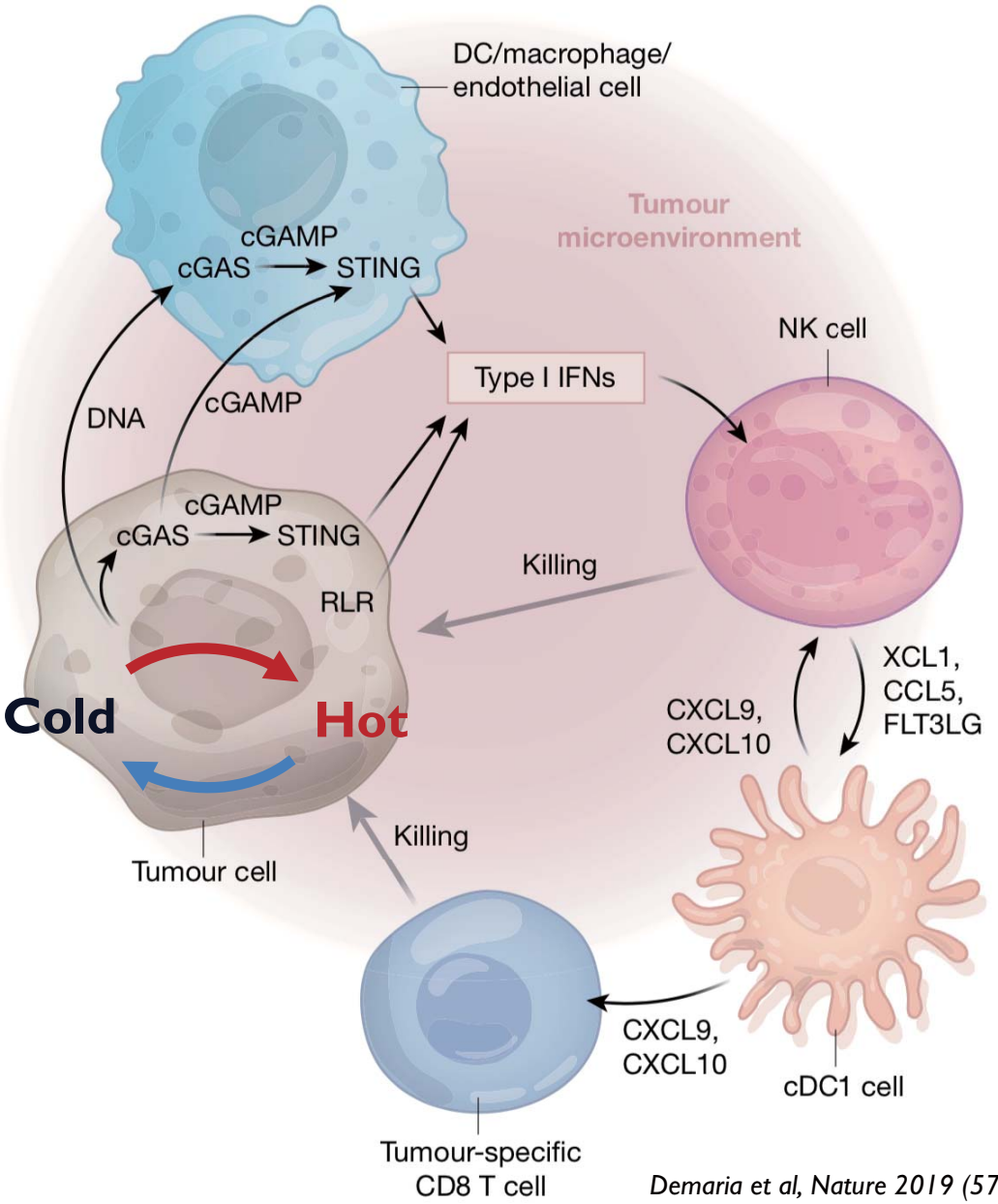
C

cGAS
STING

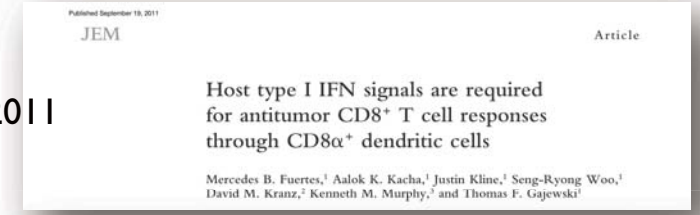
Caner



Demaria et al, Nature 2019 (574)

STING and Cancer

2011



Published September 19, 2011

JEM Article

Host type I IFN signals are required for antitumor CD8⁺ T cell responses through CD8α⁺ dendritic cells

Mercedes B. Fuertes,[1] Aalok K. Kacha,[1] Justin Kline,[1] Seng-Ryong Woo,[1] David M. Kranz,[2] Kenneth M. Murphy,[3] and Thomas F. Gajewski[1]

2014

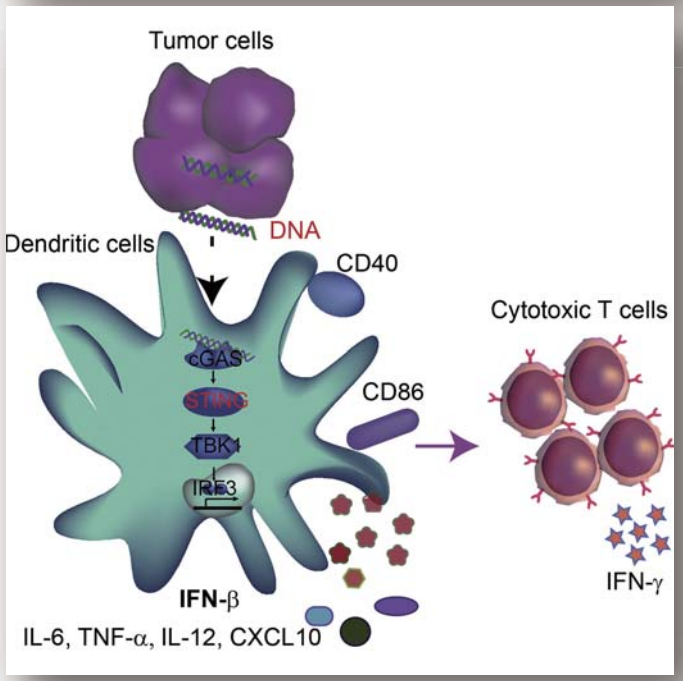
CellPress

Immunity

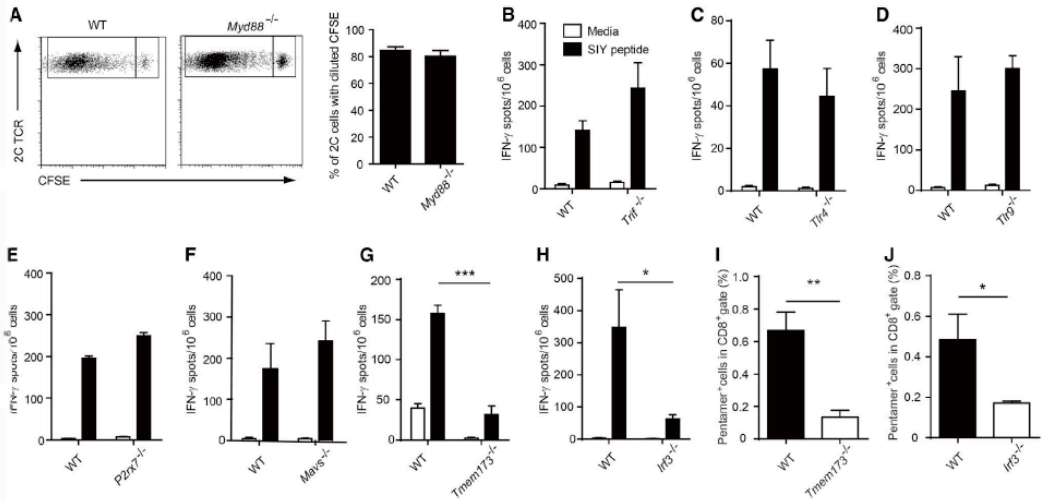
Article

STING-Dependent Cytosolic DNA Sensing Mediates Innate Immune Recognition of Immunogenic Tumors

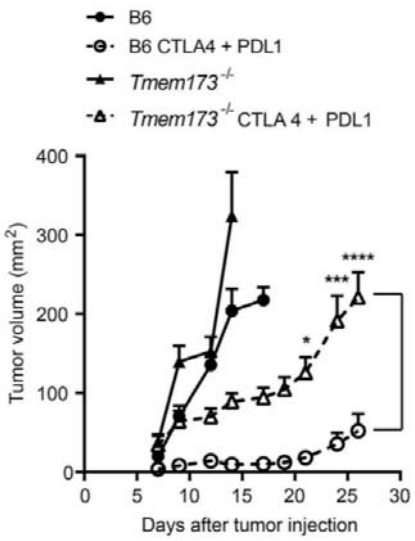
Seng-Ryong Woo,[1] Mercedes B. Fuertes,[1] Leticia Corrales,[1] Stefani Spranger,[1] Michael J. Furdyna,[1] Michael Y.K. Leung,[1] Ryan Duggan,[1] Ying Wang,[2] Glen N. Barber,[4] Katherine A. Fitzgerald,[5] Maria-Luisa Alegre,[2] and Thomas F. Gajewski[1,2,*]



Product	Cat. code
B16-Blue™ ISG Cells	bb-ifnabg



STING and IRF3 Are Required for CD8+ T Cell Priming *in vivo*



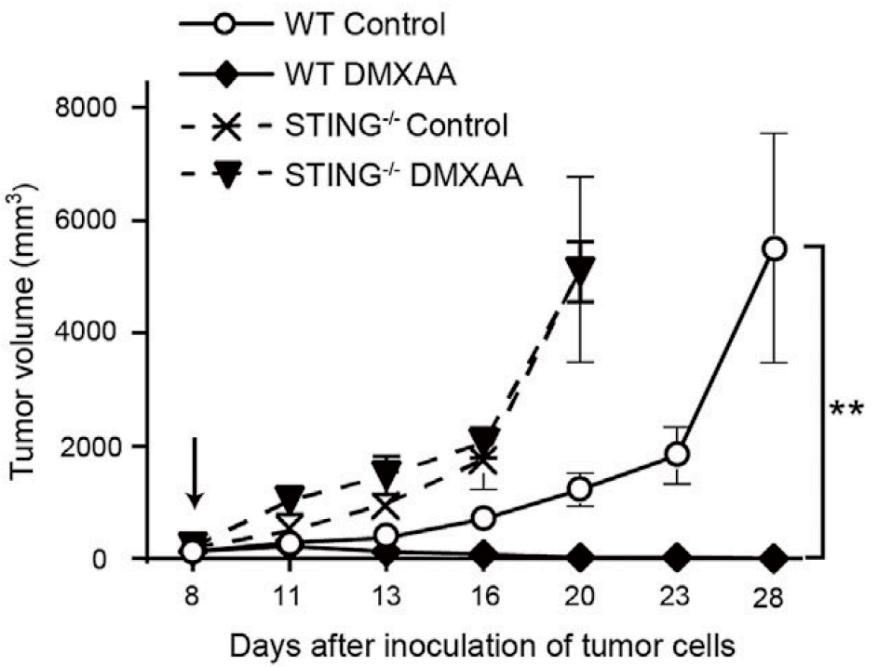
STING pathway can play a critical role in the therapeutic efficacy of cancer immunotherapies.

DMXAA in anti-tumor therapy

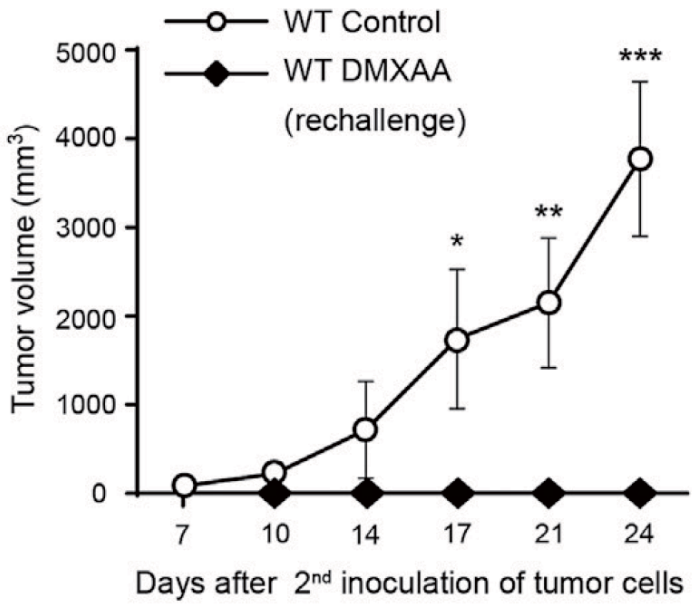
Product Box

Product	Cat. code
DMXAA	tlrl-dmx

B16.SIY tumor cells in WT C57BL/6 mice



Anti-tumor



Immunologic Memory

Leticia Corrales, et al. Cell Reports. 2015

DMXAA = 5,6-dimethylxanthenone-4-acetic acid

STING sequence	DMXAA binding affinity	Ref.
Mouse WT	Yes	Conlon *et al.*, *J Immunol*, 2013
Human WT	**No**	**Conlon *et al.*, *J Immunol*, 2013**
Human binding-site mutant S162A	Yes	Gao *et al.*, *Cell*, 2013 ; Gao et al., *Cell Reports*, 2014
Human binding-site mutant Q266I	Yes	Gao *et al.*, *Cell*, 2013 ; Gao et al., *Cell Reports*, 2014
Human lid-region mutant G230I	Yes	Gao *et al.*, *Cell*, 2013 ; Gao et al., *Cell Reports*, 2014

Stop in Phase III

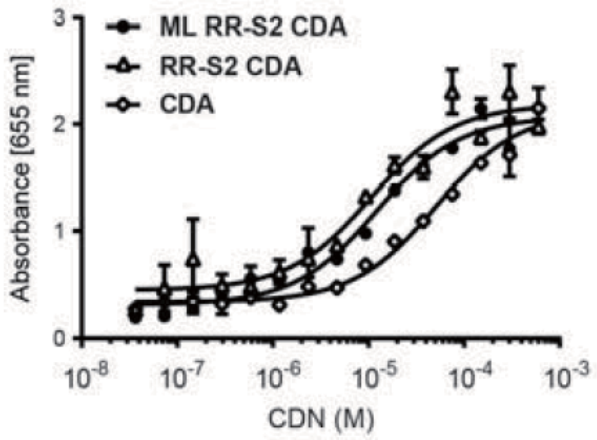
Product Box

Product	Cat. code
DMXAA	tlrl-dmx
293T-Dual™ hSTING-A162 Cells	293d-a162
THP1-Dual™ KI-hSTING-A162 Cells	thpd-a162

InvivoGen
INNOVATION WITHIN REACH

New STING ligand in anti-tumor therapy

Screened by **THP1-Blue™ ISG Cells**



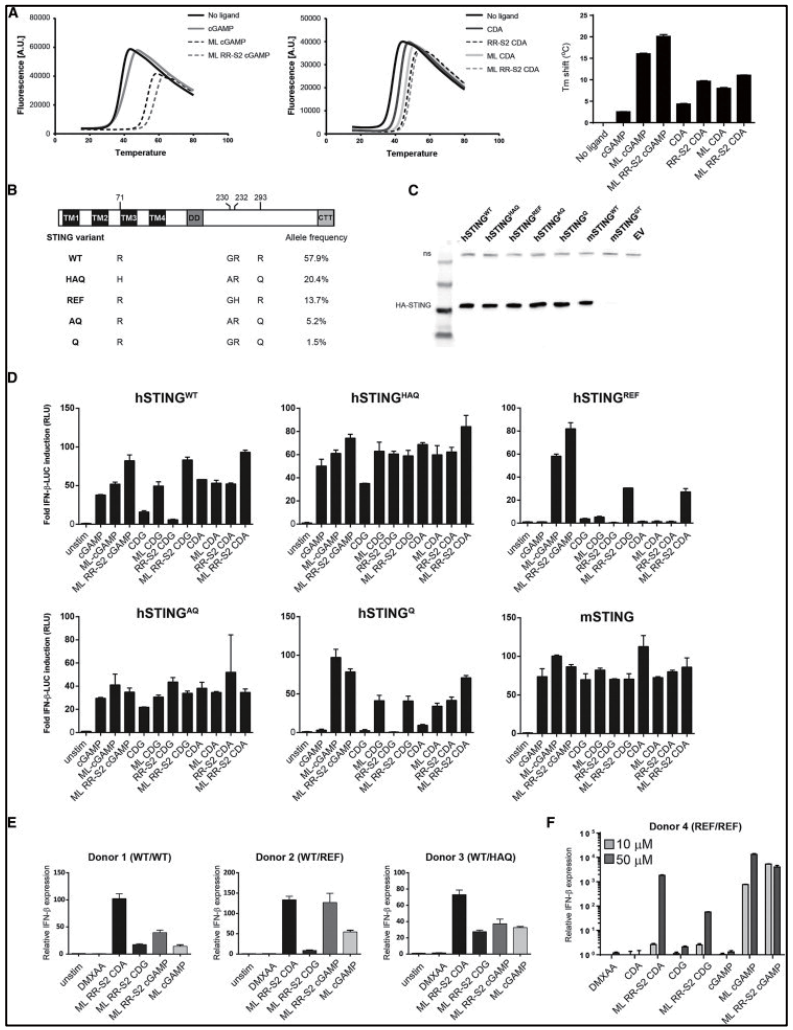
-ML RR-S2 CDA:
Mixed linkage dithio-(R_P, R_P)-[cyclic[A(2',5') pA(3',5')p

-ADU-S100 (2'3'-c-di-AM(PS)2 (Rp,Rp)): Phase I/II

Product Box

Product	Cat. code
THP1-Blue™ ISG Cells	thp-isg
QUANTI-Blue™	Rep-qb1/2
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r

Leticia Corrales, et al. Cell Reports. 2015

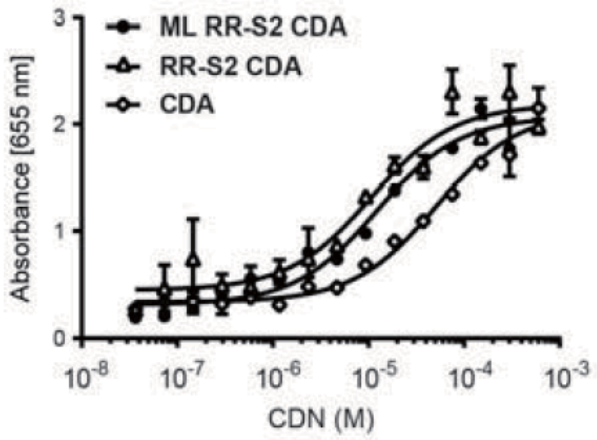


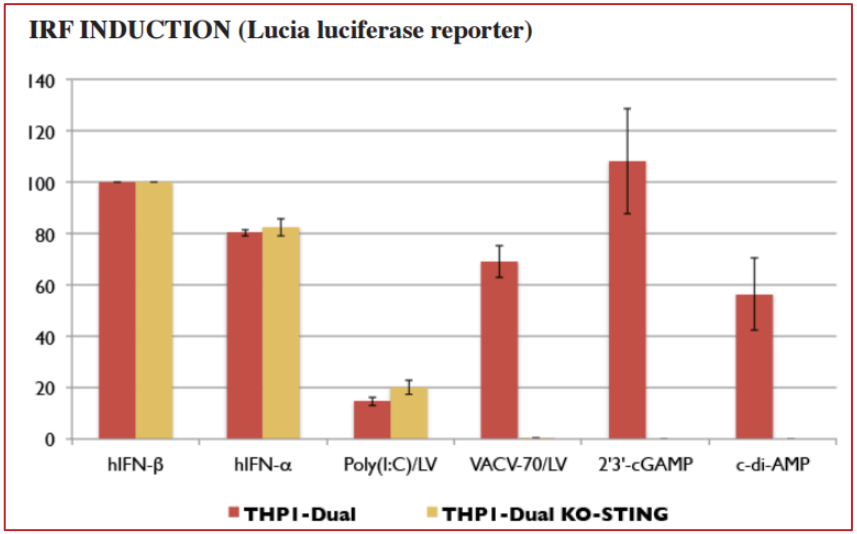
Data for proving human STING specificity

InvivoGen
INNOVATION WITHIN REACH

New STING ligand in anti-tumor therapy

Screened by THP1-Blue™ ISG Cells





-ML RR-S2 CDA:
Mixed linkage dithio-(R_P, R_P)-[cyclic[A(2',5') pA(3',5')p

-ADU-S100 (2'3'-c-di-AM(PS)2 (Rp,Rp)): Phase I/II

The author could address simple STING specific evidence through THP1-Dual KO-STING cells

Product Box

Product	Cat. code
THP1-Blue™ ISG Cells	thp-isg
QUANTI-Blue™	Rep-qb1/2
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r

Human cell line

Products	Cat.
THP1-Dual™ cells	thpd-nfis
THP1-Dual™ KO-STING cells	thpd-kostg

Leticia Corrales, et al. Cell Reports. 2015

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Also Available

Reporter cell lines

SEAP

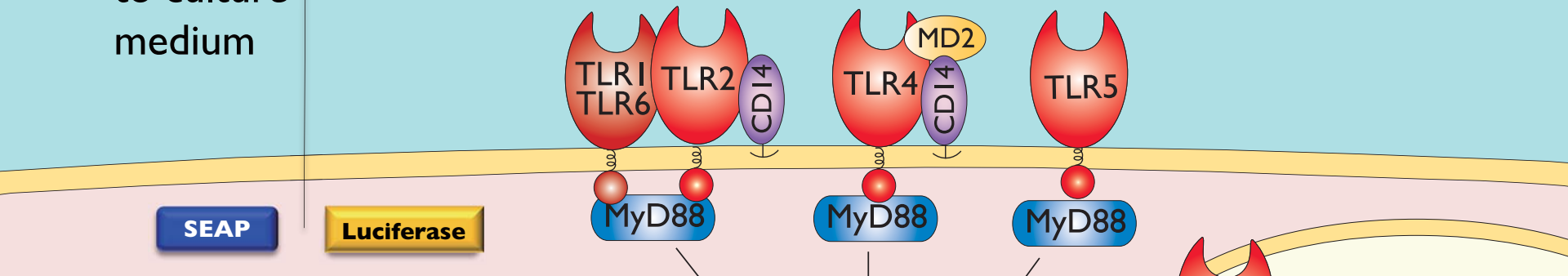
- HEK-Blue™
- QUANTI-Blue™

Luciferase

- QUANTI-Luc™

Luminometer

Secrete to culture medium



STING and Cancer

InvivoGen
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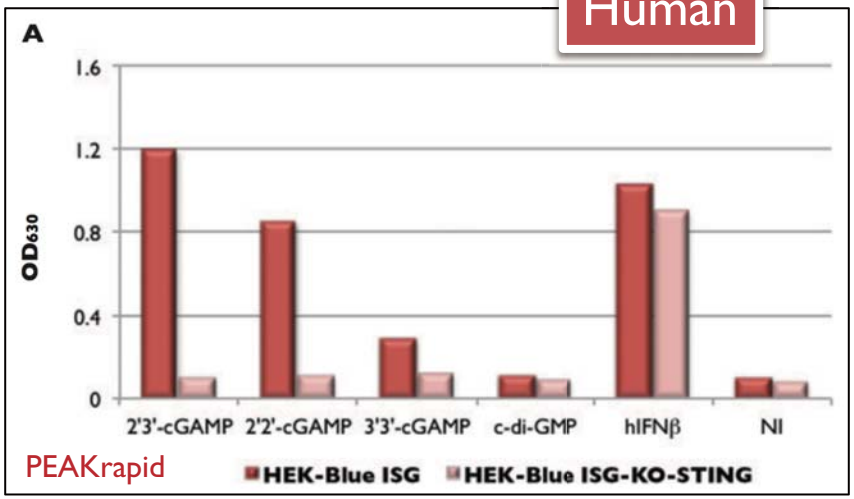
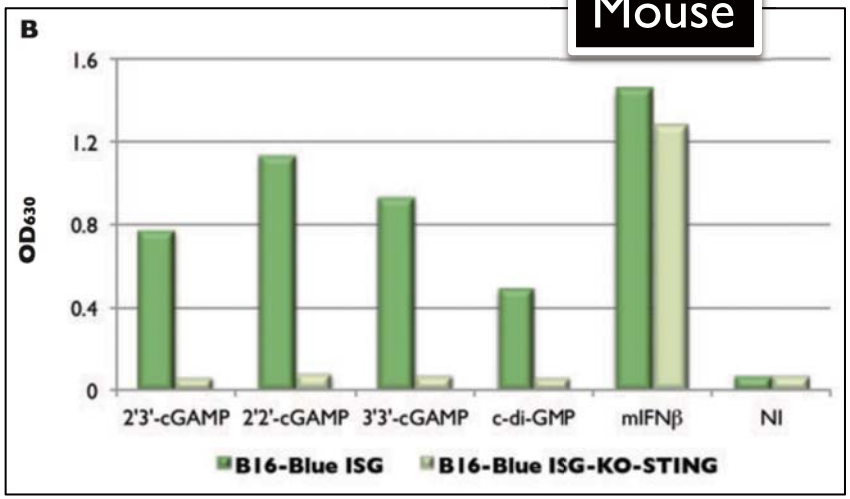
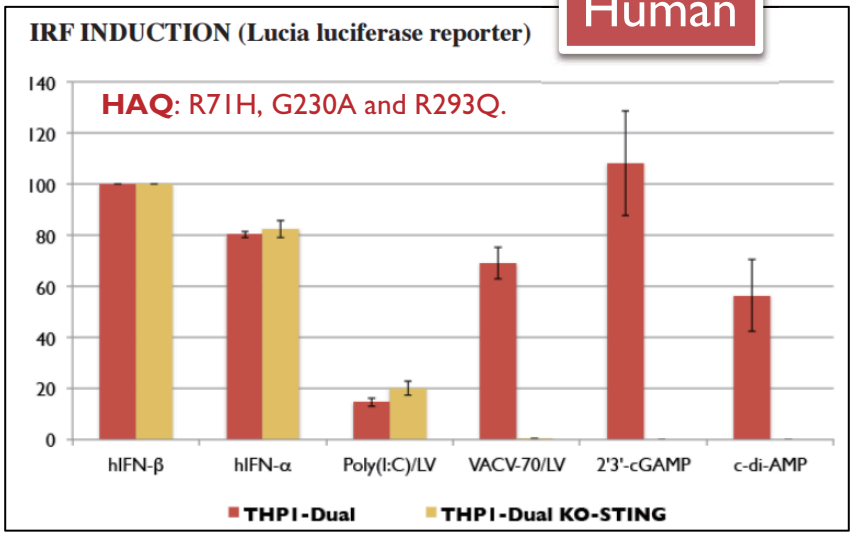
Human



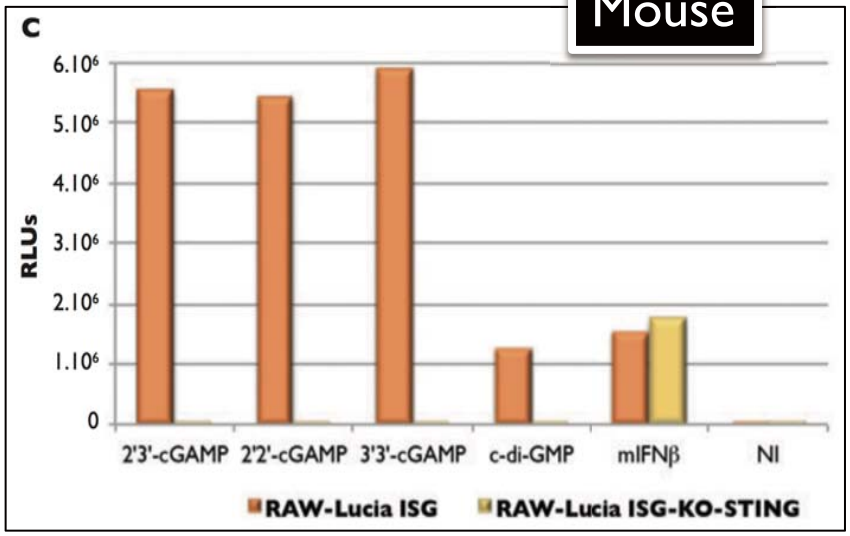
Mouse



Human



Mouse



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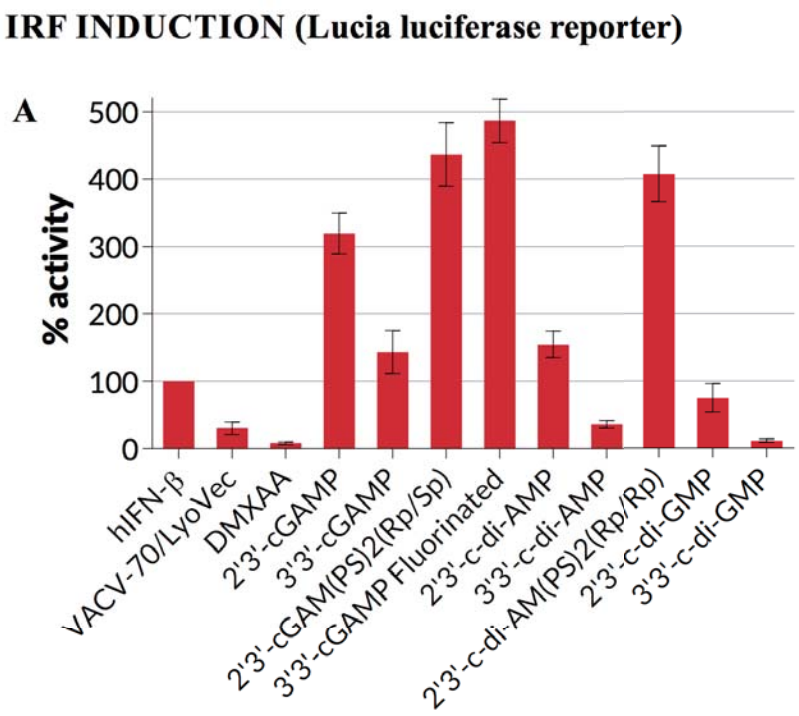
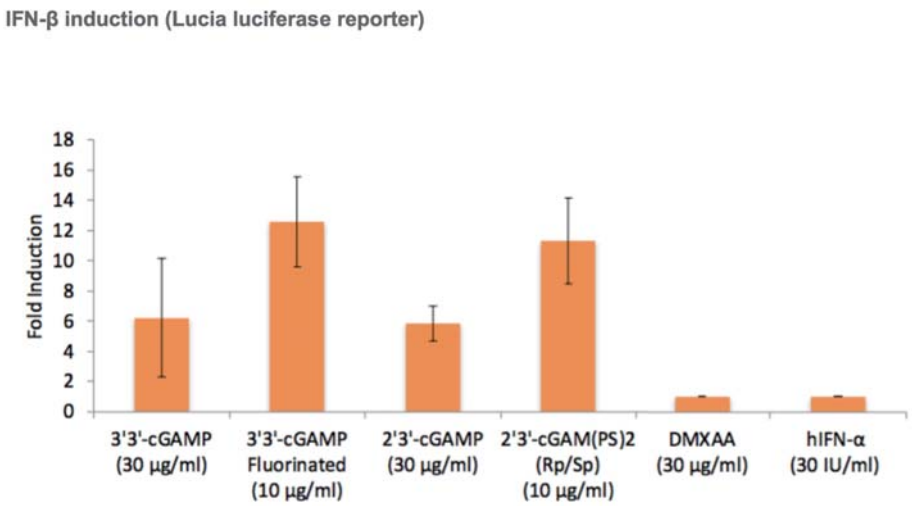
- **R232**: is the most prevalent variant with an occurrence (homozygous allele) of **~45-58%** in the human population. This isoform is preferentially activated by 2'5'linkage-containing cGAMP isomers.

THP1-Dual™ KI-hSTING-R232 Cells

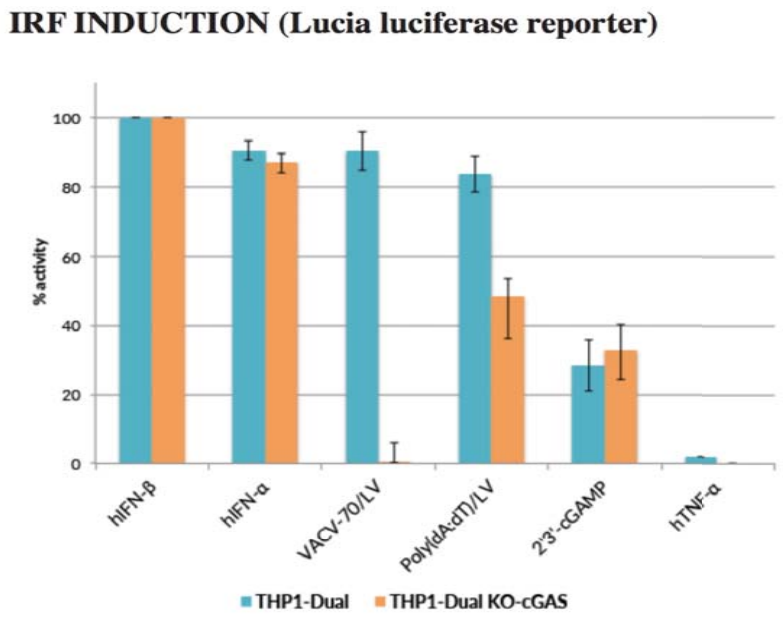


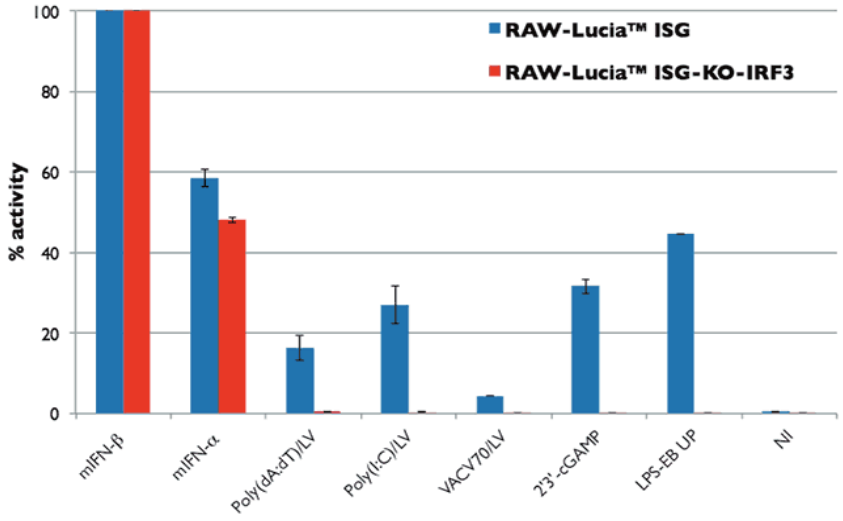
293T-Dual™ hSTING-R232 Cells



NEW !

STING and Cancer





Human cell line

Products	Cat.
THP1-Dual™ cells	thpd-nfis
THP1-Dual™ KO-STING cells	thpd-kostg
THP1-Dual™ KO-TREX1 cells	thpd-kotrex
THP1-Dual™ KO-IFI16 cells	thpd-koifi16
THP1-Dual™ KO-cGAS cells	thpd-kocgas
THP1-Dual™ KO-IFNAR2 cells	thpd-koifnar2

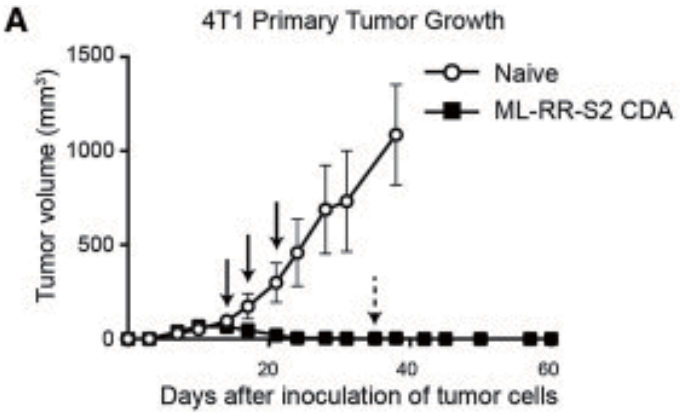
Murine cell line

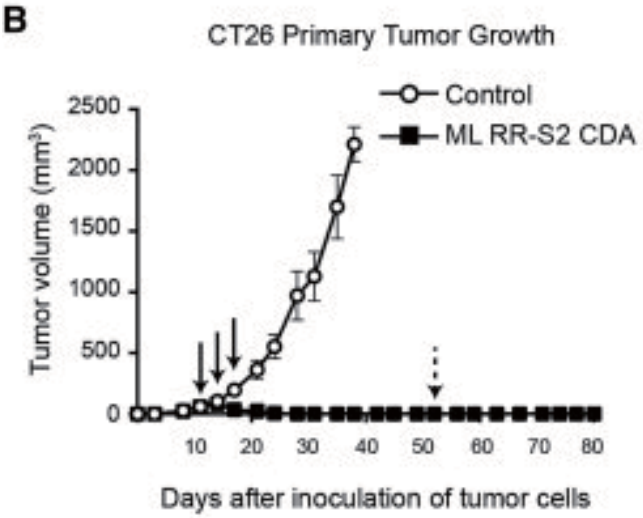
Products	Cat.
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING Cells	rawl-kostg
RAW-Lucia™ ISG-KO-cGAS Cells	rawl-kocgas
RAW-Lucia™ ISG-KO-IFI16 Cells	rawl-koif16
RAW-Lucia™ ISG-KO-TBK1 Cells	rawl-kotbk
RAW-Lucia™ ISG-KO-IRF7 Cells	rawl-koirf7
RAW-Lucia™ ISG-KO-IRF3 Cells	rawl-koirf3
RAW-Lucia™ ISG-KO-TREX1 Cells	rawl-kotrex

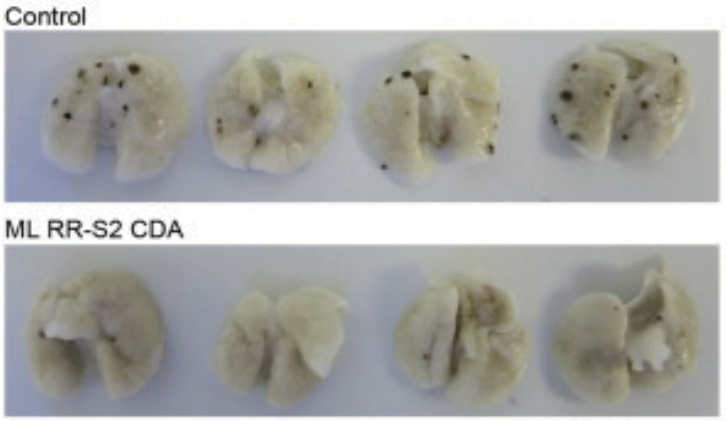
InvivoGen
INNOVATION WITHIN REACH

ML RR-S2 CDA (ADU-S100) in anti-tumor therapy

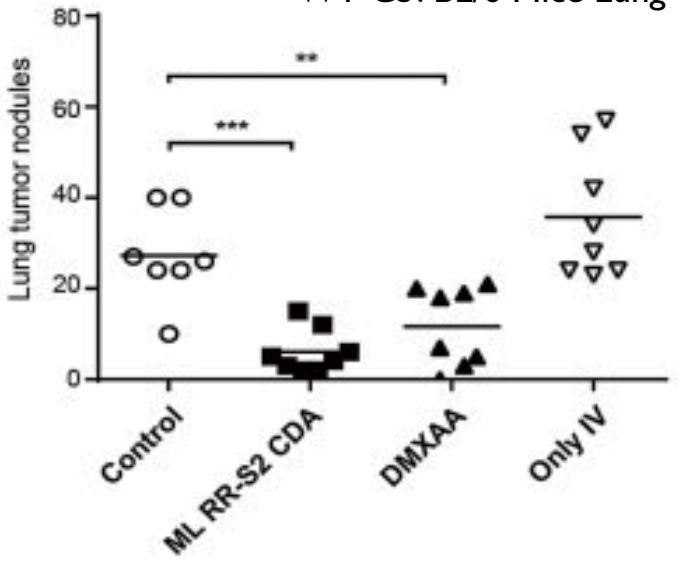
Product	Cat. code
DMXAA	tlrl-dmx
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r





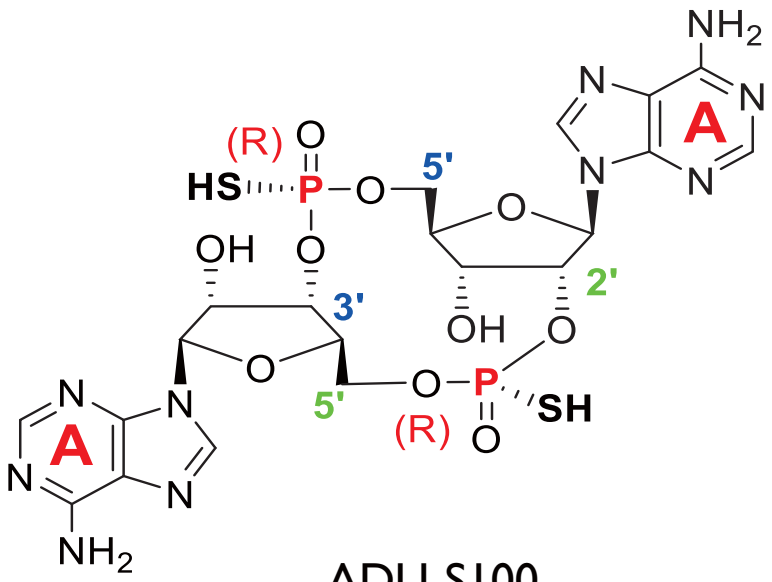


WT C57BL/6 Mice Lung

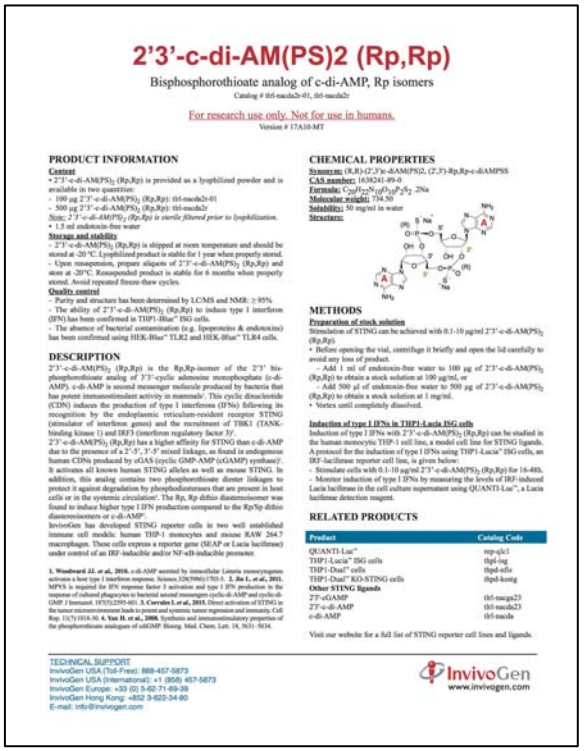


Leticia Corrales, et al. Cell Reports. 2015

STING and Cancer



ADU-S100



2'3'-c-di-AM(PS)2 (Rp,Rp)

Bisphosphorothioate analog of c-di-AMP, Rp isomers

For research use only. Not for use in humans.

PRODUCT INFORMATION

CHEMICAL PROPERTIES

METHODS

DESCRIPTION

RELATED PRODUCTS

InvivoGen

www.invivogen.com

NCT#	Start	Phase	Agent	Admin.	Cancer	Company
NCT02675439	Mar-16	I	ADUS100 ± anti-CTLA4	IT CDN	Multiple Tumors	Novartis/Aduro
NCT03172936	Sep-17	I	ADUS100 + anti-PD1	IT CDN	Multiple Tumors	Novartis/Aduro
NCT03937141	Aug-19	II	ADUS100 + anti-PD1	IT CDN	Head&Neck Cancer	Novartis/Aduro

STING and Cancer

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Clinical Trials

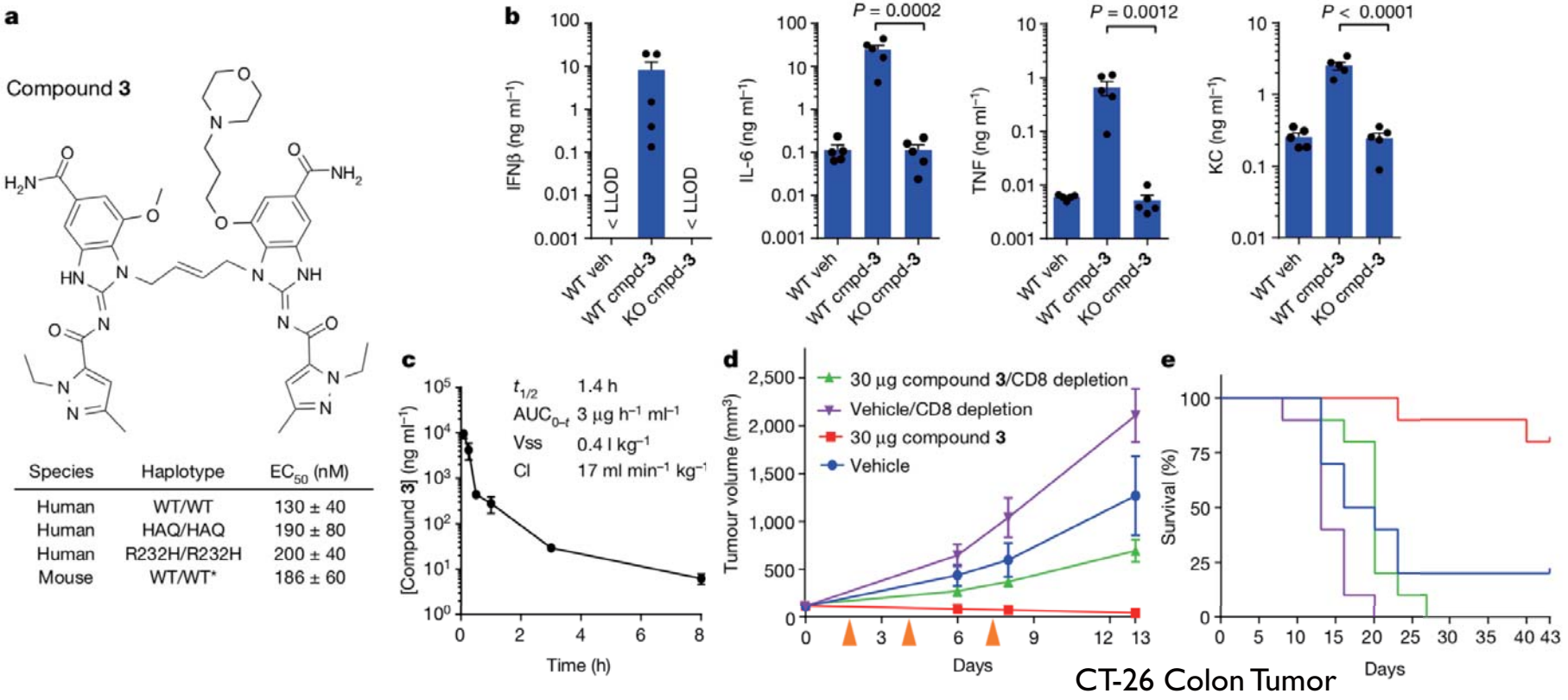
NCT#	Start	Phase	Agent	Admin.	Cancer	Company
NCT02675439	Mar-16	I	ADUS100 ± anti-CTLA4	IT CDN	Multiple Tumors	Novartis/Aduro
NCT03172936	Sep-17	I	ADUS100 + anti-PD1	IT CDN	Multiple Tumors	Novartis/Aduro
NCT03937141	Aug-19	II	ADUS100 + anti-PD1	IT CDN	H&N Cancer	Novartis/Aduro
NCT03010176	Feb-17	I	MK-1454 ± anti-PD1	IT. CDN	Multiple Tumors	Merk
NCT03249792	Sep-17	I	MK-2118 ± anti-PD1	IT. Small Molecule	Multiple Tumors	Merk
NCT04220866	Mar-20	II	MK-1454 ± anti-PD1	IT. CDN	H&N Cancer	Merk
NCT03956680	May-19	I	BMS-986301 ± Anti-PD1 & anti-CTLA4	IT CDN	Solid Tumors	BMS
NCT04144140	Oct-19	I	E7766	Intratumoral	Solid Tumors	Eisai
NCT04109092	Sep-19	I	E7766	Intravesical	Bladder Cancer	Eisai
NCT03843359	Feb-19	I	GSK3745417 ± anti-PD1	IV	Solid Tumors	GSK
NCT04096638	Sep-19	I	SB 11285 ± anti-PD1	IV	Solid Tumors	Sprink Bank
NCT04167137	Nov-19	I	SYNB1891 ± anti-PD-L1	Intratumoral E. Coli	Solid Tumors	Synlogic

STING and Cancer

- **Intravenous administration**
- **A small molecule STING agonist that is not a cyclic dinucleotide**

NCT03843359

RESEARCH LETTER



Joshi M. ramanjulu, et al. Design of amidobenzimidazole STING receptor agonists with systemic activity. Nature. 2018

synlogic

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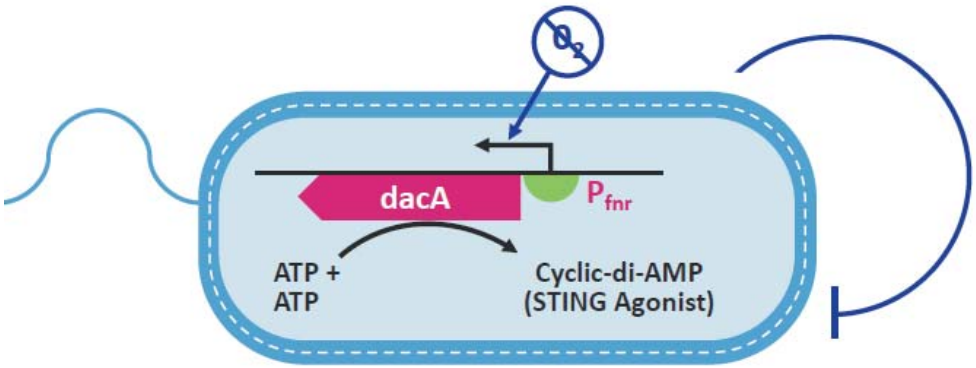
INNOVATION WITHIN REACH

Novel delivery system

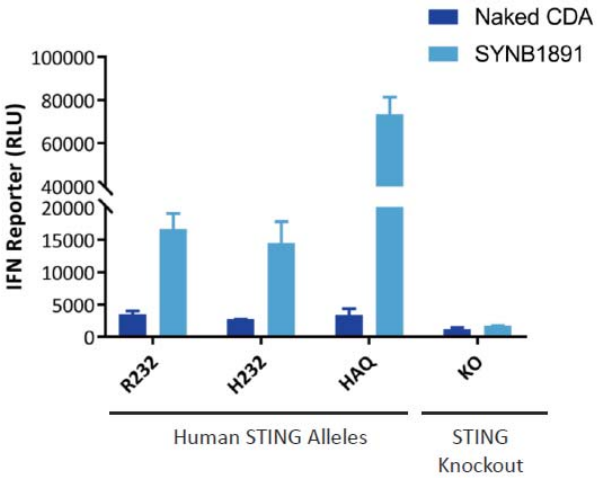
Auxotrophic *E. coli* Nissle (EcN) engineered to produce high levels of the STING agonist c-di-AMP

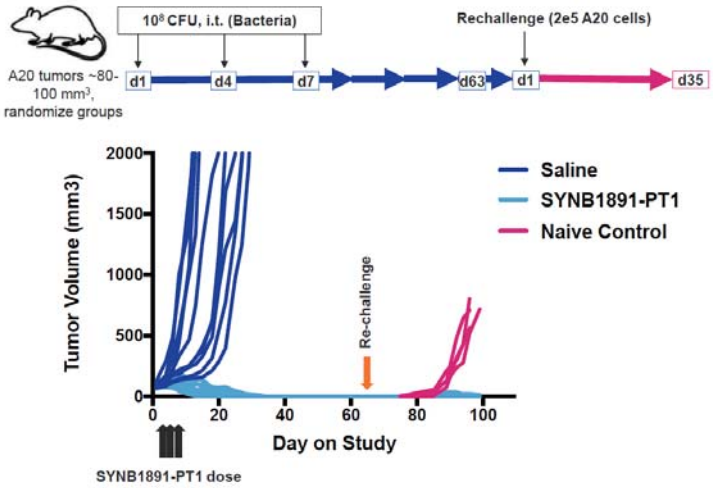


Auxotrophies

- Diaminopimelic acid (DAP)
- Thymidine

REPORTER HUMAN MONOCYTIC LINE





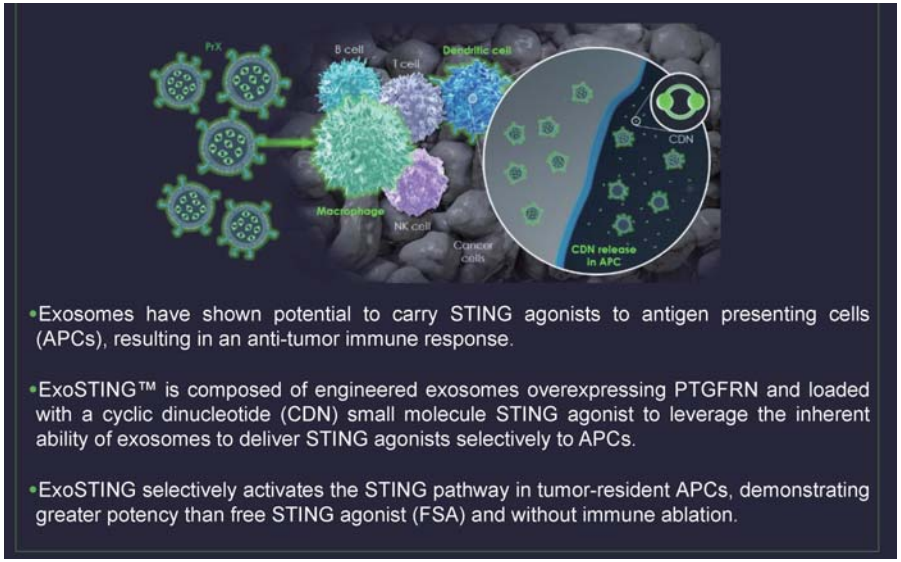
Synlogic Cooperate Presentation, 2019

Daniel S. Leventhal, et al. Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. Nature Communication. 2020

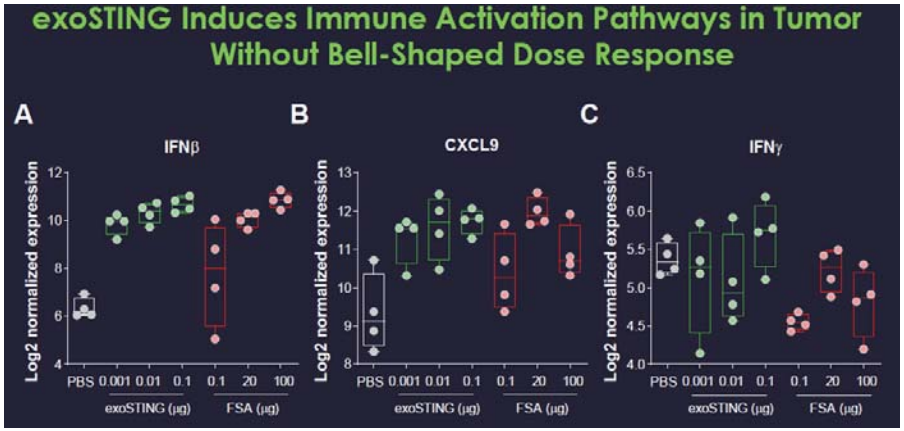
STING and Cancer

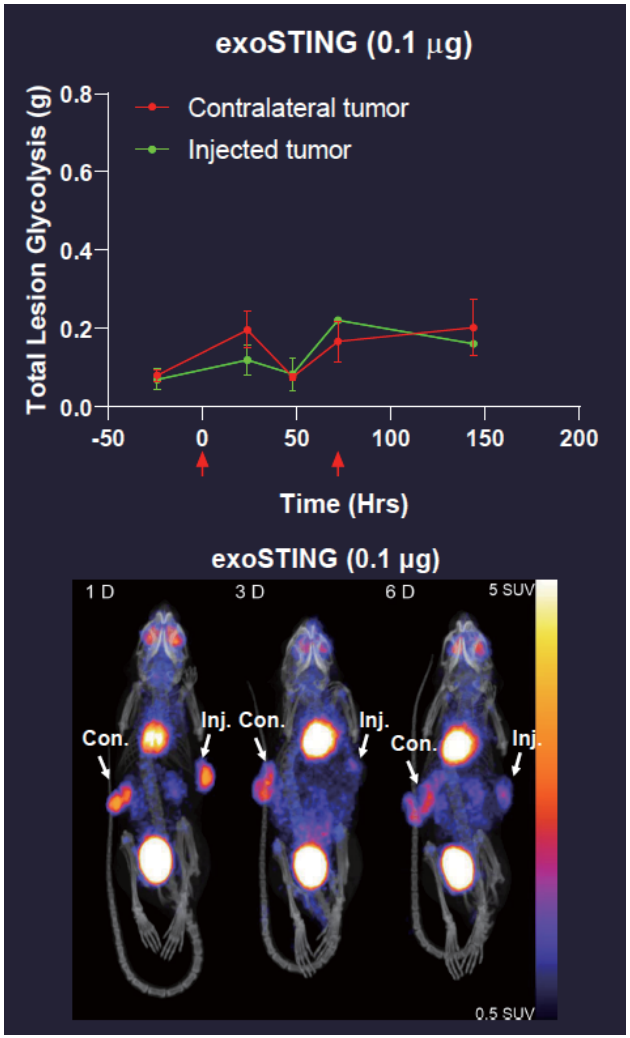
CODIAK

Exosome



- Exosomes have shown potential to carry STING agonists to antigen presenting cells (APCs), resulting in an anti-tumor immune response.
- ExoSTING™ is composed of engineered exosomes overexpressing PTGFRN and loaded with a cyclic dinucleotide (CDN) small molecule STING agonist to leverage the inherent ability of exosomes to deliver STING agonists selectively to APCs.
- ExoSTING selectively activates the STING pathway in tumor-resident APCs, demonstrating greater potency than free STING agonist (FSA) and without immune ablation.





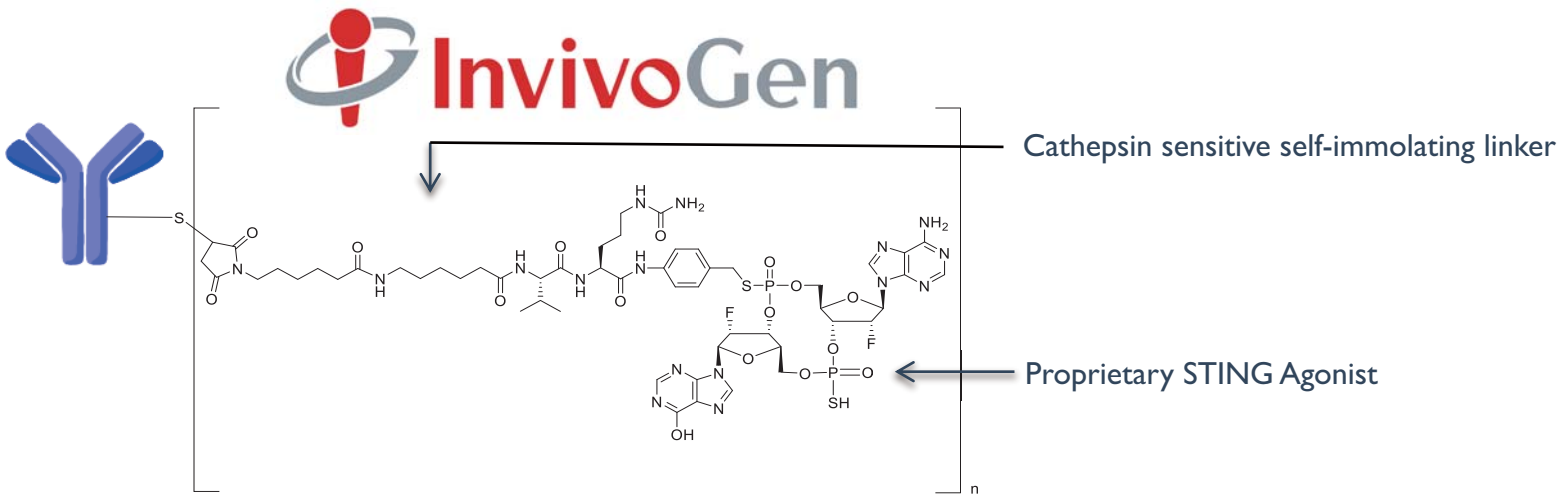
Su Chul, Jiang. Selective Activation of Antigen Presenting Cells by exoSTING Enhances Tumor Antigen-Specific Immune Response. Presented at the 34thAnnual Meeting of the Society for Immuno-Therapy of Cancer, November 6.

InvivoGen
INNOVATION WITHIN REACH

ISAC : Immune Stimulating Antibody Conjugate

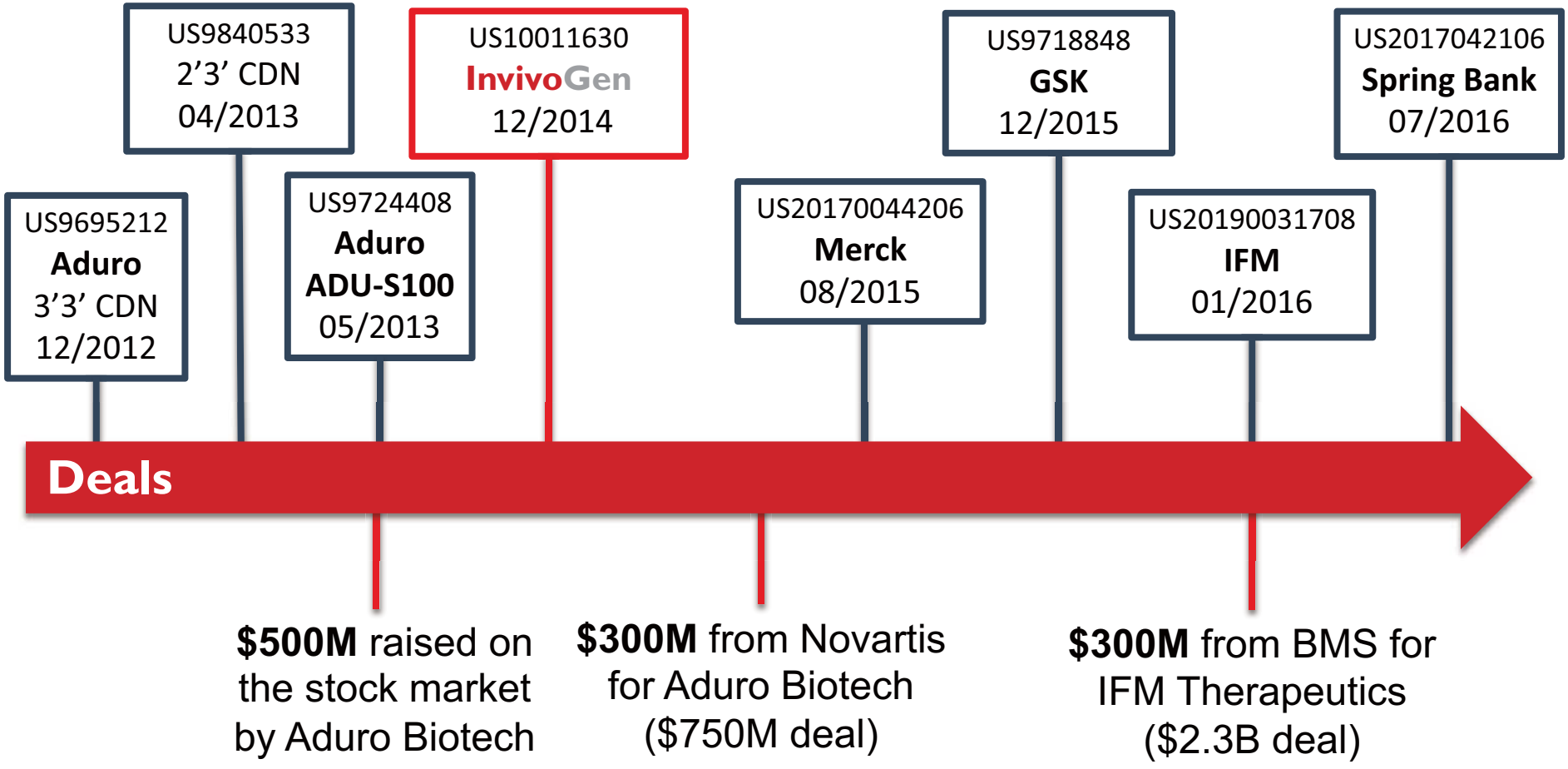
Start	Phase	Agent	Target	Company
2nd half of 2020	Discovery	Immunosynthen	Multiple Tumors	Mersana THERAPEUTICS
-	Discovery	SB11285 with ADC	Multiple Tumors	spring bank pharmaceuticals
2019	Discovery	CRD5500 conjugated to Trastuzumab	Solid Tumors	CURADEV *Let science do the talking*

- Patent application (WO2019129880) for an ADC with a proprietary CDN (*to be commercialised in 2020*)



InvivoGen
INNOVATION WITHIN REACH

Patents



Novel Cyclic Adenosine-Inosine Monophosphate Analogs

Journal of Medicinal Chemistry

Article

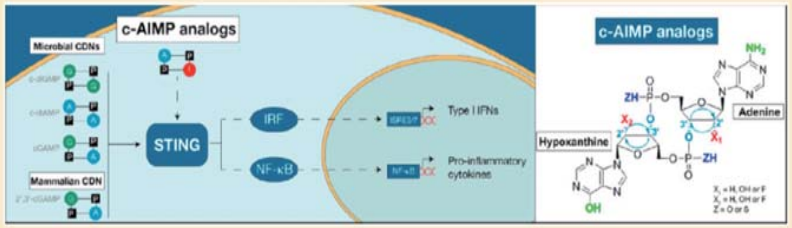
pubs.acs.org/jmc

Design, Synthesis, and Biological Evaluation of Novel Cyclic Adenosine–Inosine Monophosphate (cAIMP) Analogs That Activate Stimulator of Interferon Genes (STING)

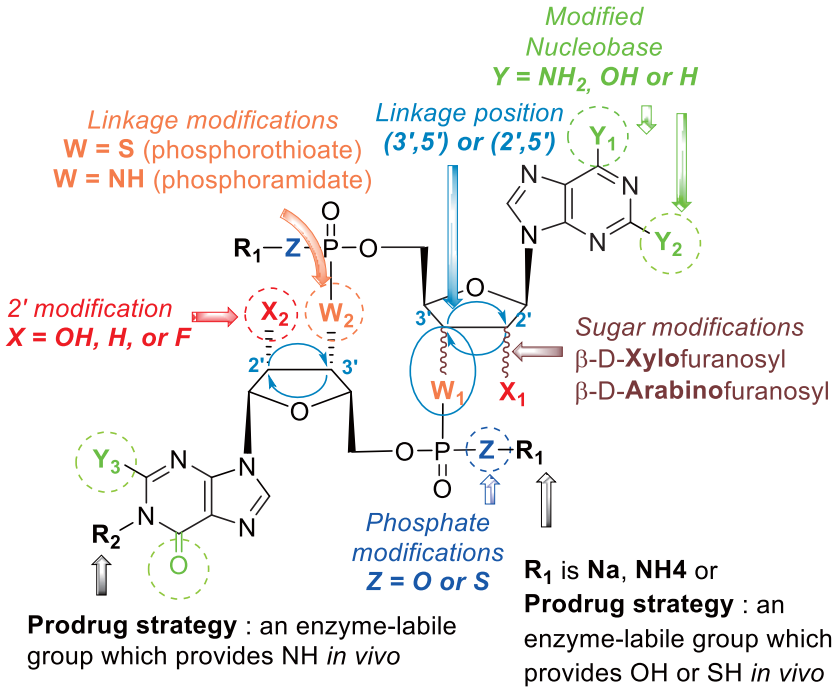
Thierry Lioux,* Marc-Antoine Mauny, Alain Lamoureux, Nicolas Bascoul, Mathieu Hays, Fabienne Vernejoul, Anne-Sophie Baudru, Cédric Boularan, Justine Lopes-Vicente, Gregory Qushair, and Gérard Tiraby

InvivoGen, 5 Rue Jean Rodier, 31400 Toulouse, France

Supporting Information



ABSTRACT: We describe novel STING-activating cyclic dinucleotides whose constituent nucleosides are adenosine and inosine and that vary by ribose substitution, internucleotide linkage position, and phosphate modification. In mammalian cells in vitro, some of these cAIMP analogs induce greater STING-dependent IRF and NF-κB pathway signaling than do the reference agonists for murine (DMXAA) or human (2′,3′-cGAMP) STING. In human blood ex vivo, they induce type I interferons (IFNs) and proinflammatory cytokines: for the former, 3′,3′-cAIMP (**9**; EC_{50} of 6.4 μM) and analogs **52**–**56** (EC_{50} of 0.4–4.7 μM), which contain one or two 2′-fluoro-2′-deoxyriboses and/or bis-phosphorothioate linkages, are more potent than 2′,3′-cGAMP (EC_{50} of 19.6 μM). Interestingly, **9** induces type I IFNs more strongly than do its linkage isomers 2′,3′-cAIMP (**10**), 3′,2′-cAIMP (**23**), and 2′,2′-cAIMP (**27**). Lastly, some of the cAIMP analogs are more resistant than 2′,3′-cGAMP to enzymatic cleavage in vitro. We hope to exploit our findings to develop STING-targeted immunotherapies.



InvivoGen literature

Lioux *et al.*, **2016**, *J. Med. Chem.* 10.1021/acs.jmedchem.6b01300

CL592 and CL656 is InvivoGen Patented CDN

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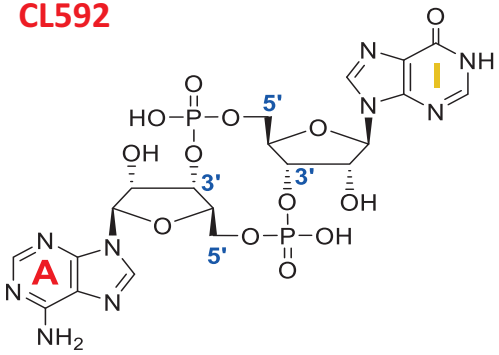
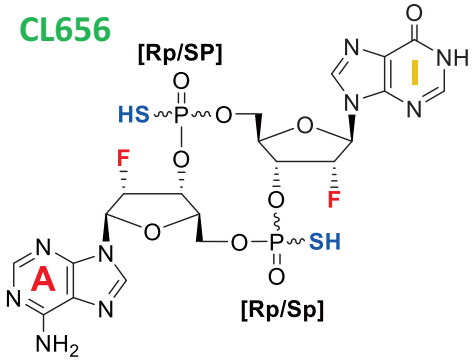
INNOVATION WITHIN REACH

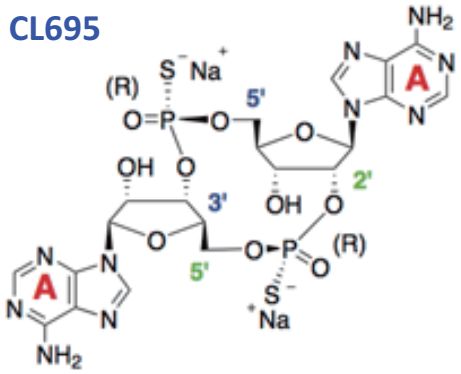
CL592



CL656



CL695



cAIMP

Synonym: 3'3'-cAIMP sodium salt, 3'3'-c(ApIp) sodium salt

Formula: $C_{20}H_{23}N_9O_{13}P_2$ .2Na

Molecular weight: 659.4 (free acid) 703.4 (sodium salt)

Purity: ≥ 95% by LC/MS & NMR.

Solubility: 50 mg/ml in water

Cat Code:

tlrl-nacai (500ug)

cAIM(PS)2 Difluor (Rp/Sp)

Synonym: (Rp/Sp) c-[2'FdAM(PS)-2'FdIM(PS)] sodium salt

Formula: $C_{20}H_{21}F_2N_9O_9P_2S_2$ .2Na

Molecular weight: 695.5 (free acid) 739.5 (sodium salt)

Purity: ≥ 95% by LC/MS & NMR.

Solubility: 50 mg/ml in water

Cat Code:

tlrl-nacairs (100ug)

2'3'-c-di-AM(PS)2 (Rp,Rp)

Synonyms: (R,R)-(2',3')c-diAM(PS)$_2$, (2',3')-Rp,Rp-c-diAMPSS.

Formula: $C_{20}H_{22}N_{10}O_{10}P_2S_2$ .2Na.

Molecular weight: 734.50.

Purity: ≥ 95% by LC/MS & NMR.

Solubility: 50 mg/ml in water.

Cat Code:

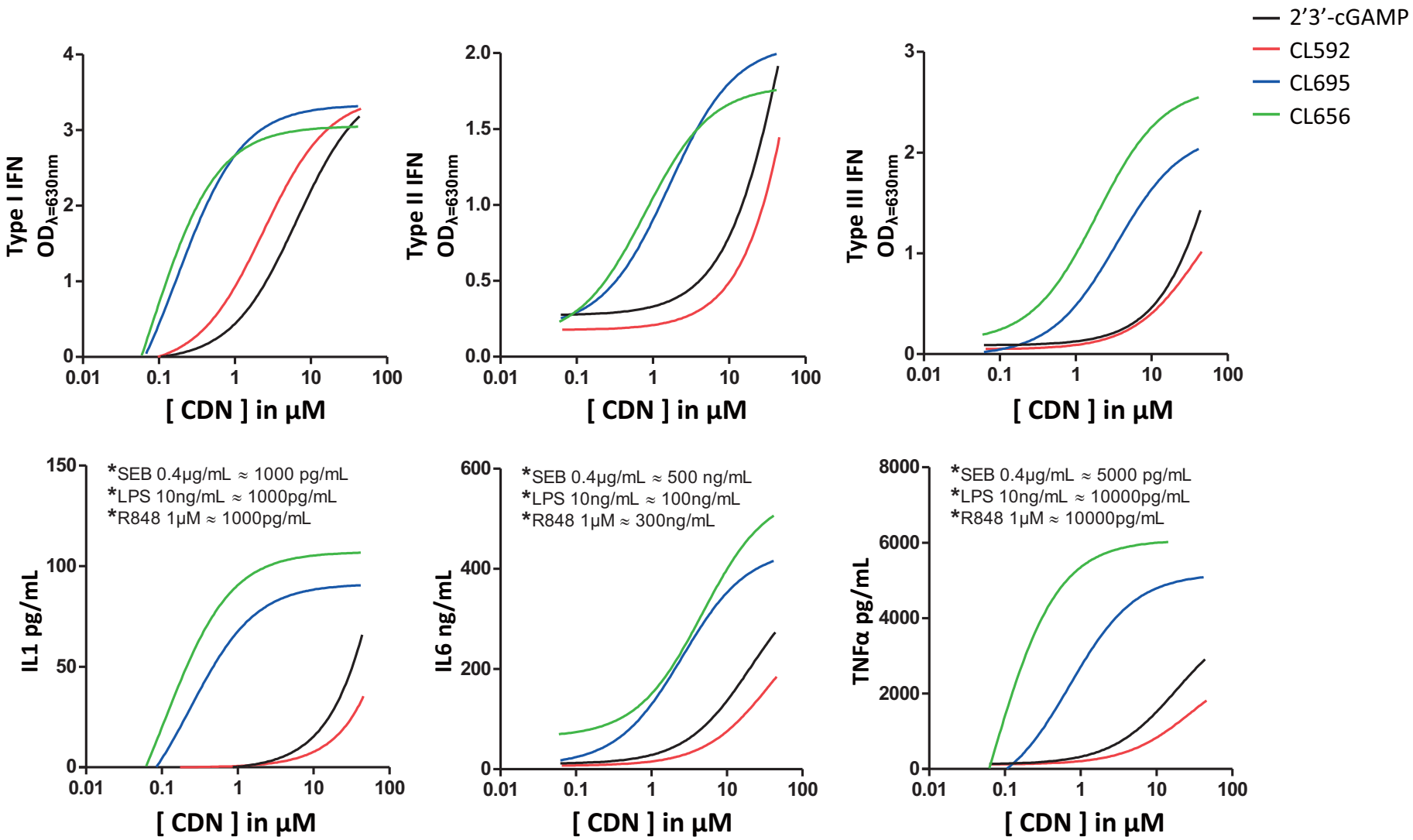
tlrl-nacda2r-01 (100ug)

tlrl-nacda2r (500ug)

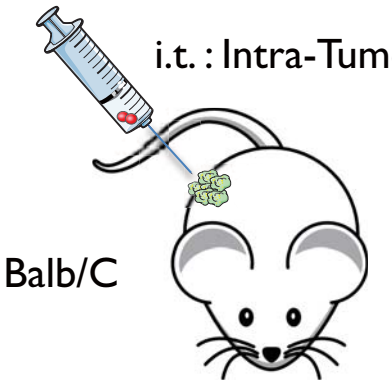
InvivoGen Patent : US20160362441; US15535864, WO/2017/186711

Aduro Biotech/Novartis

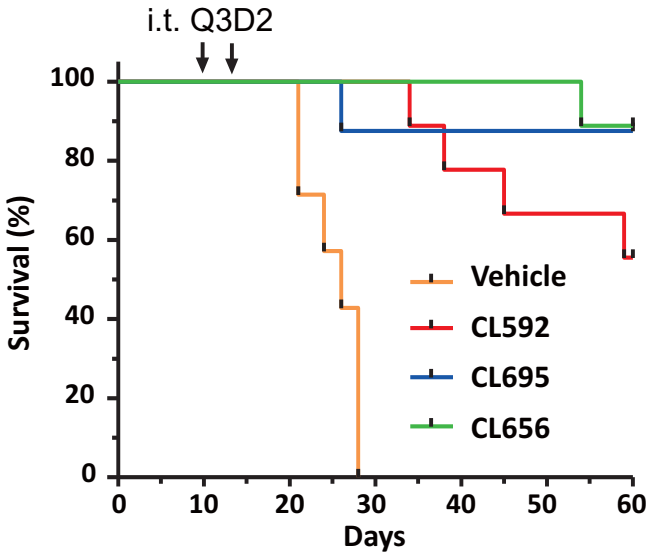
Phosphorothiotate modified molecules (CL656 and CL695) are more inflammatory than phosphodiester-based compounds

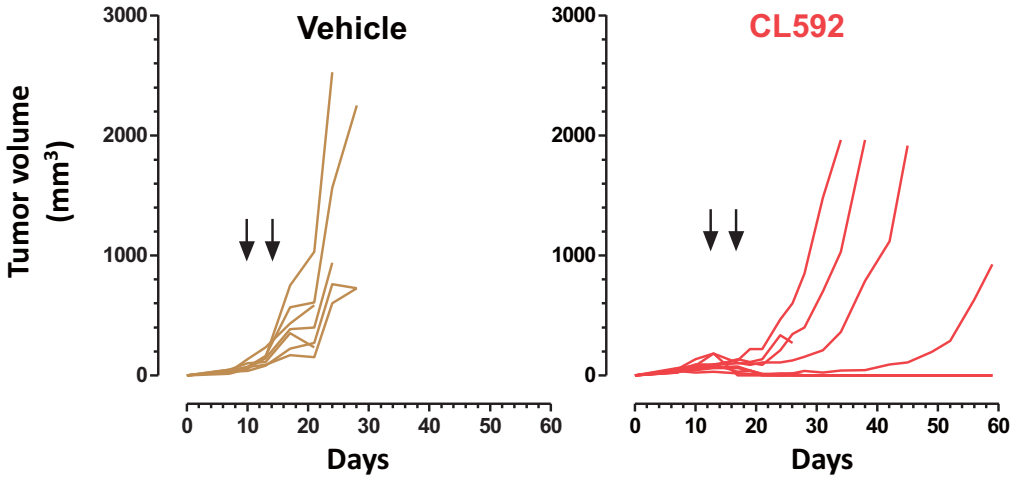


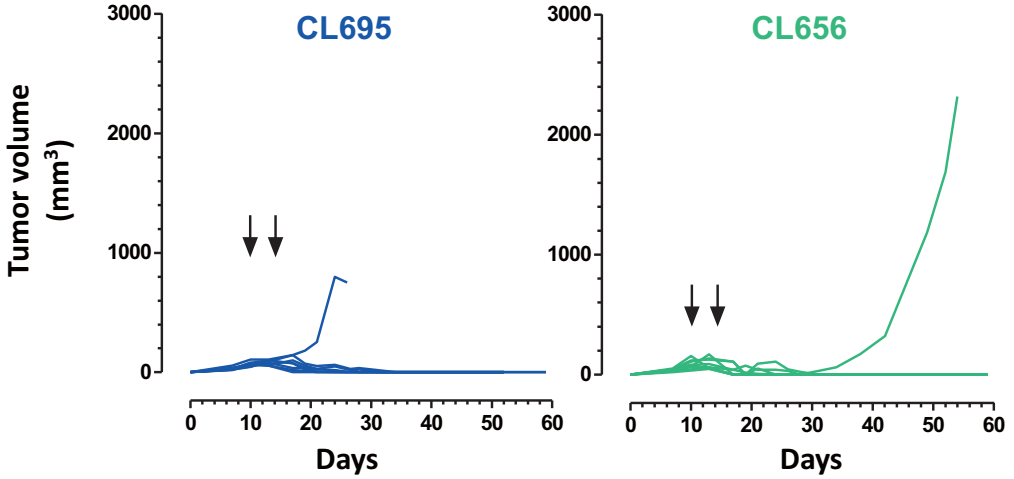
CL656 treatment decreases CT26 tumor progression and improves survival



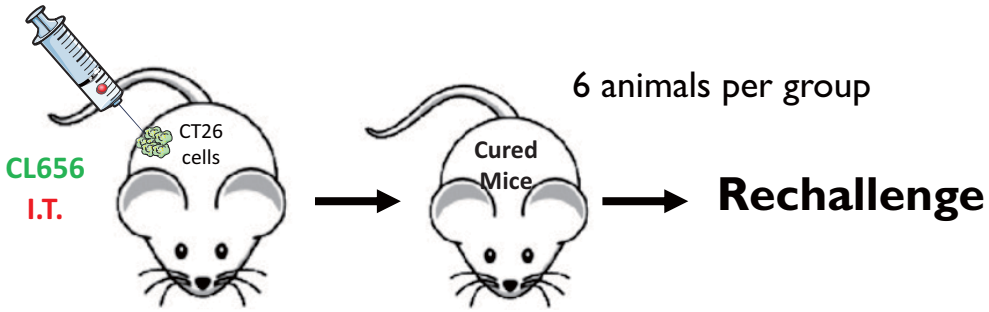
2.10^5 CT26 cells were sub-cutaneously implanted on the left flank

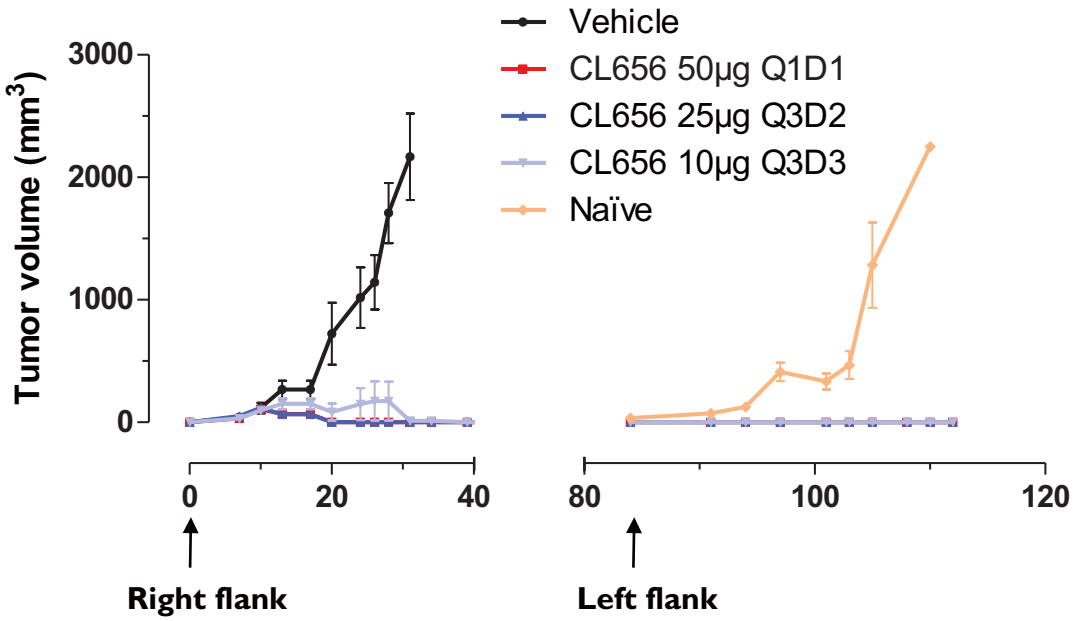


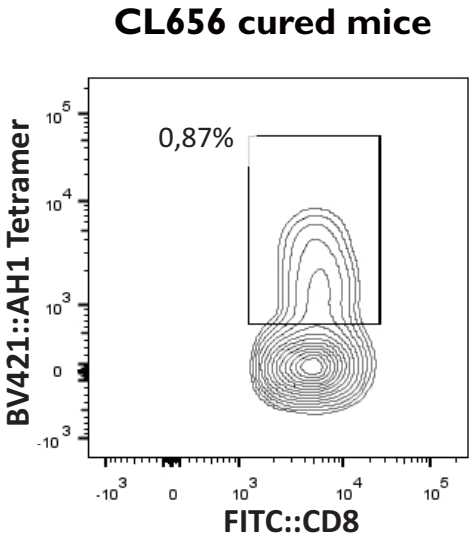


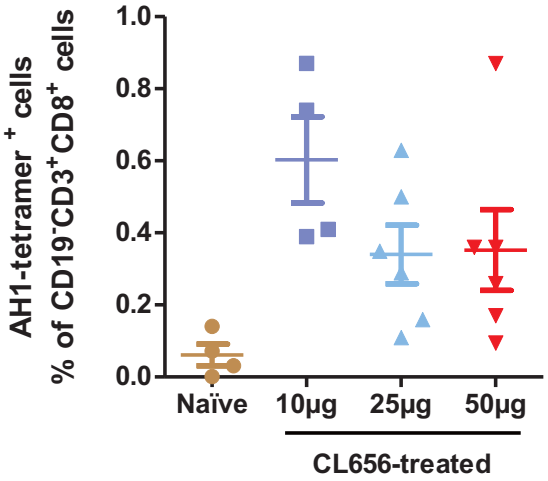


CL656 treatment leads to an adaptive immune response



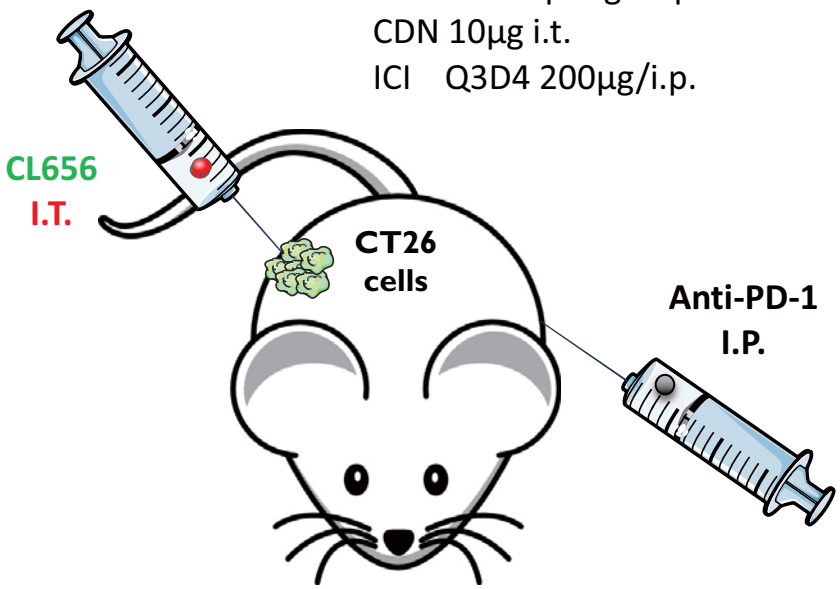




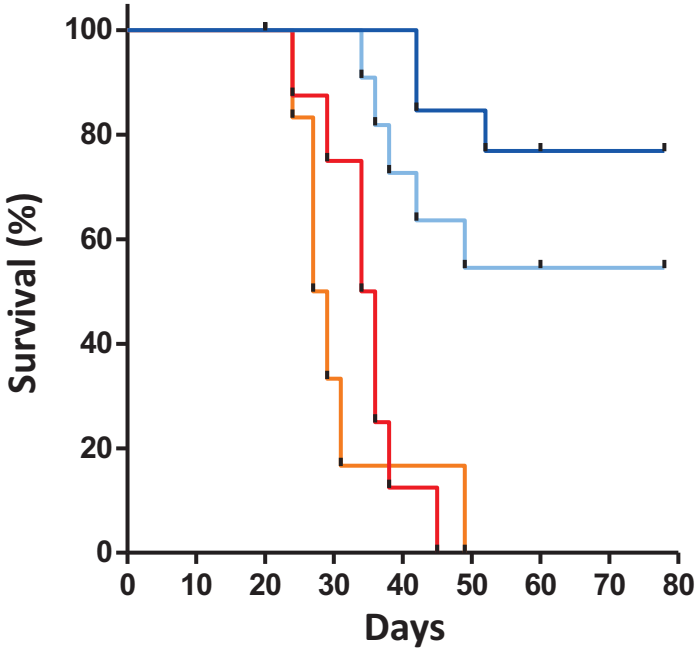


CL656 synergizes with anti PD-1 regress tumor growth

10 animals per group
CDN 10µg i.t.
ICI Q3D4 200µg/i.p.

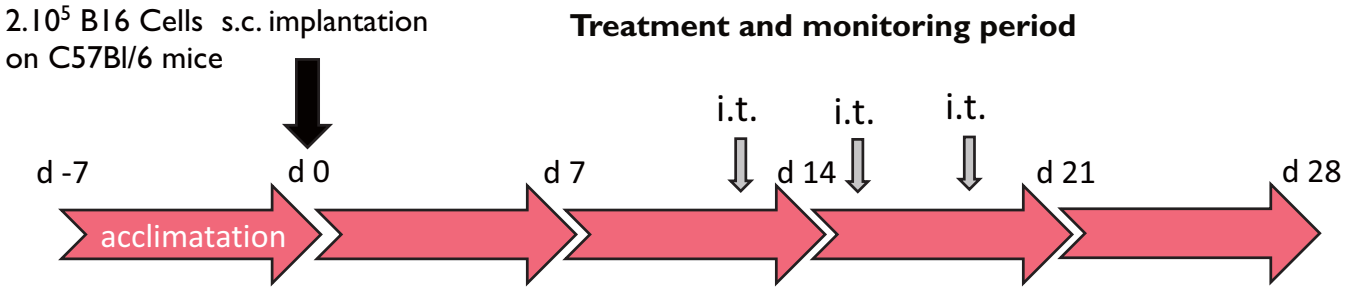


	Median Survival days
CL656 / anti PD1 (J43)	>60
CL656 / Vehicle	45.5
NaCl/ anti PD1 (J43)	35
NaCl/ Vehicle	28



ICI = Immune Checkpoint Inhibitor IT = Intra-tumoral injection IP = Intra-peritoneal injection Anti-PD-1: Clone J43

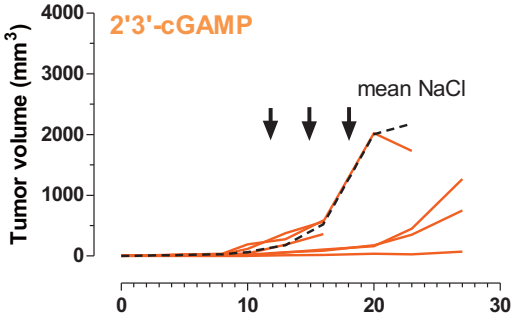
CL656 treatment leads to a better B16 tumor regression

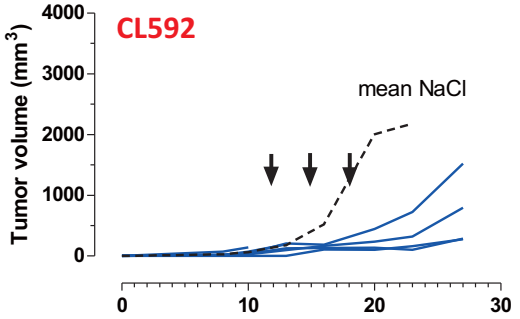


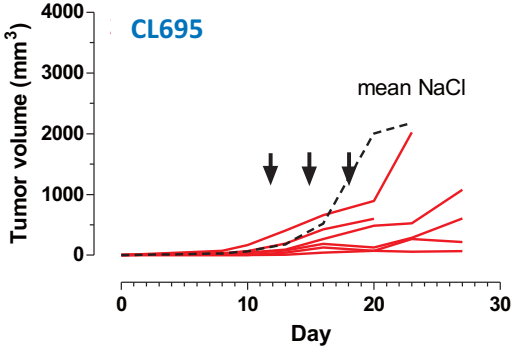
Day 17

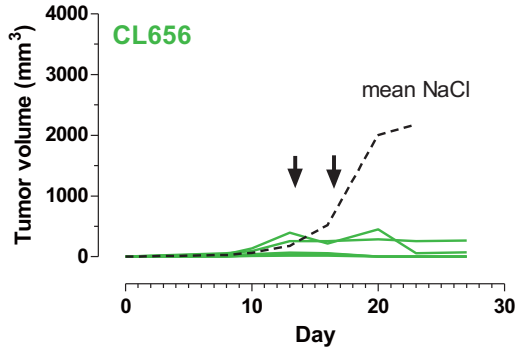
NaCl

CL656



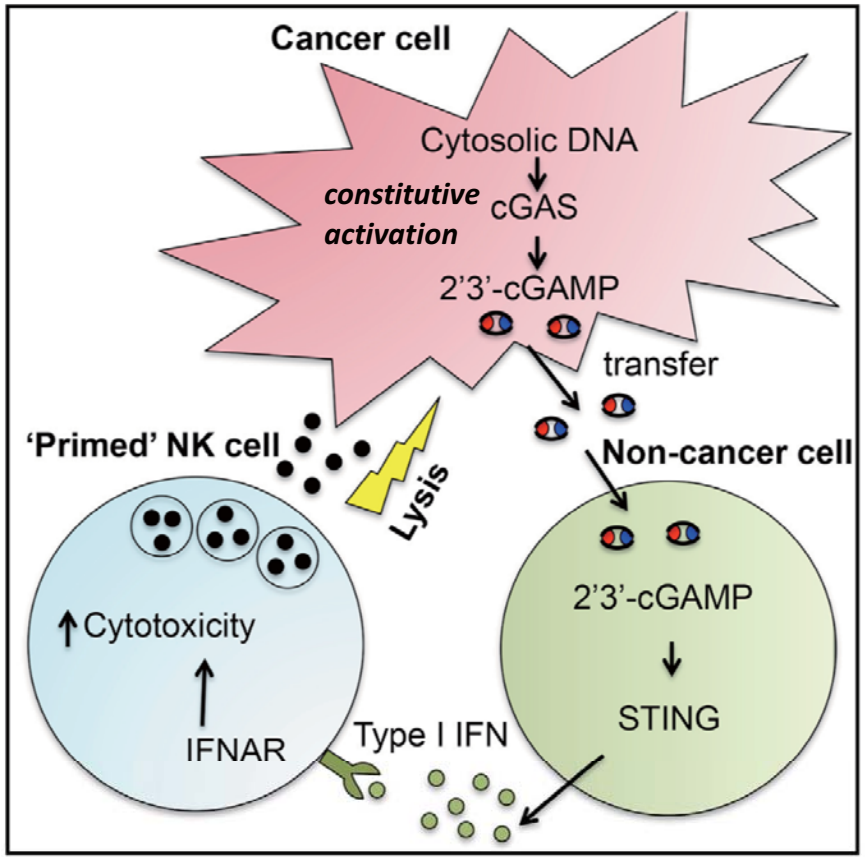


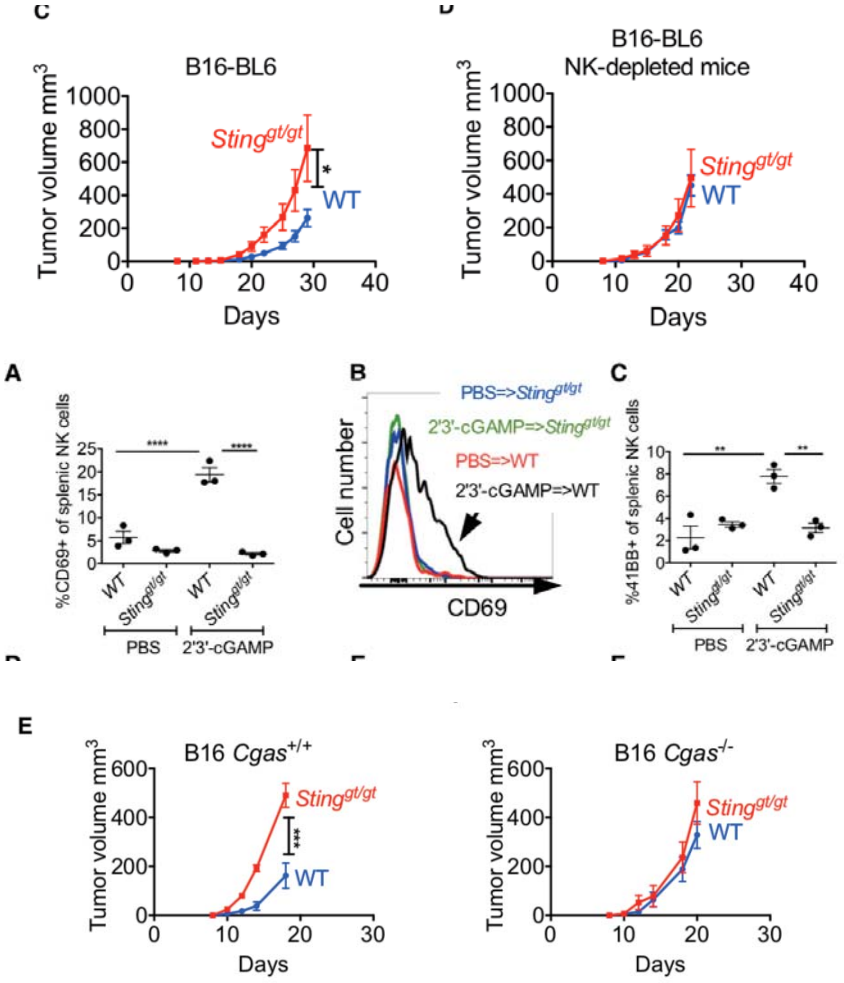




STING and Cancer

"Marker" in Tumor





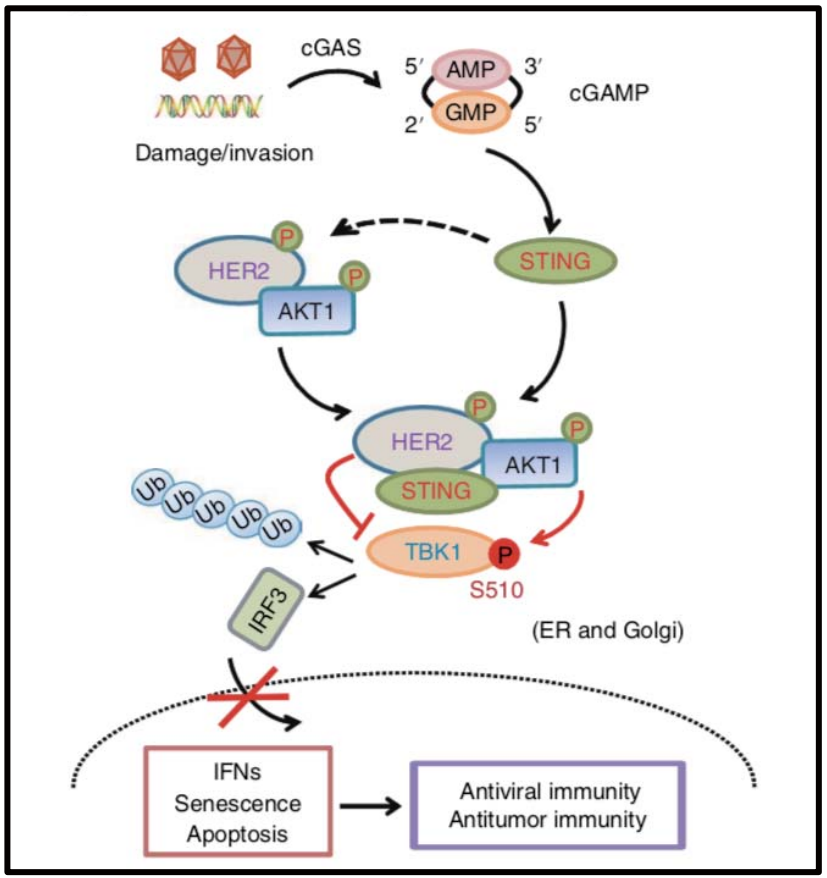
Assaf Marcus, Amy J. Mao, Monisha Lensink-Vasan, LeeAnn Wang, Russell E. Vance, David H. Raulet. Tumor-Derived cGAMP Triggers a STING-Mediated Interferon Response in Non-tumor Cells to Activate the NK Cell Response. Immunity. 2018

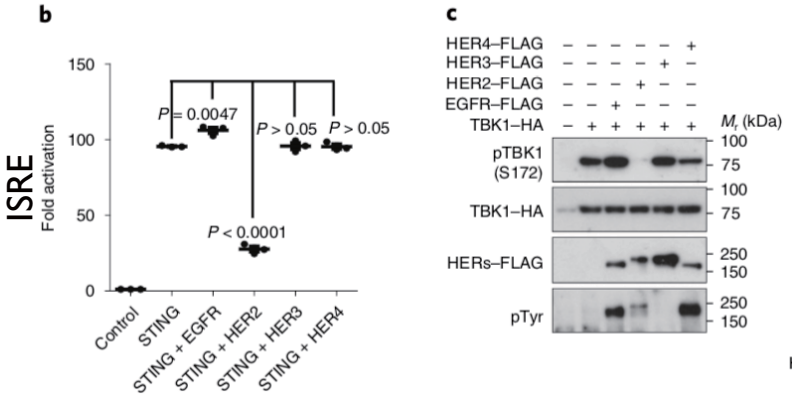
Product Box

Products	Cat.
THP1-Dual™ cells	thpd-nfis
THP1-Dual™ KO-STING cells	thpd-kostg
ISD Naked	tlrl-isdn

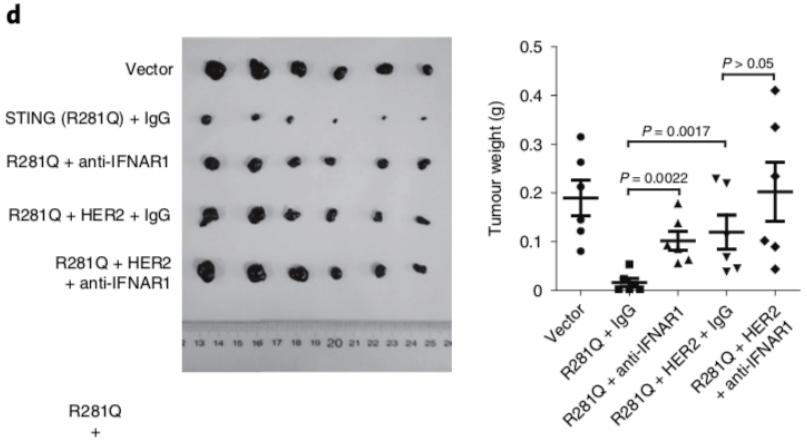
STING and Cancer

Check Point & STING





HER2 suppresses cytosolic DNA sensing



HER2 protects cancer cells from STING-mediated antitumour immunity

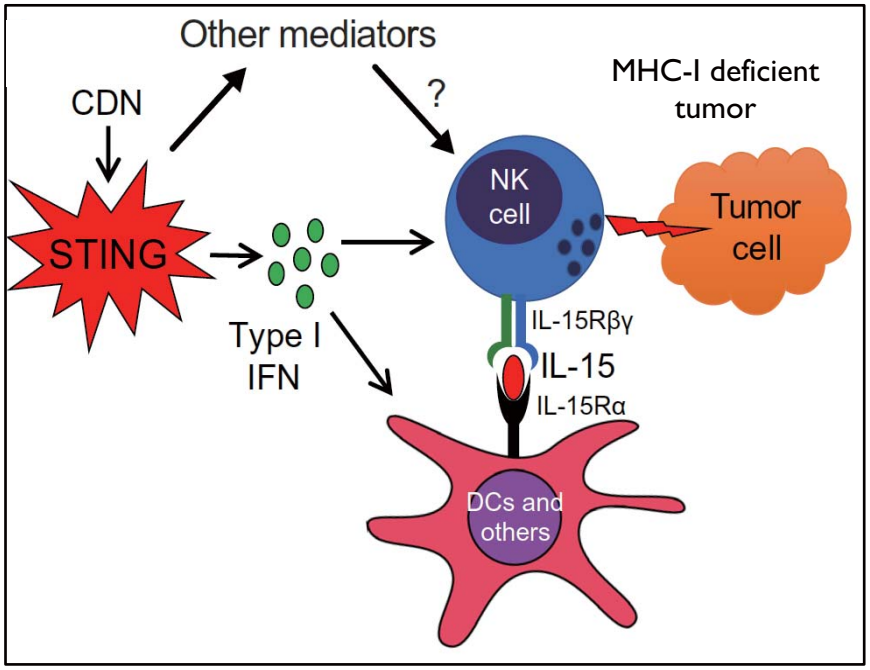
Product Box

Products	Cat.
Poly(dA:dT)	tlrl-patn-1
2'3'-cGAMP	tlrl-nacga23-1

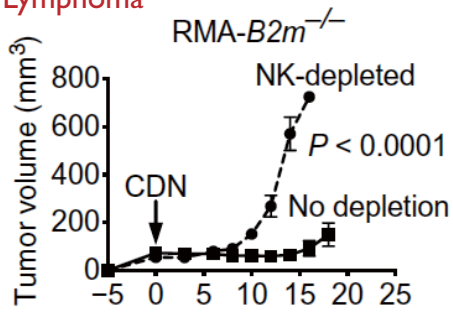
Shiying Wu, et al. HER2 recruits AKT1 to disrupt STING signalling and suppress antiviral defence and antitumour immunity. Nature Cell Biology. 2019

STING and Cancer

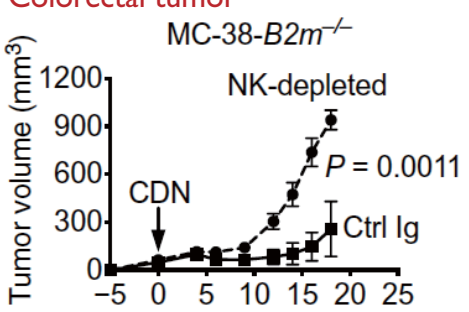
CD8+ T cell independent



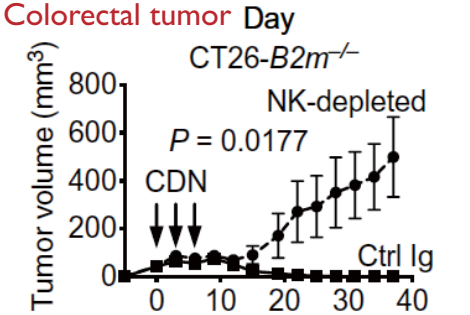
Lymphoma



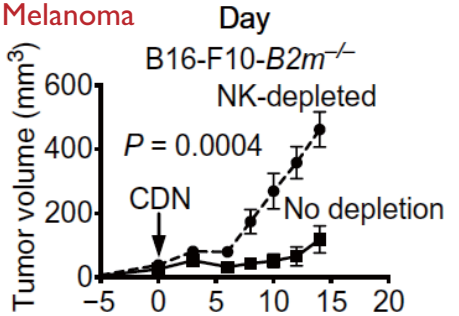
Colorectal tumor



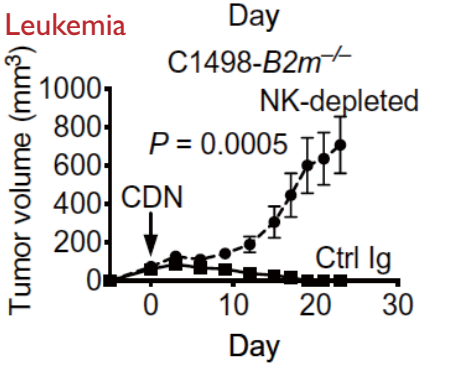
Colorectal tumor



Melanoma



Leukemia



2'3'-c-di-AM(PS)2 (Rp,Rp) induced rejection of MHC-I deficient tumors depends on NK cells

Product Box

Product	Cat. code
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r

Christopher J. Nicolai, et al. NK cells mediate clearance of CD8+ T cell–resistant tumors in response to STING agonists. Sci. Immunology. 2020

InvivoGen
INNOVATION WITHIN REACH

Microbiota

2015

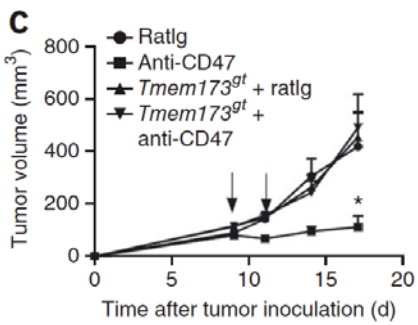
ARTICLES

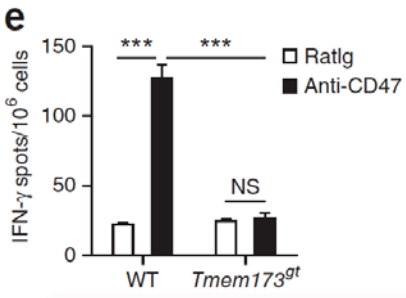
nature medicine

CD47 blockade triggers T cell–mediated destruction of immunogenic tumors

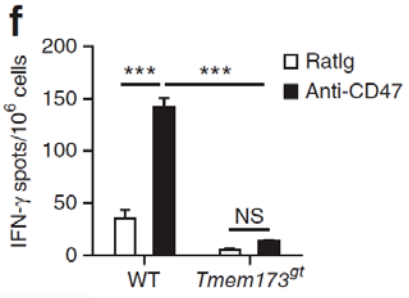
Xiaojuan Liu[1,2,6], Yang Pu[3], Kyle Cron[3], Liufu Deng[3], Justin Kline[4], William A Frazier[5], Hairong Xu[1], Hua Peng[1], Yang-Xin Fu[1,3,7] & Meng Michelle Xu[3,6,7]

MC38 Tumor





BMDCs coculture with OTI cell



CD8+ T cells coculture with MC38 cells

2020

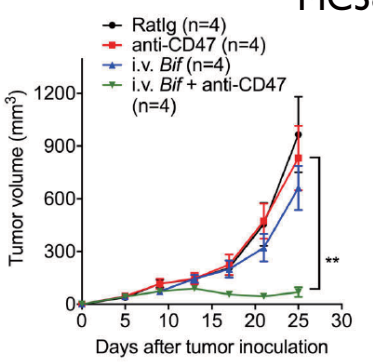
JEM Journal of Experimental Medicine

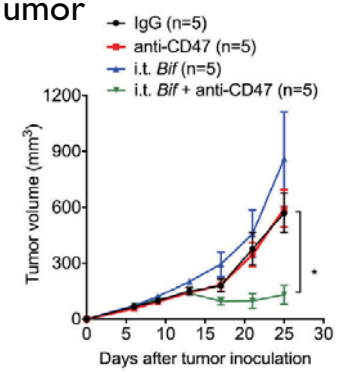
BRIEF DEFINITIVE REPORT

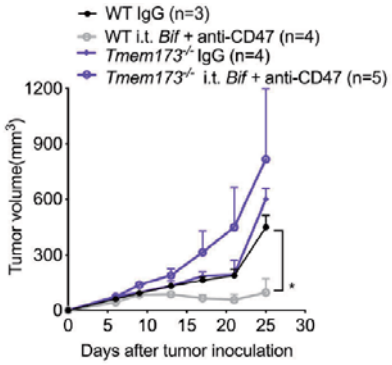
Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling

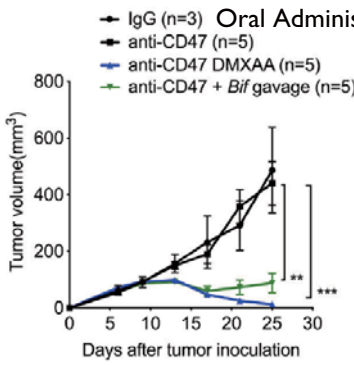
Yaoyao Shi[1,2*], Wenxin Zheng[1,2*], Kaiting Yang[1,2], Katharine G. Harris[3], Kaiyuan Ni[4], Lai Xue[1,2,5], Wenbin Lin[1,2,4], Eugene B. Chang[3], Ralph R. Weichselbaum[1,2], and Yang-Xin Fu[6]

MC38 Tumor

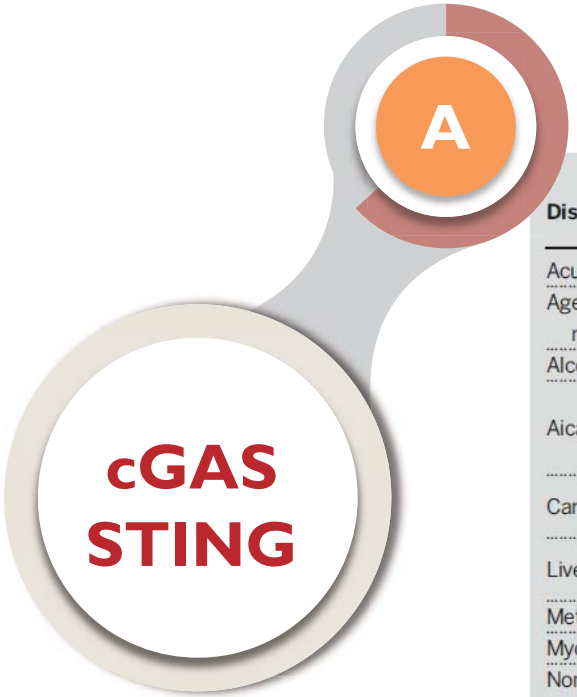








Bifidobacterium



Autoimmunity

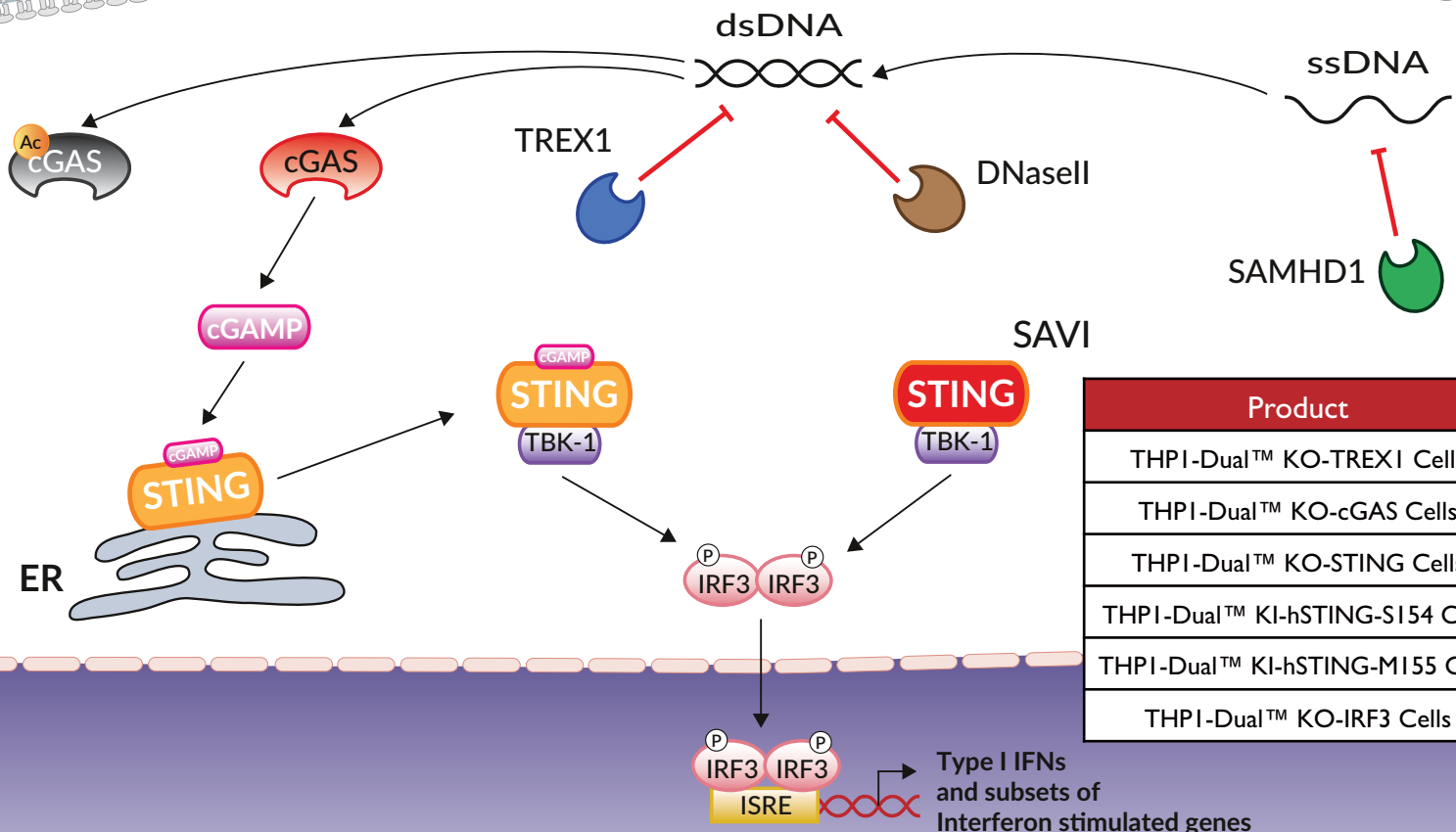
Disease	Animal model/ human data	Proposed trigger	Improved in cGAS KO?	Improved in STING KO?
Acute pancreatitis	Cerulein model	Unknown	Yes	Yes
Age-dependent macular degeneration	*Alu* transcript induced RPE death	Mitochondrial DNA	Yes	Yes
Alcoholic liver disease	Chronic ALD model	Unknown	Unknown	Yes
Aicardi–Goutières syndrome*	*Trex1*$^{-/-}$, *RnaseH2*$^{-/-}$, *Samhd1*$^{-/-}$ mice; Human data	Nuclear DNA	Yes	Yes
Cancer	DMBA-induced skin cancer model	Nuclear DNA	Unknown	Yes
Liver fibrosis	CCl_4 administration	Unknown	Unknown	Yes
Metastasis	Distinct metastatic models	Nuclear DNA	Unknown	Yes
Myocardial infarction	Permanent ligation model	Extracellular DNA	Yes	Yes
Nonalcoholic steatohepatitis (NASH)	MCD, HFD	Mitochondrial DNA	Unknown	Yes
Parkinson's disease	*Prkn*$^{-/-}$, *Pink1*$^{-/-}$ upon exercise and *Prkn*$^{-/-}$;*mutator* mice	Mitochondrial DNA	Unknown	Yes
Polyarthritis/fetal and neonatal anemia	*Dnase2a*$^{-/-}$ model; biallelic mutations in *DNASE2A* in humans	Extracellular and nuclear DNA	Yes	Yes
Sepsis	LPS shock model, cecal ligation and puncture (CLP) model	Unknown	Unknown	Yes
SAVI	Human data	NA	NA	NA
Systemic lupus erythematosus	Increase in cGAMP levels in human SLE PBMCs	Unknown	NA	NA

* Human data show an increased type I IFN signature.

Andrea Ablasser and Zhijian J. Chen. cGAS in action: Expanding roles in immunity and inflammation. Science. 2019

STING and Autoimmunity

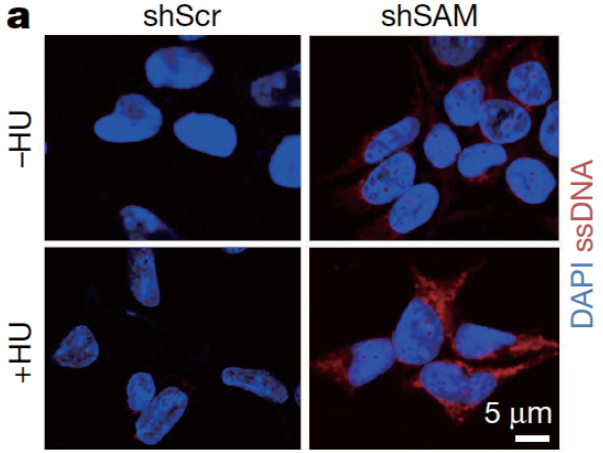
InvivoGen
INNOVATION WITHIN REACH

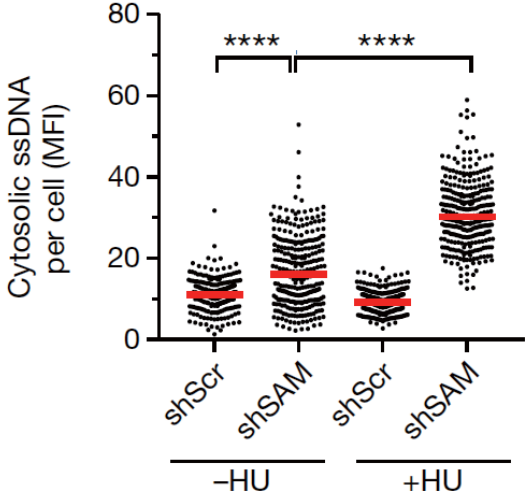


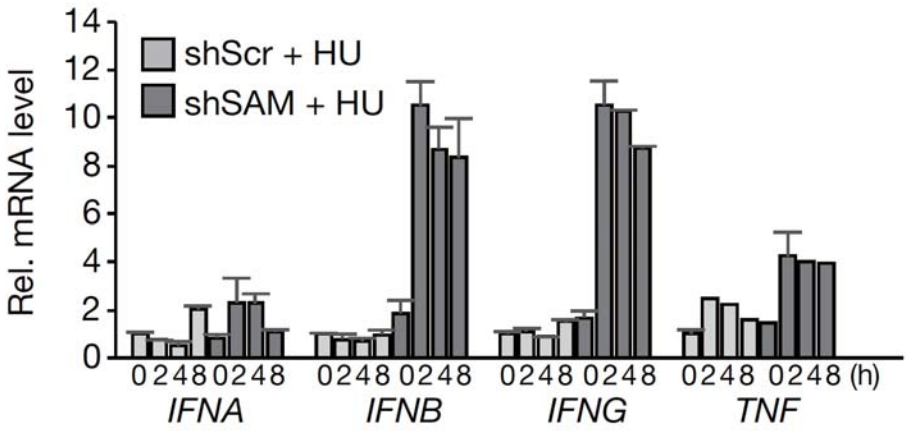
Product	Cat. code
THP1-Dual™ KO-TREX1 Cells	thpd-kotrex
THP1-Dual™ KO-cGAS Cells	thpd-kocgas
THP1-Dual™ KO-STING Cells	thpd-kostg
THP1-Dual™ KI-hSTING-S154 Cells	thpd-s154
THP1-Dual™ KI-hSTING-M155 Cells	thpd-m155
THP1-Dual™ KO-IRF3 Cells	thpd-koirf3

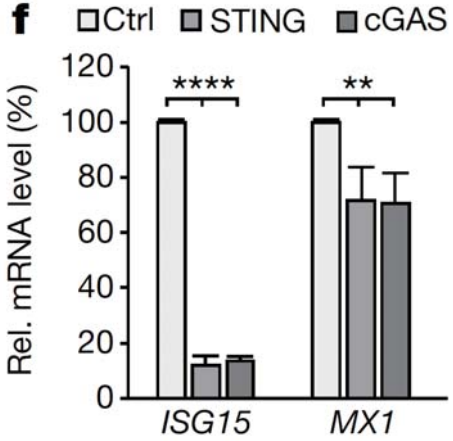
InvivoGen
INNOVATION WITHIN REACH

SAMHD1 and Aicardi-Goutieres Syndrome (AGS)







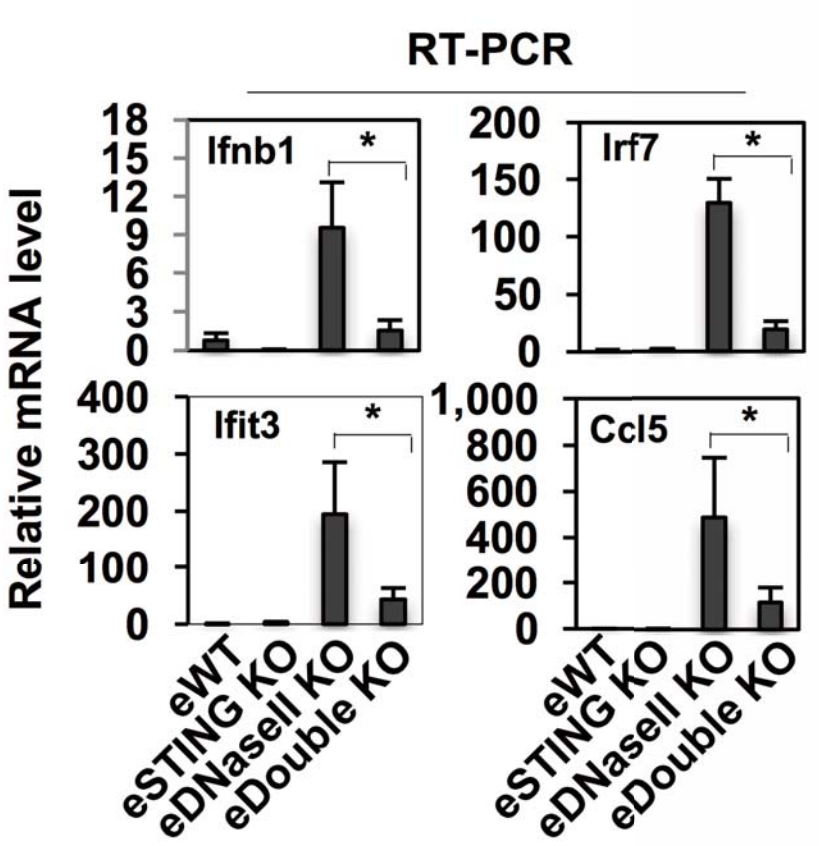


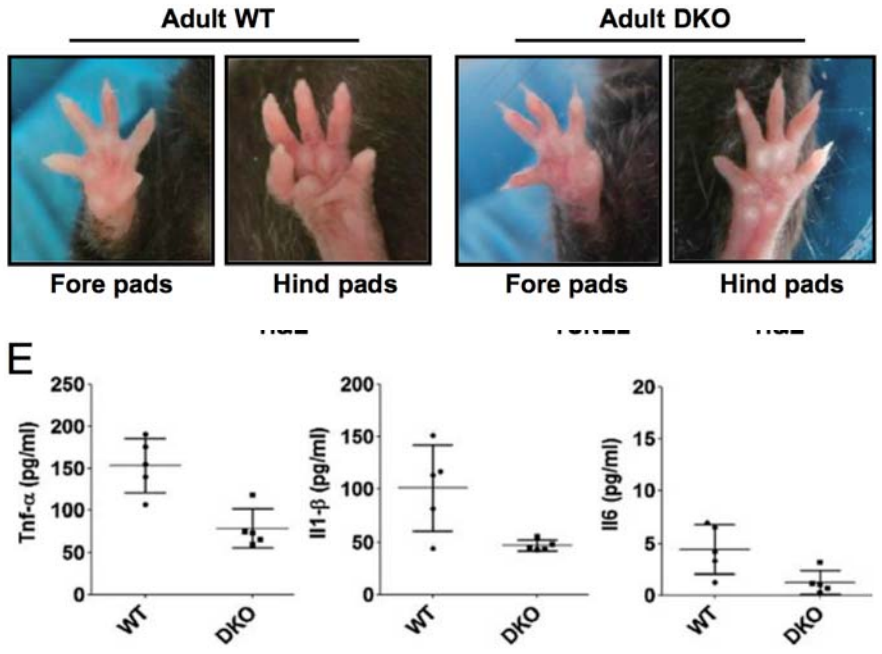
SAMHD1 prevents accumulation of cytosolic ssDNA and induction of type I IFNs mediated by cGAS-STING pathway.

Flavie Coquel, et. al. SAMHD1 acts at stalled replication forks to prevent interferon induction. Nature. 2018

DNaseII$^{-/-}$ and Polyarthritis

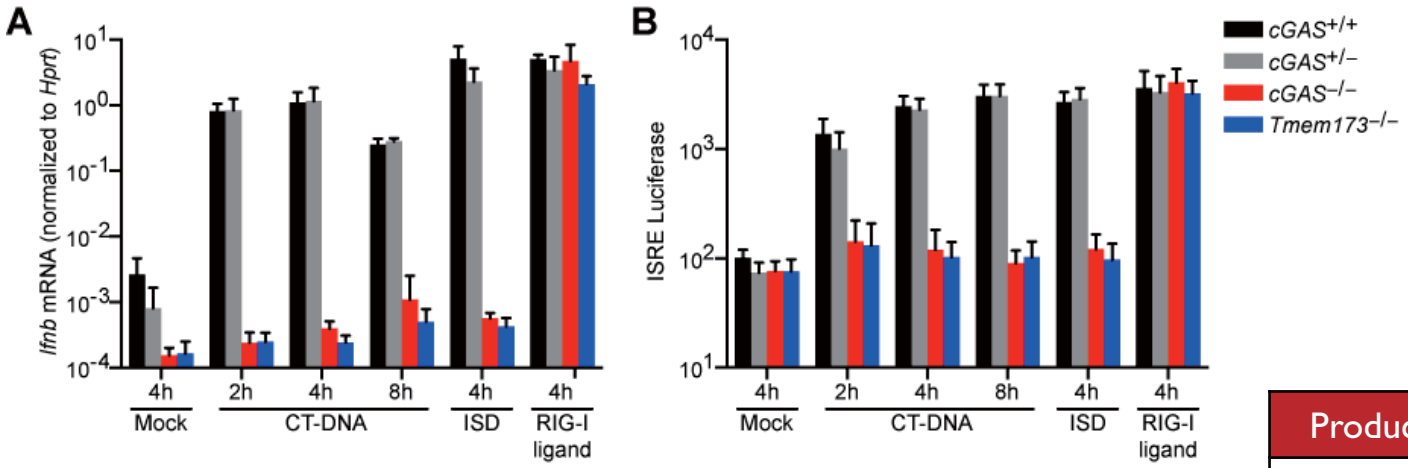
WT: DNase II$^{-/-}$ Ifnar I $^{-/-}$
DKO: DNase II$^{-/-}$ STING$^{-/-}$





Ahn, J., Gutman, D., Saijo, S. & Barber, G. N. STING manifests self DNA-dependent inflammatory disease. Proc. Natl Acad. Sci. USA 109, 19386–19391 (2012).

STING and Autoimmunity

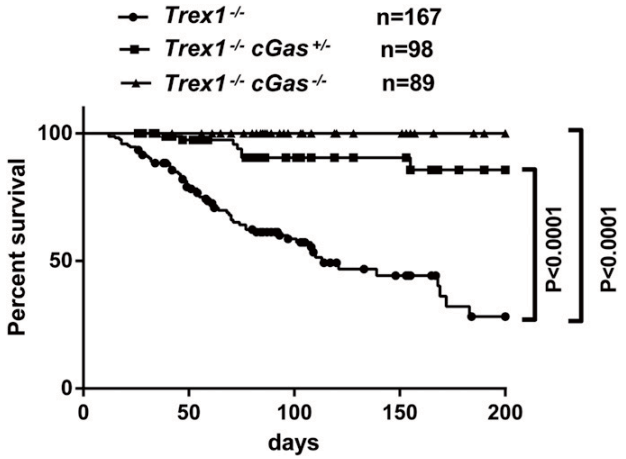


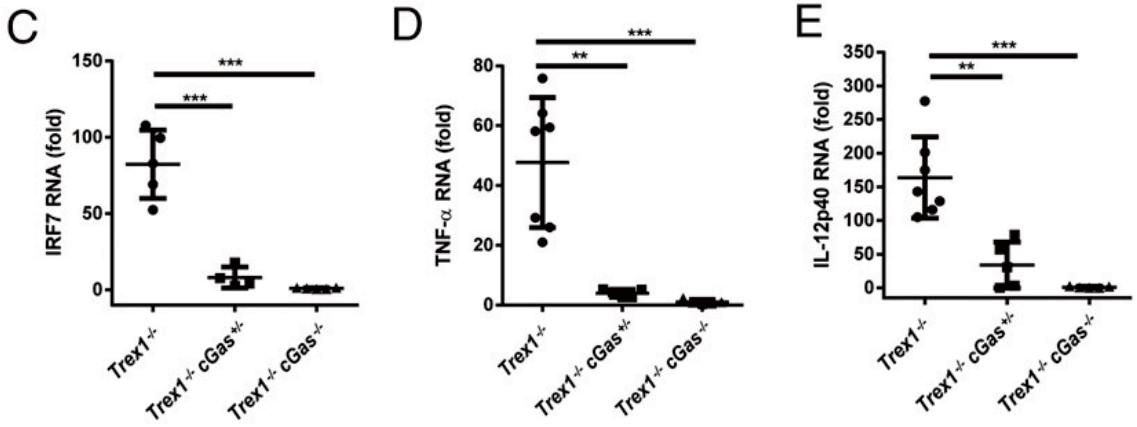
Elizabeth E. Gray, et al. Journal of Immunology. 2015

Trex1$^{-/-}$ and Aicardi-Goutieres Syndrome (AGS)

Product Box

Product	Cat. code	Sensor
5'ppp-dsRNA	tlrl-3prna	RIG-I
ISD Naked	tlrl-isdn	cGAS
THP1-Dual KO-TREX1 Cells		thpd-kotrex

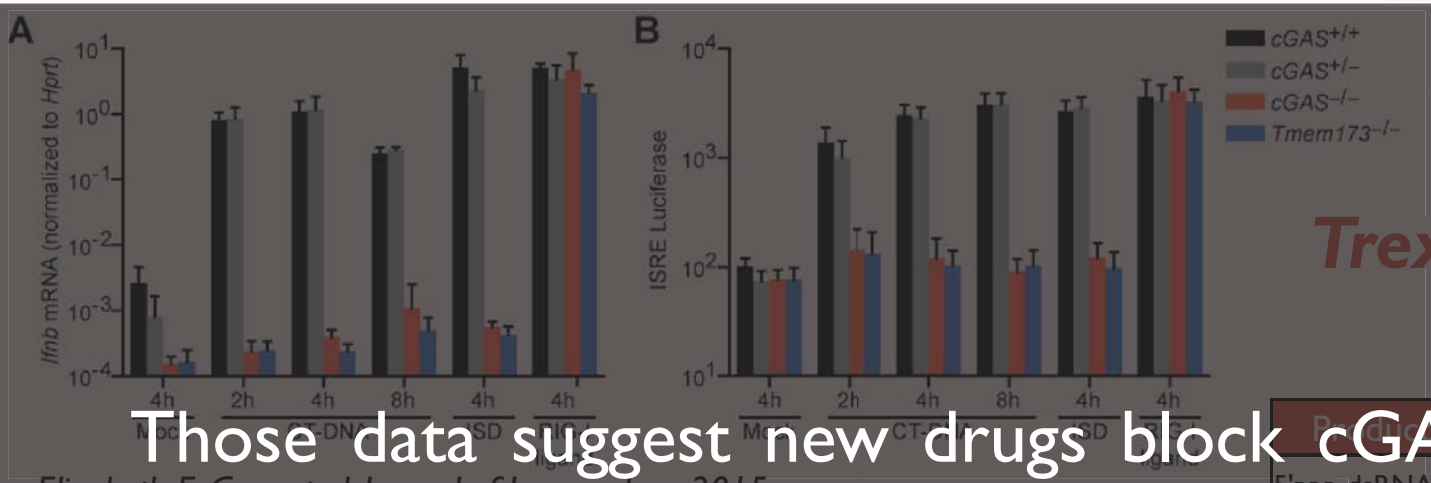




Daxing Gao, et al. Proc Natl Acad Sci. 2015

STING and Autoimmunity

InvivoGen
INNOVATION WITHIN REACH



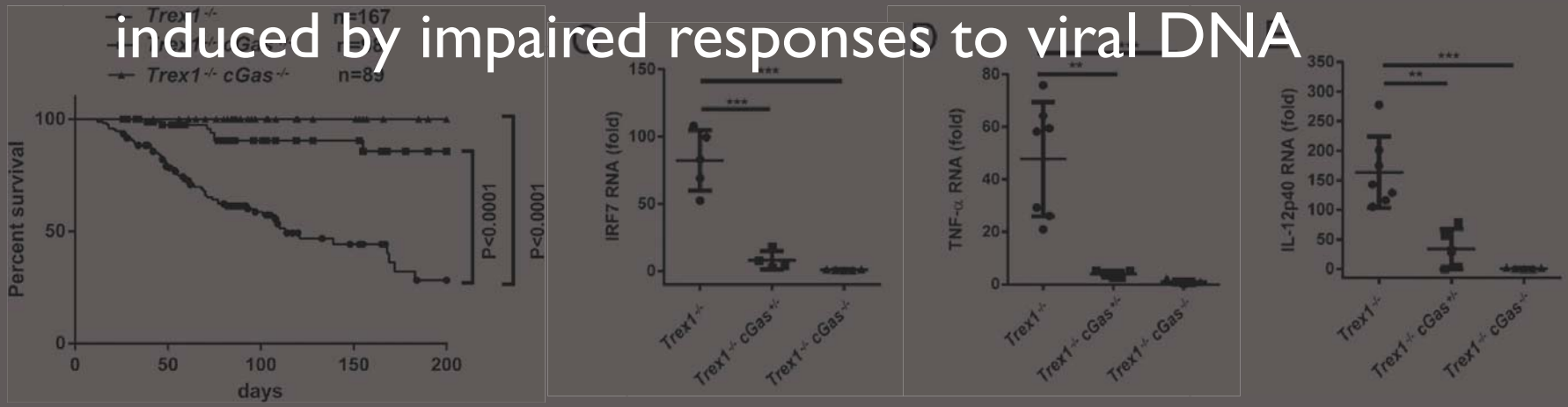
Elizabeth E. Gray, et al. Journal of Immunology. 2015

Trex1$^{-/-}$ and AGS

Those data suggest new drugs block cGAS at dose dependent manner might be efficacious on autoimmune disease, and avoid the immunodeficiency induced by impaired responses to viral DNA

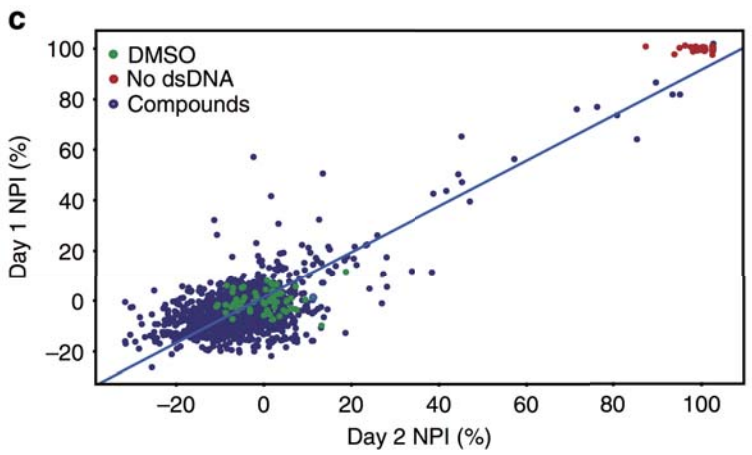
Product Box

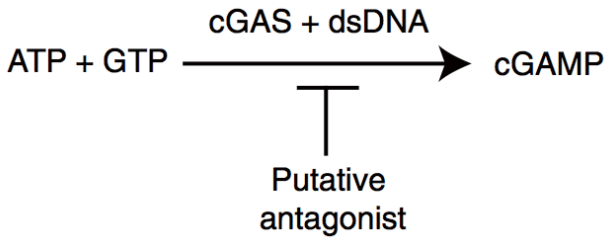
Pro-Drug	Control	Sensor
5'ppp-dsRNA	tlrl-3prna	RIG-I
		cGAS
THP1-Dual KO-TREX1 Cells		thp1kotrex



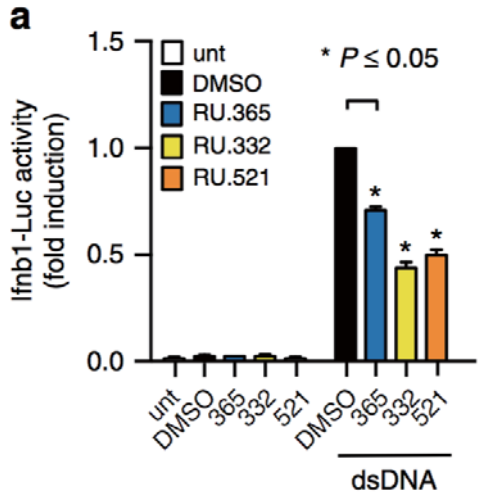
Daxing Gao, et al. Proc Natl Acad Sci. 2015

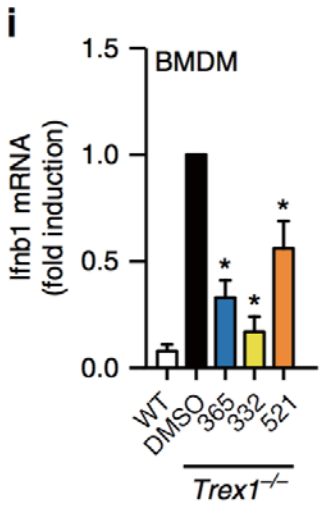
cGAS inhibitor





Identification of cGAS inhibitors





Inhibition of cGAS activity in RAW macrophage and BMDM cells from an Aicardi-Goutières Syndrome mouse model.

Product Box

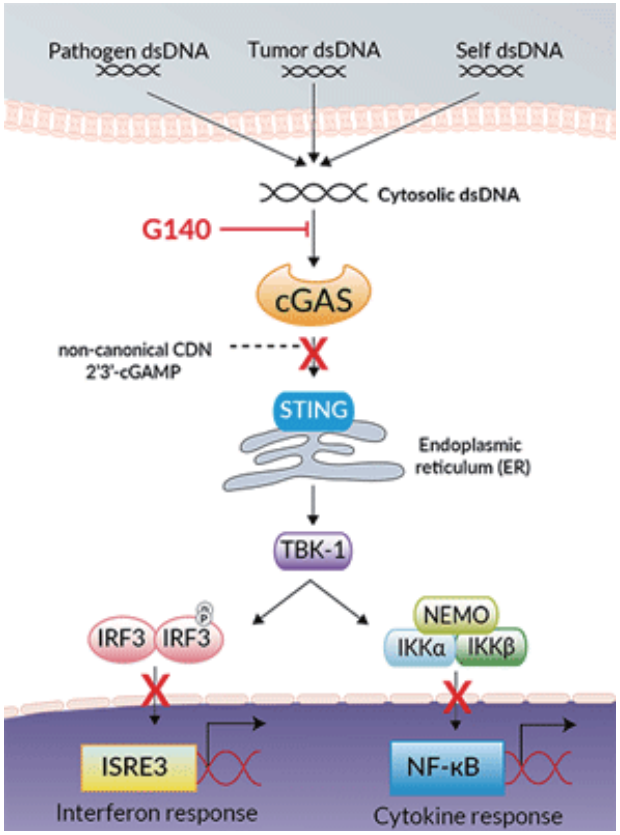
Product	Cat. code
ISD Naked	tlrl-isdn
RAW-Lucia ISG	rawl-isg
RAW-Lucia ISG-KO- TREX1	rawl-kotrex
RAW-Lucia ISG-KO-cGAS	rawl-kocgas
RU.521	inh-ru521

Jessica Vincent, et al. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. Nature Communication.2017

STING and Autoimmunity

InvivoGen

cGAS inhibitor



Product	Cat. code
THP1-Dual cells	thpd-nfis
RAW-Lucia cells	rawl-isg
Poly(I:C)	tlrl-pic
G140 **[NEW]**	inh-g140

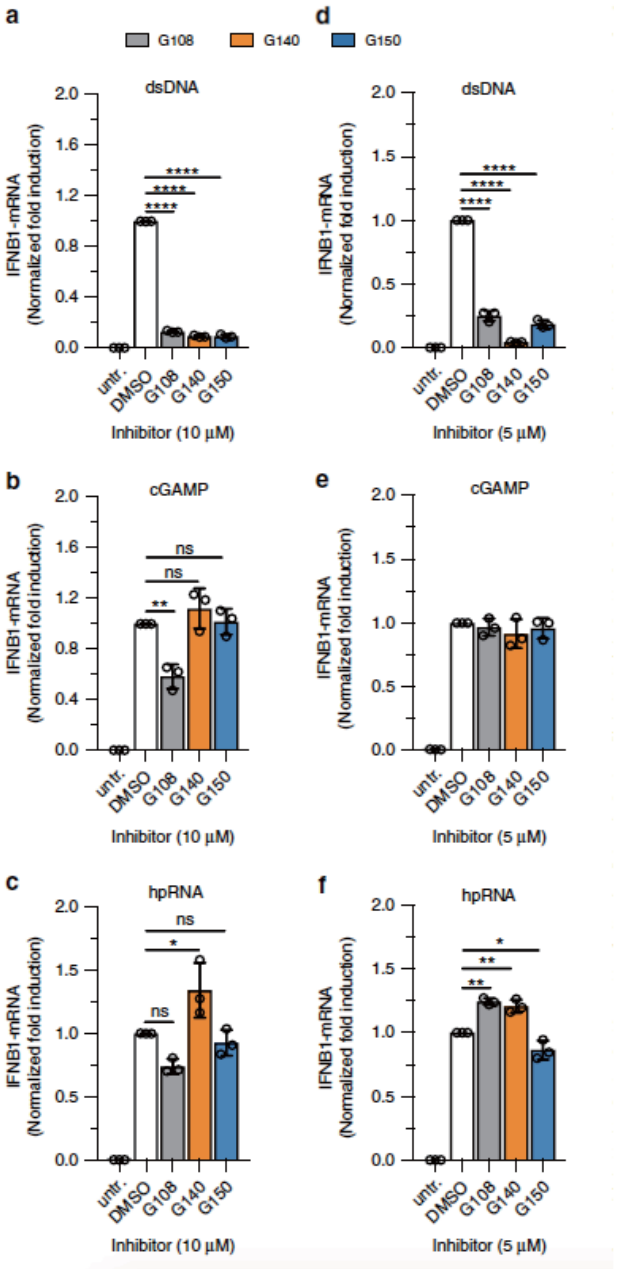
Key features of G140:

•G140 specifically inhibits human cGAS in a dose-dependent manner.

•G140 inhibits both cGAS-mediated IRF and NF-κB signaling.

•Each lot of G140 is highly pure (>95%) and functionally tested.

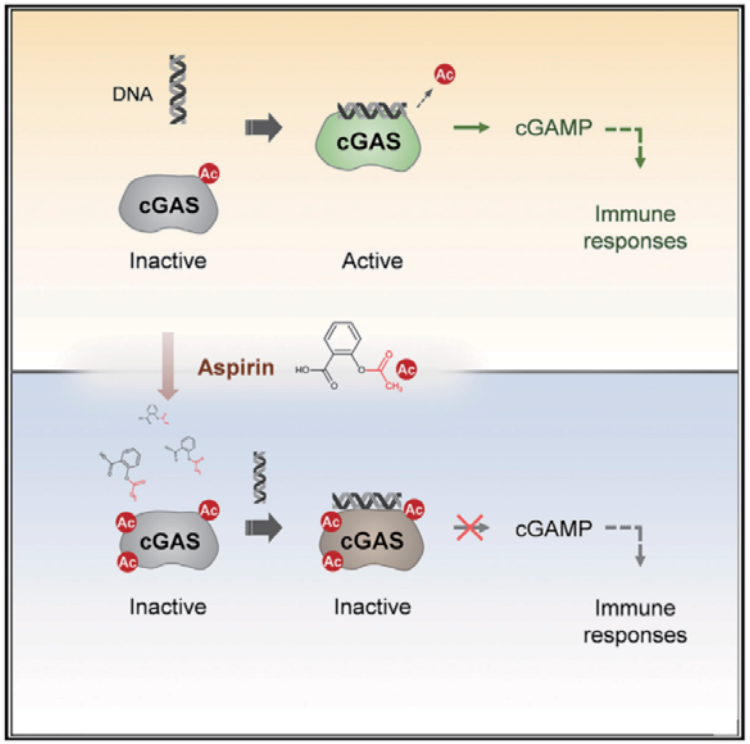
Lodoe Lama, et al. Development of human cGAS-specific small molecule inhibitors for repression of dsDNA triggered interferon expression .Nature Communication.2019



STING and Autoimmunity

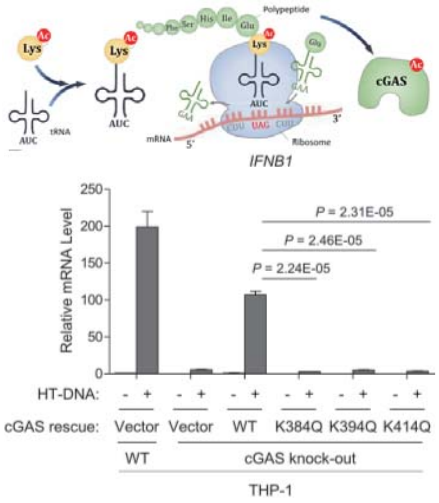
InvivoGen
INNOVATION WITHIN REACH

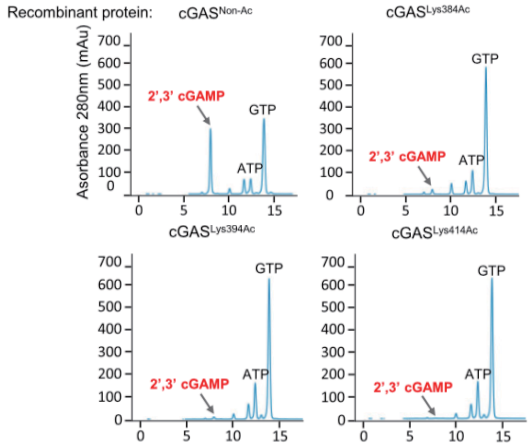
cGAS inhibitor



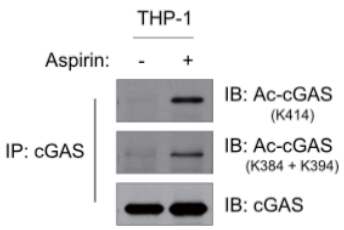
Product	Cat. code
c-di-AMP	tlrl-nacdg
2'3'-cGAMP	tlrl-nacga23
Poly (I:C)	tlrl-pic
BX795	tlrl-bx7

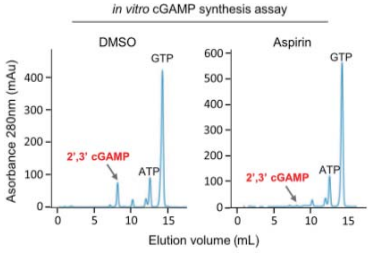
Jiang Dai, et al. Acetylation Blocks cGAS Activity and Inhibits Self-DNA-Induced Autoimmunity. Cell. 2019

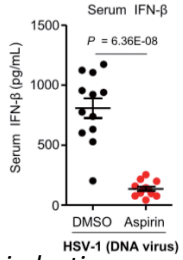




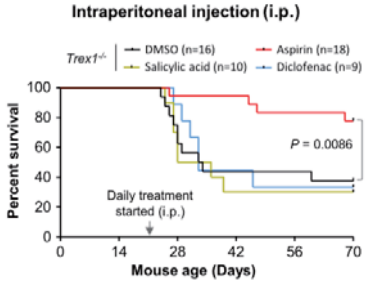
- Acetylation suppresses cGAS activity and type I IFN induction

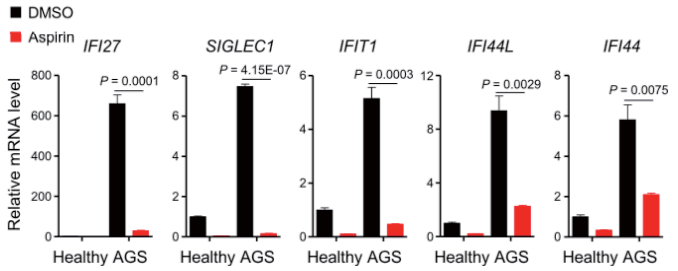






- Aspirin directly acetylates cGAS and inhibit type I IFN induction



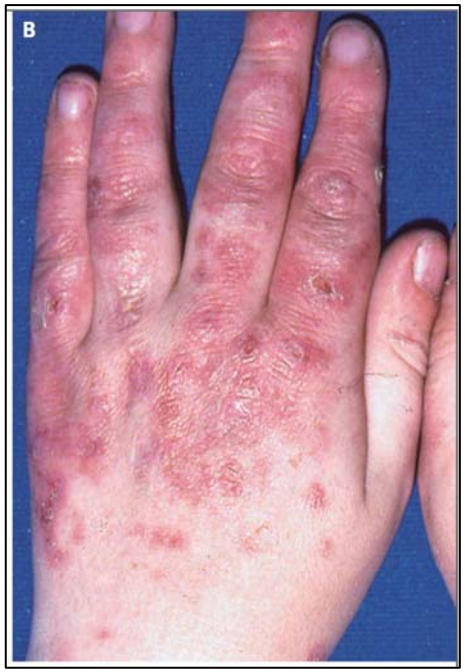


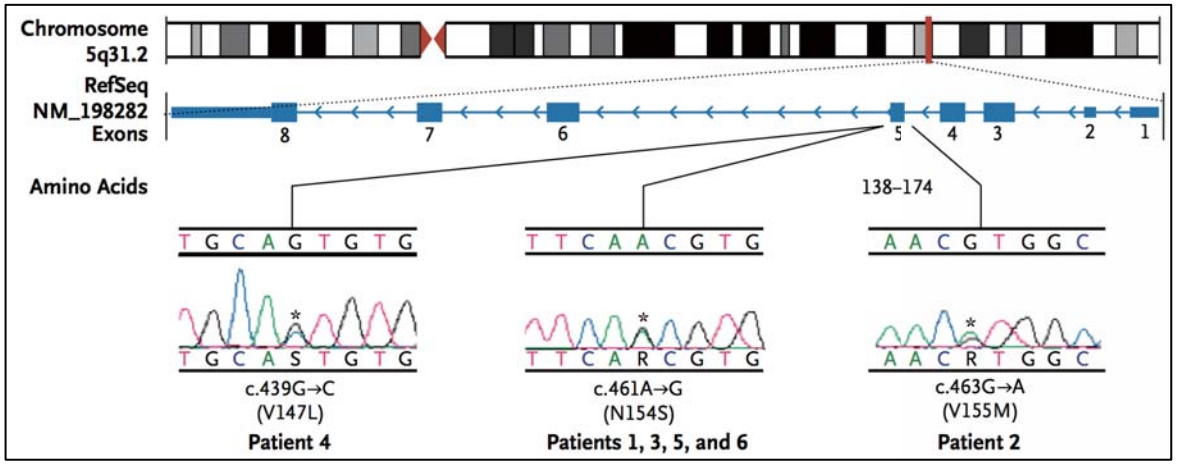
- Aspirin suppresses autoimmunity in mice(left) and human patient cells(right)

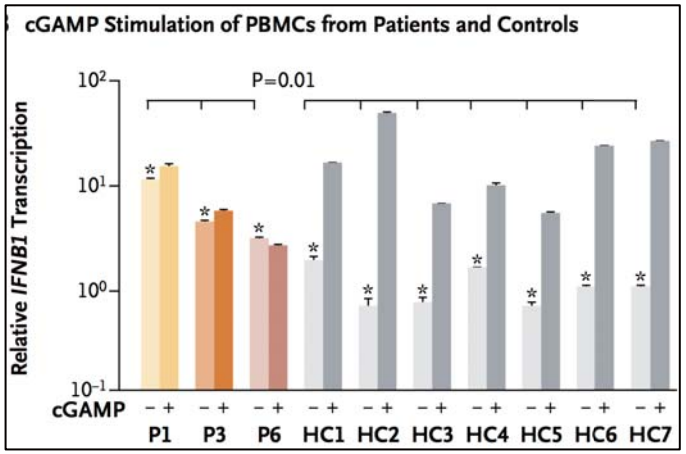
InvivoGen
INNOVATION WITHIN REACH

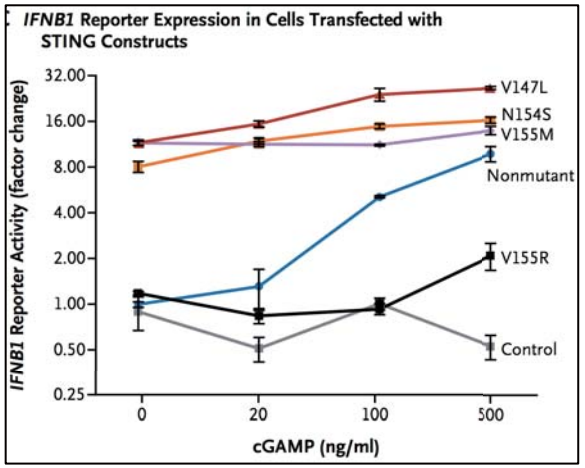
Stimulator of Interferon Genes (STING)–Associated Vasculopathy with Onset in Infancy (SAVI).

STING Variants









Y.Liu, et al. Activated STING in a Vascular and Pulmonary Syndrome. N ENGL J MED. 2014

STING and Autoimmunity

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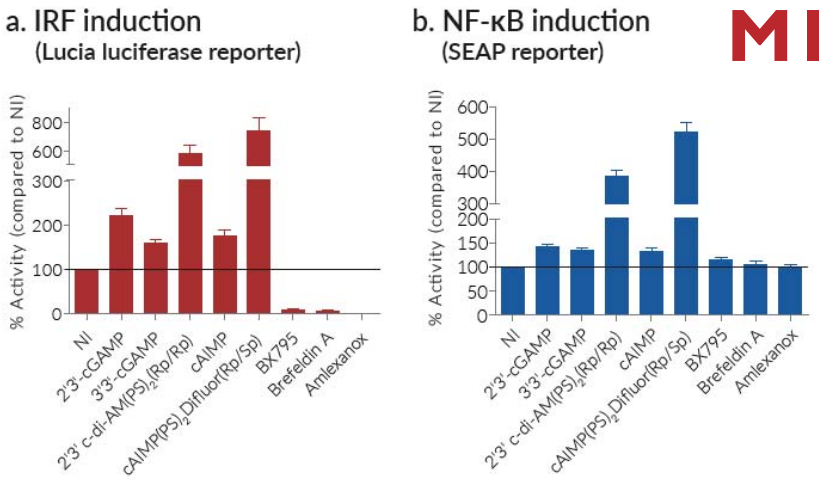
INNOVATION WITHIN REACH

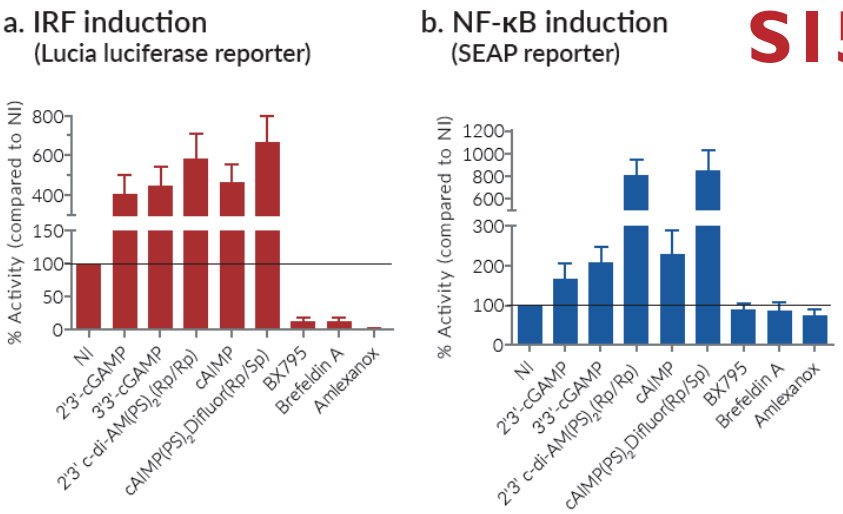
NEW !

M155



THP1-Dual™ KI-hSTING-M155 cells were stimulated with 10 µg/ml of various cyclic dinucleotides (2'3'-cGAMP, 3'3'-cGAMP, 2'3' c-di-AM(PS)2(Rp/Rp), cAIMP or cAIMP(PS)2Difluor(Rp/Sp), 30 µM BX795, 10 µM Brefeldin A, or 300 µg/ml Amlexanox. After overnight incubation, the IRF (panel a) and NF-κB(panel b) responses were determined using QUANTI-Luc™ and QUANTI-Blue™ respectively. Bars represent the % activity on the signal in non-induced (NI) cells.

S154



THP1-Dual™ KI-hSTING-S154 cells were stimulated with 10 µg/ml of various cyclic dinucleotides (2'3'-cGAMP, 3'3'-cGAMP, 2'3' c-di-AM(PS)2(Rp/Rp), cAIMP or cAIMP(PS)2Difluor(Rp/Sp), 30 µM BX795, 10 µM Brefeldin A, or 300 µg/ml Amlexanox. After overnight incubation, the IRF (panel a) and NF-κB (panel b) responses were determined using QUANTI-Luc™ and QUANTI-BlueT™ respectively. Bars represent the % activity on the signal in non-induced (NI) cells.

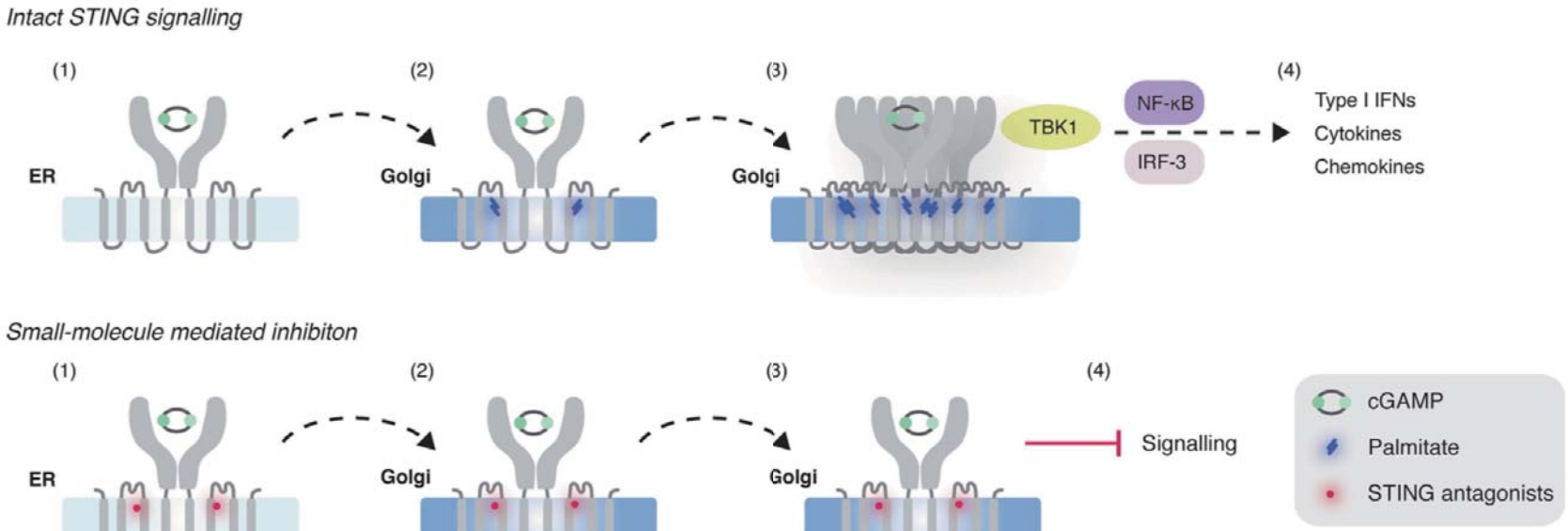
STING SAVI Reporter Cells

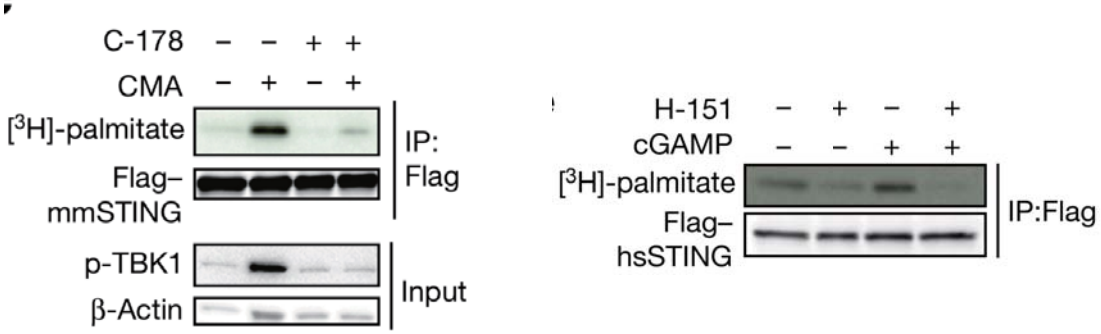
InvivoGen provides a collection of STING variant reporter cells derived from the human THP-1 monocytic cell line. Among them, two express a STING gain-of-function (S154 and M155) responsible for the STING-associated vasculopathy with onset in infancy (SAVI). SAVI-patients display a single-point mutation in the STING protein leading to its constitutive activation and excessive activation of ISGs. While S154 results from a de novo germline mutation1, M155 is an inherited mutation2. STING SAVI reporter cells are convenient and powerful tools for antagonist screening in the STING pathway.

Products	Cat.
THP1-Dual™ KI-hSTING-S154 Cells	thpd-s154
THP1-Dual™ KI-hSTING-M155 Cells	thpd-m155
BX795	tlrl-bx7

InvivoGen
INNOVATION WITHIN REACH

STING inhibitor





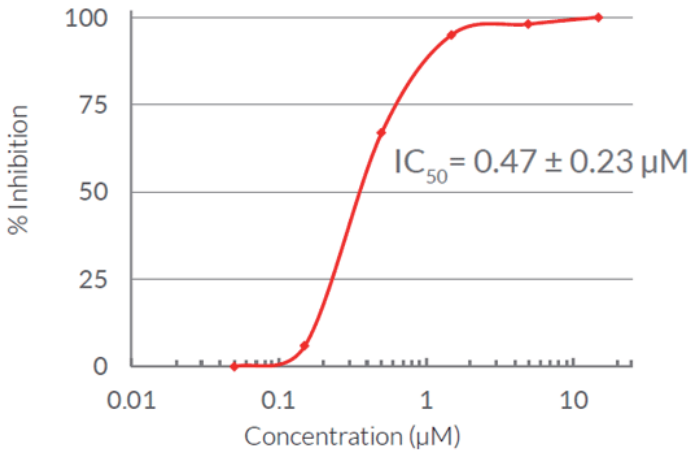
Product Box

Product	Cat. code
c-di-AMP	tlrl-nacda
DMXAA	tlrl-dmx
5'ppp-dsRNA	tlrl-3prna-100
H-151	inh-h151

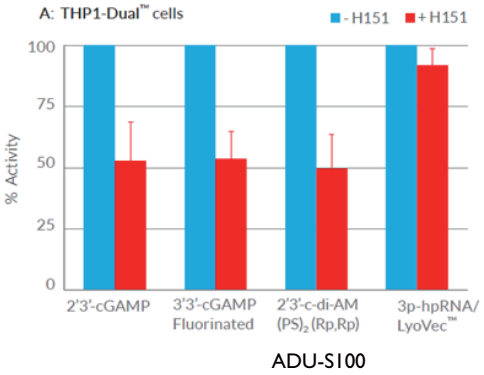
Simone M. Haag et al. Targeting STING with covalent small-molecule inhibitors. Nature. 2018

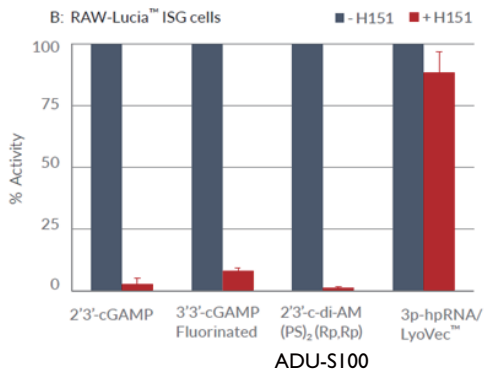
InvivoGen
INNOVATION WITHIN REACH

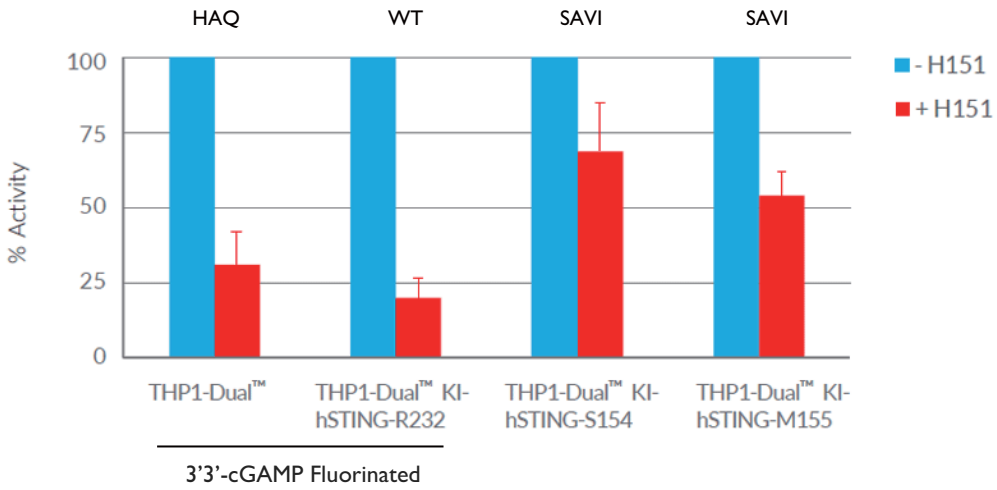
STING inhibitor: The best STING inhibitor by far



Effect of H-151 on the IRF response of THP1-Dual™ cells to 2'3'-cGAMP.







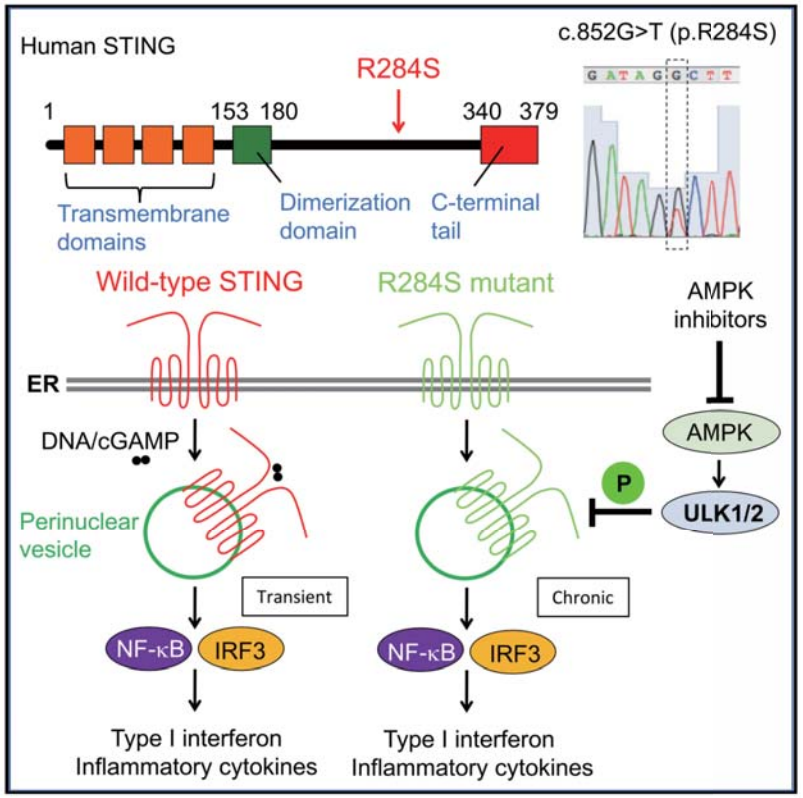
H-151 NEW

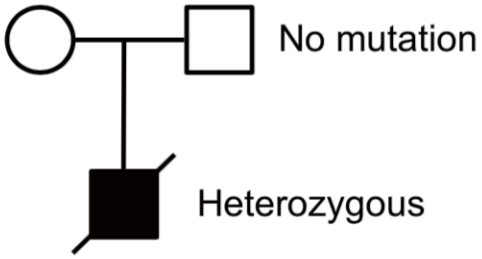
H-151 NEW	Unit size	Cat. code	Docs	Qty
Synthetic Indole Derivative - STING Inhibitor	10 mg	inh-h151	TDS MSDS DATA	1

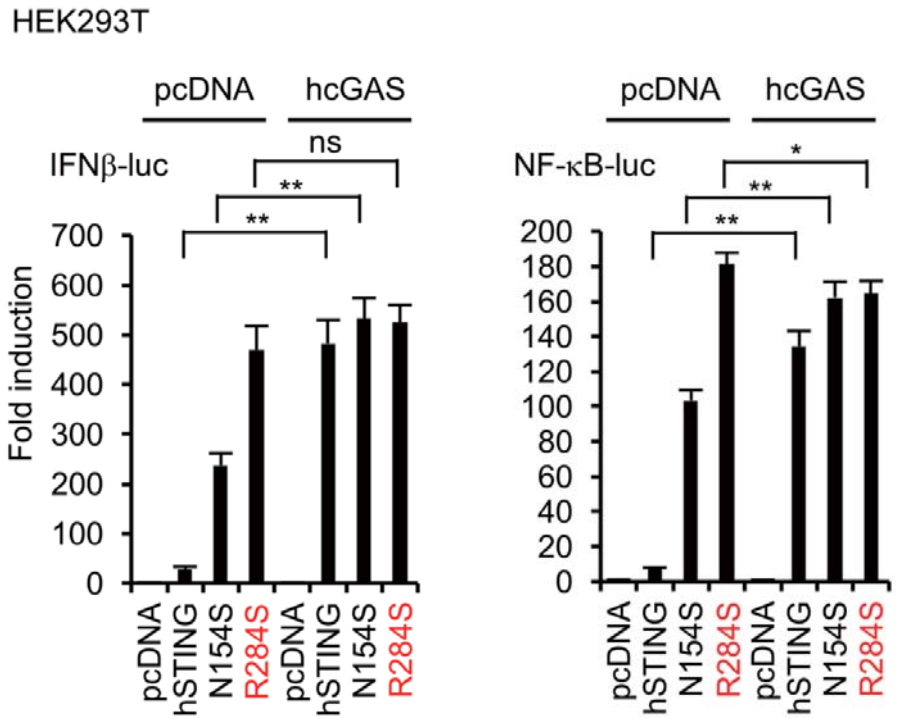
Simone M. Haag et al. Targeting STING with covalent small-molecule inhibitors. Nature. 2018

InvivoGen
INNOVATION WITHIN REACH

STING Variants







Hiroyasu Konno, et al. Pro-inflammation Associated with a Gain-of-Function Mutation (R284S) in the Innate Immune Sensor STING. Cell Reports. 2018

AMPK Inhibitors: **Doxorubicin and GSK 690693**

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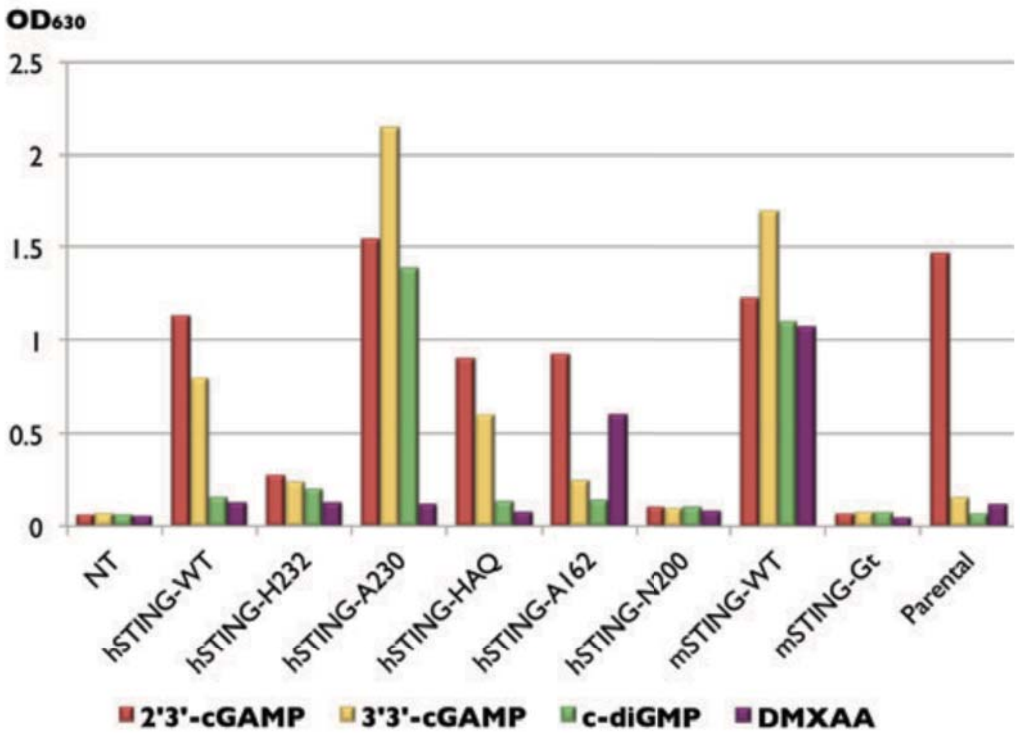
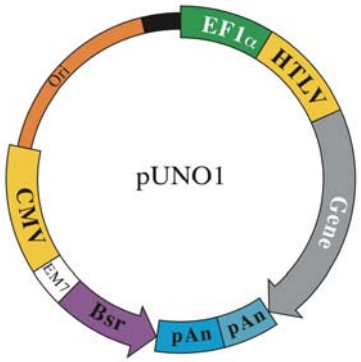
INNOVATION WITHIN REACH

STING Variant



KO-STING cells transfected with WT or mutant STING and HEK-Blue™ ISG cells (parental) were stimulated with 10 mg/ml of 2'3'-cGAMP, 3'3'-cGAMP, c-di-GMP or DMXAA. After 24h incubation, the levels of IRF-induced SEAP were determined using QUANTI-Blue™.

STING Isoforms	Cat. Code
hSTING	puno1-hstingwt
mSTING	puno1-mstingwt
hSTING-A162	puno1-hsting-a162
hSTING-A230	puno1-hsting-a230
hSTING-H232	puno1-hsting-h232
hSTING-HAQ	puno1-hsting-haq
hSTING-M155	puno1-hsting-m155
hSTING-N200	puno1-hsting-n200
mSTING-Gt	puno1-msting-gt



Contents

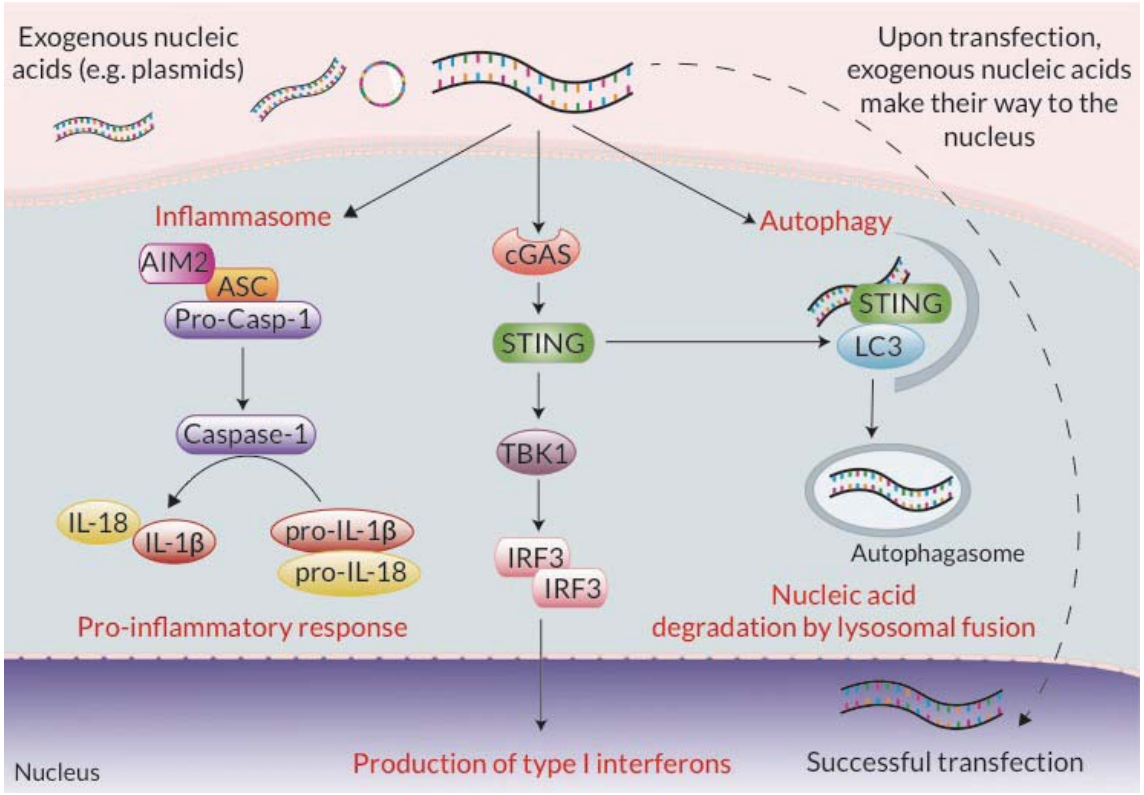
STING variants are provided in the pUNO1 plasmid as 20 µg of lyophilized DNA. Each plasmid is supplied with 4 pouches of Fast-media® Blas and 1 ml blasticidin at 10 mg/ml.

InvivoGen
INNOVATION WITHIN REACH

NATE™

Nucleic Acid Transfection Enhancer NEW

InvivoGen now offers the NATE™ reagent, a nucleic acid transfection enhancer, to boost both transient and stable transfection efficiencies in hard-to-transfect cell lines such as monocytes(THP-1) and macrophages(RAW 264.7).

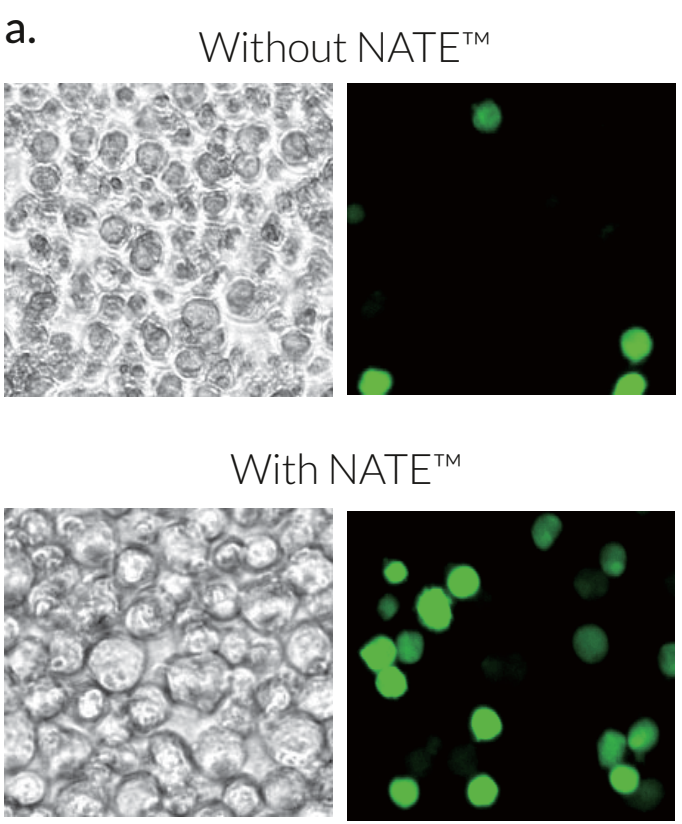


- Exogenous nucleic acids are detected by cytosolic sensors such as cGAS/STING, AIM2 inflammasome, and LC3 mediated autophagy.
- The NATE™ reagent inhibits a number of nucleic acid sensing pathways, thereby protecting exogenous DNA and facilitating its expression.

InvivoGen
INNOVATION WITHIN REACH

NATE™

Increased DNA expression in NATE™ treated cells



NATE™ enhances transient DNA expression in human THP-1 monocytes:

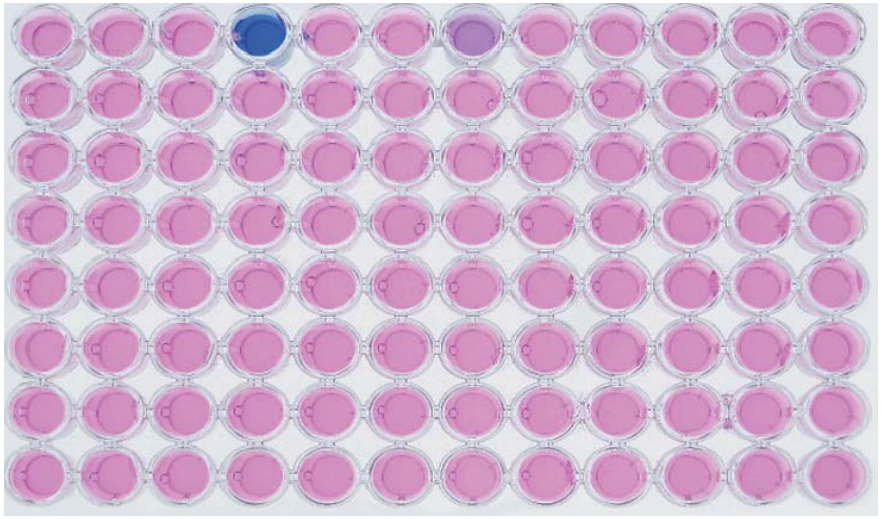
Transfection of a ~3 kb GFP-expressing plasmid into THP-1 cells was performed using GeneXPlus without (a:top; b:yellow) or with (a:bottom; b:green) the NATE™ reagent. After 48 hours, cells were visualized by fluorescence microscopy (a), and transfection efficiency was measured using flow cytometry (b). Data are presented as a fold change normalized to the transfection efficiency without NATE™ reagent.

InvivoGen
INNOVATION WITHIN REACH

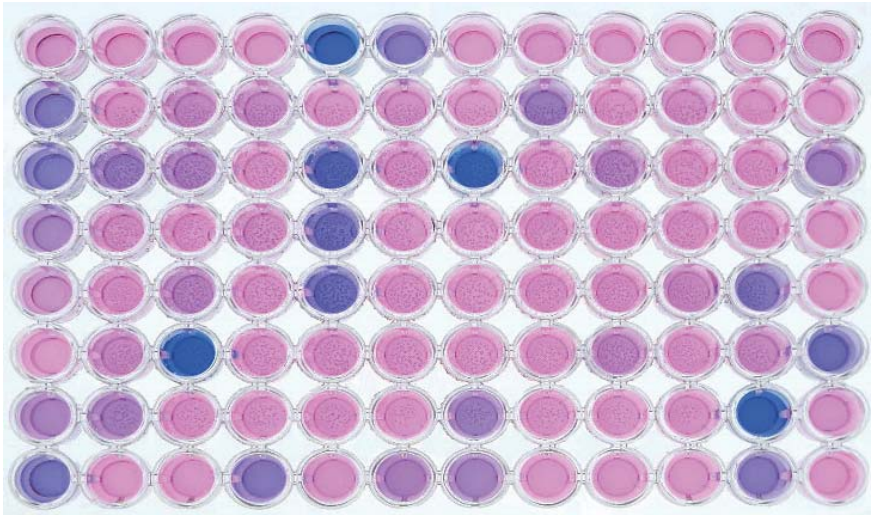
NATE™

Increased rate of stably transfected cells with NATE™

Without NATE™



With NATE™



No clone Stable clone

NATE™ increases the number of stable SEAP-expressing clones in murine RAW 264.7 macrophages:

Transfection of a ~10 kb SEAP-expressing plasmid into RAW 264.7 cells was performed using Lipofectamine® LTX, without (left) or with (right) the NATE™ reagent. After 10 days in selection with Blasticidin, the number of stables clones expressing SEAP (blue wells) was readily visualized using QUANTI- Blue™ Solution detection reagent.

InvivoGen
INNOVATION WITHIN REACH

Also Available

Do you realize that antibiotics can stimulate immune responses

Molecular patterns of microorganisms can stimulate immune cells is common wisdom. Antibiotics are produced from microorganisms, therefore they may contain the component of microorganisms. As an immune specialist, InvivoGen is the only provider test microorganisms contamination in selective antibiotics.

Selective Antibotics	Solvent
Blasticidin	HEPES
Zeocin™ [1,2]	HEPES
Puromycin	HEPES
G418	H_2O
Hygromycin B Gold™	HEPES
Phleomycin	HEPES

[1] Gatignol, A., Durand, H. & Tiraby, G. Bleomycin resistance conferred by a drug-binding protein. FEBS Lett. ***230****: 171–175. 1988.*
[2] Mulsant P, Gatignol A, Dalens M, Tiraby G. Phleomycin resistance as a dominant selectable marker in CHO cells. Somatic Cell and Molecular Genetics. ***14*** *(3): 243–52. 1988.*

STING and Autoimmunity

IN this section

Around the world in a month p201

First anti-CD123 oncology drug p202

CRISPR target prediction in the clinic p204

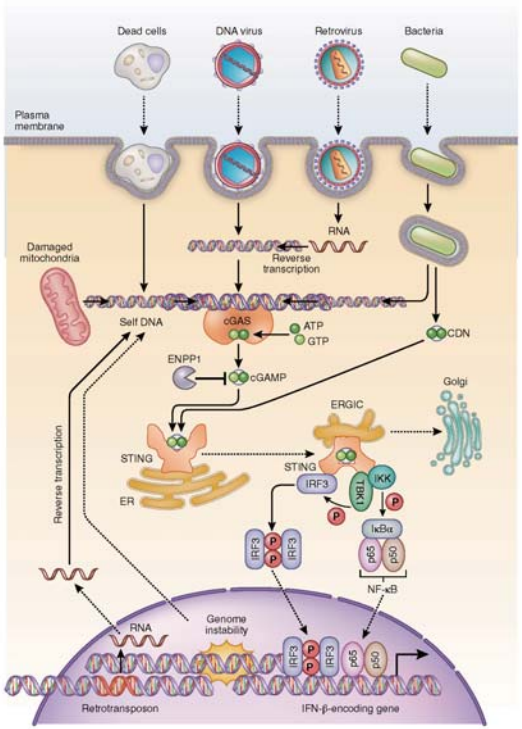
Drug developers switch gears to inhibit STING

Several biotechs are exploring STING inhibitors as a means to control innate immunity and inflammation.

Eli Lilly is the latest large pharma company to set its sights on cGAS–STING, the mechanism used by cells to sense DNA danger signals and trigger the fast-acting innate immune response. In December, Lilly entered a pact with Aduro Biotech to develop cGAS–STING inhibitors as anti-inflammatory agents. The deal involves a modest $12 million up front but a hefty $620 million per product in potential development and commercial milestones. It reflects growing industry interest in the therapeutic possibilities of shutting down a signal cascade that leads to immune activation. Such efforts complement existing industry programs that seek to instead upregulate STING as a means to potentiate checkpoint inhibitor treatments in cancer (Box 1).

The presence of DNA in the cytosol is a warning signal to the innate immune system. It indicates invasion by pathogens, cancer, or cellular breakdown. That DNA is sensed by the enzyme cGAS (cyclic GMP-AMP synthase). Once cGAS is activated by binding DNA, it catalyzes the synthesis of a cyclic dinucleotide second messenger, guanosine monophosphate–adenosine monophosphate (cGAMP), from adenosine triphosphate (ATP) and guanosine triphosphate (GTP), which in turn activates the adaptor protein STING (stimulator of interferon genes) on the endoplasmic reticulum. STING then transmits a signal that ultimately leads to the production of type I interferons and other inflammatory cytokines, including tumor necrosis factor-α (TNF-α).

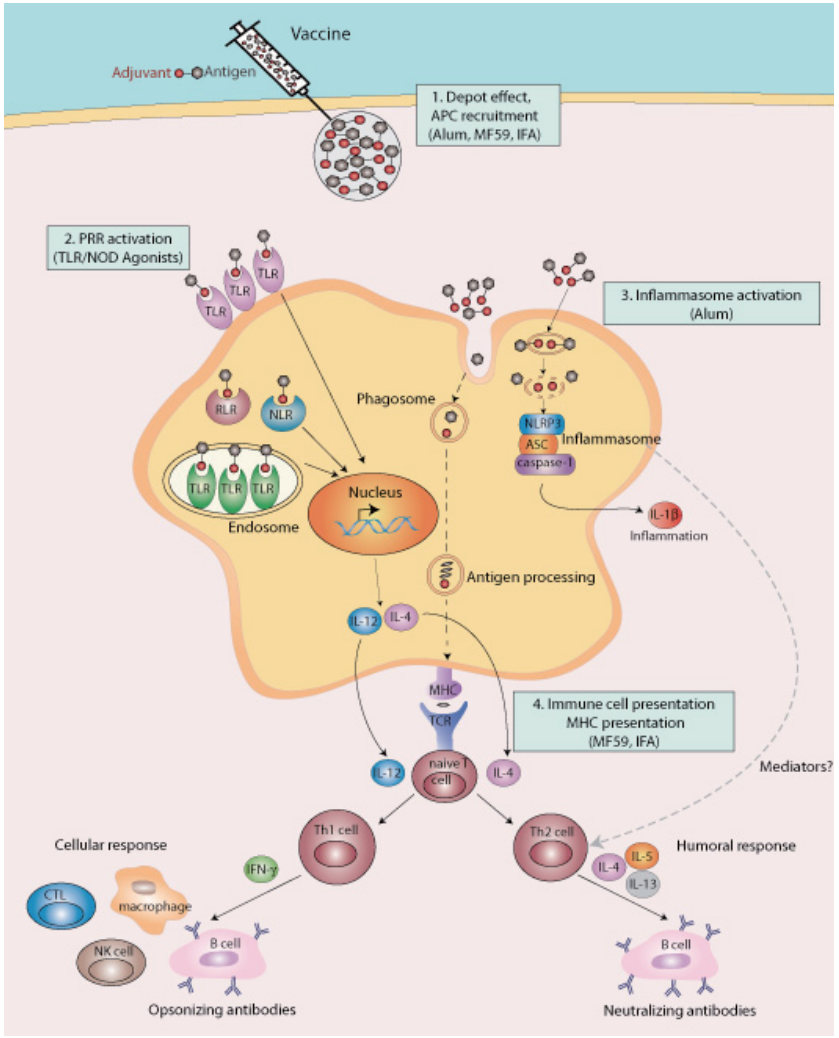
Excessive signaling through cGAS–STING is linked to a range of chronic and rare diseases, ranging from Parkinson's disease through certain forms of systemic lupus erythematosus (SLE), lupus nephritis, and nonalcoholic steatohepatitis (NASH) to interferonopathies, a set of rare genetic conditions characterized by interferon overproduction. These include a systemic vasculopathy with



The warning signal that rouses the innate immune system is out-of-place DNA. The sensor is cGAS–STING, which, on detecting cytosolic DNA, ramps up interferon (IFN) production. Reprinted with permission from *Nature Immunology* **17**, 1142–1149, 2016, Springer Nature.

Phase	Agent	Target	Company
Discovery	cCAS inhibitor	Inflammation, autoimmunity, neuroinflammation	IFM and Novartis
Discovery	STING inhibitor	Inflammation, autoimmunity, neuroinflammation	IFM and Novartis
Preclinical	SB11736 (Orally available STING antagonist)	Autoimmunity & Inflammatory Diseases	Spring Bank
Discovery	STING inhibitor	Inflammation	CURADEV
Discovery	STING inhibitor	Autoimmune disorders	Nimbus Therapeutics

Adjuvant

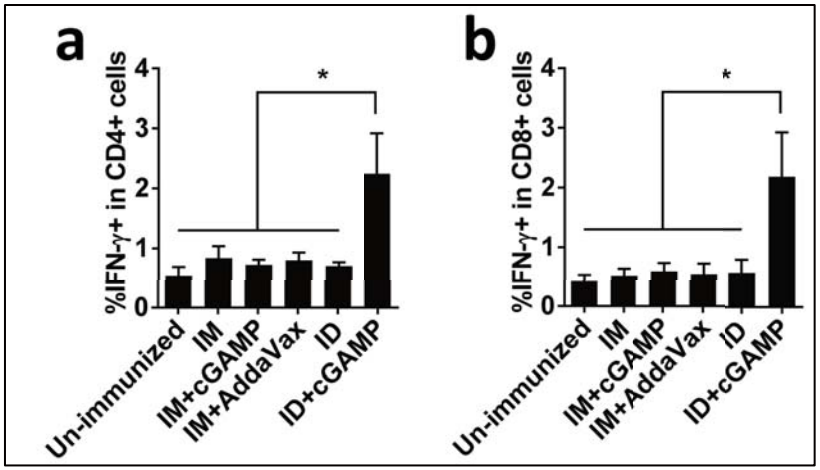


Vaccine against intracellular pathogen and cancer has been considered as cellular immunity. Adjuvant that initiate antigen-specific effector and long-lived memory CD4 and CD8 T cell responses is becoming a hot topic

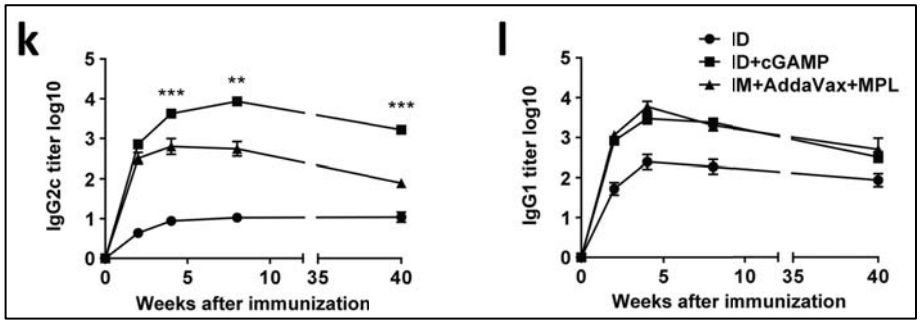
InvivoGen
INNOVATION WITHIN REACH

STING vs Influenza Vaccine Adjuvant

Intradermally (ID) / Intramuscularly (IM)



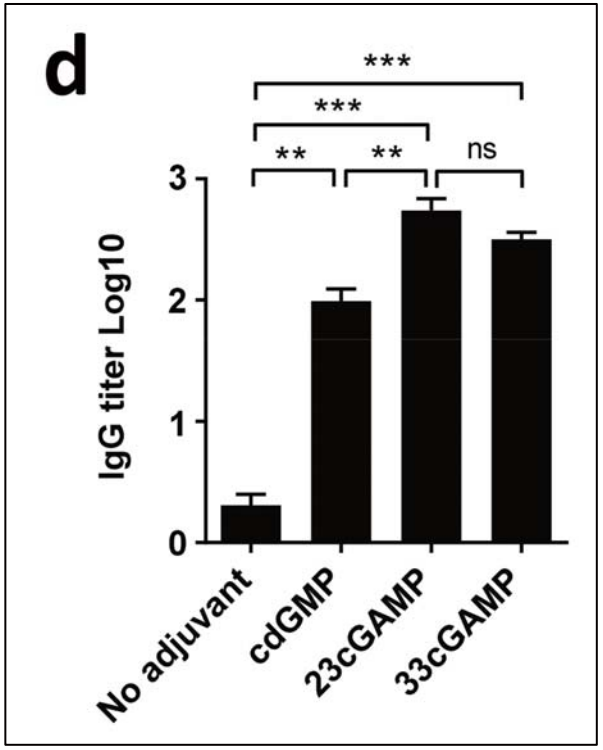
-cGAMP shows superior adjuvant effect on cutaneous vaccination



-cGAMP augments intradermally H5N1 vaccine immunization

Product Box

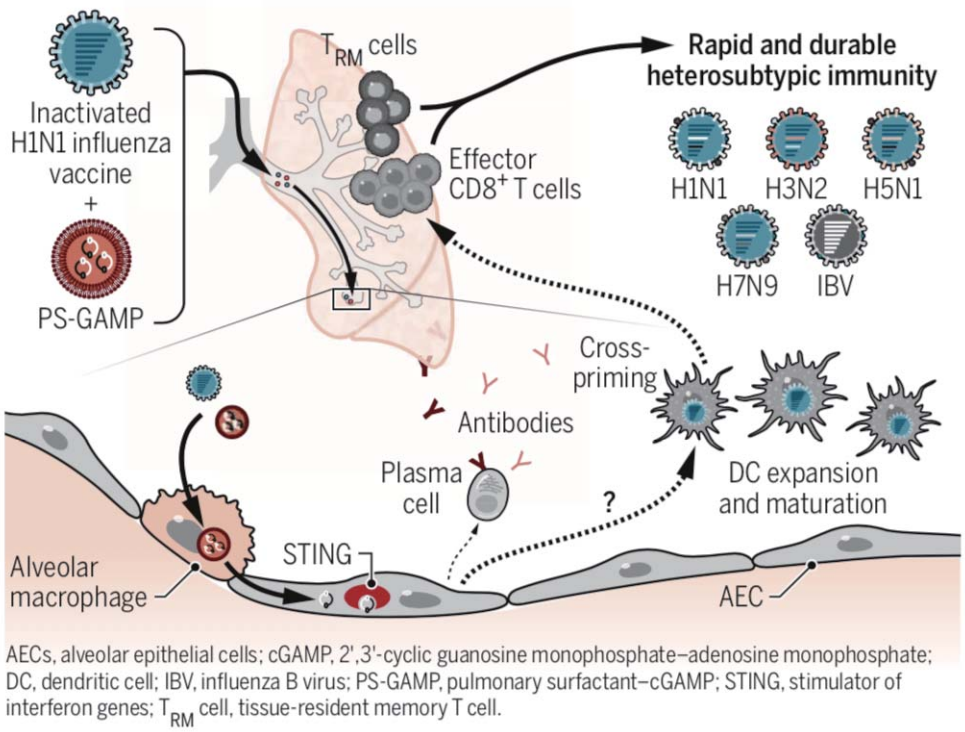
Products	Cat.
AddaVax™	vac-adx-10
c-di-GMP VacciGrade™	vac-cdg
2'3'-cGAMP VacciGrade™	vac-nacga23
3'3'-cGAMP VacciGrade™	vac-nacga
MPLA-SM VacciGrade™	vac-mpla



Ji Wang, et al. The Journal of Investigative Dermatology. *2016*

STING and Adjuvant

STING vs Influenza Vaccine Adjuvant

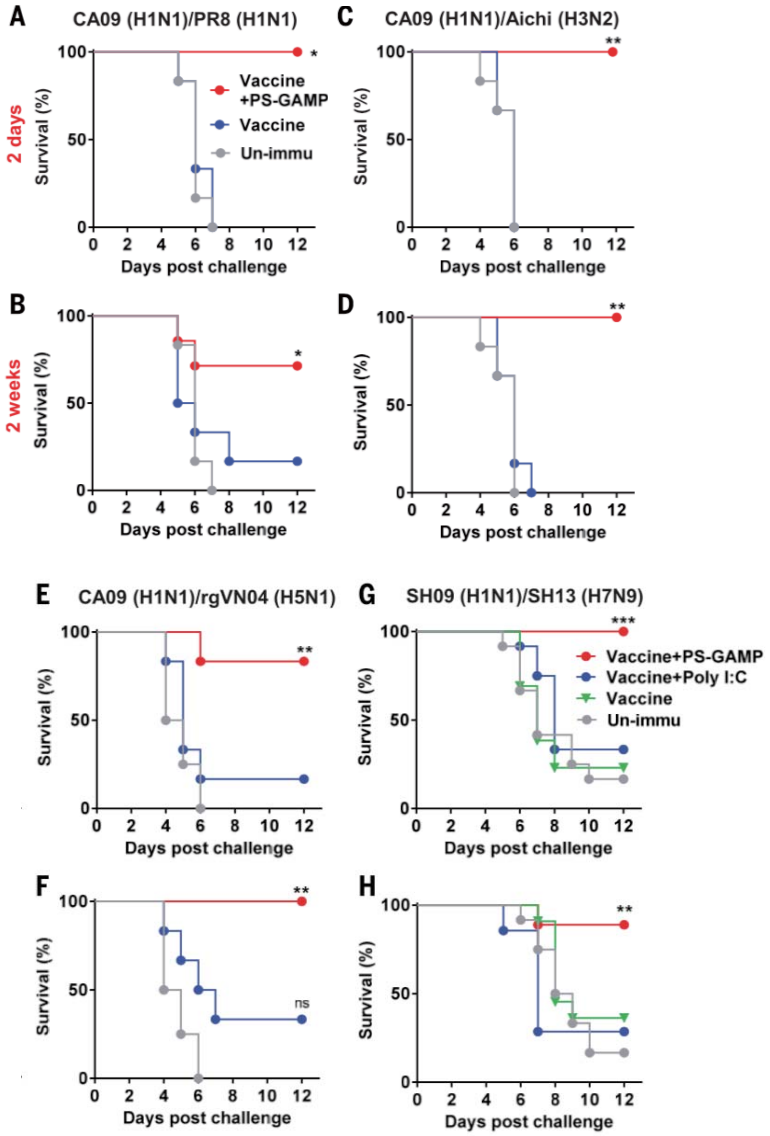


AECs, alveolar epithelial cells; cGAMP, 2',3'-cyclic guanosine monophosphate–adenosine monophosphate; DC, dendritic cell; IBV, influenza B virus; PS-GAMP, pulmonary surfactant–cGAMP; STING, stimulator of interferon genes; T_{RM} cell, tissue-resident memory T cell.

Susanne Herold and Leif-Erik Sander. Toward a universal flu vaccine. Science. 2020

Product Box

Products	Cat.
Poly (I:C)	tlrl-pic
Pam3CSK3	tlrl-pms
LumiKine™ Xpress mIFN-β 2.0	luex-mifnbv2



Ji Wang, et al. Pulmonary surfactant–biomimetic nanoparticles potentiate heterosubtypic influenza immunity. Science. 2020

InvivoGen
INNOVATION WITHIN REACH

STING & HSV

RESEARCH ARTICLE

STING agonists enable antiviral cross-talk between human cells and confer protection against genital herpes in mice

Morten K. Skouboe[1], Alice Knudsen[1], Line S. Reinert[1], Cedric Boularan[2], Thierry Lioux[2], Eric Perouzel[2], Martin K. Thomsen[3☯*], Søren R. Paludan[1☯*]

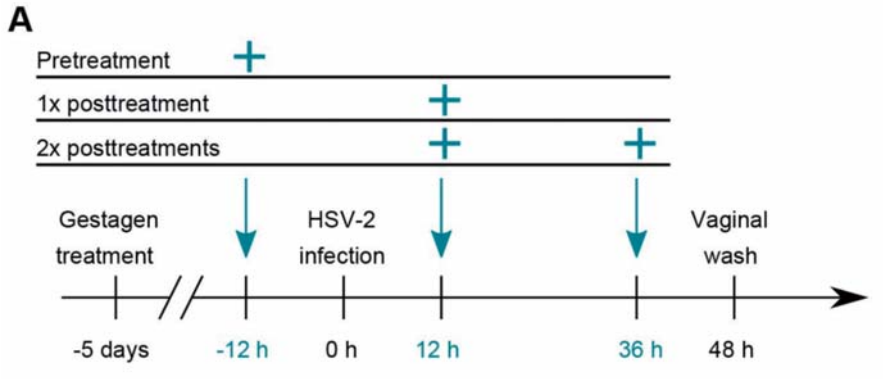
1 Department of Biomedicine, Aarhus University, Denmark, 2 InvivoGen, Toulouse France, 3 Department of Clinical Medicine, Aarhus University, Denmark

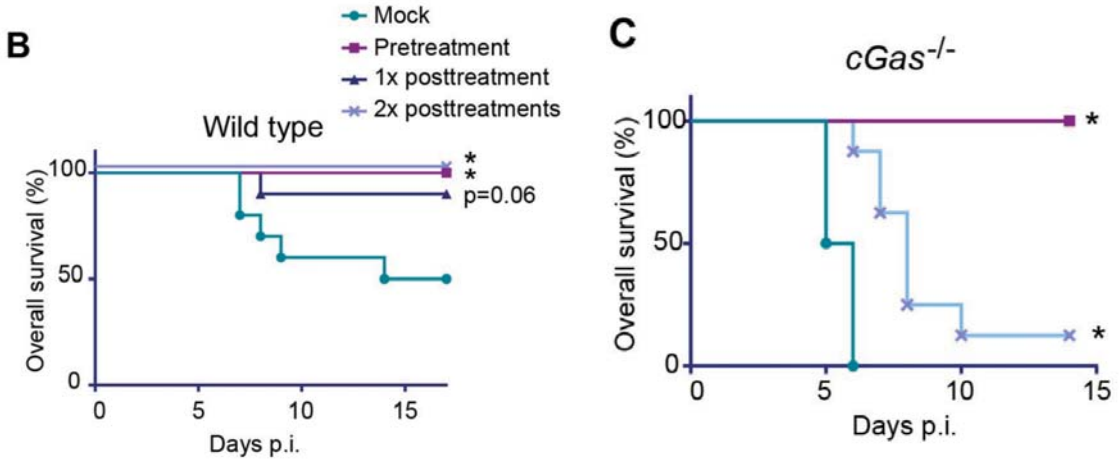
☯ These authors contributed equally to this work.
* srp@biomed.au.dk (SRP); mkt@clin.au.dk (MKT)

Funding: This work was funded by The Danish Medical Research Council (grants no: 12-124330 and DFF – 6110-00068), The Lundbeck Foundation (grant no R198-2015-171) and an unrestricted research grant from InvivoGen (all to SRP). MKT was funded by a fellowship sponsored by InvivoGen, Danish cancer society (R146-A9394-16-S2) and AUFF NOVA (E-2o15-FLS-9-8). AK is

Morten K. Skouboe, Alice Knudsen, Line S. Reinert, ***Cedric Boularan, Thierry Lioux, Eric Perouzel****, Martin K. Thomsen, Søren R. Paludan. STING agonists enable antiviral cross-talk between human cells and confer protection against genital herpes in mice. PLOS Pathogens. 2018*

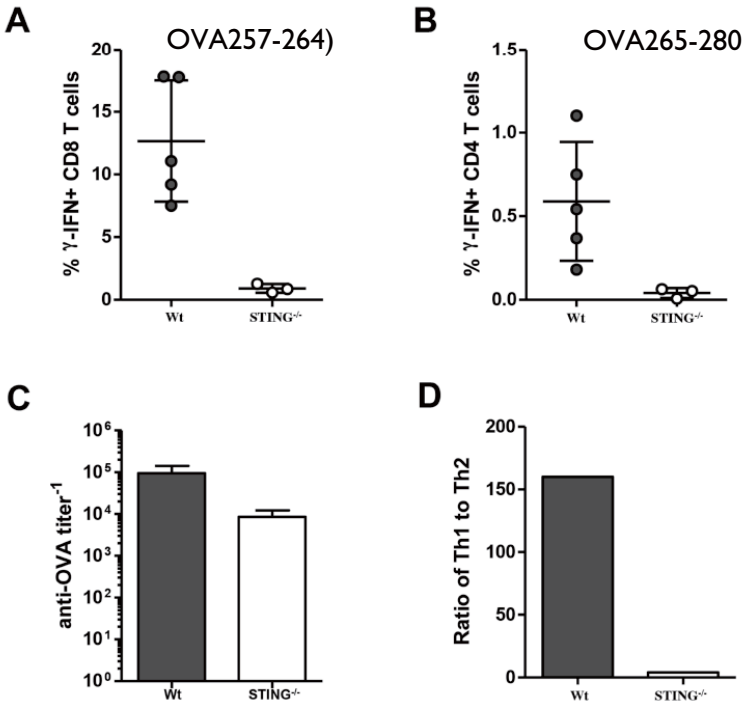
2'3'-cGAM $(PS)_2$ (Rp/Sp) (125 µg/mouse)
Cat Code: tlrl-nacga2srs





Systemic administration of STING agonists improves the survival of mice infected with HSV2

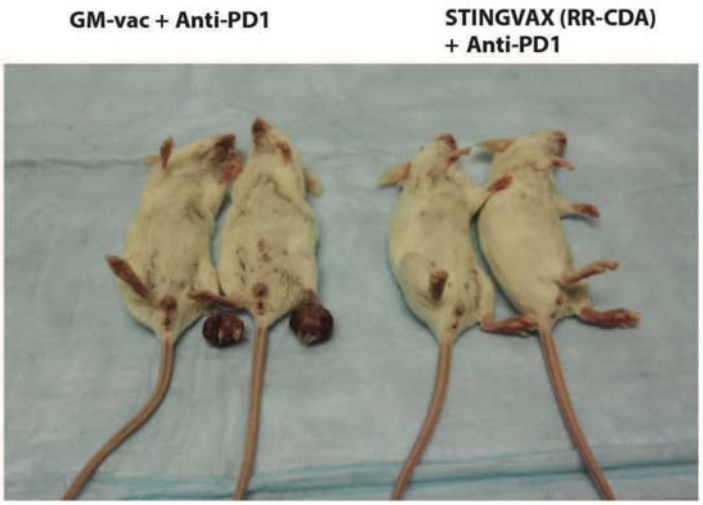
STING vs Anti-tumor Vaccine

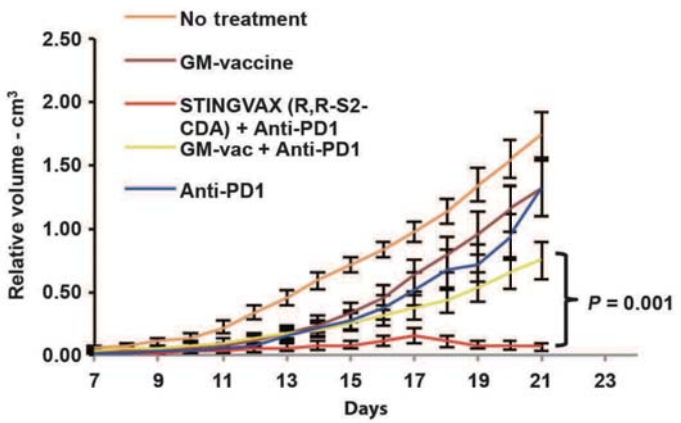


CDNs induced potent STING-dependent CD4+, CD8+, and T helper 1 (TH1)–biased humoral immunity that was specific for the coformulated ovalbumin (OVA) antigens

Product Box

Product	Cat. code
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r



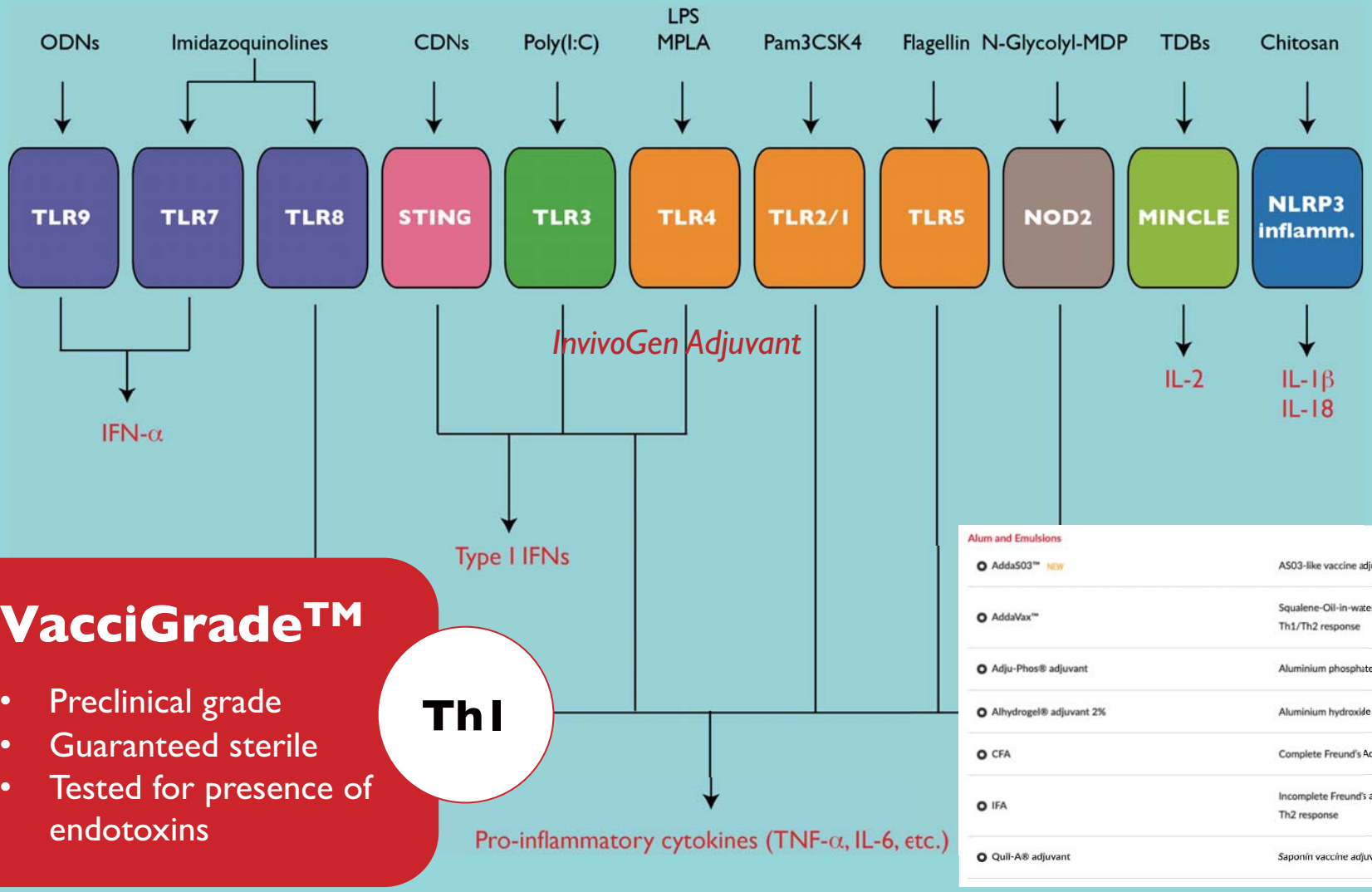


ML RR-S2 CDA (ADU-S100) with PD-1 blockade cures mice bearing established tumors

Fu J, et al. STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. Sci Transl Med. 2015.

InvivoGen
INNOVATION WITHIN REACH

VacciGrade™ Ligands as adjuvants for Th1 response

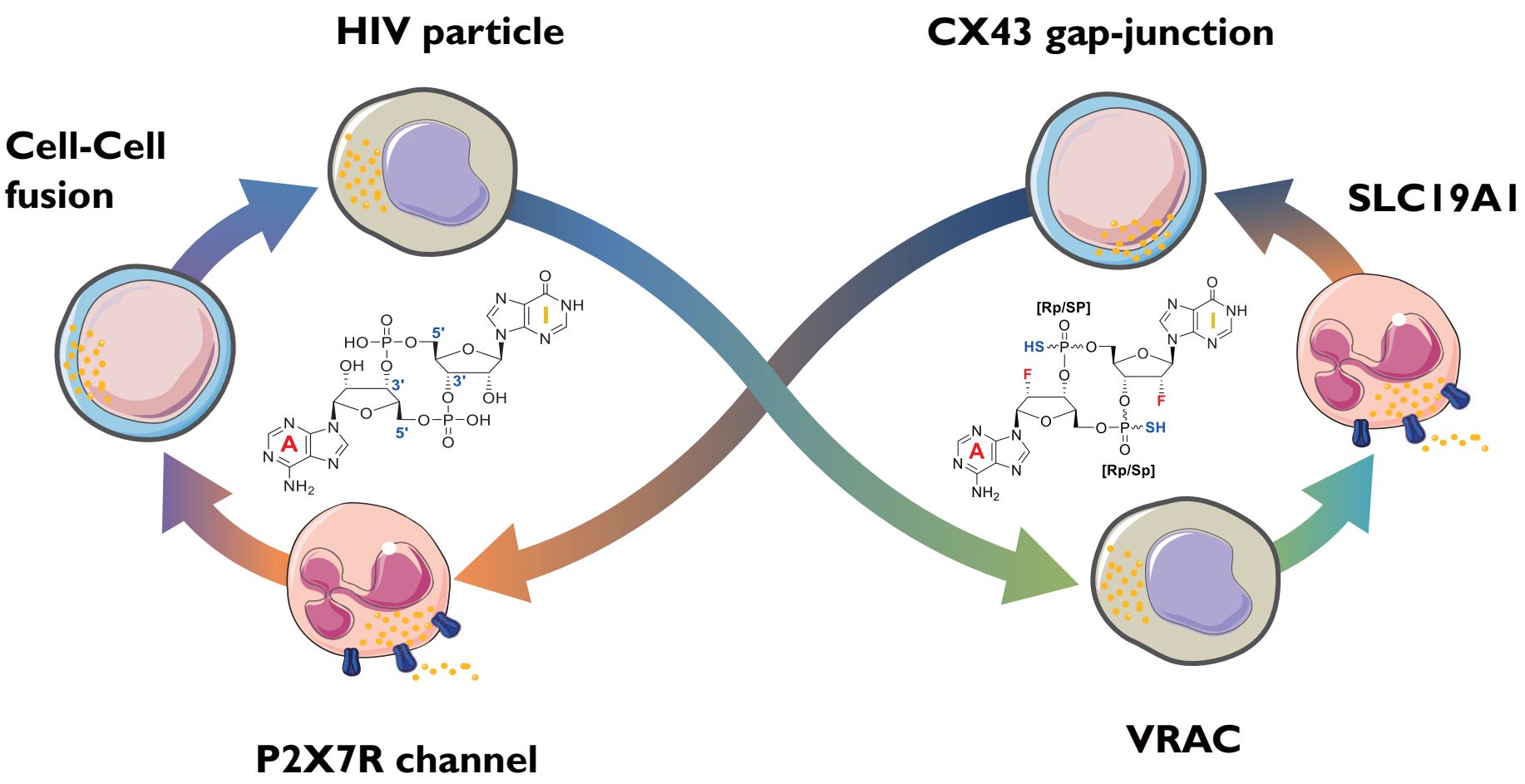


VacciGrade™

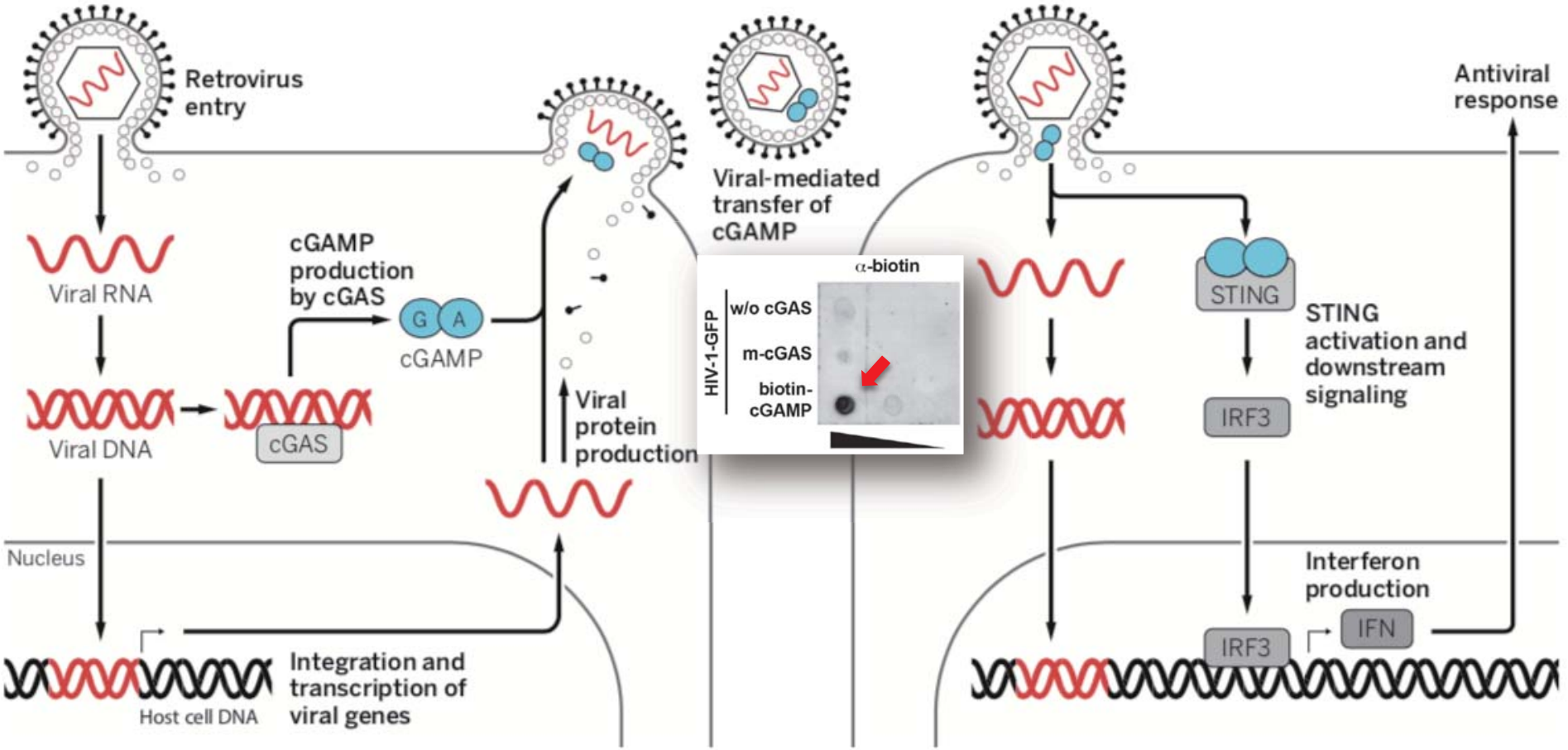
- Preclinical grade
- Guaranteed sterile
- Tested for presence of endotoxins

Alum and Emulsions	
AddaS03™ NEW	AS03-like vaccine adjuvant
AddaVax™	Squalene-Oil-in-water Th1/Th2 response
Adju-Phos® adjuvant	Aluminium phosphate gel | Th2 Response
Alhydrogel® adjuvant 2%	Aluminium hydroxide gel | Th2 Response
CFA	Complete Freund's Adjuvant | Th1 response
IFA	Incomplete Freund's adjuvant Th2 response
Quil-A® adjuvant	Saponin vaccine adjuvant

cGAMP Transfer



cGAMP Transfer

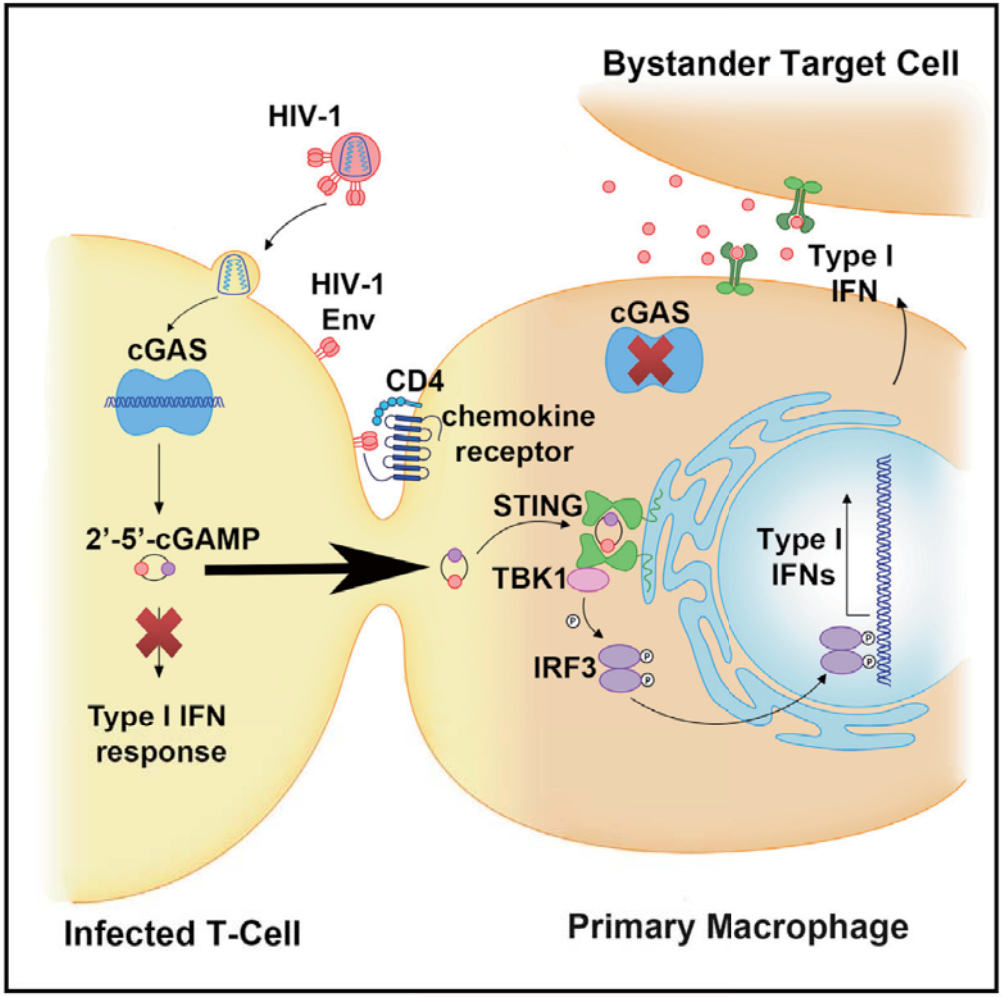


Stowaway. Viral DNA generated during a viral infection is sensed by cGAS, which produces the cGAMP dinucleotide. cGAMP is incorporated as cargo into newly formed virions that exit the cell and infect nearby cells. cGAMP is released into the cytosol of the newly infected cell, binds STING, and triggers downstream antiviral signaling. IRF3, interferon regulatory factor 3.

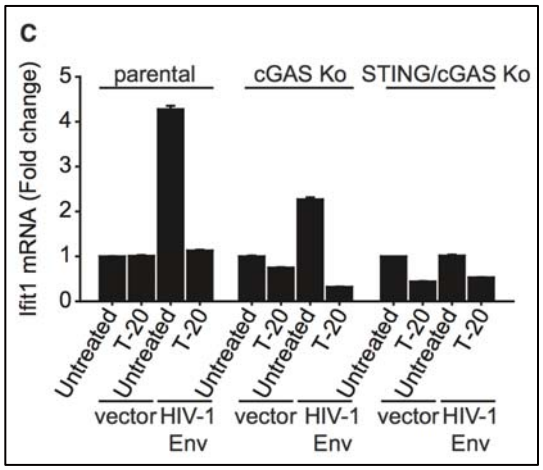
A. Bridgeman, et al.Viruses transfer the antiviral second messenger cGAMP between cells. Science. 2015

InvivoGen
INNOVATION WITHIN REACH

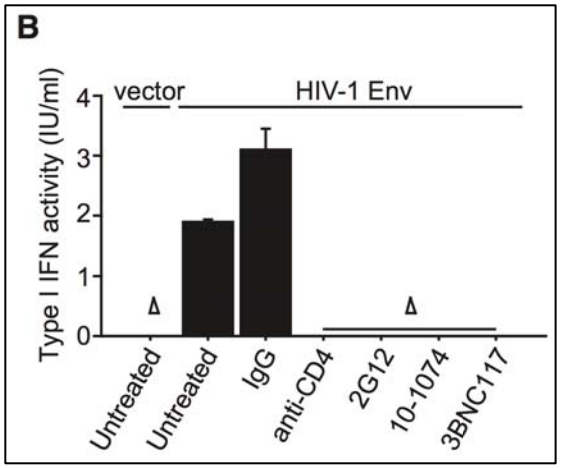
Cell-Cell Fusion



Shuting Xu , et al. cGAS-Mediated Innate Immunity Spreads Intercellularly through HIV-1 Env-Induced Membrane Fusion Sites. Cell Host & Microbe.2016

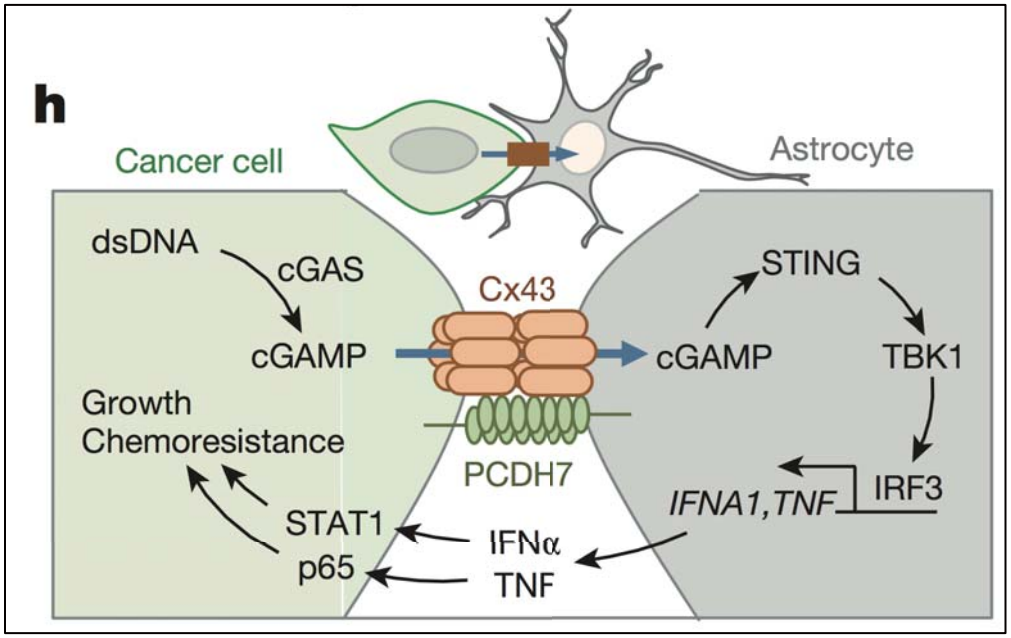


Membrane Fusion-Enabled Spread of Innate Immunity Requires Functional STING, but Not cGAS, in Target Cells



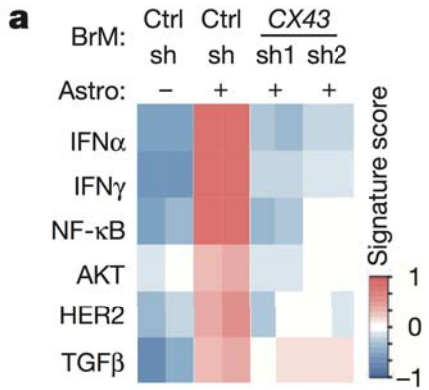
Mounting of Coculture-Induced Type I IFN Requires HIV-1 Env and CD4/Coreceptor-Mediated Membrane Fusion and Direct Cell-Cell Contacts.

cGAMP Transfer

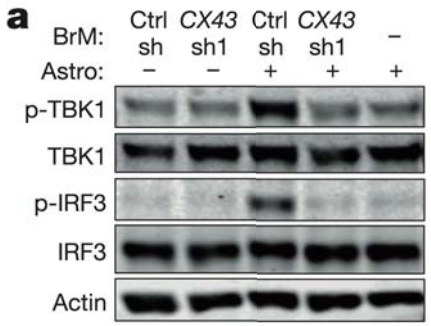


Gap-Junction - Connexin

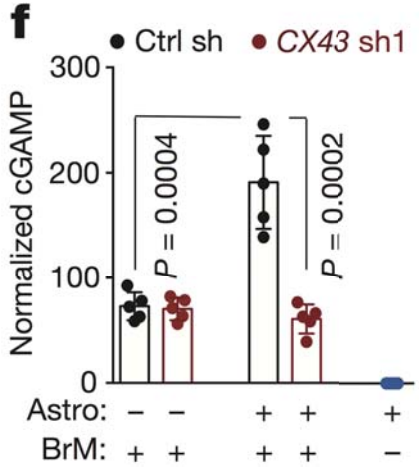
Qing chen, et al. Carcinoma–astrocyte gap junctions promote brain metastasis by cGAMP transfer. Nature. 2016



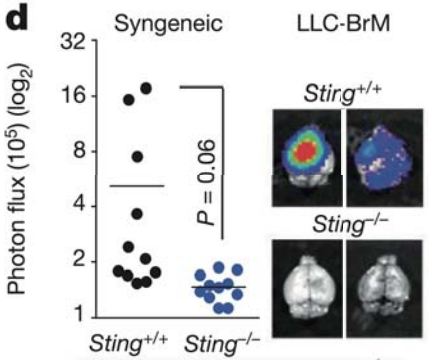
-Cancer cells trigger astrocyte cytokine release via CX43



-CX43-dependent phosphorylation of TBK1 and IRF3



-Cancer cells transfer cGAMP to astrocytes via CX43.



-STING promote brain metastasis

cGAMP Transfer

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INNOVATION WITHIN REACH

Molecular Cell
Article

SLC19A1 Is an Importer of the Immunotransmitter cGAMP

2019

Christopher Ritchie,[1,3,4] Anthony F. Cordova,[1,3,4] Gaelen T. Hess,[2,3] Michael C. Bassik,[2,3] and Lingyin Li[1,3,5,*]
[1]Department of Biochemistry, Stanford University, Stanford, CA 94305, USA
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA
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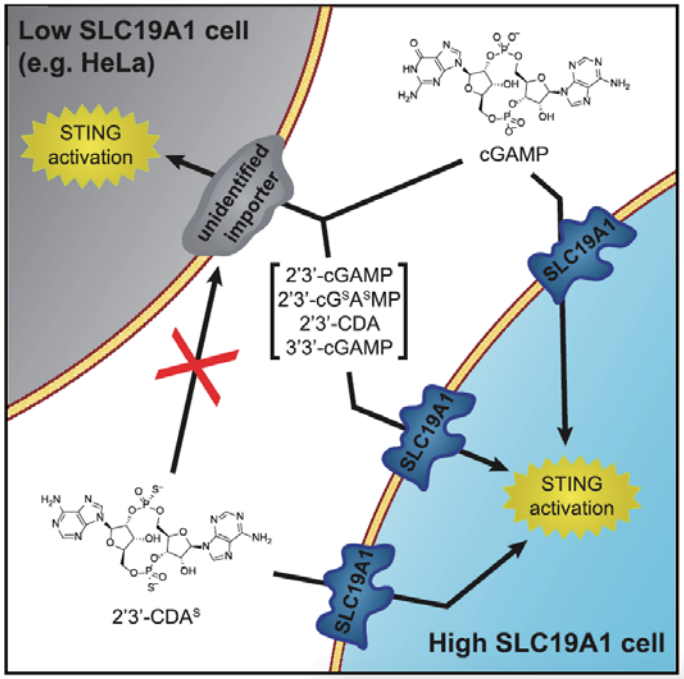
[4]These authors contributed equally
[5]Lead Contact
*Correspondence: lingyinl@stanford.edu
https://doi.org/10.1016/j.molcel.2019.05.006



SLC19A1 is folate transporter

FDA approved drug methotrexate and sulfasalazine block the SLC19A1 channel

Product Box

Product	Cat. code
3'3'-cGAMP	tlrl-nacga
2'3'-cGAMP	tlrl-nacga23
2'2'-cGAMP	tlrl-nacga22
2'3'-c-di-AM(PS)2 (Rp,Rp)	tlrl-nacda2r

CellPress

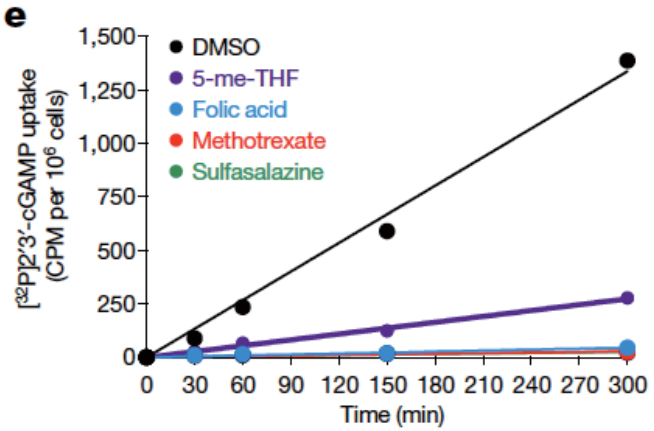
LETTER

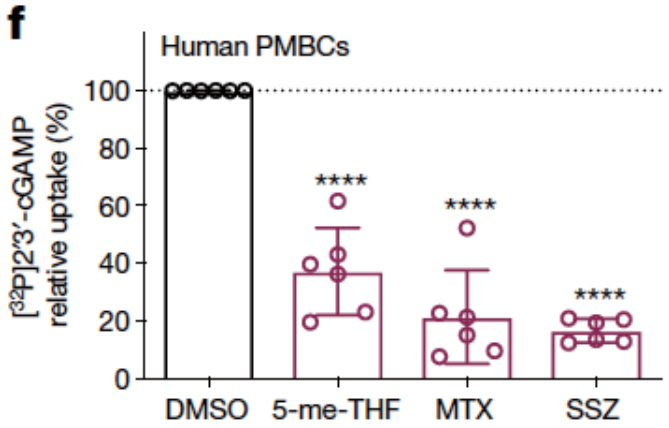
https://doi.org/10.1038/s41586-019-15

SLC19A1 transports immunoreactive cyclic dinucleotides

2019

Rutger D. Luteijn[1,7], Shivam A. Zaver[2,7], Benjamin G. Gowen[3,4], Stacia K. Wyman[3], Nick E. Garelis[1], Liberty Onia[1], Sarah M. McWhirter[5], George E. Katibah[5], Jacob E. Corn[3,4,6], Joshua J. Woodward[2] & David H. Raulet[1]*





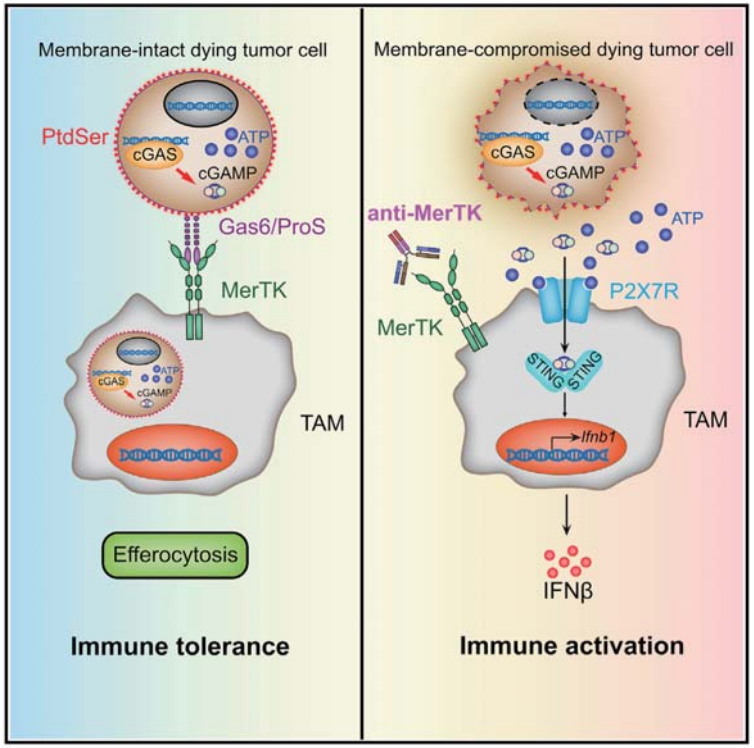
cGAMP Transfer

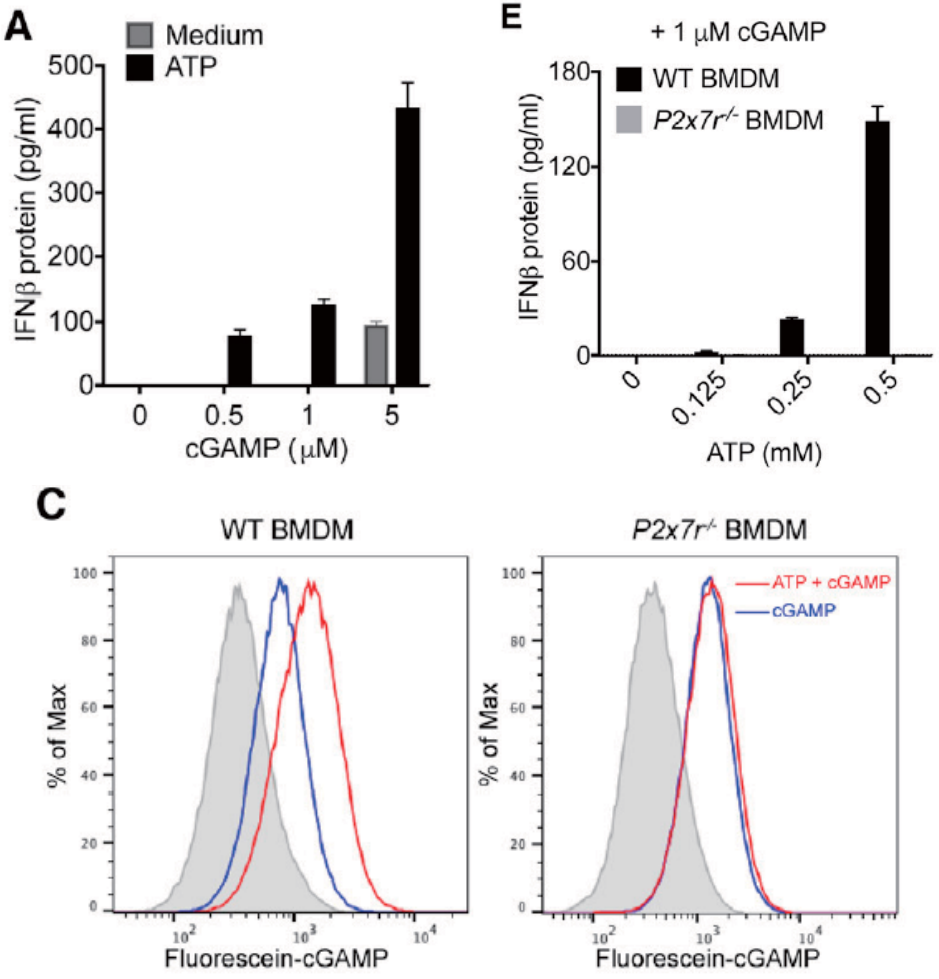
Article

Immunity

Blockade of the Phagocytic Receptor MerTK on Tumor-Associated Macrophages Enhances P2X7R-Dependent STING Activation by Tumor-Derived cGAMP

2020





Product Box

Product	Cat. code
5'ppp-dsRNA	tlrl-3prna-100
2'3'-cGAMP	tlrl-nacga23
DMXAA	tlrl-dmx
ATP	tlrl-atpl

The entry of tumor-cell-produced cGAMP into host cells was facilitated by the ATP-gated P2X purinoceptor 7 (P2X7) receptor (P2X7R).

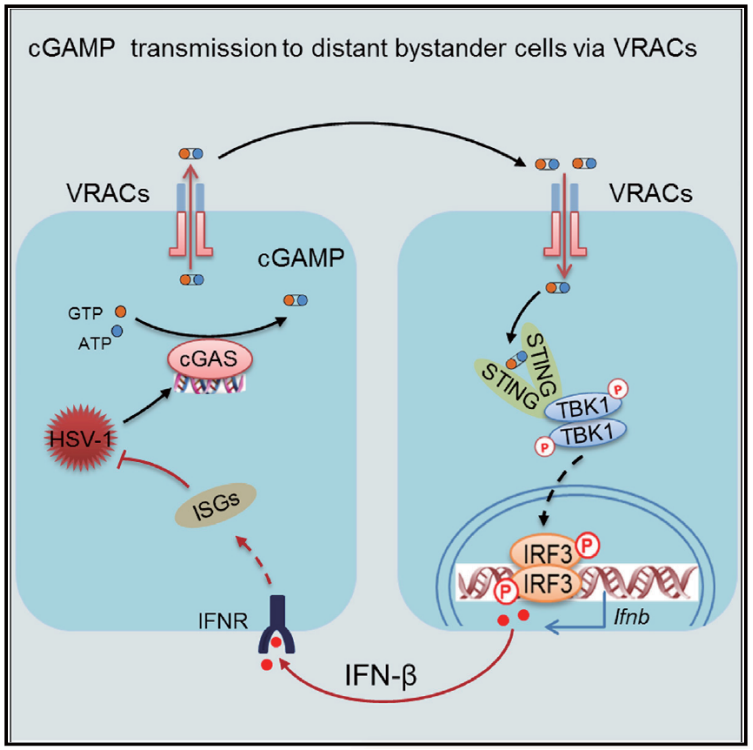
cGAMP Transfer

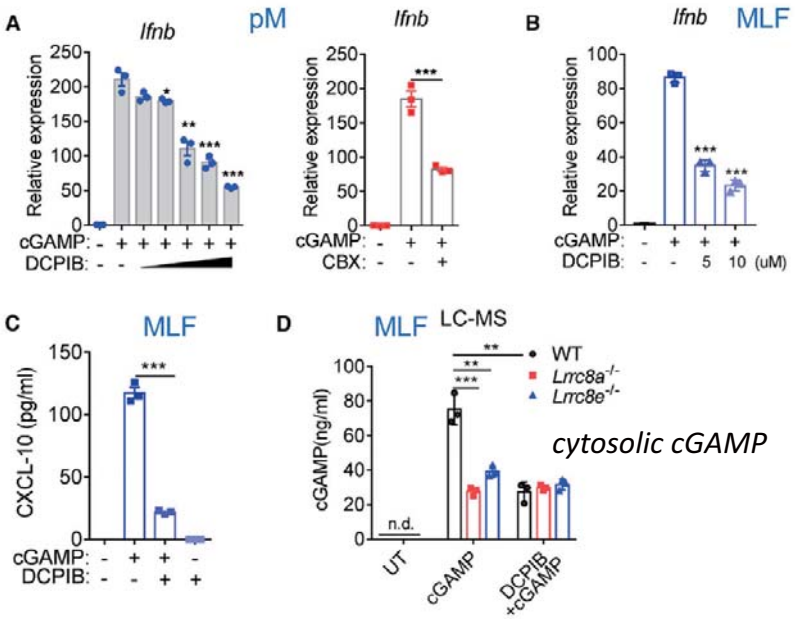
InvivoGen
INNOVATION WITHIN REACH

2020 Article

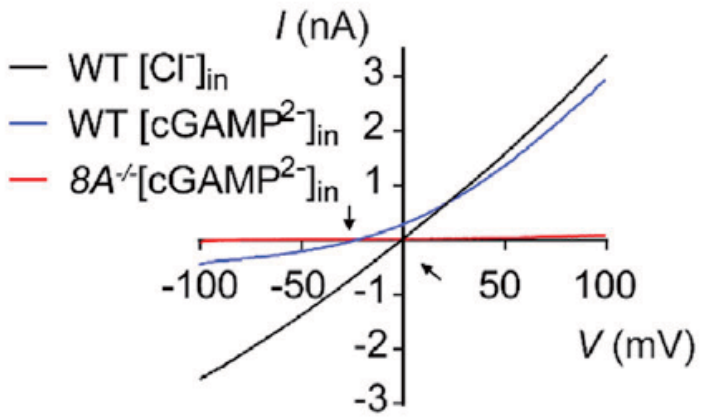
Immunity

Transfer of cGAMP into Bystander Cells via LRRC8 Volume-Regulated Anion Channels Augments STING-Mediated Interferon Responses and Anti-viral Immunity





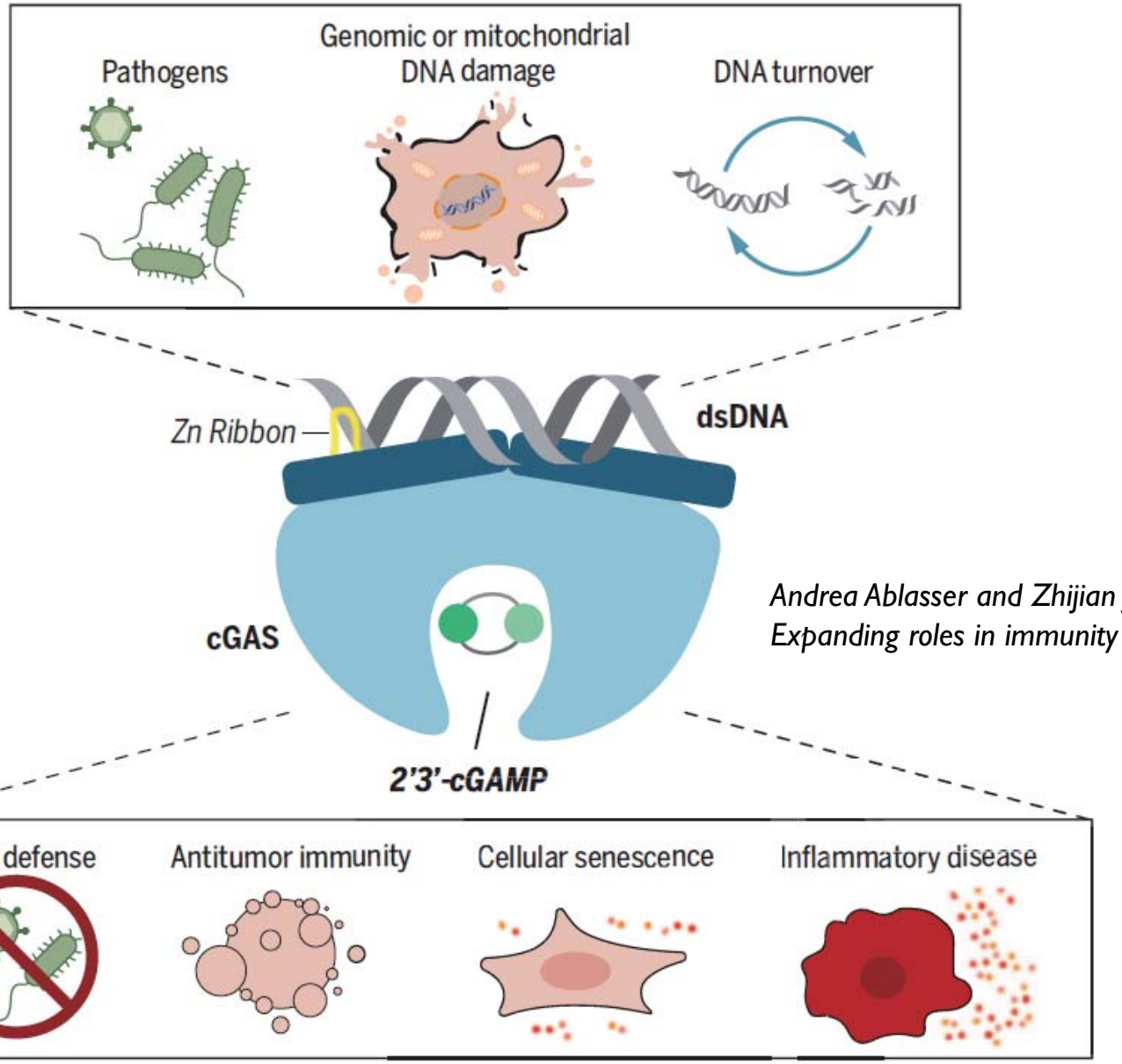
VRAC Channel Activity Positively Correlates with Extracellular cGAMP Responses



LRRC8 VRACs Directly Transport cGAMP

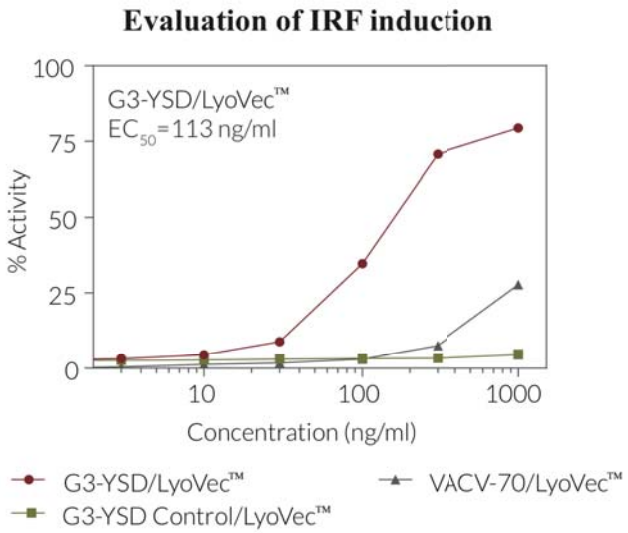
Product Box

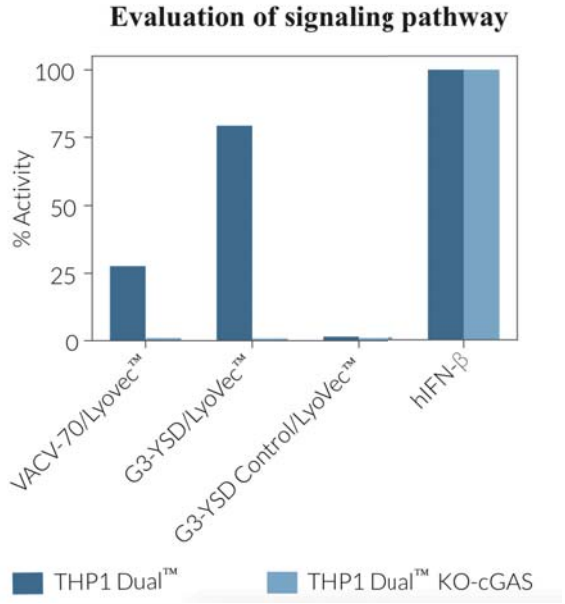
Product	Cat. code
3'3'-cGAMP	tlrl-nacga
2'3'-cGAMP	tlrl-nacga23-5
Poly(I:C) LMW	tlrl-picw
LPS-EK	tlrl-eklps



Andrea Ablasser and Zhijian J. Chen. cGAS in action: Expanding roles in immunity and inflammation. Science. 2019

Comparative Potency of cGAS Ligands





G3-YSD is a 26-mer DNA sequence derived from the HIV-1 RNA genome. This short DNA displays a Y-shape arising from its palindromic sequence flanked by unpaired guanosine trimers (G3). The guanosine overhangs in this Y-form DNA have been identified as minimal recognition motifs for cGAS (cyclic GMP-AMP synthase, cGAMP synthase), a critical cytosolic DNA sensor.

Herzner AM. et al., 2015. Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. Nat Immunol. 16(10):1025-33.

VACV-70 is a 70 bp oligonucleotides containing poxviral genome DNA motifs. VACV-70 derives from the vaccinia virus DNA. Transfected VACV-70 was shown to potently induce IFN-β in a TLR-, DAI- and RNA Pol III-independent, but STING-, TBK1- and IRF3-dependent manner.

Unterholzner L. et al., 2010. IFI16 is an innate immune sensor for intracellular DNA. Nat Immunol. 11(11):997-1004.

Product Name	Cat. code
G3-YSD	tlrl-ydna
G3-YSD Control	tlrl-ydnac
VACV-70 Naked	tlrl-vav70n
VACV-70/LyoVec™	tlrl-vav70c

Endotoxin Free Ligands

InvivoGen
INNOVATION WITHIN REACH

IMMUNOLOGY

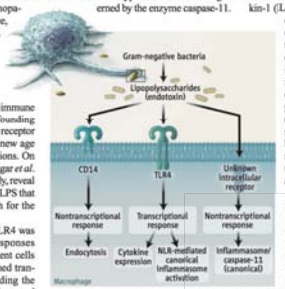
Sensing Endotoxins from Within

Jonathan C. Kagan

Macrophages respond to bacteria through a protein complex that promotes inflammation when activated by internalized bacterial endotoxin.

The human innate immune system identifies Gram-negative bacteria by recognizing lipopolysaccharides (LPS), components of the microbial cell wall (*1*). This detection triggers massive inflammatory responses that help eradicate infections, but may also result in immunopathology if regulated improperly. Hence, LPS is also referred to as endotoxin. More than a century after its discovery, the molecular basis for the inflammatory activity of endotoxin was finally revealed by the discovery that Toll-like receptor 4 (TLR4) induces innate and adaptive immune responses to LPS (*2*). TLR4 is the founding member of the mammalian Toll-like receptor family, and its discovery heralded a new age in the study of host-microbe interactions. On pages 1250 and 1246 of this issue, Hagar *et al.* (*3*) and Kayagaki *et al.* (*4*), respectively, reveal the existence of cellular responses to LPS that do not depend on TLR4. The search for the new LPS receptor can now begin.

For years, it was assumed that TLR4 was solely responsible for cellular responses induced by LPS (*5*, *6*). TLR4-deficient cells are defective for all classically defined transcriptional responses to LPS, including the expression of inflammatory cytokines and interferons (*7*). However, LPS can also induce nontranscriptional cellular responses, such as autophagy, endocytosis, phagocytosis, and oxidative bursts (*8*–*11*). Hagar *et al.* and Kayagaki *et al.* add to this list of atypical responses to LPS, by showing that LPS activates the formation of an atypical inflammasome governed by the enzyme caspase-11.



Sensing lipopolysaccharides. Macrophages respond to Gram-negative bacteria by activating transcriptional and non-transcriptional immune responses. Some of these responses are handled by protein complexes called inflammasomes. An intracellular sensor for lipopolysaccharides was revealed, but its identity has yet to be determined.

Inflammasomes are protein complexes that are assembled in the cytosol of macrophages in response to a variety of extracellular stimuli (*12*). The best-defined function of inflammasomes is to promote the processing and secretion of inflammatory cytokines of the interleukin-1 (IL-1) family. At the center of the best-characterized inflammasomes is the enzyme caspase-1, which cleaves the precursor of IL-1 in the cytosol of macrophages. Cleaved IL-1 family members are then secreted to induce inflammation. A second class of inflammasomes also requires caspase-11 to promote IL-1 cleavage (*13*). These noncanonical inflammasomes are activated by intracellular bacteria and contribute to the phenotypes associated with sepsis. How these noncanonical inflammasomes are activated remains unclear.

Hagar *et al.* and Kayagaki *et al.* recognized that several species of Gram-negative bacteria can activate caspase-11–dependent IL-1 secretion (*13*). Thus, a molecule common to Gram-negative bacteria must be responsible for activating caspase-11. Both research groups show that LPS is the molecule of interest. For example, Hagar *et al.* show that when transfected into macrophages, cell lysates derived from Gram-negative bacteria, but not Gram-positive bacteria (which contain no LPS), can

Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston, MA 02115, USA. E-mail: jonathan.kagan@childrens.harvard.edu

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PERSPECTIVES

TIMELINE

Innate immune sensing and its roots: the story of endotoxin

Bruce Beutler and Ernst Th. Rietschel

How does the host sense pathogens? Our present concepts grew directly from longstanding efforts to understand infectious disease: how microbes harm the host, what molecules are sensed and, ultimately, the nature of the receptors that the host uses. The discovery of the host sensors — the Toll-like receptors — was rooted in chemical, biological and genetic analyses that centred on a bacterial poison, termed endotoxin.

formed the conceptual framework within which early workers sought to identify it.

Impressed by the malodorous exhalations of patients suffering from plague and their similarity to the foul vapours emanating from marshes, physicians came to believe that the putative poison was generated by putrefaction (from the Greek for sepsis) of organic matter present in sick people or in locations such as swamps. The bad air of the marshes (nowadays still present in the term malaria = mal'aria) was named miasma (from the

not undergone decomposition failed to have such effects. In attempts to isolate and characterize the poisonous material, Peter L. Panum (1820–1885) could be considered a pioneer. He showed that putrid fluids contained a water-soluble, but alcohol-insoluble, heat-resistant, non-volatile substance, which was lethal to dogs[3]. Also, Ernst von Bergmann (1836–1906) believed that a chemically defined substance was responsible for putrid intoxication, which he termed sepsin[4].

Of course, the contagionists could not explain how a single contact with putrid fluids or a sick patient could transmit so much poison that not only the affected person, but also thousands of other people, would die. It was, therefore, an intellectual breakthrough to postulate that the putrid venom communicated by miasma or contagion could reproduce in the affected individual, thereby having attributes of a living organism. This revolutionary idea was formulated by Jacob Henle (1809–1885), who without knowing

frontiers in Immunology

Bacterial Exotoxins and the Inflammasome

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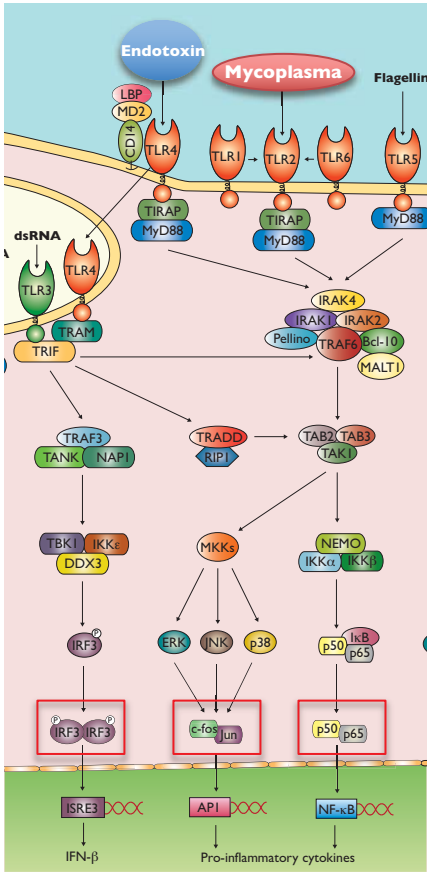
The inflammasomes are intracellular protein complexes that play an important role in innate immune sensing. Activation of inflammasomes leads to activation of caspase-1 and maturation and secretion of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18. In certain myeloid cells, this activation can also lead to an inflammatory cell death (pyroptosis). Inflammasome sensor proteins have evolved to detect a range of microbial ligands and bacterial exotoxins either through direct interaction or by detection of host cell changes

Endotoxin: False positive results

RIG-1, STING, TLRs, NOD1/NOD2, Inflammasome



Biological Activity Tested

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Certificate of Analysis

PRODUCT INFORMATION

Product: 2'-3'-cGAMP
Cat. code: tlrl-cga23-s
Lot number: GA3-36-09
Quantity: 500µg
Storage temperature: -20°C
Recommended Retest Date: Dec. 2016

QUALITY CONTROL

	Specifications	Results
Physico-chemical characteristics		
• *Appearance*	White lyophilizate	Conform
• *Solubility (in physiological water)*	1 mg / ml	Conform
Biological assay*		
• *TLR2 activity at 30 µg / ml*	Negative	Conform
• *TLR4 activity at 30 µg / ml*	Negative	Conform
• *ISG activity at 1 µg / ml*	Positive	Conform

* TLR activity tested on HEK-Blue™ TLR cells and ISG tested on THP1-Blue™ ISG cells

Date: 22 Dec. 2014

Reviewed by QA department:

Nicolas RICARD

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Caution – Not fully tested. For research use only. Not for human use.

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Product Box

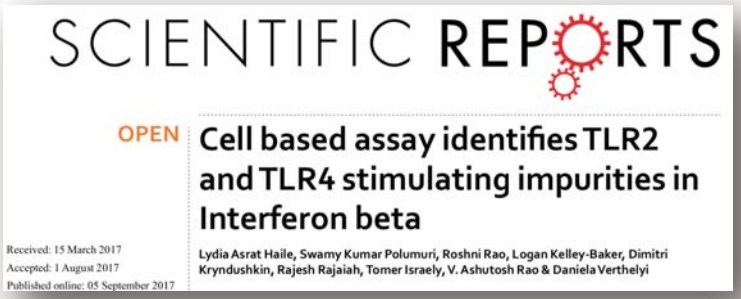
Product	Cat. code	Sensor
2'3'-cGAMP	tlrl-cga23	STING

TLR2,TLR4 Negative:
Avoid the contamination of
Bacteria, Mycoplasma, Fungi

TLR4 Negative:
Endotoxin Free

THP1-Blue™ Positive:
Biological activity confirmed

Anti-Contamination – Detection


SCIENTIFIC REPORTS

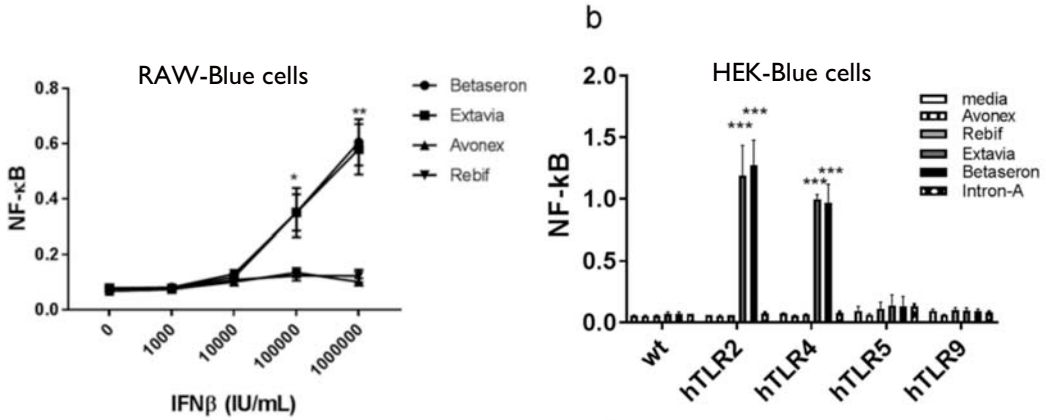
OPEN

Cell based assay identifies TLR2 and TLR4 stimulating impurities in Interferon beta

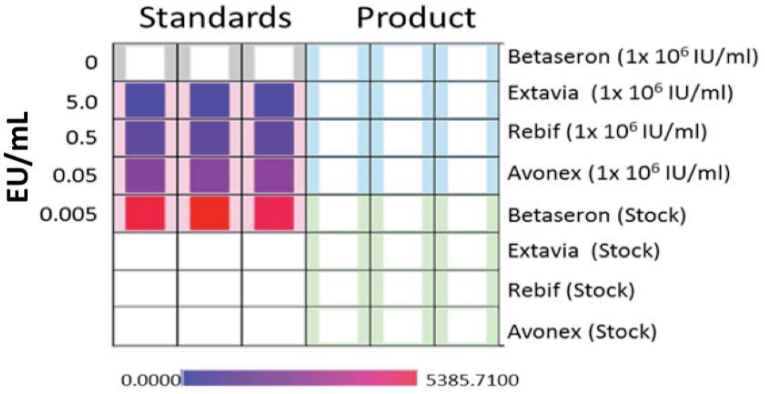
Received: 15 March 2017
Accepted: 1 August 2017
Published online: 05 September 2017

Lydia Asrat Haile, Swamy Kumar Polumuri, Roshni Rao, Logan Kelley-Baker, Dimitri Kryndushkin, Rajesh Rajaiah, Tomer Israely, V. Ashutosh Rao & Daniela Verthelyi

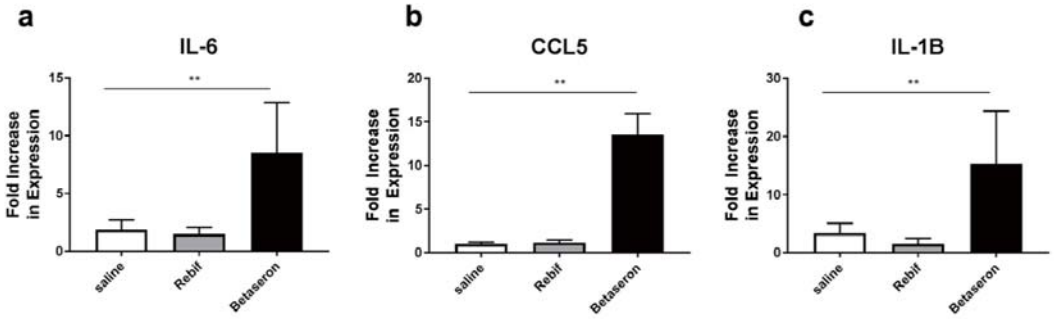
FDA tested TLR2/TLR4 contamination in commercial IFNb products



InvivoGen reporter cells suggest Betaseron contains TLR2/4 impurities



Commercial LAL-based tests showed no endotoxin (TLR4) contamination



Mice experiments suggest InvivoGen cell lines are accurate

InvivoGen cell lines detect low levels of impurities that are not evident in LAL assay

Anti-Contamination

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	Normocin™
	Primocin™
	Fungin™
Detection	PlasmoTest™
Elimination	Plasmocin™ treatment
	Plasmocure™
	Normocure™
	Fungin™

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Normocin™

	Mycoplasma	Bacteria	Fungi
Normocin™	✔	✔	✔
Pen/Strep	✗	✔	✗

Normocin™

PROTOCOL

Genome engineering using the CRISPR-Cas9 system

F Ann Ran[1–5,8], Patrick D Hsu[1–5,8], Jason Wright[1], Vineeta Agarwala[1,6,7], David A Scott[1–4] & Feng Zhang[1–4]

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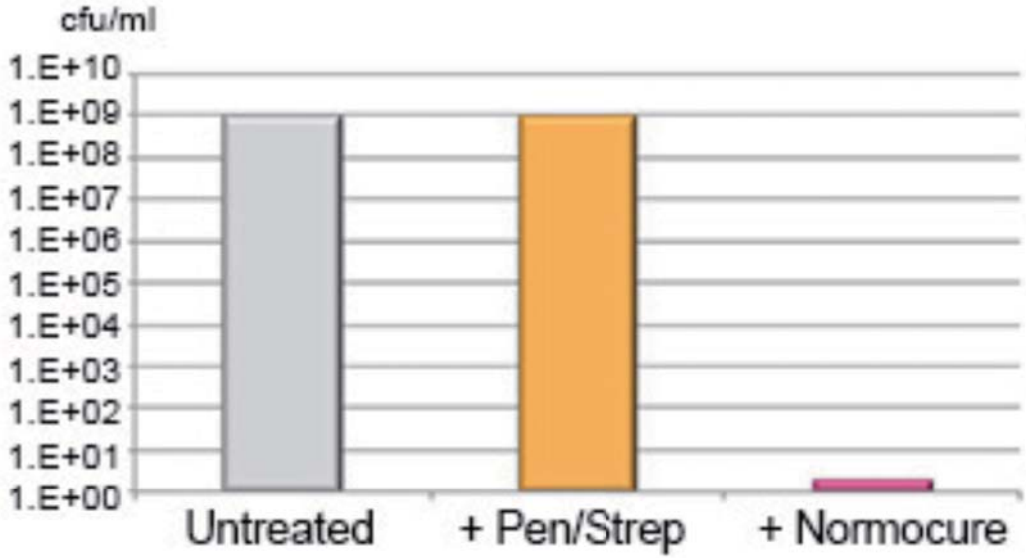
Normocure™

Anti - Bacteria



HEK293 cells ($3x10^5$ cells/ml) were spiked with a mixture of Gram- non-fermenting bacilli (*Pseudomonas aeruginosa*, *Alcaligenes xylosodans*, *Achromobacter sp*. and *Stenotrophomonas maltophilia*) at the concentration of 10^5 colony forming units (cfu)/ml, and were then either left untreated, or treated with 100 U/ml penicillin and 100 µg/ml streptomycin or with 100 µg/ml Normocure™, After 4 days at 37° C, 5% CO_2, the bacteria were quantified (cfu per ml).

SARS-CoV-2 Related Products

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COVID-19

SARS-CoV-2 (2019-nCoV) Research - Latest Insights & Products

The ongoing coronavirus disease-19 **(COVID-19)** pandemic is caused by the severe acute respiratory syndrome coronavirus-2, SARS-CoV-2 (or 2019-nCoV), a β-coronavirus that has crossed species barriers to infect humans [1, 2]. A similar disease was provoked in the past by two other β-coronaviruses, SARS-CoV in 2002 [3] and MERS-CoV in 2012 [4]. SARS-CoV-2 exhibits a high infectious rate and provokes a wide-array of symptoms (no clinical signs, mild, acute, or life-threatening). Today it represents a real burden not only for human health, but also for civil societies, and the global economy.

This is the third outbreak due to a highly-pathogenic β-coronavirus in only two decades. This points towards the tremendous need to acquire in-depth knowledge of the virus infectious cycle among species, the cellular pathways involved in the viral replication, and the mounting of innate and adaptive immune responses. Answers to these fundamental questions shall help in the design of safe and efficient therapeutics, as well as prophylactic vaccines.

InvivoGen offers an expanding set of tools to foster research on SARS-CoV-2 infection and immune responses:

COVID-19-RELATED GENES NEW

- SARS-CoV-2 Cellular Receptor Genes
- SARS-CoV-2 Structural Genes

COVID-19-RELATED ANTIBODIES NEW

- Anti-SARS-CoV-2 Antibodies

COVID-19-RELATED PROTEINS NEW

- Spike
- Human ACE2

COVID-19-RELATED INHIBITORS

- Viral RdRp Inhibitor NEW
  - Remdesivir
- JAK/STAT Signaling Inhibitors
  - Ruxolitinib
- Endosomal Acidification Inhibitors
  - Chloroquine
  - Bafilomycin A1

OTHER COVID-19-RELATED PRODUCTS

- Type I IFN Inducers
  - Poly(I:C)
  - 3p-hpRNA
- Fc-Fusion
  - pFUSE-Fc plasmids
- Antibody Generation
  - pFUSE
  - pTRIOZ
- Vaccine Adjuvants
  - Alum, emulsions, and PRR agonists

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Thank you !

tech.hk@invivogen.com