InvivoGen Insight
FALL 2012

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Cytosolic DNA Sensors (CDSs): a STING in the tail

The innate immune system provides the first line of defense against infectious pathogens and serves to limit their early proliferation. It is also vital in priming and activating the adaptive immune system. Innate immune detection of intracellular DNA derived from viruses and invasive bacteria is important to initiate an effective protective response. This crucial step depends on cytosolic DNA sensors (CDSs), which upon activation trigger the production of type I interferons (IFNs) and the induction of IFN-responsive genes and proinflammatory chemokines. Although the identity of these CDSs is not fully uncovered, much progress has been made in understanding the signaling pathways triggered by these sensors.

Cytosolic DNA-mediated production of type I IFNs (mainly IFN-β) requires the transcription factor IFN regulatory factor 3 (IRF3), which is activated upon phosphorylation by TANK-binding-kinase-1 (TBK1)[1]. Recently, a new molecule, STING (stimulator of IFN genes), has been shown to be essential for the TBK1-IRF3-dependent induction of IFN-β by transfected DNA ligands and intracellular DNA produced by pathogens after infection[2, 3]. STING (also known as MITA, MPYS and ERIS) is a transmembrane protein that resides in the endoplasmic reticulum (ER)[2-6]. In response to cytosolic DNA, STING forms dimers and translocates from the ER to the Golgi then to punctate cytosolic structures where it colocalizes with TBK-1, leading to the phosphorylation of IRF3. How STING stimulates TBK1-dependent IRF3 activation was recently elucidated by Tanaka and Chen. They found that, upon cytosolic DNA sensing, the C-terminal tail of STING acts as a scaffold protein to promote the phosphorylation of IRF3 by TBK1[7].

Until very recently, STING in addition to its role as an adaptor protein was also thought to function as a sensor of cyclic dinucleotides. Burdette *et al.* first demonstrated that STING binds directly to the bacterial molecule cyclic diguanylate monophosphate (c-di-GMP)[8]. This finding was confirmed by several teams who examined the structure of STING bound to c-di-GMP[9-11], including Cheng and colleagues, however their data suggest that STING is not the primary sensor of c-di-GMP[12]. Rather, they indicate that DDX41, an identified CDS (see next page), functions as a direct receptor for cyclic dinucleotides upstream of STING. The authors hypothesized that DDX41 binds to c-di-GMP then forms a complex with STING to activate the IFN response.

Exciting new developments reveal that STING participates in another aspect of innate immunity, autophagy. Autophagy plays a critical role in host defense responses to pathogens by promoting the elimination of microbes that enter into the cytosol by their sequestration into autophagosomes and their delivery to the lysosome. Recent studies have reported that DNA viruses and intracellular bacteria induce autophagy and that this process is dependent on cytosolic genomic DNA and STING[13-15]. Robust induction of autophagy was also observed after transfection of various double stranded (ds) DNA species, such as poly(dA:dT), poly(dG:dC) or plasmid DNA, but not single stranded (ss) DNA, dsRNA or ssRNA[16]. Interestingly, activated STING was shown to relocate to unidentified membrame-bound compartments where it colocalizes with LC3, a hallmark of autophagy, and ATg9a. The latter protein was reported to regulate the interaction between STING and TBK1 after dsDNA stimulation[16]. The E3 ubiquitin ligases TRIM 56 and TRIM32 were also found to regulate STING by mediating its dimerization through K63-linked ubiquitination[17, 18].

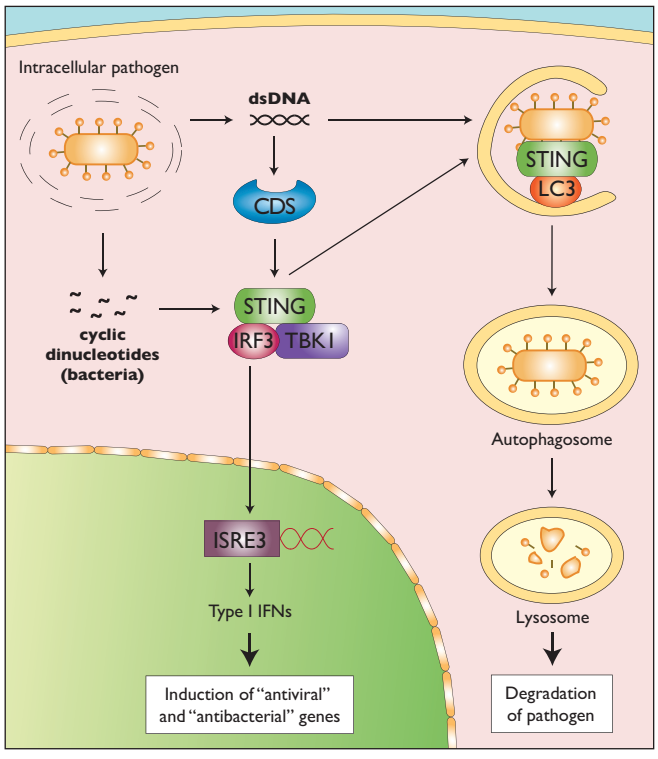
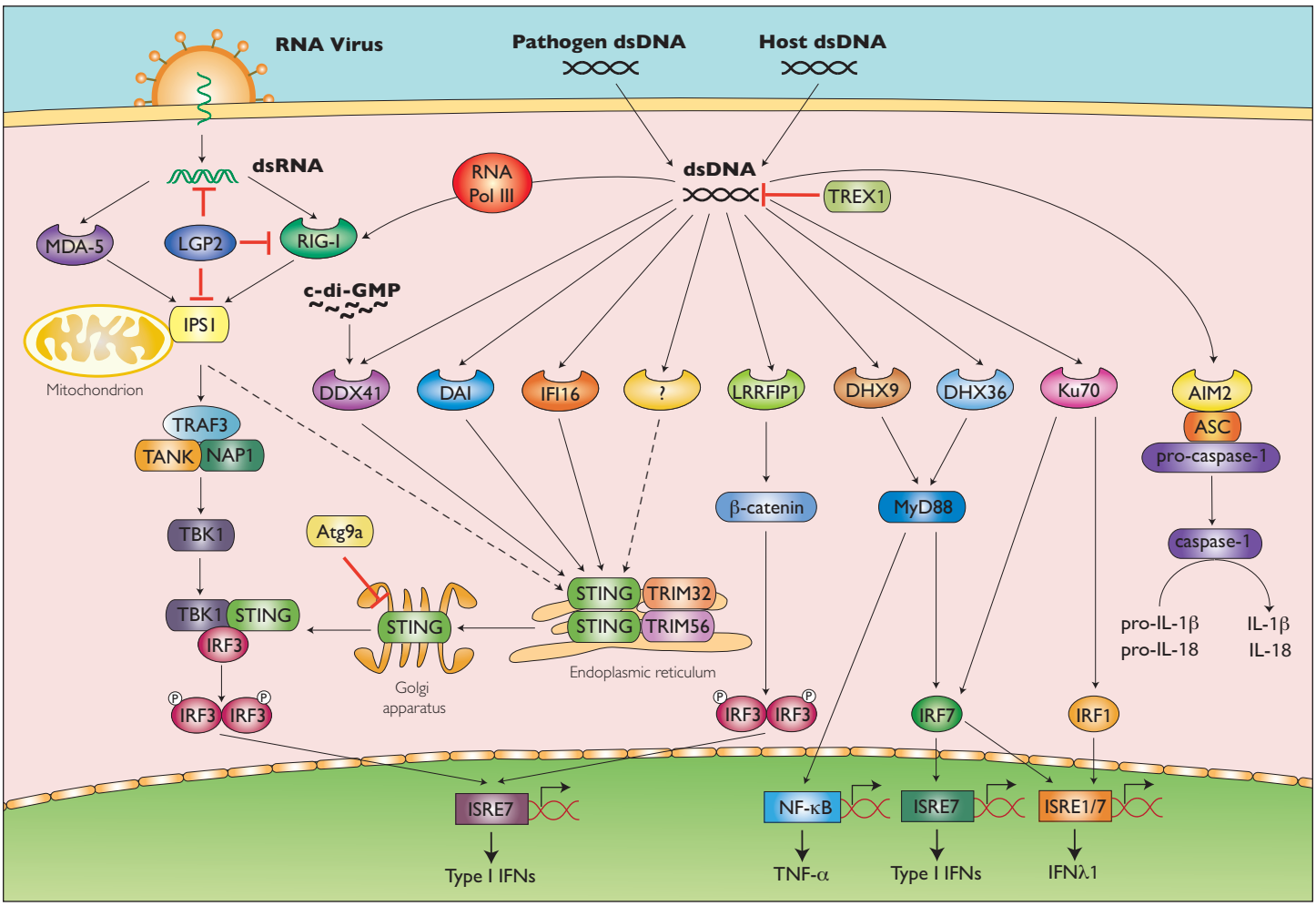


Figure 1: STING in the host response to intracellular pathogens. Linking type I IFN response and autophagy for better defense

Several cytosolic DNA sensors upstream of STING have been proposed. DNA-dependent activator of IRFs (DAI) was the first CDS discovered based on the ability of transfected poly(dA:dT) to induce IFN-β[19]. However, the role of DAI has been shown to be very cell-type specific and cells derived from DAI-deficient mice responded normally to dsDNA ligands[20]. While analyzing immune responses to dsDNA regions derived from vaccinia virus (VACV-70) or Herpes simplex virus 1 (HSV-60) genomes, Unterholzner *et al.* identified IFI16 as a DNA binding protein mediating IFN-β induction[21]. Interestingly, IFI16 belongs to a new family of pattern recognition receptors that contain the pyrin and HIN domain (PYHIN), termed AIM2-like receptors (ALRs). AIM2 is a STING-independent cytosolic DNA sensor that forms an inflammasome with ASC to trigger caspase-1 activation and the secretion of the pro-inflammatory cytokines IL-1β and IL-18[20]. Members of the DExD/H-box helicase superfamily have also been reported to function as cytosolic DNA sensors. While DHX36 and DHX9 were identified as STING-independent but MyD88-dependent sensors of CpG-containing DNA in plasmacytoid dendritic cells, DDX41 was found to bind various dsDNA ligands and localize with STING to promote IFN-β expression[22]. Other CDSs have been reported to function independently of STING: RNA Pol III, LRRFIP1 and Ku70[20].

Unlike cytosolic RNA sensors (RIG-I, MDA-5), which detect structural moieties specific to pathogen RNA, such as 5'-triphosphates, it is not clear whether cytosolic DNA sensors can recognize any particular structural motif of DNA that would discriminate between self and non-self. This suggests that CDSs may have a role not only in anti-microbial innate immune responses but also in autoimmunity. A multitude of CDSs have been described but whether they are all true receptors remains an open question.



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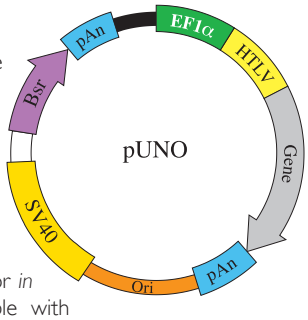
Nucleic Acid Sensors: Expression and Silencing

InvivoGen provides a comprehensive collection of genes, as well as shRNAs, that target genes involved in the signaling mediated by nucleic acid sensors (see illustration on opposite page). The genes are available in an expression vector with a strong and ubiquitous mammalian promoter, ready-to-use in protein expression studies. Similarly, short hairpin RNAs (shRNAs) are available as ready-made psiRNA plasmids validated for gene silencing.

Genes in pUNO Plasmids

Description



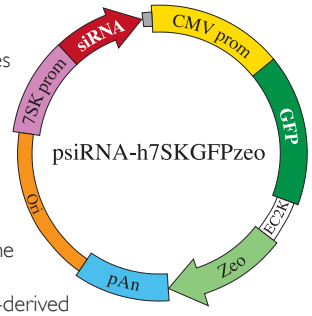
Genes involved in nucleic acid sensing are available in the pUNO plasmid. The genes are cloned from the ATG to the Stop codon, excluding introns and untranslated regions, and are expressed constitutively at high levels by the EF1α/HTLV composite promoter. All genes are fully sequenced, and their sequences are available on our website.
pUNO plasmids can be used directly for *in vitro* or *in vivo* transfection experiments. They are selectable with blasticidin in both *E. coli* and mammalian cells. To facilitate the excision and subcloning of the gene of interest into another vector, each gene is flanked by unique restriction sites that are compatible with many others.

Contents and Storage

Each pUNO plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least 1 year when properly stored. Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

shRNAs in psiRNA Plasmids

Description



Short hairpin RNAs (shRNAs) targeting the genes involved in nucleic acid sensing are expressed by a family of ready-made psiRNA plasmids. The functionality of these shRNAs has been described in the literature or validated in house. Ready-made psiRNA plasmids eliminate the need to design and clone several siRNA sequences before identifying an effective one. They express shRNAs that silence the expression of a target gene by >70%.
Ready-made psiRNAs are psiRNA-h7SKGFPzeo-derived plasmids that express high amounts of shRNAs through the human 7SK RNA Pol III promoter. They feature a GFP::Zeo fusion gene which confers both reporter and antibiotic resistance activities allowing simple monitoring of transfection efficiency and selection with Zeocin™ in both *E. coli* and mammalian cells.

Contents and Storage

Ready-made psiRNA plasmids are available alone or in a kit.
Ready-made psiRNA plasmids are provided as 20 µg of lyophilized DNA.
Ready-made psiRNA kits contain 20 µg of a Ready-made psiRNA plasmid, 20 µg of a control psiRNA plasmid (psiRNA-Luc), 1 vial of LyoComp GT116, 4 pouches of Fast-Media® Zeo. Products are shipped at room temperature. Store at -20°C.

GENE NAME	SPECIES	pUNO	psiRNA*
AIM2	Human Mouse	puno1-haim2 puno1-maim2	psirna42-haim2 psirna42-maim2
ASC	Human Mouse	puno-hasca puno1-masc	psirna42-hasc psirna42-masc
Caspase 1	Human Mouse	porf-hcasp1 porf-mcasp1	psirna42-hcasp1 psirna42-mcasp1
DAI / ZBP1	Human Mouse	puno1-hzbp1 puno1-mzbp1a	psirna42-hdai psirna42-mdai
DDX41	Human Mouse	puno1-hddx41 puno1-mddx41	psirna42-hddx41 psirna42-mddx41
DHX9	Human	puno1-hdhx9	psirna42-hdhx9
DHX36	Mouse	puno1-mdhx36	-
IFI16	Human Mouse	puno1-hifi16 puno1-mifi16	psirna42-hifi16 psirna42-mifi16
IPS-1	Human Mouse	puno-hips1 puno-mips1	psirna42-hips1 psirna42-mips1
IRF1	Human Mouse	puno-hirf1 puno-mirf1	psirna42-hirf1 psirna42-mirf1
IRF3	Human Mouse	puno-hirf3 puno-mirf3	psirna42-hirf3 psirna42-mirf3
IRF7	Human Mouse	puno1-hirf7 puno-mirf7	psirna42-hirf7 psirna42-mirf7
Ku70/XRCC6	Human Mouse	puno1-hxrcc6 puno1-mxrcc6	psirna42-hku70 psirna72-mku70
LGP2	Human Mouse	puno1-hlgp2 puno1-mlgp2	psirna42-hlgp2 psirna42-mlgp2
LRRFIP1	Human Mouse	puno1-hLRRFIP1b -	psirna42-hlrrfip1 psirna42-mlrrfip1
MDA-5	Human Mouse	puno1-hmda5 puno1-mmda5	psirna42-hmda5 psirna42-mmda5
NAP1	Human Mouse	puno-hnap1 puno-mnap1	psirna42-hnap1 psirna42-mnap1
RIG-I	Human Mouse	puno1-hrigi puno-mrigi	psirna42-hrigi psirna42-mrigi
STING	Human Mouse	puno1-hsting puno1-msting	psirna42-hsting psirna42-msting
TANK	Human Mouse	puno1-htank puno1-mtankb	psirna42-htank psirna42-mtank
TBK1	Human Mouse	puno-htbk1 puno-mtbk1	psirna42-htbk1 psirna42-mtbk1
TRAF3	Human Mouse	puno-htraf3 puno-mtraf3	psirna42-htraf3 psirna42-mtraf3
TREX1	Human Mouse	puno-htrex1 puno-mtrex1	psirna42-htrex1 psirna42-mtrex1
TRIM32	Human	puno1-htrim32	psirna42-htrim32
TRIM56	Human Mouse	puno1-htrim56 puno1-mtrim56	psirna42-htrim56 psirna42-mtrim56

* For the catalog code of the kit, replace psirna42 by ksirna42

CDS and STING Ligands

Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA. InvivoGen provides a comprehensive collection of double-stranded DNA ligands known to stimulate CDSs and single-stranded DNA that serve as negative controls. These ligands are provided either naked or already complexed to a transfection reagent (LyoVec™).

➢ CDS Ligands

HSV-60 and VACV-70 NEW

HSV-60 and VACV-70 are 60 bp and 70 bp oligonucleotides, respectively, containing viral DNA motifs[1]. HSV-60 derives from the herpes simplex virus 1 genome, while VACV-70 derives from the vaccinia virus DNA. Transfected HSV-60 and VACV-70 were shown to potently induce IFN-β in a TLR-, DAI- and RNA Pol III-independent, but STING-, TBK1- and IRF3-dependent manner. Both oligonucleotides are recognized by IFI16[1].

HSV-60c and VACV-70c are single-stranded oligonucleotides which, unlike their double-stranded counterparts, HSV-60 and VACV-70, respectively, are not IFN-inducers[1].

ISD NEW

ISD (interferon stimulatory DNA) is a 45-bp non-CpG oligomer from the *Listeria monocytogenes* genome. When transfected into various cell types, including plasmacytoid and conventional DCs, macrophages and murine embryonic fibroblasts, ISD strongly enhances the expression of IFN-β[2]. This ISD-induced response is mediated by the STING-TBK1-IRF3 signaling axis[2-3].

ISD Control is a non-immunostimulatory single-stranded oligonucleotide with the same sequence as its double-stranded counterpart.

pCpGfree-giant

pCpGfree-giant is a CpG-free non-coding high molecular weight (~15 kb) plasmid containing AT-rich regions. When transfected in ISG-reporter cells (see next page), pCpGfree-giant induces strong reporter activity. This activity requires STING as cells deficient for STING fail to respond to transfected pCpGfree-Giant (see data next page). The CDSs of pCpGfree-giant have not been identified yet, but they probably include IFI16 and DDX41.

Poly(dA:dT) & poly(dG:dC)

Poly(dA:dT) and poly(dG:dC) are repetitive synthetic double-stranded DNA sequences of poly(dA-dT)•poly(dT-dA) and poly(dG-dC)•poly(dC-dG), respectively. Poly(dA:dT) is a synthetic analog of the B-DNA form while poly(dG:dC) is a synthetic analog of the Z-DNA form. Poly(dA:dT) is recognized by several sensors, including DAI[4], IFI16[1], LRRFIP1[5] and DDX41[6]. So far, only LRRFIP1 has been clearly identified as a sensor for poly(dG:dC)[5].

Poly(dA) is a repetitive synthetic single-stranded DNA sequence of polydeoxyadenylic acid with no IFN stimulatory property.

➢ STING Ligands

c-di-AMP and c-di-GMP NEW

Cyclic diadenylate monophosphate (c-di-AMP) and cyclic diguanylate monophosphate (c-di-GMP) are second messenger molecules produced in bacteria but not in mammals. Both c-di-AMP and c-di-GMP can induce a strong immune response *in vitro* and *in vivo*. It was recently found that these cyclic dinucleotides induce the production of type I interferons in a STING dependent manner[7]. Additionally, STING was shown to directly sense these cyclic dinucleotides[8], although a very recent study suggests that DDX41 may function appstream of STING[9].

PRODUCT	QTY	CAT. CODE
CDS Ligands		
HSV-60 Naked	200 µg	tlrl-hsv60n
HSV-60 / LyoVec	100 µg	tlrl-hsv60c
HSV-60c Naked (control)	200 µg	tlrl-hsv60cn
HSV-60c / LyoVec (control)	100 µg	tlrl-hsv60cc
ISD Naked	200 µg	tlrl-isdn
ISD / LyoVec	100 µg	tlrl-isdc
ISD Control Naked	200 µg	tlrl-isdcn
ISD Control / LyoVec	100 µg	tlrl-isdcc
pCpGfree-giant Naked	200 µg	tlrl-cpgfn
pCpGfree-giant / LyoVec	100 µg	tlrl-cpgfc
Poly(dA) Naked	200 µg	tlrl-pan
Poly(dA) / LyoVec	100 µg	tlrl-pac
Poly(dA:dT) Naked	200 µg	tlrl-patn
Poly(dA:dT) / LyoVec	100 µg	tlrl-patc
Poly(dG:dC) Naked	200 µg	tlrl-pgcn
Poly(dG:dC) / LyoVec	100 µg	tlrl-pgcc
VACV-70 Naked	200 µg	tlrl-vav70n
VACV-70 / LyoVec	100 µg	tlrl-vav70c
VACV-70c Naked (control)	200 µg	tlrl-vav70cn
VACV-70c / LyoVec (control)	100 µg	tlrl-vav70cc
STING Ligands		
c-di-AMP	1 mg	tlrl-cda
c-di-GMP	1 mg	tlrl-cdg

Contents and Storage

All CDS ligands are available **naked or complexed** with the cationic lipid LyoVec™ to facilitate their uptake. STING ligands are provided naked.
CDS and STING ligands are provided lyophilized. Products are shipped at room temperature and should be stored at -20°C.

Related Products

5' Triphosphate blunt-end double-stranded (ds) RNA is a ligand for RIG-I, unlike blunt-end dsRNA, which thus serves as a negative control. InvivoGen provides both products naked or complexed with LyoVec™.
5'ppp-dsRNA Naked, 100 µg (tlrl-3prna-100)
5'ppp-dsRNA / LyoVec, 100 µg (tlrl-3prnalv-100)
5'ppp-dsRNA Control Naked, 100 µg (tlrl-3prnac-100)
5'ppp-dsRNA Control / LyoVec, 100 µg (tlrl-3prnaclv-100)
Products are also available as 25 µg units.

CDS Reporter Cell Lines

CDS and STING ligands trigger the production of type I IFNs and the induction of interferon stimulated genes (ISG) through interferon regulatory factors (IRFs). In order to facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages. They feature a novel reporter gene encoding a secreted luciferase, Lucia®.

➤ ISG-Luciferase Reporter Cells

THP1-Lucia™ ISG Cells NEW

THP1-Lucia™ ISG cells were derived from the human monocytic cell line THP-1, which represents a model of choice to study the activation and signaling of CDSs. Indeed, THP-1 cells have been shown to express all the CDSs identified so far[6,10,11], with the exception of DAI[12].

THP1-Lucia™ ISG cells express the secreted luciferase Lucia® reporter gene under the control of an IRF-inducible promoter. This composite promoter is comprised of five IFN-stimulated response elements (ISRE) fused to an ISG54 minimal promoter, which is unresponsive to activators of the NF-κB or AP-1 pathways. As a result, THP1-Lucia™ ISG cells allow the monitoring of the IRF pathway by determining the activity of Lucia®. The levels of IRF-induced Lucia® in the cell culture supernatant are readily assessed with QUANTI-Luc™, a Lucia® detection reagent. THP1-Lucia™ ISG cells are resistant to Zeocin™.

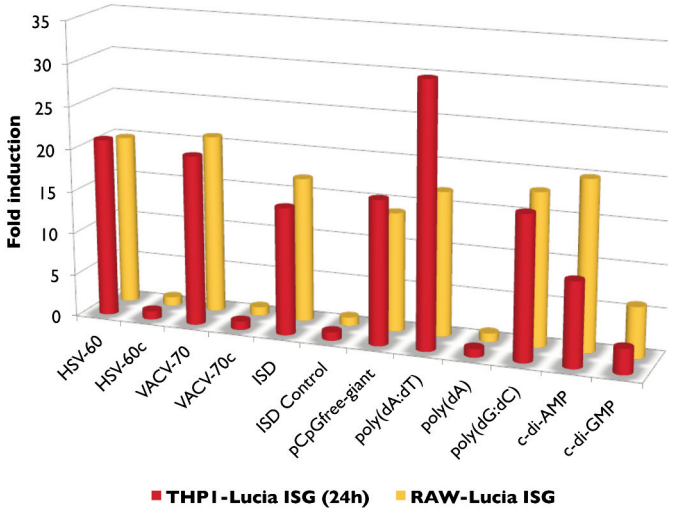
THP1-Lucia™ ISG cells are highly responsive to transfected double-stranded nucleic acids, such as CDS and STING ligands (see figure).

RAW-Lucia™ ISG Cells NEW

RAW-Lucia™ ISG cells were generated from the murine RAW 264.7 macrophage cell line by stable integration of an interferon regulatory factor (IRF)-inducible Lucia® reporter construct. RAW 264.7 have been reported to express IFI16[13], DDX41 and AIM2[14].

RAW-Lucia™ ISG cells express the Lucia™ gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. Thus, RAW-Lucia™ ISG cells allow the monitoring of IRF activation by determining the activity of Lucia®. The levels of IRF-induced Lucia® in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a Lucia® detection reagent. RAW-Lucia™ ISG cells are resistant to Zeocin™.

RAW-Lucia™ ISG cells are responsive to murine IFN-α and IFN-β but do not respond to their human counterparts. They are also responsive to PRR ligands that trigger the IFN signaling pathway, such as transfected double-stranded nucleic acids (see figure).



IRF response of THP1-Lucia™ ISG and RAW-Lucia™ ISG cells. Cells were transfected with CDS ligands (1 µg/ml of HSV-60, HSV-60c, VACV-70, VACV-70c, ISD, ISD Control or 0.3 µg/ml pCpGfree-giant, poly(dA:dT), poly(dA), poly(dG:dC) using LyoVec™ or directly stimulated with 10 µg/ml c-di-AMP or c-di-GMP. After 24h incubation, the levels of IRF-induced Lucia® were determined using QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Lucia™ ISG Cells	5-7 x 10^6 cells	thpl-isg
RAW-Lucia™ ISG Cells	5-7 x 10^6 cells	rawl-isg

Contents

THP1-Lucia™ ISG cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. RAW-Lucia™ ISG cells are grown in DMEM medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™ and 200 µg/ml Zeocin™. THP1-Lucia™ ISG and RAW-Lucia™ ISG cells are provided frozen in a cryotube containing 5-7 x 10^6 cells and supplied with 50 mg of Normocin™, 10 mg Zeocin™ and 1 pouch of QUANTI-Luc™.

Also Available

THP1-Blue™ ISG and **RAW-Blue™ ISG cells** feature an IRF-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene which expression is driven by the same promoter construct than their Lucia® counterparts. Levels of SEAP in the cell supernatant can be determined using QUANTI-Blue™.

Coming Soon

THP1-Blue™ defSTING and **THP1-Blue™ defTBK1** are ISG-SEAP reporter cells knock-down for STING or TBK1 expression, respectively. SEAP production is highly reduced in response to cytosolic nucleic acids compared to the parental cell line, THP1-Blue™ ISG.

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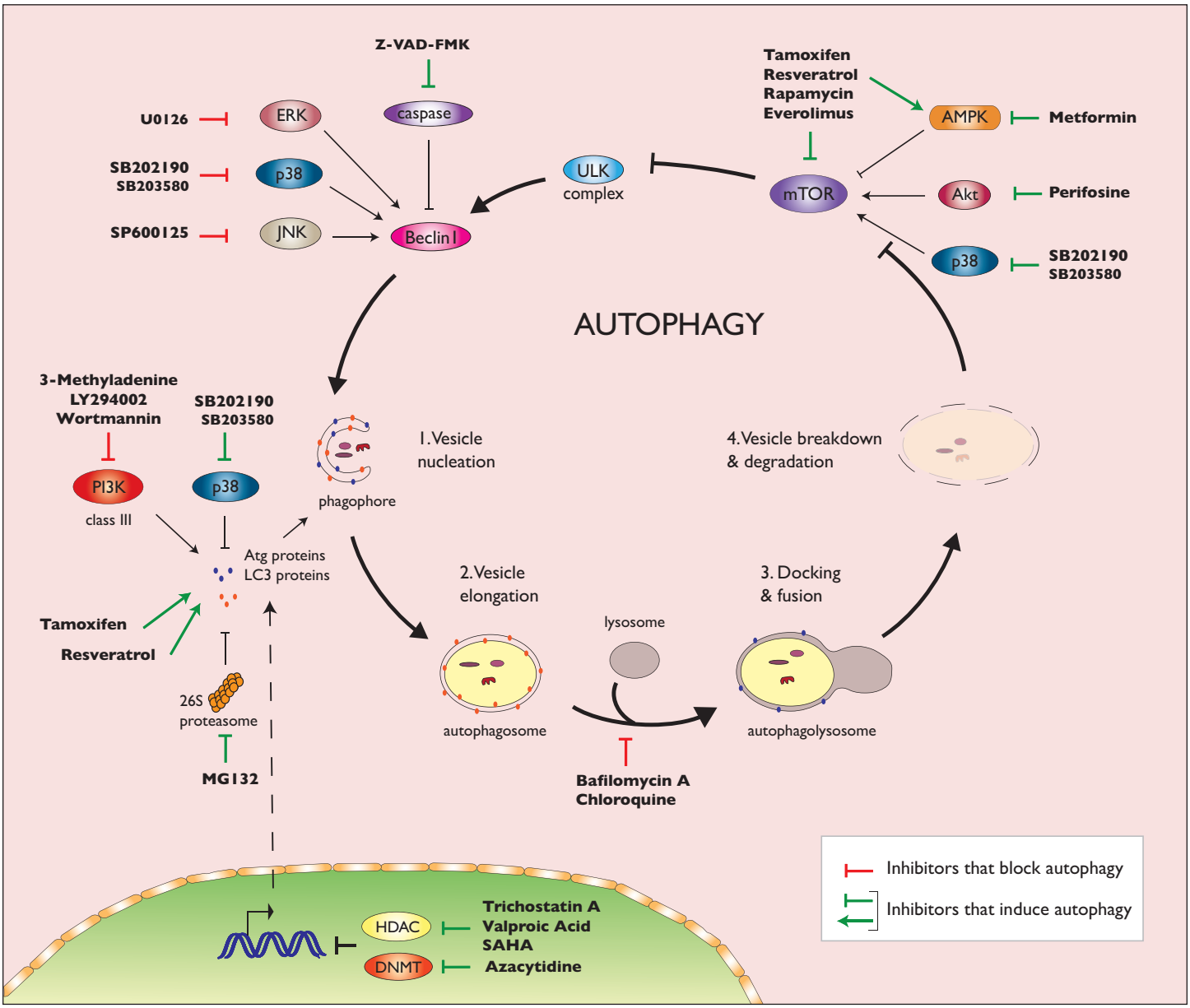
Autophagy

Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles. The autophagic process is also used to remove intracellular microbial pathogens. Several signaling pathways sense different types of cell stress, ranging from nutrient deprivation to microbial invasion, and converge to regulate autophagy at multiple stages of the process. A number of inhibitors that target molecules involved in these pathways can impact autophagy at different levels of the process, as depicted in the illustration below, and can be used to study autophagy in cells.



Inhibitors that block autophagy

The primary step in inducing autophagy involves membrane nucleation, controlled by ULK complex and Beclin1. Inhibitors of positive regulators of of the ULK complex and Beclin1 have been demonstrated to block autophagy. These include inhibitors to the MAP kinases, JNK1, ERK and p38. The induction of Atg protein and LC3 proteins is required for vesicle expansion and formation. Inhibitors of the class III PI3 kinases can block autophagy. In a later step of the autophagic process, inhibitors that inhibit lysosome acidification essentially block the formation of autophagosome and autophagic degradation.

Inhibitors that induce autophagy

mTOR is a major negative regulatory axis of autophagy and is influenced by several nutrient signaling pathways. Direct inhibitors of mTOR and those of pathways activating mTOR, subsequently induce autophagy by inhibiting mTOR. In addition, Beclin1 is negatively regulated by caspases, the inhibitors of which act to promote Beclin1 action to induce the initial stages of autophagy. Furthermore, inhibitors of the 26S proteasome and the epigenetic regulators, HDACs and DNMTs, result in the increase of Atg and LC3 proteins levels essential to the process of autophagy.

➢ Inhibitors of Autophagy

Inhibitors of Beclin1

SP600125 - JNK Inhibitor
SP600125 is a potent, and selective inhibitor of c-Jun N-terminal kinase (JNK). Inhibition of JNK activity by SP600125 is usually associated with downregulation of Beclin-1 and reduced autophagy.

U0126 - MEK1/MEK2 Inhibitor
U0126 is a selective inhibitor of the MAP kinase kinases, MEK1 and MEK2, and thus of ERK activation. U0126 can be used to study the role of ERK, a MAPK involved in the induction of autophagy.

Inhibitors of autophagosome / autophagolysosome formation

3-Methyladenine, LY294002 & Wortmannin - PI3K Inhibitors
The PI3K inhibitors, 3-methyladenine (3MA), LY294002 and wortmannin have a net inhibitory effect on autophagy through blocking the class III PI3Ks that are critical during the late stage of vesicle expansion.

SB203580 & SB202190 - p38 MAP Kinase Inhibitors
SB203580 and its close relative, SB202190, are widely used to assess the physiological roles of p38α and p38β MAPKs. Several studies suggest that p38 MAPKs regulate distinct phases of autophagy. p38 can elicit autophagy via Beclin1. Contrarily, p38α has also been reported to inhibit autophagy by interfering with the trafficking of Atg9.

Bafilomycin A1 & Chloroquine - Endosomal Acidification Inhibitor
Bafilomycin A1 and chloroquine are known inhibitors of the late phase of autophagy. They both cause a disruption in the pH of acidic vesicles preventing the fusion of autophagosomes with lysosomes

PRODUCT	QUANTITY	CAT. CODE
3-Methyladenine	50 mg	tlrl-3ma
5-Aza-cytidine	100 mg	inh-aza
Bafilomycin A1	10 µg	tlrl-baf
Chloroquine	250 mg	tlrl-chq
Everolimus	5 mg	tlrl-eve
LY294002	5 mg	tlrl-ly29
Metformin	1 g	tlrl-metf
MG-132	5 mg	tlrl-mg132
Perifosine	5 mg	tlrl-peri
Rapamycin	5 mg	tlrl-rap
Resveratrol	100 mg	tlrl-resv
SAHA	25 mg	inh-saha
SB202190	5 mg	tlrl-sb90
SB203580	5 mg	tlrl-sb20
SP600125	10 mg	tlrl-sp60
Tamoxifen	200 mg	tlrl-txf
Trichostatin A	1 mg	met-tsa-1
U0126	5 mg	tlrl-u0126
Valproic acid	5 g	inh-vpa
Wortmannin	5 mg	tlrl-wtm
Z-VAD-FMK	1 mg	tlrl-vad

➢ Activators of Autophagy

Inhibitors of mTOR activation

Metformin - AMPK Activator
Metformin activates adenosine monophosphate-activated protein kinase (AMPK), an enzyme that coordinates control of cell growth and autophagy. Metformin can be used to induce autophagy in an AMPK-dependent manner.

Perifosine - Akt Inhibitor
Perifosine is the best characterized inhibitor of Akt. mTOR is a target for Akt, the activation of which suppresses autophagy. Therefore, treatment with perifosine, alone or in combination with rapamycin, induces autophagy.

Rapamycin & Everolimus - mTOR Inhibitors
Rapamycin (sirolimus) and its analog, everolimus (RAD001), are potent inhibitors of mTOR, the master negative regulator of autophagy. Both inhibitors are powerful inducers of autophagy.

Resveratrol - mTOR Inhibitor
Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol found in plants displaying autophagy-promoting activities. Resveratrol induces autophagy through its capacity to both inhibit mTOR and activate AMPK.

Tamoxifen - Estrogen Receptor Antagonist
Tamoxifen is an antagonist of the estrogen receptor known to induce autophagy. Tamoxifen stimulates autophagy by increasing the intracellular level of ceramide, which inhibits mTOR activation and/or stimulates expression of Atg genes.

Activators of autophagosome formation

MG-132 - 26S Proteasome Inhibitor
MG-132 selectively blocks the proteolytic activity of the 26S proteasome and is used to disrupt the proteasome-regulated degradation of proteins. Recent studies suggest that inhibition of the proteasome by MG-132 can induce autophagy by stabilizing Atg protein levels.

SAHA, Trichostatin A & Valproic acid - HDAC Inhibitors
SAHA (Vorinostat), Trichostatin A and Valproic Acid are histone deacetylase (HDAC) inhibitors. HDACs epigenetically silence the transcription of Atg and LC3 genes. Thus, HDAC inhibitors can lead to an increase of Atg and LC3 proteins levels and promote autophagy.

5-Aza-cytidine - DNA Methyltransferase Inhibitor
5-aza-cytidine (AZA) is a potent inhibitor of DNA methyltransferase 1 (DNMT1). Similar to HDAC inhibitors, AZA can enhance the expression of Atg and LC3 and hence induce autophagy.

Z-VAD-FMK - Caspase Inhibitor
Z-VAD-FMK is a cell-permeable pan-caspase inhibitor that irreversibly binds to the catalytic site of caspase proteases. Through the inhibition of caspases 3, 7 and 8, Beclin1 is stabilized and autophagy is promoted.

Warning: The net effect of many inhibitors on autophagy is dependent on the induction cue, cell-type and the stage of the autophagic process. Differential or off-target effects by any one particular inhibitor may be seen. It is advisable to confirm effects through use of multiple inhibitors.

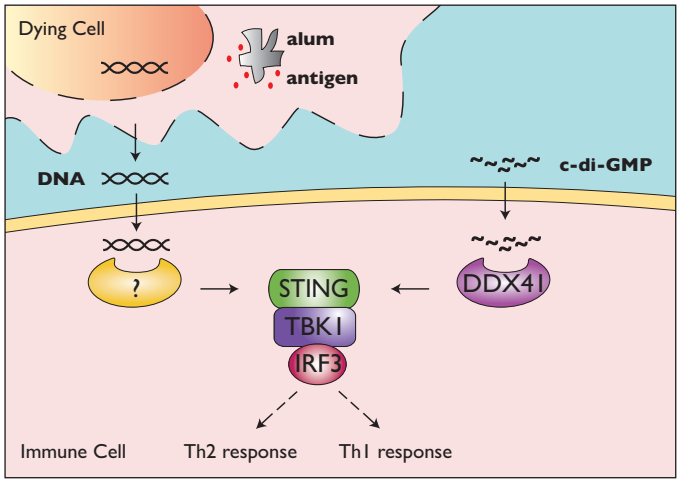
Contents and Storage

Products are provided as solids and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label. Products are stable at least 6 months.

STING-in Vaccine Adjuvants

Adjuvants enhance and direct the adaptive immune response to vaccine antigens through various mechanisms, some of which are still poorly understood. Recently, STING and more generally the host nucleic acid sensing machinery were shown to play an essential role in vaccination. Aluminum salt formulations (referred to as alum) have been used for decades as effective and safe vaccine adjuvants. Alum mostly potentiates IgG1 and IgE production through the promotion of Th2 cell responses. It has long been known that alum causes cell death and the release of numerous molecules that act as damage-associated molecular patterns (DAMPs). Two of these DAMPs, which are both connected to nucleic acid biology, have been identified. Uric acid[1], which is the end product of the degradation of purines, and host cell DNA[2] have been shown to accumulate at sites of alum injection and to induce T cell responses and the production of IgG1 and IgE. The signaling pathways activated by uric acid in this context have not been identified, nor the cytosolic DNA sensor(s) triggered by host DNA in alum immunization[1,2]. However, the (STING)-TBK1-IRF3 axis has been shown to control the IgE response[2].

The STING-TBK1-IRF3 axis has also been involved in the adjuvant activity of c-di-GMP. Cyclic-di-GMP is a bacterial signaling molecule with strong immunostimulatory activity, which is currently being investigated as a mucosal adjuvant[3,4]. Several studies have shown that intranasal immunization with c-di-GMP promotes predominantly Th1 responses, essential for the elimination of intracellular pathogens. Although its adjuvancy mechanism is not yet understood, c-di-GMP is known to activate the innate immune system[5] and induce the production of type I IFNs through STING-TBK1-IRF3[6], the same signaling pathway used by intracellular DNA. According to a very recent study, c-di-GMP is recognized by the cytosolic DNA sensor DDX41[7] and not by STING directly, as previously thought[8]. The data suggest that DDX41 binds to c-di-GMP then forms a complex with STING to facilitate downstream signaling and the activation of type IFNs. It is intriguing that the STING-TBK1-IRF3 signaling pathway can promote two distinct Th responses.



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InvivoGen provides different classes of vaccine adjuvants that are either already approved for use in human vaccination or under investigation. InvivoGen's adjuvants are VacciGrade™, a specific grade for *in vivo* studies. They are prepared under strict aseptic conditions. They are guaranteed sterile and thoroughly tested for the presence of endotoxins.

Alhydrogel® 2%

Alhydrogel® 2% is an aluminium hydroxide (alum) wet gel suspension and the most widely used adjuvant in licensed human vaccines. Alum causes the adsorption of protein antigens, slowing their release from the injection site (the "depot effect") and facilitating their uptake by antigen-presenting cells. Alum increases Th2 antibodies but does not promote significant Th1 cellular response. Alum has long been known to trigger the production of the proinflammatory cytokine IL-1β which was recently found to be mediated by the NLRP3 inflammasome[1]. However, the contribution of the NLRP3 inflammasome in the adjuvant effect of alum remains unclear.

c-di-GMP & c-di-AMP VacciGrade™

Cyclic diguanylate monophosphate (c-di-GMP) and cyclic diadenylate monophosphate (c-di-AMP) are intracellular signaling molecules produced by bacteria. Both molecules have been shown to activate the host innate immune system, which has prompted their evaluation as vaccine adjuvants. Preclinical studies have proven that co-administered c-di-GMP elicits a Th1-biased response after systemic or mucosal vaccination[2], while recent data suggest that c-di-AMP promotes a balanced Th1/Th2/Th17 reponse pattern[3].

Contents and Storage

Alhydrogel 2% is provided as a ready-to-use, sterile wet gel suspension. c-di-GMP and c-di-AMP VacciGrade™ are provided lyophilized. Products are shipped at room temperature. Store at 4°C or -20°C according to the product label.

PRODUCT	QUANTITY	CAT. CODE
Alhydrogel® 2% (Alum)	250 ml	vac-alu-250
c-di-AMP VacciGrade™	1 mg	vac-cda
c-di-GMP VacciGrade™	1 mg	vac-cdg

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Check our website for the complete list of InvivoGen's vaccine adjuvants
http://www.invivogen.com/vaccine-adjuvants

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