InvivoGen
INNOVATION WITHIN REACH

Inflammasome:
The New Spotlight of Immunity

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- RIG-I/PACT and Measles Virus

SCHOOL OF BIOMEDICAL SCIENCES
THE UNIVERSITY OF HONG KONG
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InvivoGen Limited

Technical Sales Specialist
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History of InvivoGen Product Family

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

History of InvivoGen Product Family

1970s

Gerard Tiraby discovered antibiotics resistant gene: *Sh ble*

1977

Zeocin™

1980s

Selective Antibiotics

\+ Anti-contamination Antibiotics

1997

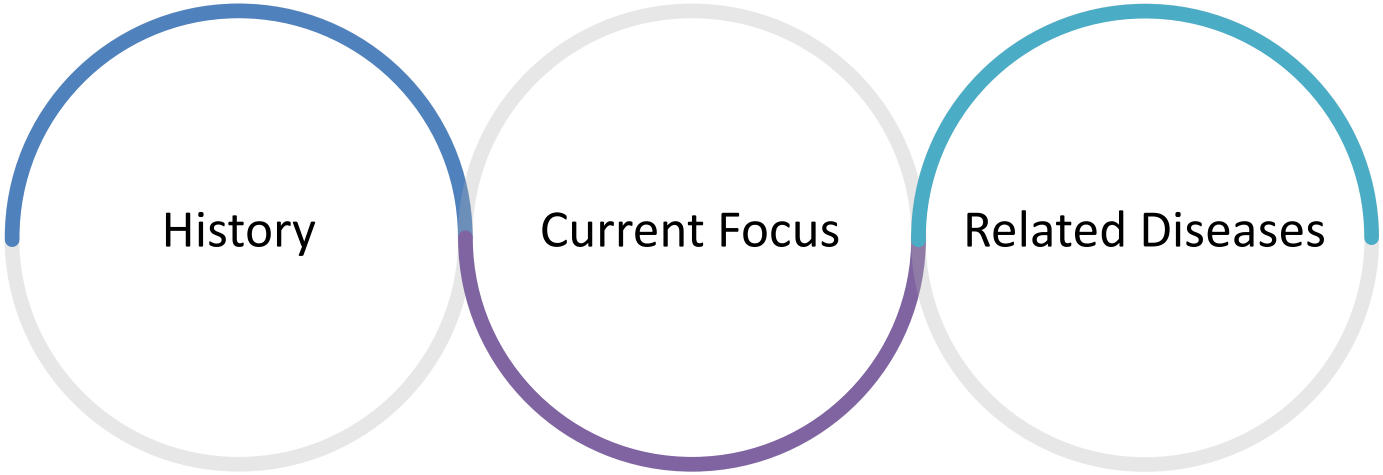
Innate Immunity

Pattern Recognition Receptors (PRRs) and Pathogen Associated Molecular Pattern (PAMPs)

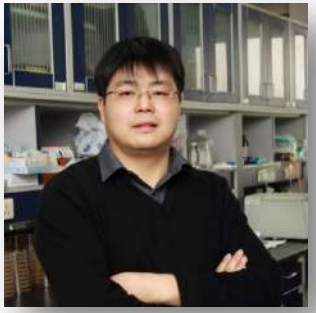
2000

Innate Immunity-related Products

Inflammasome



History of Inflammasome



Cloning and characterization of IL-1β
Auron, PNAS 1984

1984

Identification of NALP1 protein (NLRP1)
Bertin, Cell Death Differ. 2000

2000

Discovery of NLRP3 inflammasome by Jürg Tschopp
Agostini, Immunity 2004
and discovery of NLRC4 inflammasome by Vishva Dixit
Mariathansan, Nature 2004

2004

Discovery of GasderminD by Vishva Dixit
Kayagaki, Nature 2015
and Feng Shao
Shi, Nature 2015

2015

1992

Identification of Caspase1 as IL-1-converting enzyme
Thornberry, Nature 1992

2002

Discovery of NLRP1 inflammasome by Jürg Tschopp
Martinon, Mol Cell 2002

2011

Discovery of Non-canonical inflammasome pathway by Vishva Dixit
Kayagaki, Science 2011

Discovery of Inflammasome

Jürg Tschopp,
University of Lausanne,
Switzerland

LPS

?

Casp-1

IL-1β

Immune response

NBS family

NALP3

Apaf-1

NOD

NALP1

High expression in spleen and thymus

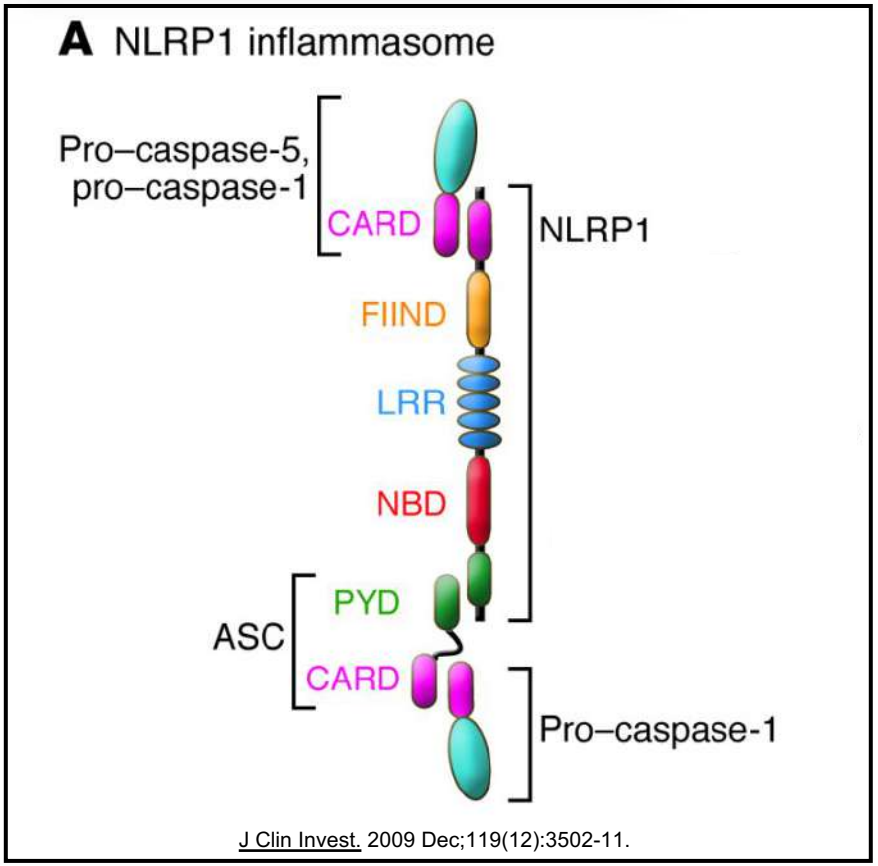
Caspase activation

Immune response?

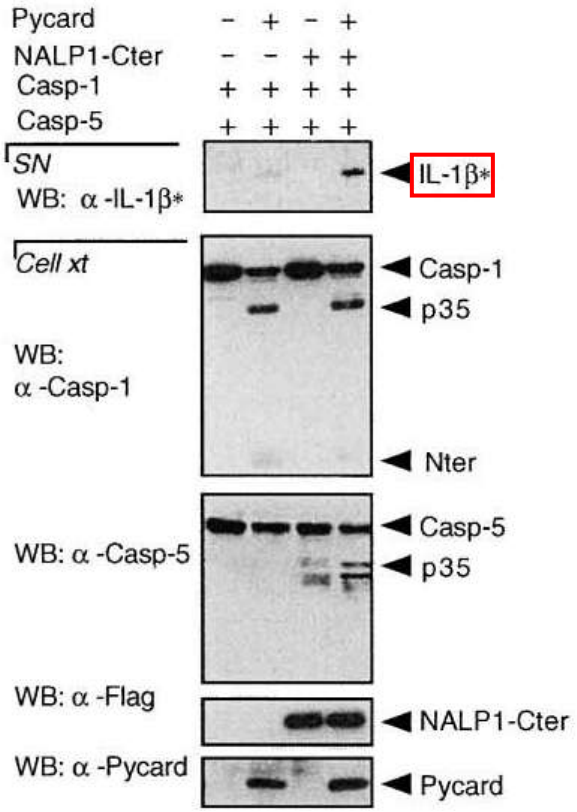
Discovery of Inflammasome (NLRP1) - 2002

Molecular Cell, Vol. 10, 417–426, August, 2002, Copyright ©2002 by Cell Press

The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-β



J Clin Invest. 2009 Dec;119(12):3502-11.



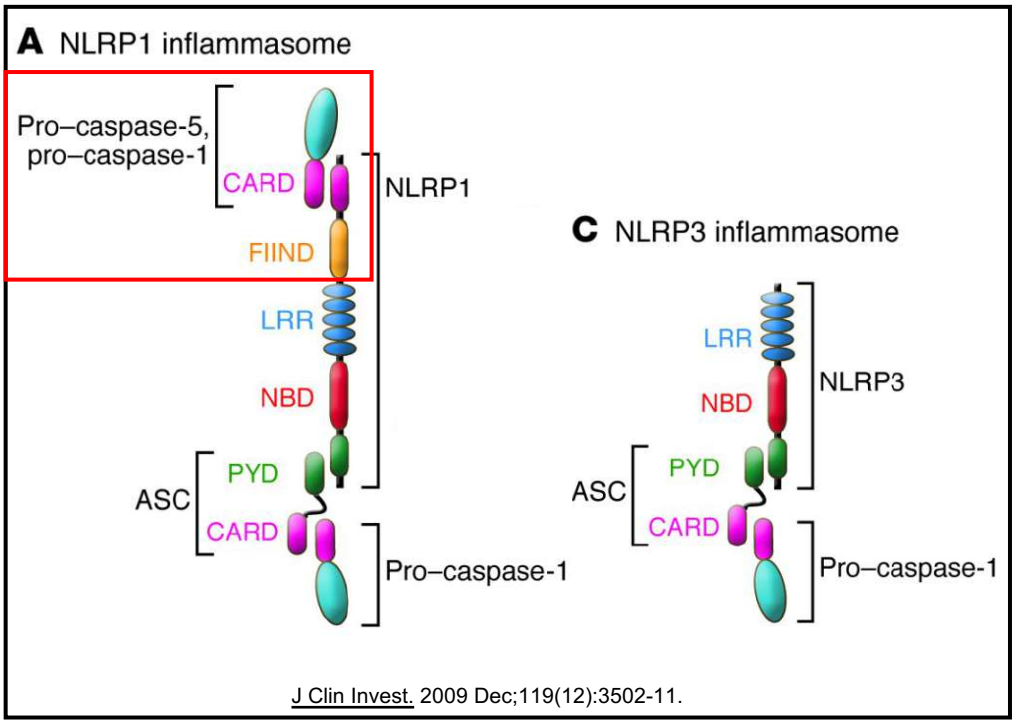
Expression of ASC, NALP1, Casp-1 and Casp-5 activates IL-1β

Discovery of NLRP3 Inflammasome - 2004

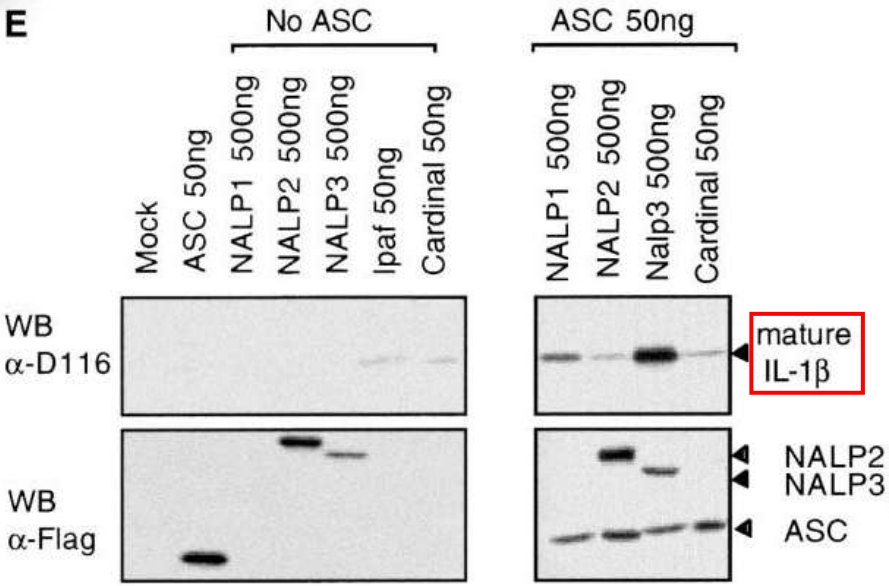
Immunity, Vol. 20, 319–325, March, 2004, Copyright ©2004 by Cell Press

NALP3 Forms an IL-1β-Processing Inflammasome with Increased Activity in Muckle-Wells Autoinflammatory Disorder

What about NLRP3 without CARD domain (no Casp5 binding)?



J Clin Invest. 2009 Dec;119(12):3502-11.



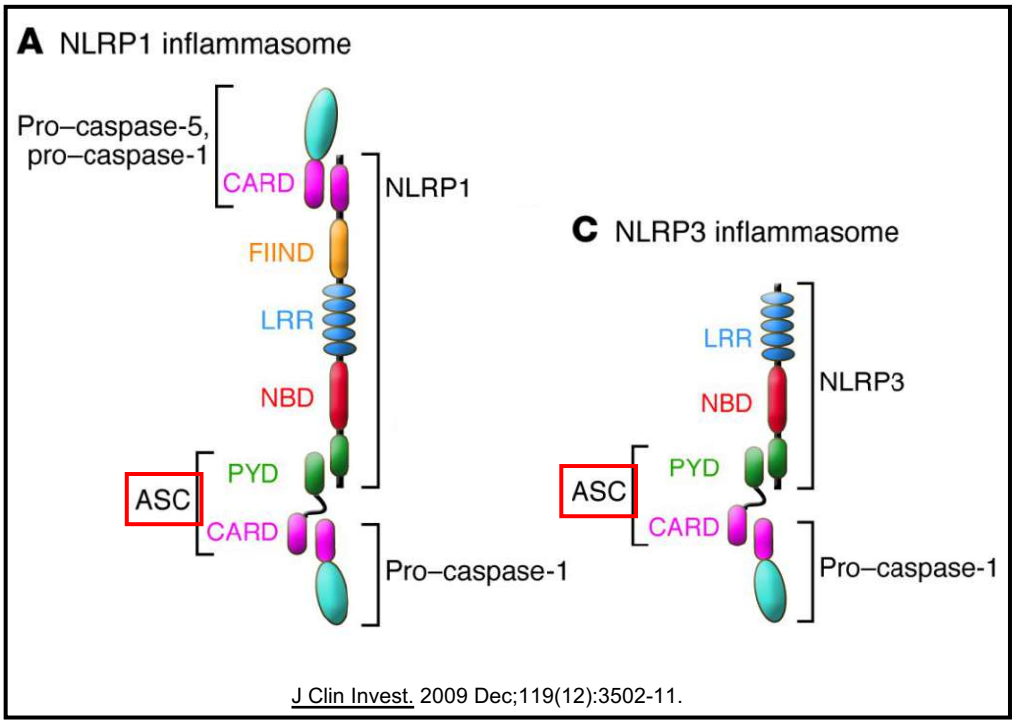
Expression of ASC, NLRP3 and Casp-1 activates IL-1β

ASC as an Essential Component for Inflammasome - 2004

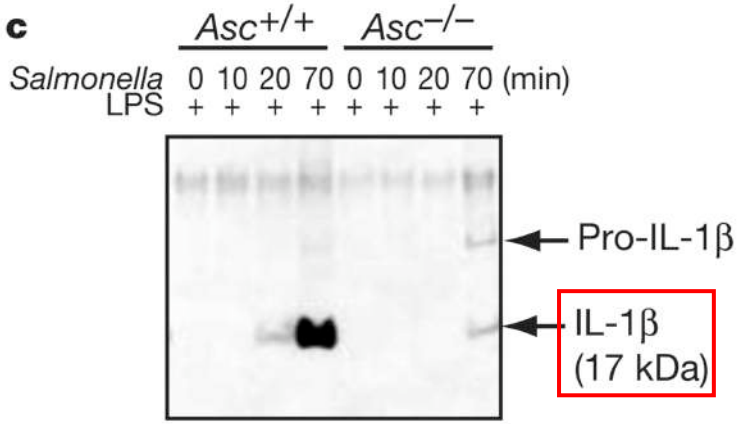
Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf

Sanjeev Mariathasan[1], Kim Newton[1], Denise M. Monack[4], Domagoj Vucic[1], Dorothy M. French[1], Wyne P. Lee[2], Meron Roose-Girma[3], Sharon Erickson[3] & Vishva M. Dixit[1]

Is ASC essential in physiological setting?



J Clin Invest. 2009 Dec;119(12):3502-11.



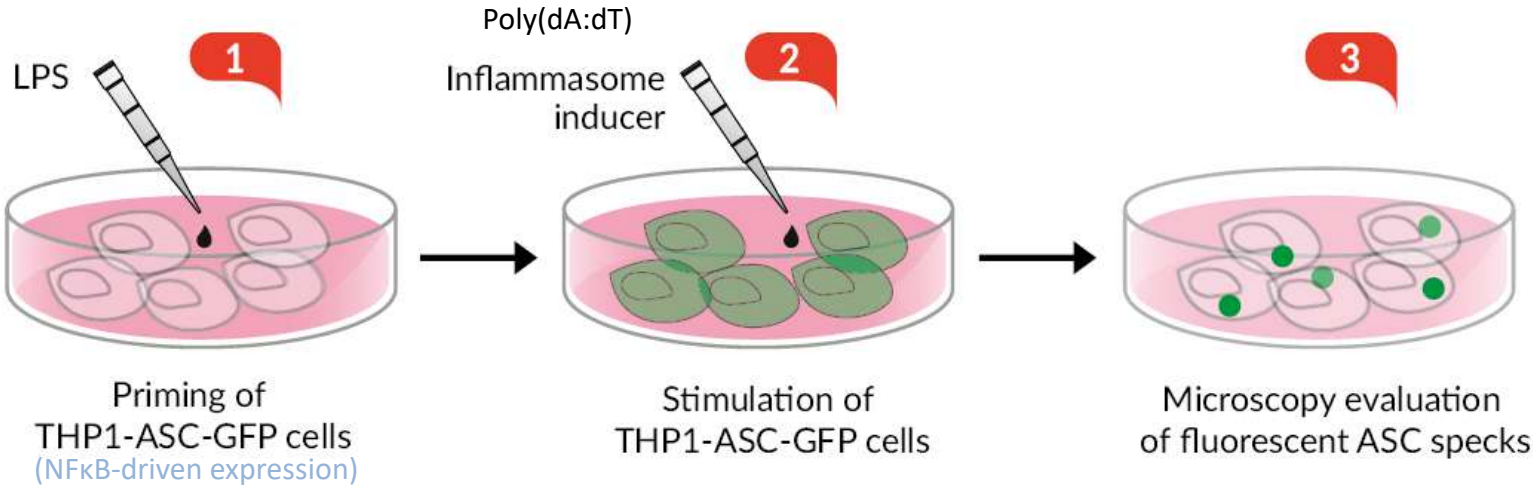
ASC is essential for IL-1β processing in peritoneal macrophages

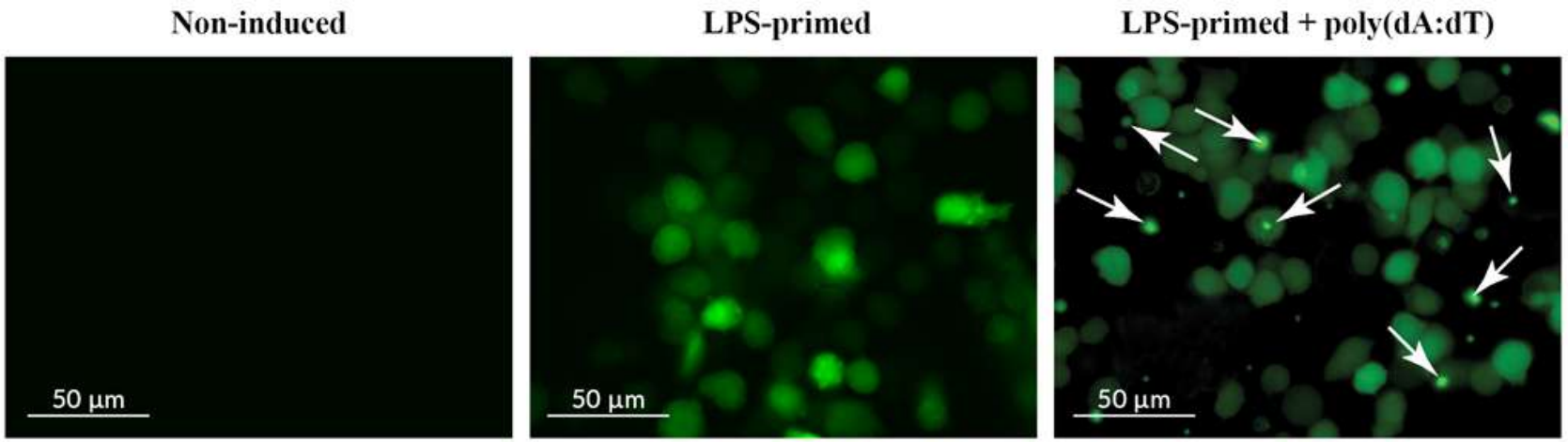
(ASC: Apoptosis-associated Speck-like protein containing a CARD)

Product Used	Cat. Code
Heat Killed Listeria monocytogenes	tlrl-hklm
Lipoteichoic acid from B.subtilis	tlrl-lta
Peptidoglycan from S.aureus	tlrl-sipgn
LPS-EB	tlrl-eblps
R848 (Resiquimod)	tlrl-r848

Real-time Monitoring of ASC-dependent Inflammasomes

Product	Unit Size	Cat. Code
THP1-ASC-GFP	3-7 x 10^6 cells	thp-ascgfp



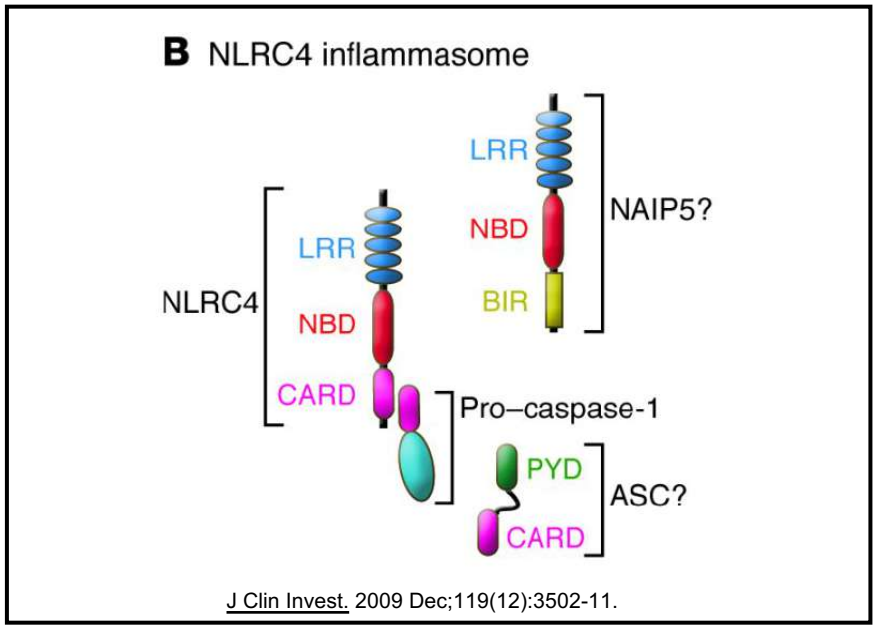


Discovery of NLRC4 Inflammasome - 2004

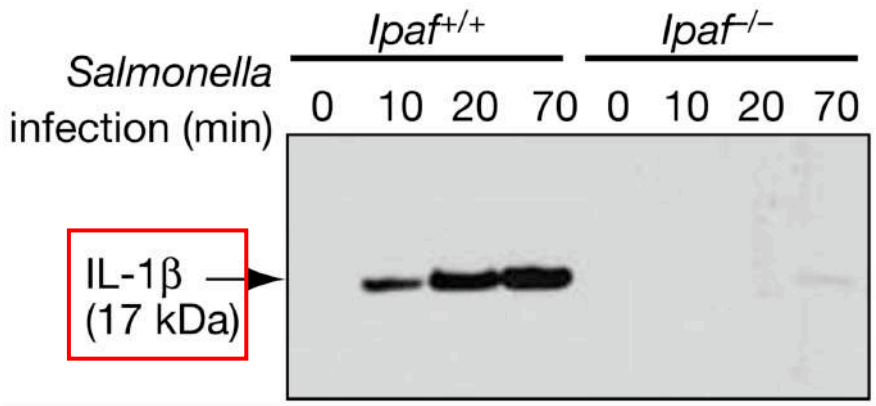
Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf

Sanjeev Mariathasan[1], Kim Newton[1], Denise M. Monack[4], Domagoj Vucic[1], Dorothy M. French[1], Wyne P. Lee[2], Meron Roose-Girma[3], Sharon Erickson[3] & Vishva M. Dixit[1]

Ipaf binds Casp-1, What is the role of Ipaf in Casp-1 activation?



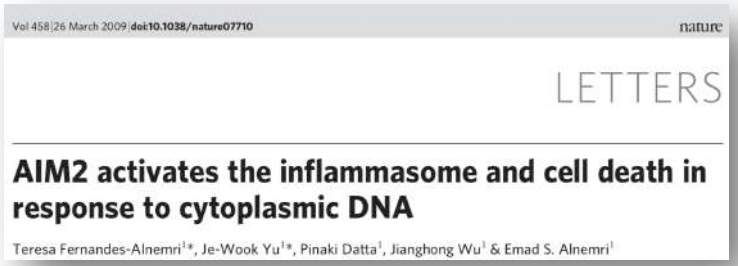
J Clin Invest. 2009 Dec;119(12):3502-11.



Ipaf(NLRC4) is essential for IL-1β activation

Product Used	Cat. Code
Heat Killed Listeria monocytogenes	tlrl-hklm
Lipoteichoic acid from B.subtilis	tlrl-lta
Peptidoglycan from S.aureus	tlrl-sipgn
LPS-EB	tlrl-eblps
R848 (Resiquimod)	tlrl-r848

Discovery of AIM2 Inflammasome - 2009

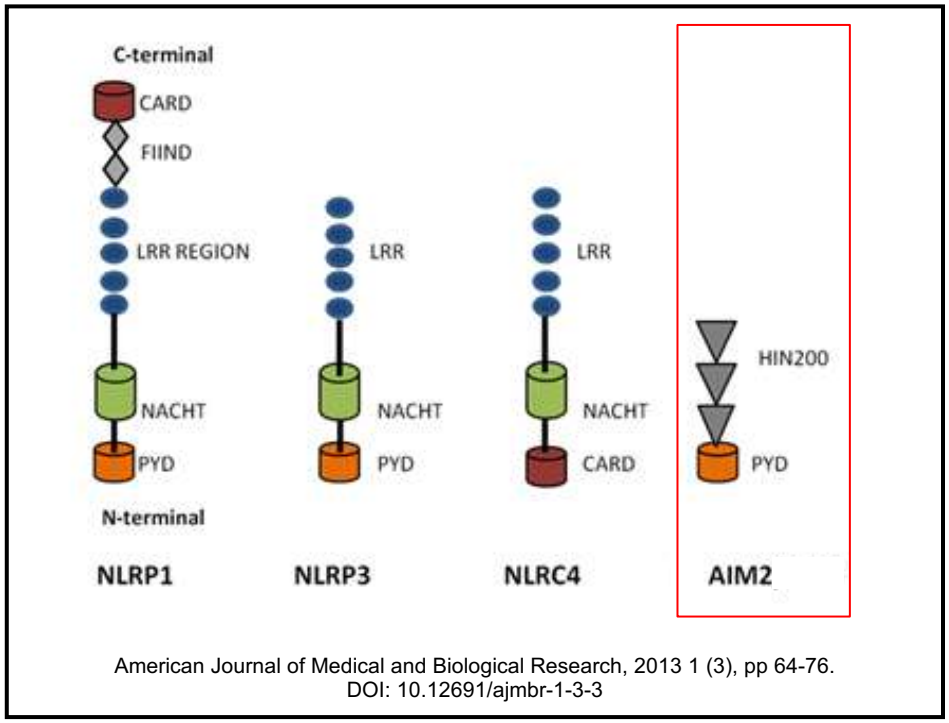

Vol 458|26 March 2009|doi:10.1038/nature07710 nature

LETTERS

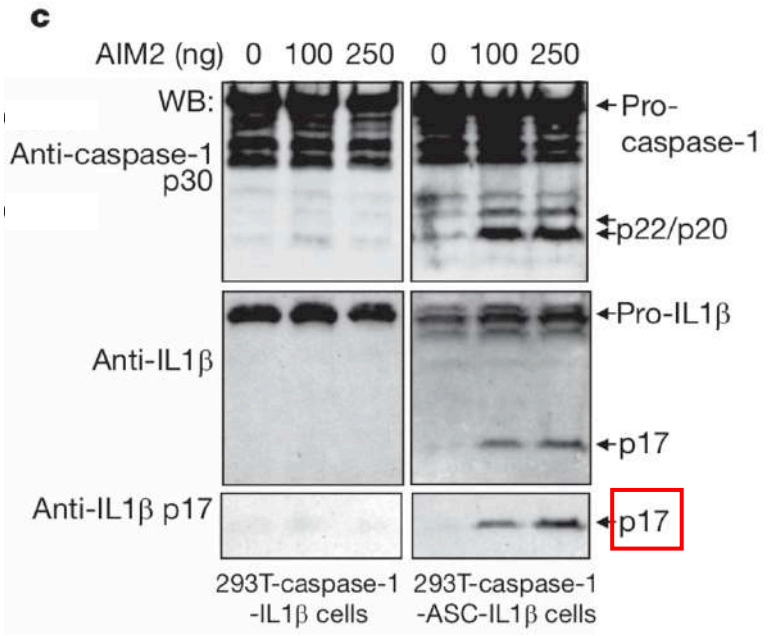
AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA

Teresa Fernandes-Alnemri[1]*, Je-Wook Yu[1]*, Pinaki Datta[1], Jianghong Wu[1] & Emad S. Alnemri[1]

DNA binding inflammasome?



American Journal of Medical and Biological Research, 2013 1 (3), pp 64-76.
DOI: 10.12691/ajmbr-1-3-3

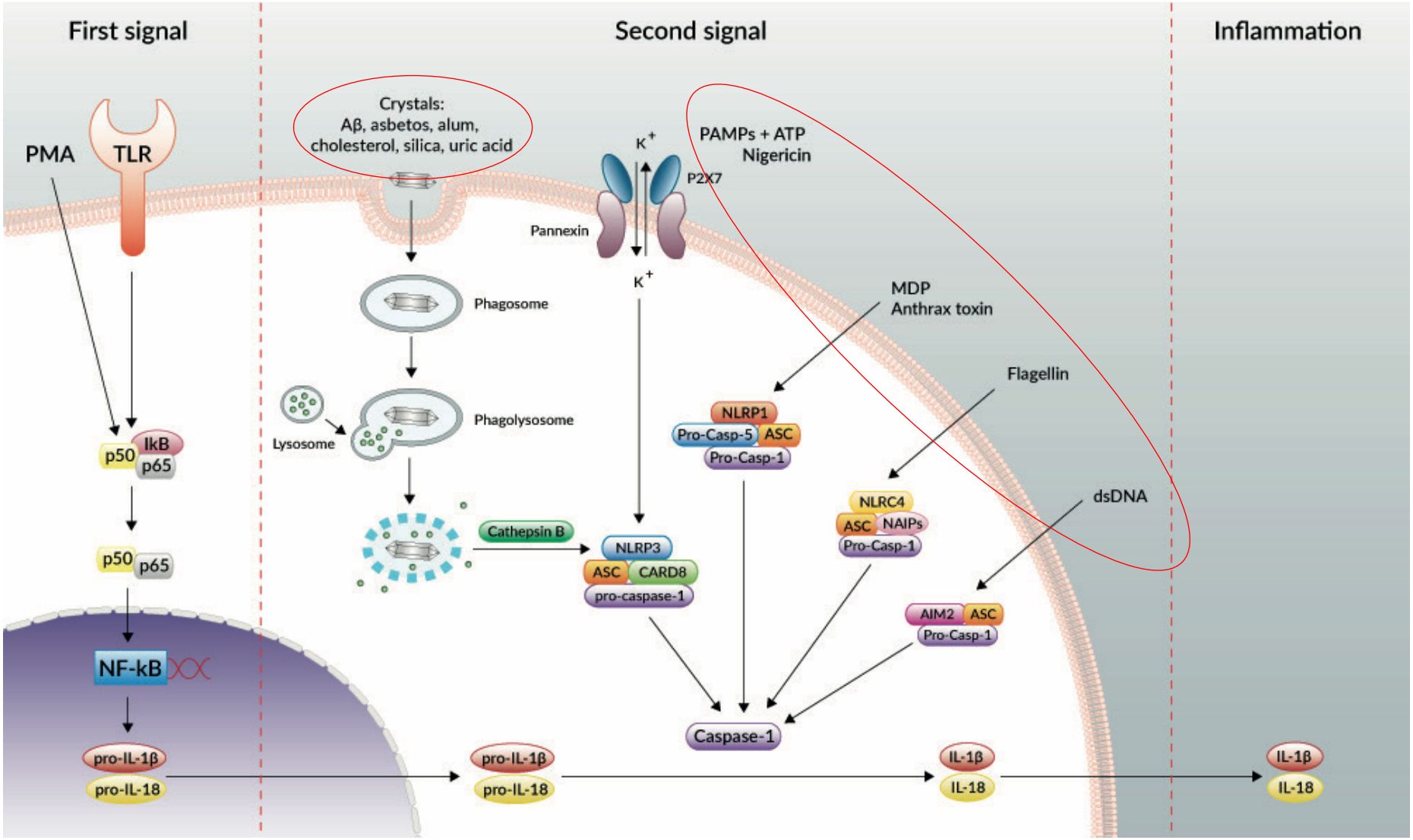


AIM2 activates IL-1β
ASC is essential for IL-1β activation

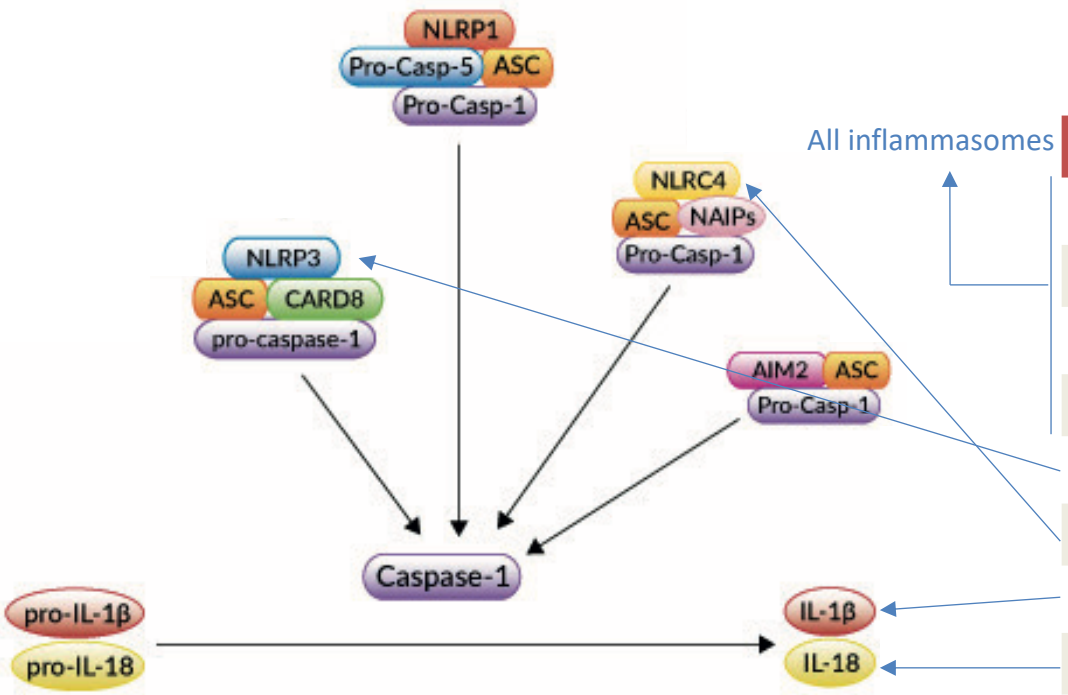
(AIM2: Absent in Malenoma2)

Related Product	Cat. Code
Poly(dA:dT)	tlrl-patn
ODN TTAGGG (A151)	ttrl-ttag151

Overview of Canonical Inflammasome



Canonical Inflammasome and Test Cells



Canonical Inflammasomes

All inflammasomes

Product	Unit Size	Cat. Code
THP1-ASC-GFP	3-7 x 10^6 cells	thp-ascgfp
THP1-Null	3-7 x 10^6 cells	thp-null
THP1-defASC	3-7 x 10^6 cells	thp-dasc
THP1-defCASP1	3-7 x 10^6 cells	thp-dcasp1
THP1-defNLRP3	3-7 x 10^6 cells	thp-dnlp
THP1-NLRC4 Cells	3-7 x 10^6 cells	thp-nlrc4
HEK-Blue™ IL-1β	3-7 x 10^6 cells	hkb-il1b
HEK-Blue™ IL-18	3-7 x 10^6 cells	hkb-hmil18
RAW-mASC		
THP1 KO NLRP3		
THP1 KO ASC		

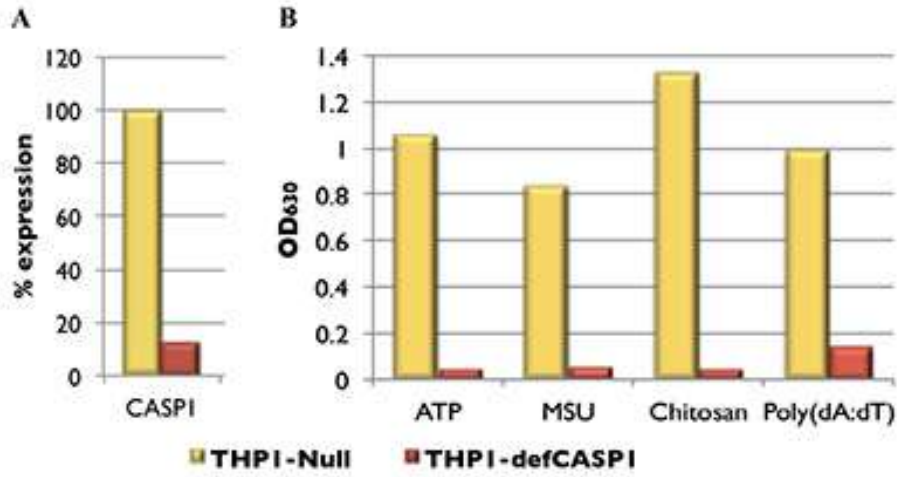
NEW!

Cell Lines with Reduced Inflammasome Activity

Product	Unit Size	Cat. Code
THP1-defCASP1	3-7 x 10^6 cells	thp-dcasp1
THP1-defASC	3-7 x 10^6 cells	thp-dasc
THP1-defNLRP3	3-7 x 10^6 cells	thp-dnlp
THP1-Null	3-7 x 10^6 cells	thp-null

Expression

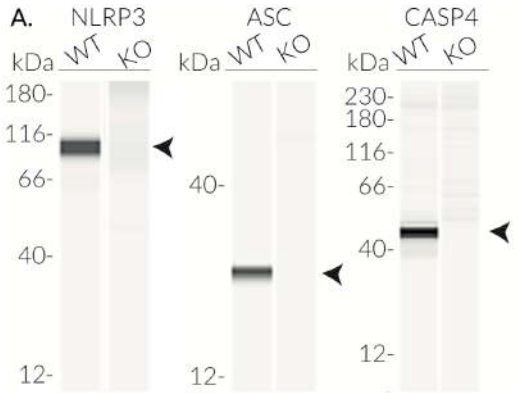
Activity



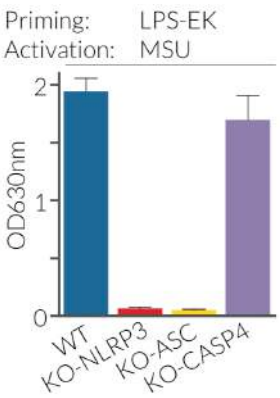
NEW!

Product	Unit Size	Cat. Code
THP1-KO-NLRP3	3-7 x 10^6 cells	thp-konlrp3
THP1-KO-ASC	3-7 x 10^6 cells	thp-koasc
THP1-KO-CASP4	3-7 x 10^6 cells	thp-kocasp4

Expression

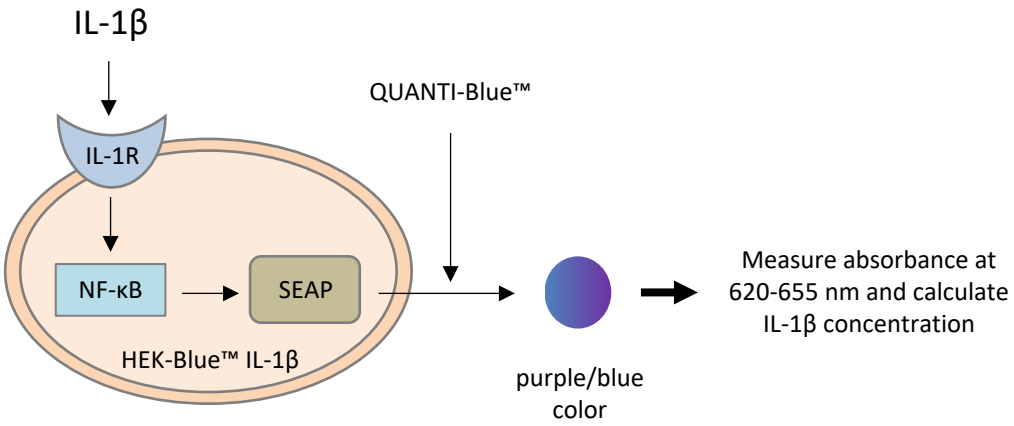


Activity



SEAP and Alkaline Phosphatase Detection

SEAP: Secreted Embryonic Alkaline Phosphatase

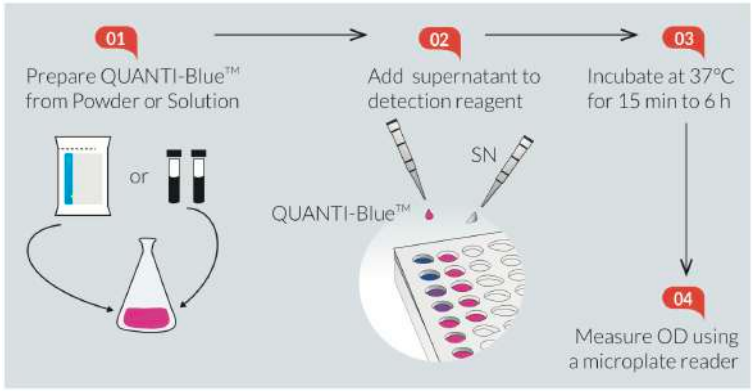


No need to lyse cells!!

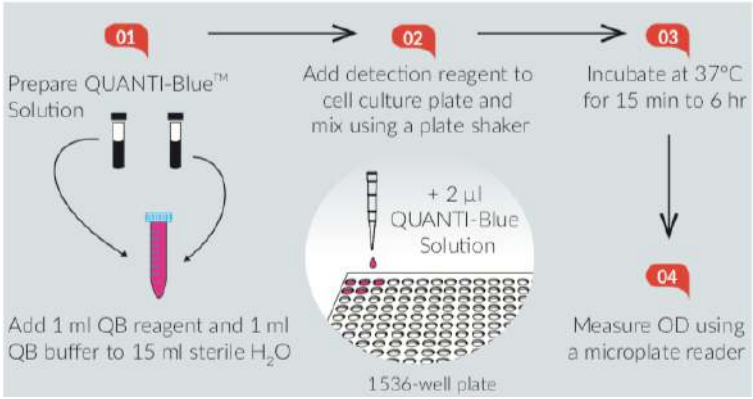
2019 Nat Commun. DOI: 10.1038/s41467-018-08265-9
Efficient oral vaccination by bioengineering virus-like particles with protozoan surface proteins.
Serradell M.C. et al.

2019 Nat. Immunol. DOI: 10.1038/s41565-018-0342-5
Endosomolytic polymersomes increase the activity of cyclic dinucleotide STING agonists to enhance cancer immunotherapy.
Shae D. et al.

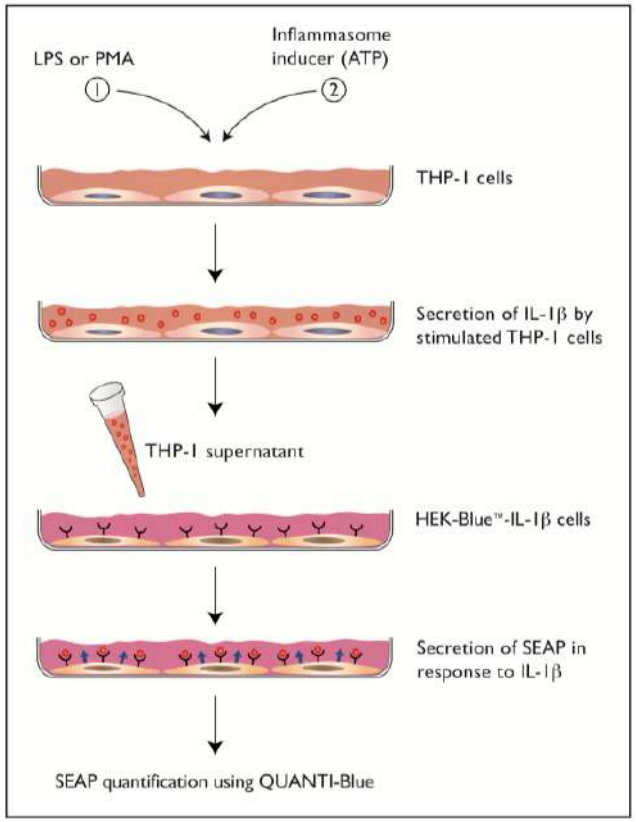
Normal Setup



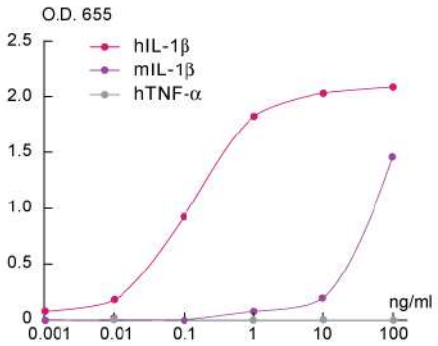
High-throughput Screening



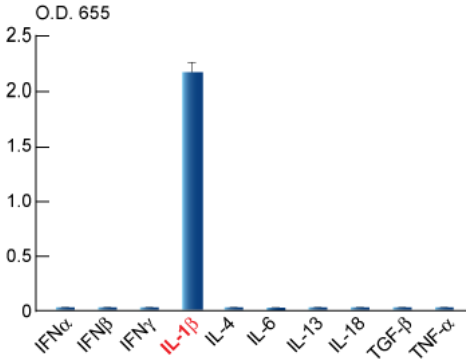
IL-1β Sensor Cells



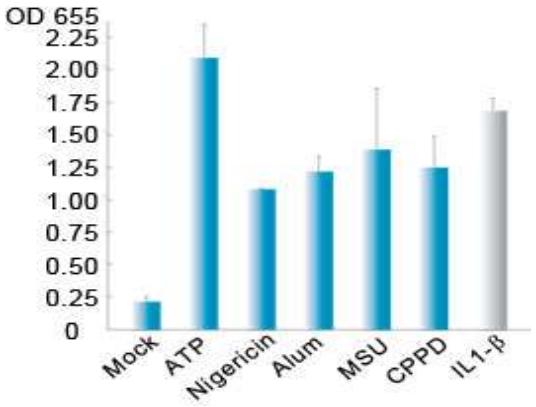
Sensitivity



Specificity

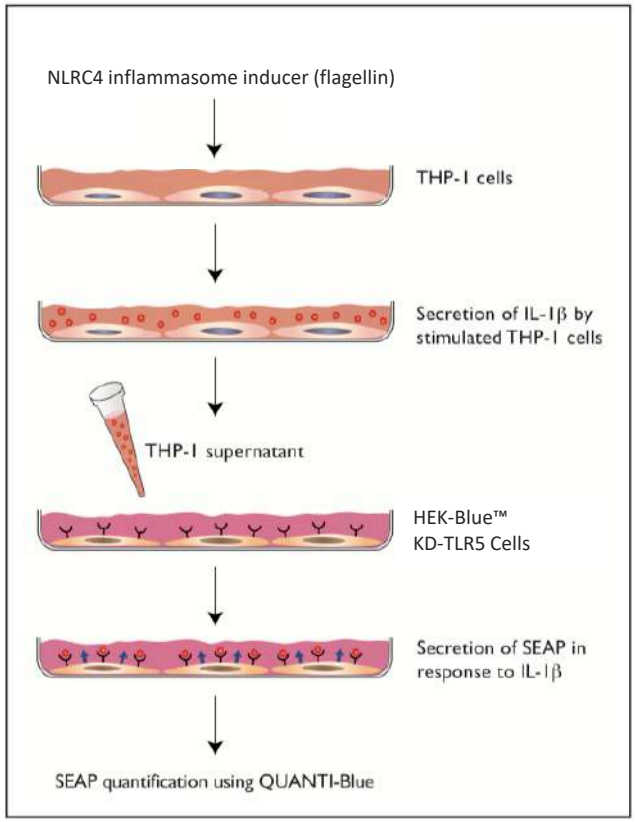


Activation by Different Inducers



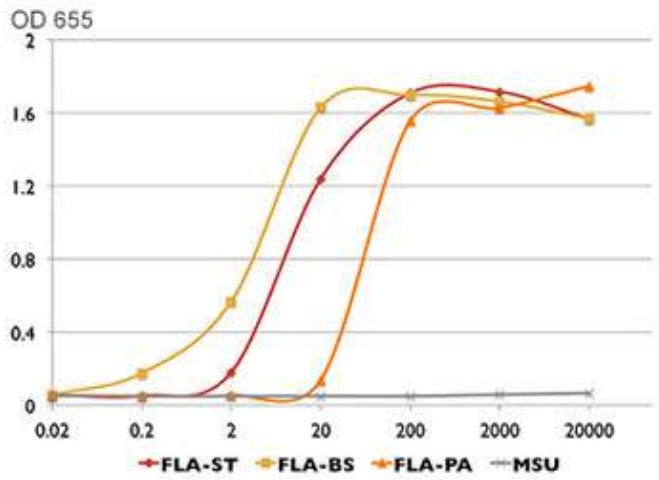
Product	Unit Size	Cat. Code
HEK-Blue IL-1β	3-7 x 10^6 cells	hkb-il1b
QUANTI-Blue Solution	5 ml	rep-qbs
Recombinant human IL-1β	10 µg	rcyec-hil1b

NLRC4 Inflammasome Monitoring Assay

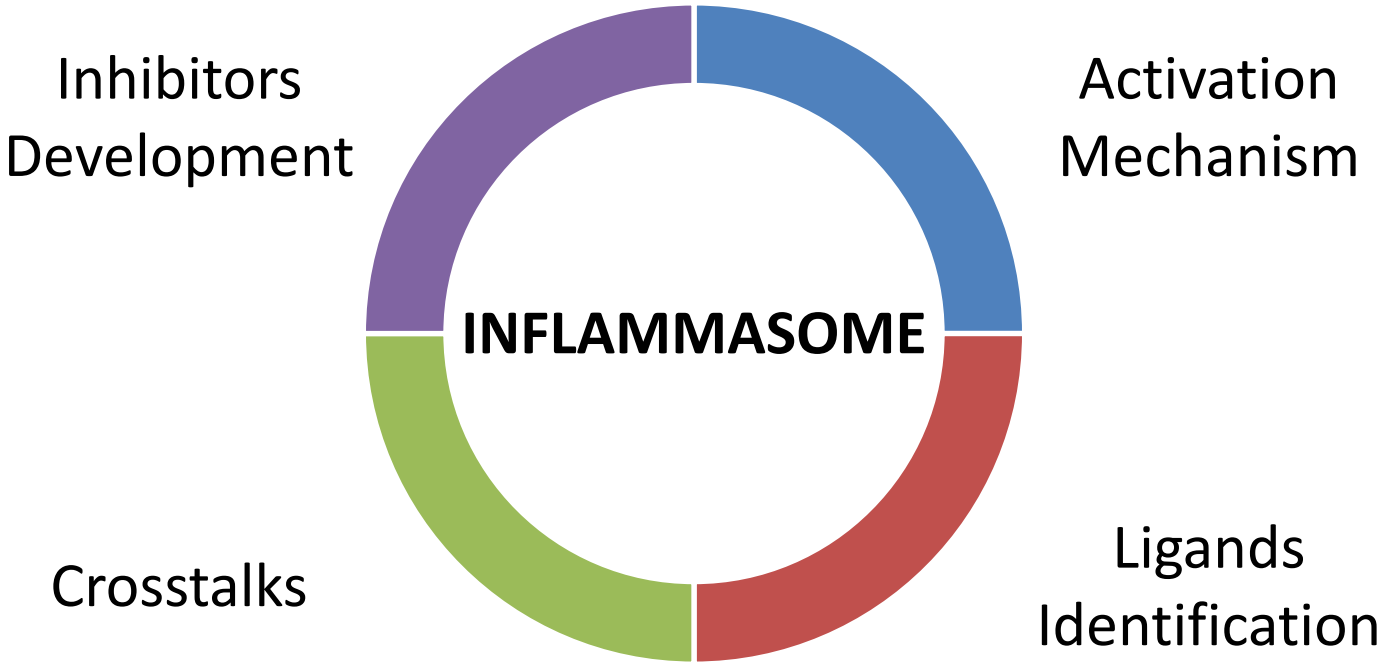


Product	Unit Size	Cat. Code
THP1-NLRC4 Cells	3-7 x 10^6 cells	thp-nlrc4
TLR5 deficient - IL-1β SEAP reporter HEK293 cells	3-7 x 10^6 cells	hkb-kdtlr5

- THP1 cells stably expressing NLRC4 for flagellin-mediated inflammasome response
- HEK-Blue™ IL-1β SEAP/KD-TLR5 cells to prevent NFκB activation by flagellin-induced TLR5 stimulation



Inflammasome – Recent Focus



Now (1) Mechanism – functional degradation

Science RESEARCH ARTICLES

2019

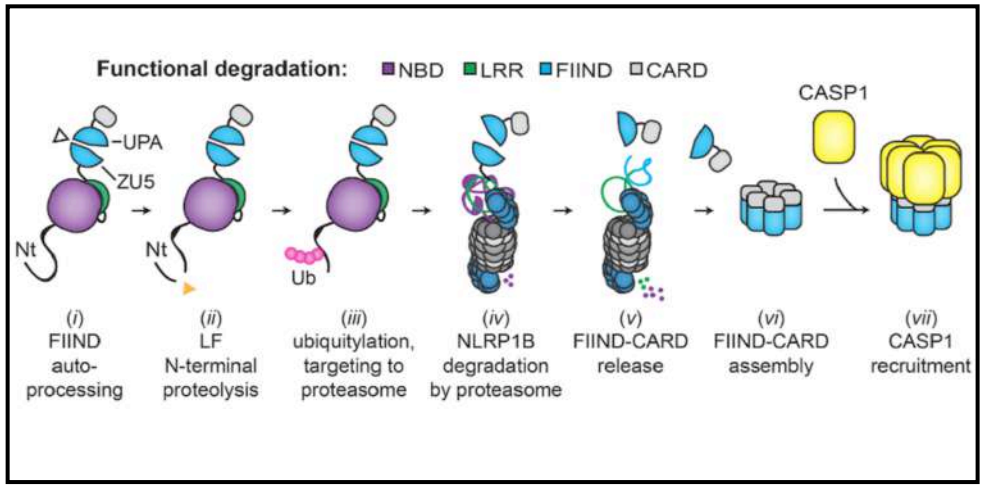
Cite as: A. Sandstrom *et al.*, *Science* 10.1126/science.aau1330 (2019).

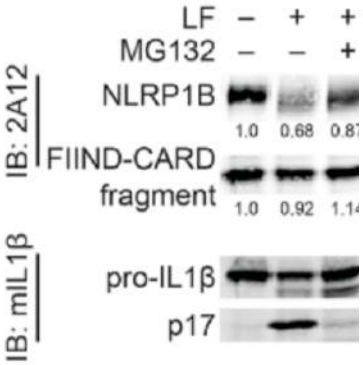
Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes

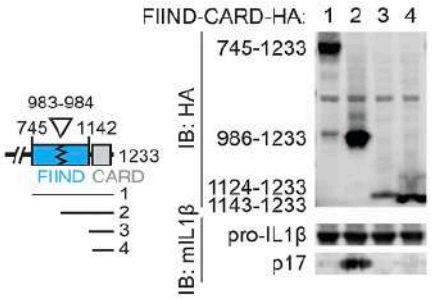
Andrew Sandstrom[1,3]*, Patrick S. Mitchell[1]*, Lisa Goers[2,4,5], Edward W. Mu[1], Cammie F. Lesser[2,4,5], Russell E. Vance[1,2]†

[1]Division of Immunology & Pathogenesis, Department of Molecular & Cell Biology, and Cancer Research Laboratory, University of California, Berkeley, CA, USA. [2]Howard Hughes Medical Institute, University of California, Berkeley, CA, USA. [3]Department of Microbiology, Harvard Medical School, Boston, MA, USA. [4]Broad Institute of Harvard and MIT, Cambridge, MA, USA. [5]Department of Medicine, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA.

[How proteolysis activates NLRP1?]







IL-1β activated by NLRP1 degradation

NLRP1 C-terminal is sufficient for IL-1β activation

Product Used	Cat. Code
Pam3CSK4	tlrl-pms

Now (1) Mechanism – functional degradation


Science

REPORTS

2019

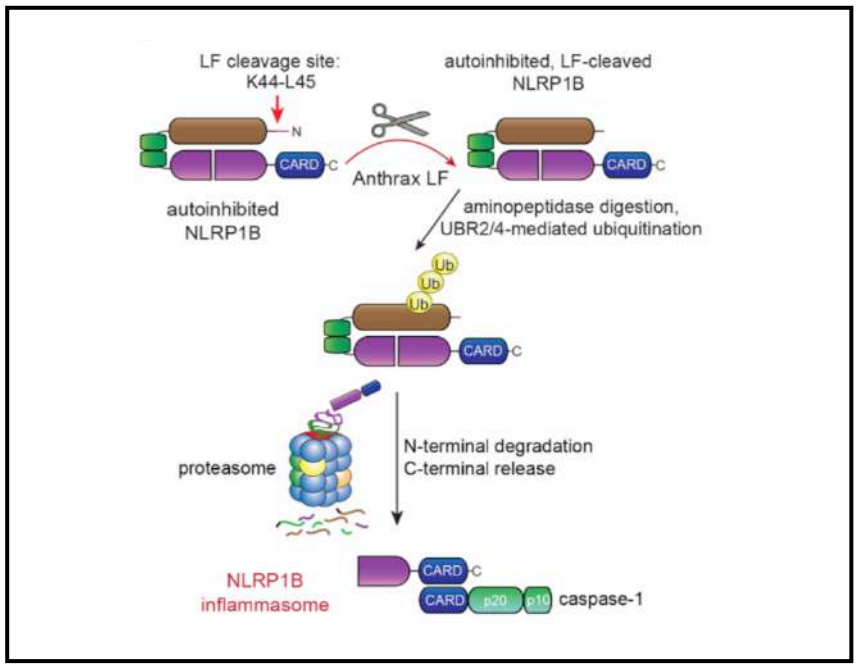
Cite as: A. J. Chui *et al.*, *Science* 10.1126/science.aau1208 (2019).

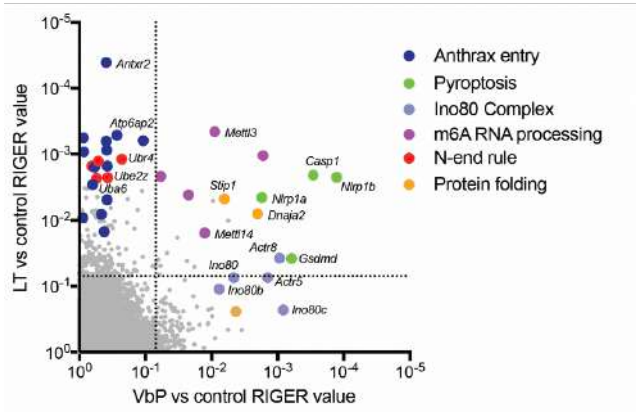
N-terminal degradation activates the NLRP1B inflammasome

Ashley J. Chui[1*], Marian C. Okondo[2*], Sahana D. Rao[1*], Kuo Gai[2], Andrew R. Griswold[3], Darren C. Johnson[1], Daniel P. Ball[2], Cornelius Y. Taabazuing[2], Elizabeth L. Orth[1], Brooke A. Vittimberga[2], Daniel A. Bachovchin[1,2,3]†

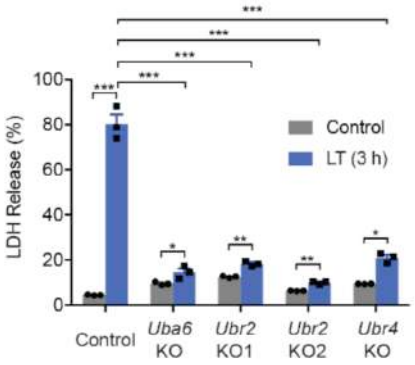
[1]Tri-Institutional PhD Program in Chemical Biology, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA. [2]Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA. [3]Pharmacology Program of the Weill Cornell Graduate School of Medical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA.

[CRISPR screen to identify NLPR1 activation mechanism]





N-end rule identified to be involved in NLRP1B-mediated pyroptosis



NLRP1 activated by N-terminal degradation

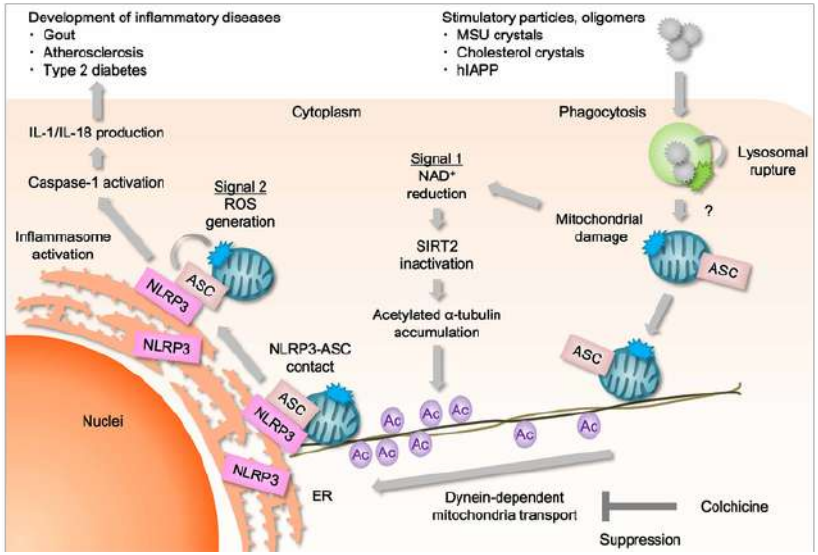
Now (1) Mechanism – Microtubule

2013

nature immunology

Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome

Takuma Misawa[1,2], Michihiro Takahama[1,2], Tatsuya Kozaki[1,2], Hanna Lee[1,2], Jian Zou[1,2], Tatsuya Saitoh[1,2] & Shizuo Akira[1,2]



ASC is brought to NLRP3 on ER through microtubule for interaction

Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Pam3CSK4	tlrl-pms
FLA-PA Ultrapure	tlrl-pafla

2017

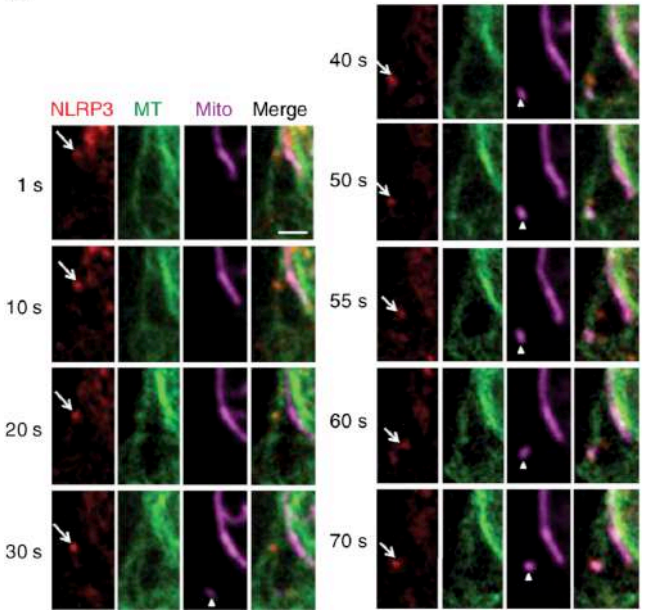
nature COMMUNICATIONS

ARTICLE

Received 6 Mar 2017 | Accepted 17 May 2017 | Published 28 Jun 2017 DOI: 10.1038/ncomms15986 OPEN

MARK4 regulates NLRP3 positioning and inflammasome activation through a microtubule-dependent mechanism

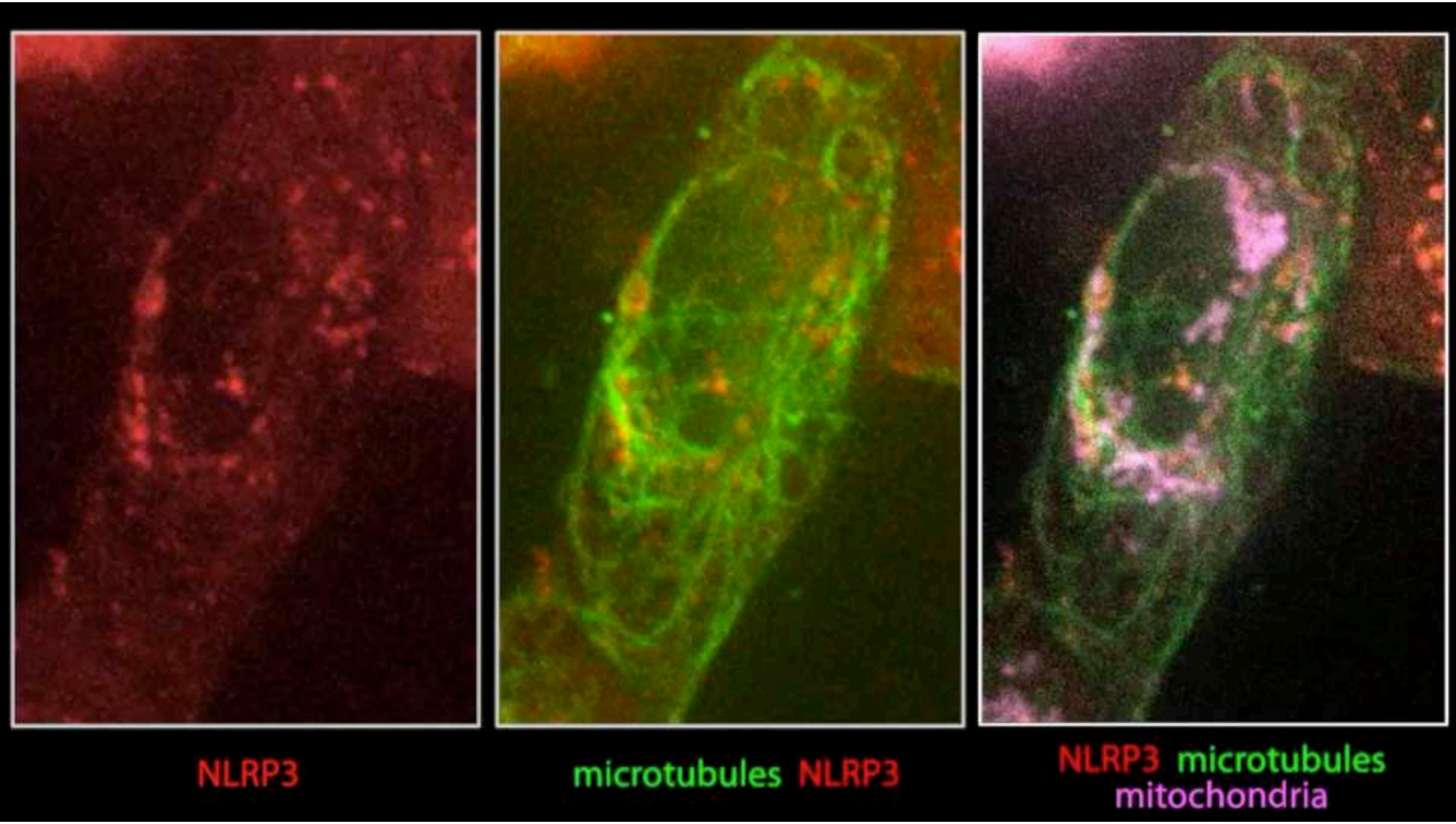
Xuan Li[1], Sarah Thome[1], Xiaodan Ma[2], Mamta Amrute-Nayak[3], Alison Finigan[1], Lauren Kitt[1], Leanne Masters[1], John R. James[4], Yuguang Shi[5], Guoyu Meng[2] & Ziad Mallat[1,6]



NLRP3 uses microtubule for positioning

Product Used	Cat. Code
iE-DAP	tlrl-dap

Now (1) Mechanism – Microtubule



Now (1) Mechanism – trans-Golgi network

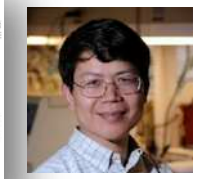
ARTICLE

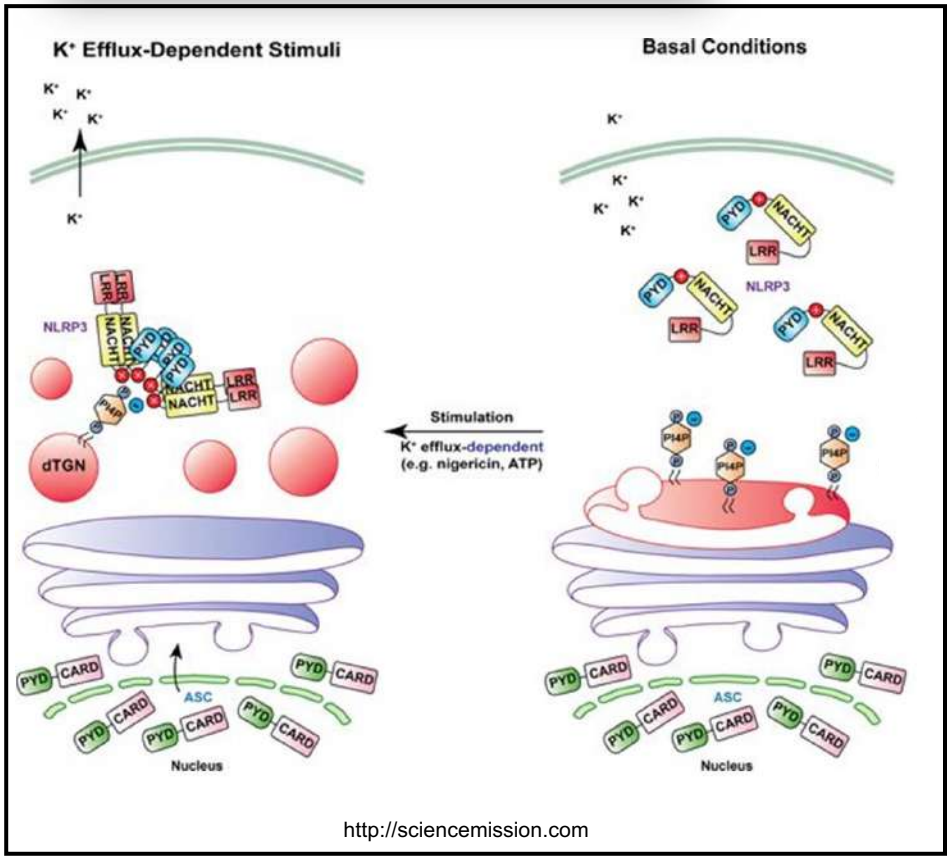
https://doi.org/10.1038/s41586-018-0761-3

PtdIns4P on dispersed *trans*-Golgi network mediates NLRP3 inflammasome activation

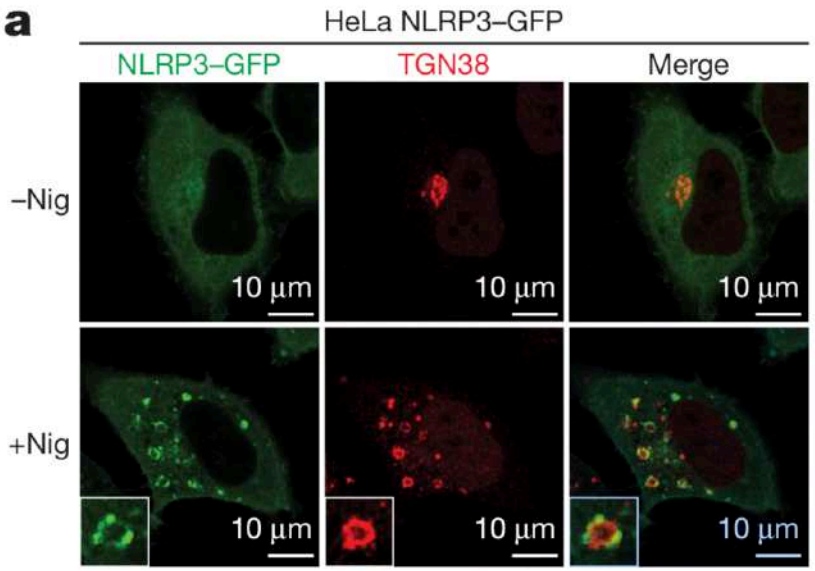
2018

Jueqi Chen[1] & Zhijian J. Chen[1,2]*





http://sciencemission.com

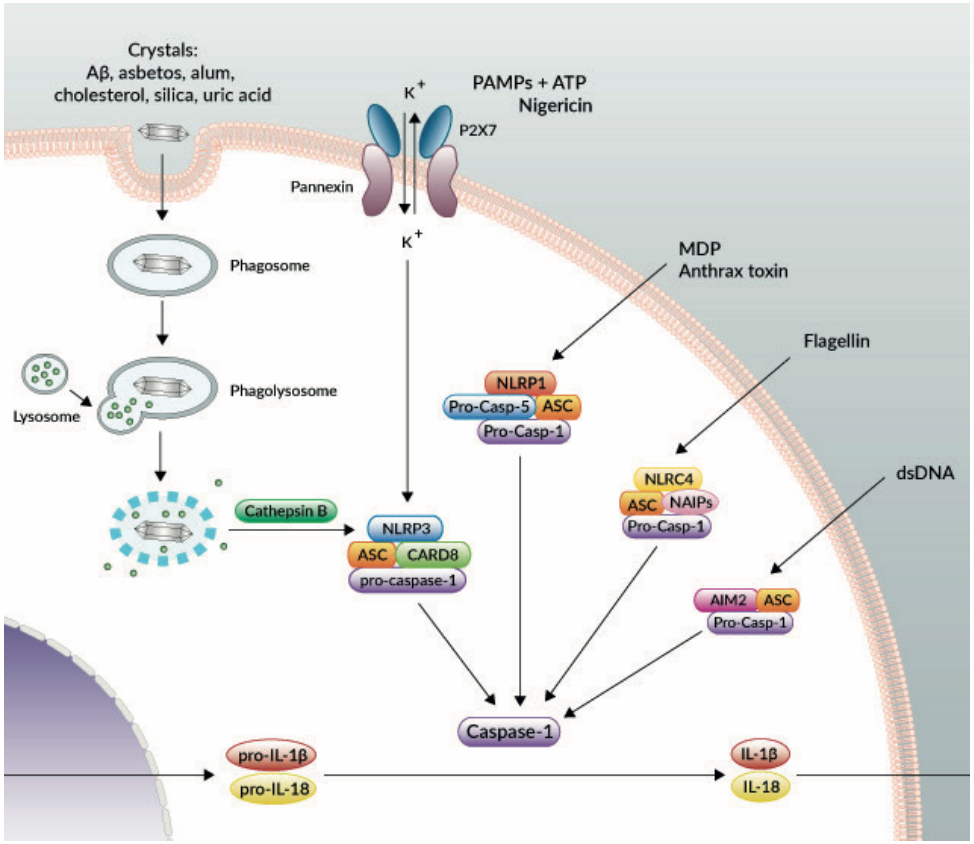


TGN as scaffold for NLRP3 aggregation

Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Imiquimod	tlrl-imq
Normocin	ant-nr-1

Now (2) Ligands – K^+

AIM2	dsDNA
NLRC4	Flagellin
NLRP1	degradation
NLRP3	?



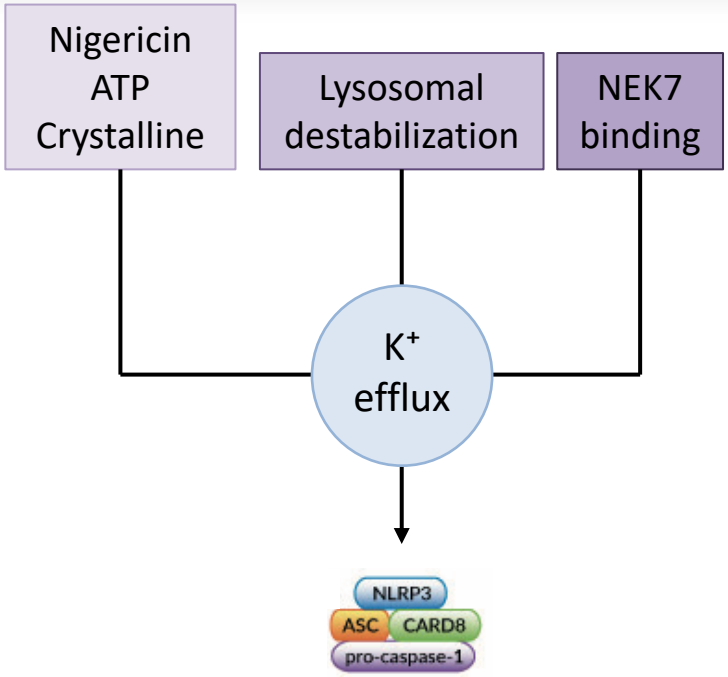
Yang et al. *Cell Death and Disease* (2019)10:128
https://doi.org/10.1038/s41419-019-1413-8

2018 Cell Death & Disease

REVIEW ARTICLE Open Access

Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors

Yang Yang[1], Huanan Wang[2], Mohammed Kouadir[3], Houhui Song[1] and Fushan Shi[2]



Now (2) Ligands

LETTER 2012

doi:10.1038/nature11351

Rapid induction of inflammatory lipid mediators by the inflammasome *in vivo*

Jakob von Moltke[1], Norver J. Trinidad[1], Mahtab Moayeri[4], Alexander F. Kintzer[2], Samantha B. Wang[3], Nico van Rooijen[5], Charles R. Brown[6], Bryan A. Krantz[2], Stephen H. Leppla[4], Karsten Gronert[3] & Russell E. Vance[1]

Legionella pneumophila flagellin

FlaA – LFn

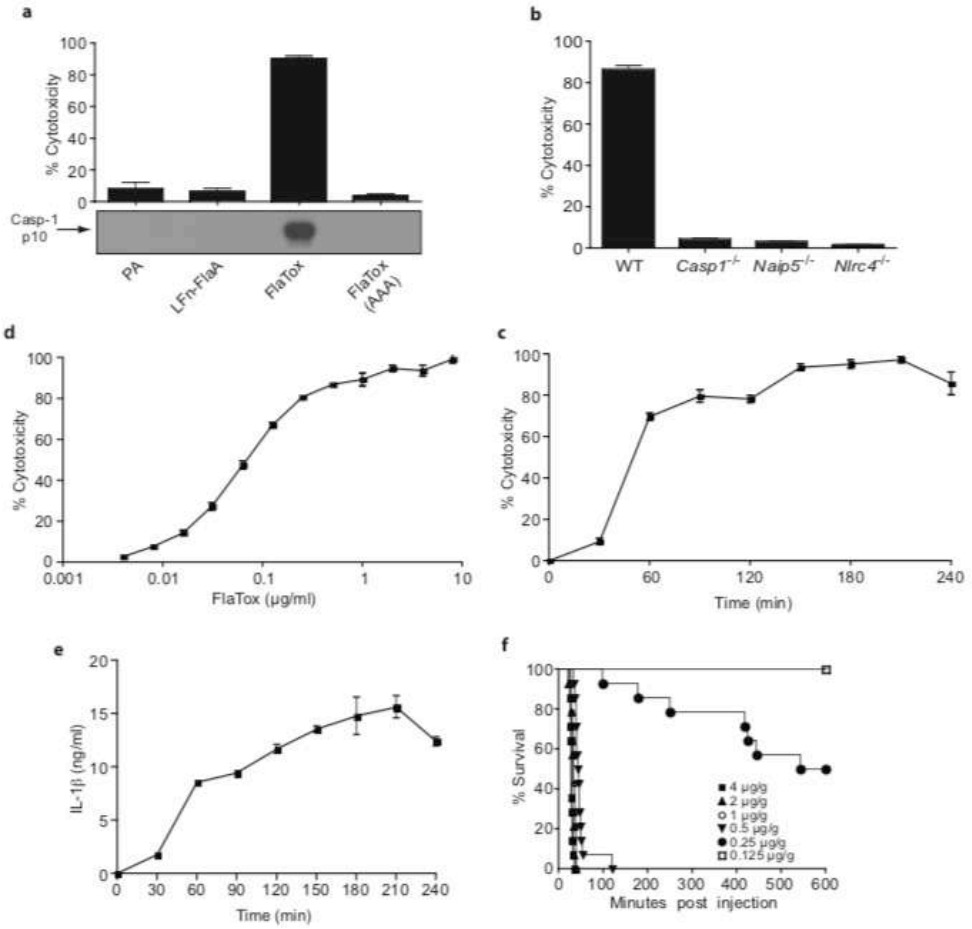
Bacillus anthracis lethal factor

\+ PA

anthrax protective antigen channel

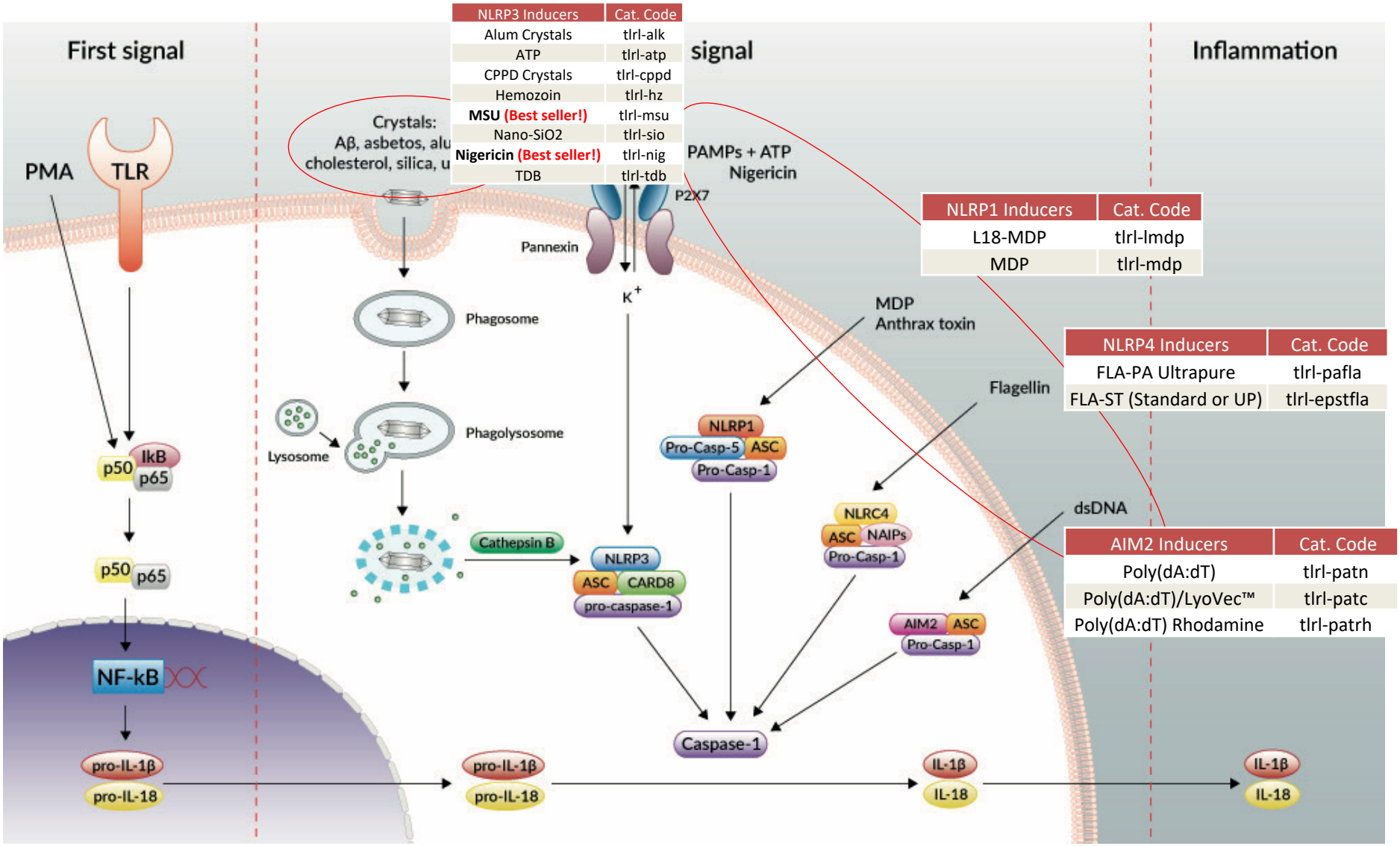
= FlaTox (cytosolic flagellin)

For NLRC4 activation



Coming Soon!

Canonical Inflammasome Inducers



NLRP3 Inducers	Cat. Code
Alum Crystals	tlrl-alk
ATP	tlrl-atp
CPPD Crystals	tlrl-cppd
Hemozoin	tlrl-hz
MSU (Best seller!)	tlrl-msu
Nano-SiO2	tlrl-sio
Nigericin (Best seller!)	tlrl-nig
TDB	tlrl-tdb

NLRP1 Inducers	Cat. Code
L18-MDP	tlrl-lmdp
MDP	tlrl-mdp

NLRP4 Inducers	Cat. Code
FLA-PA Ultrapure	tlrl-pafla
FLA-ST (Standard or UP)	tlrl-epstfla

AIM2 Inducers	Cat. Code
Poly(dA:dT)	tlrl-patn
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(dA:dT) Rhodamine	tlrl-patrh

Now (3) Crosstalks – STING

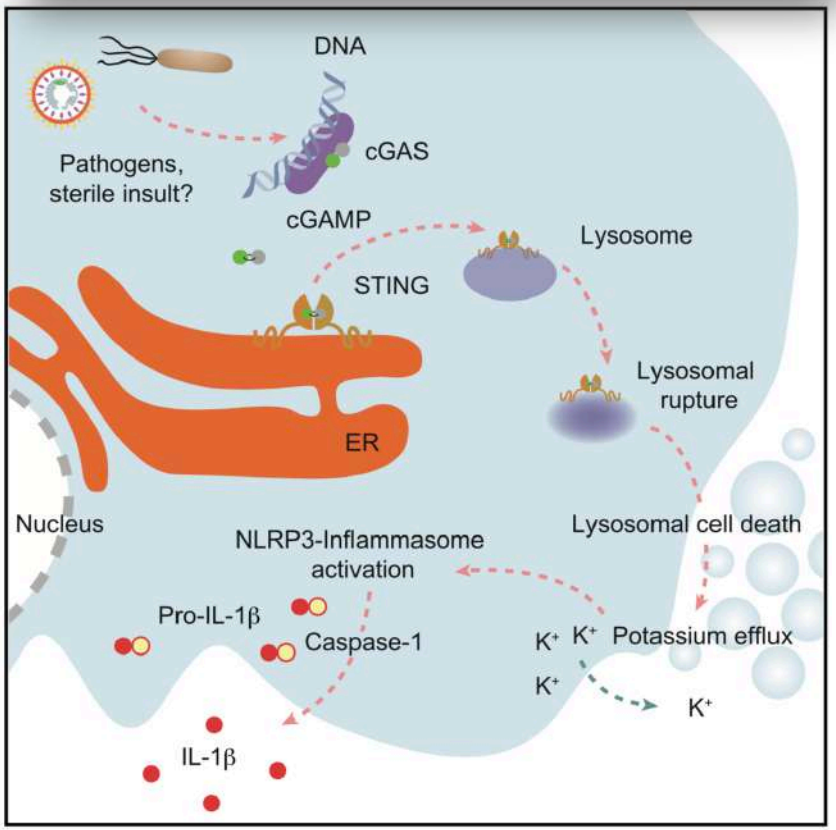

Cell

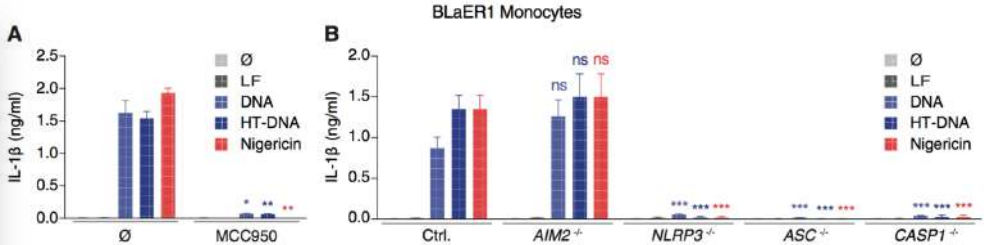
2017

Article

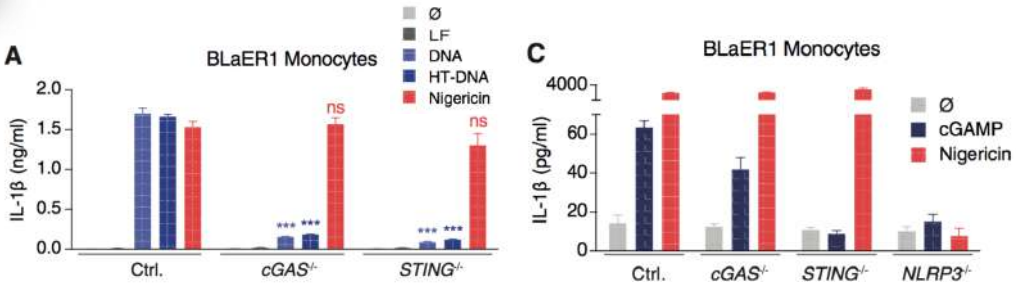
The DNA Inflammasome in Human Myeloid Cells Is Initiated by a STING-Cell Death Program Upstream of NLRP3

Moritz M. Gaidt,[1] Thomas S. Ebert,[1] Dhruv Chauhan,[1] Katharina Ramshorn,[1] Francesca Pinci,[1] Sarah Zuber,[1] Fionan O'Duill,[1] Jonathan L. Schmid-Burgk,[1,6] Florian Hoss,[2] Raymund Buhmann,[3] Georg Wittmann,[3] Eicke Latz,[2,4] Marion Subklewe,[5,6] and Veit Hornung[1,7,8,*]

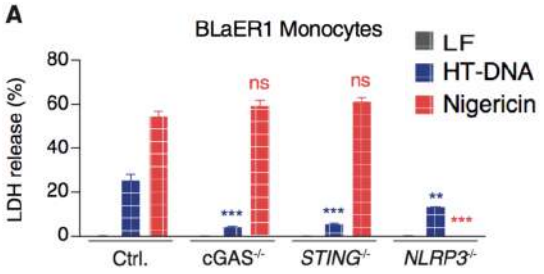




DNA-Mediated NLRP3-Inflammasome Activation Independently of AIM2



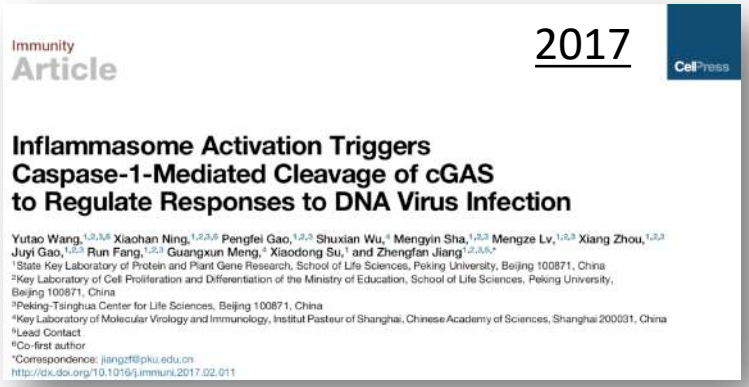
cGAS/STING Signaling Activates NLRP3 inflammasome



STING and NLRP3 Activation Leads to a Lytic Cell Death.

Product Used	Cat. Code
2'3'-cGAM(PS)2 (Rp/Sp)	tlrl-nacga2srs
2'3'-cGAMP	tlrl-nacga23-1
Pam3CSK4	tlrl-pms
LPS-EB Ultrapure	tlrl-3pelps

Now (3) Crosstalks – cGAS



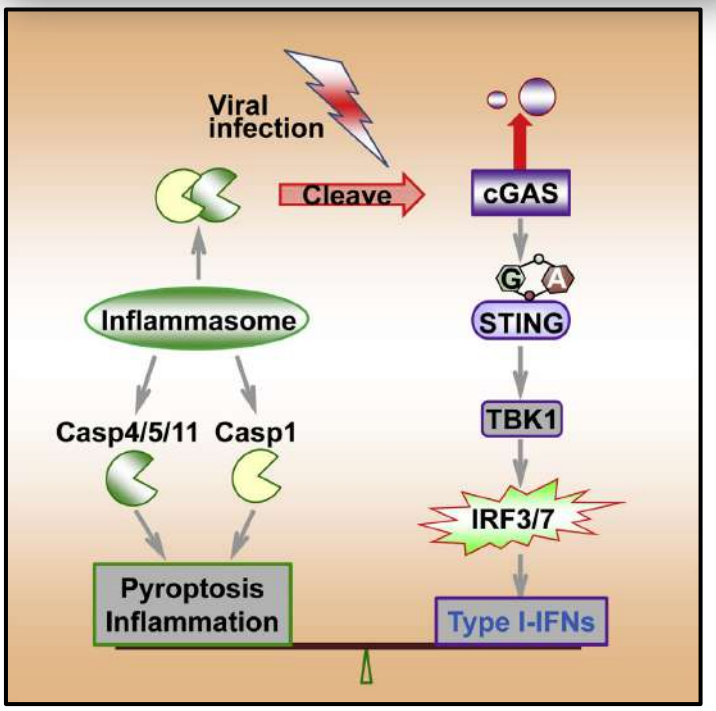
Immunity
Article

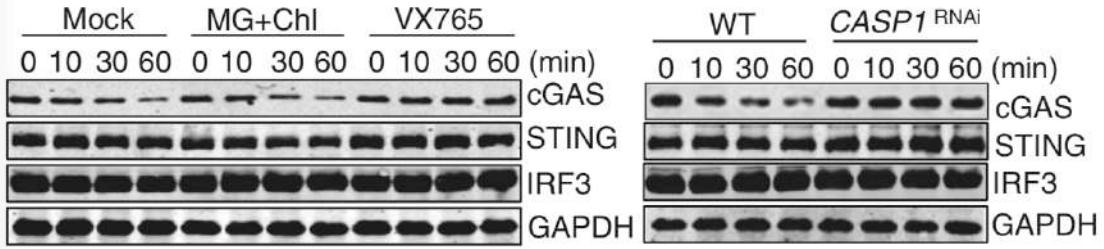
2017

CellPress

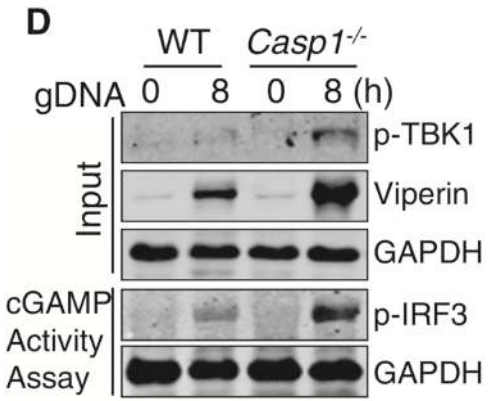
Inflammasome Activation Triggers Caspase-1-Mediated Cleavage of cGAS to Regulate Responses to DNA Virus Infection

Yutao Wang,[1,2,3,6] Xiaohan Ning,[1,2,3,6] Pengfei Gao,[1,2,3] Shuxian Wu,[4] Mengyin Sha,[1,2,3] Mengze Lv,[1,2,3] Xiang Zhou,[1,2,3] Juyi Gao,[1,2,3] Run Fang,[1,2,3] Guangxun Meng,[4] Xiaodong Su,[1] and Zhengfan Jiang[1,2,3,5,*]
[1]State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing 100871, China
[2]Key Laboratory of Cell Proliferation and Differentiation of the Ministry of Education, School of Life Sciences, Peking University, Beijing 100871, China
[3]Peking-Tsinghua Center for Life Sciences, Beijing 100871, China
[4]Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai 200031, China
[5]Lead Contact
[6]Co-first author
*Correspondence: jiangzf@pku.edu.cn
http://dx.doi.org/10.1016/j.immuni.2017.02.011





Inflammatory Caspases cleave cGAS



Increased cGAMP level in Casp KO cells

Related Product	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
ATP	tlrl-atpl
VX-765	inh-vx765i-1

Now (3) Crosstalks – Immune Checkpoint

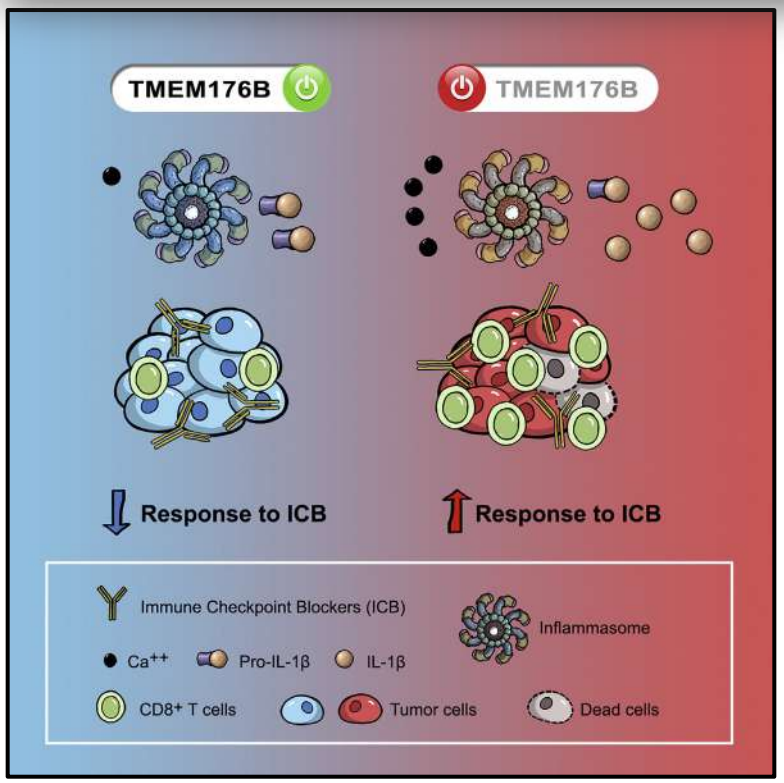
Cancer Cell
Article

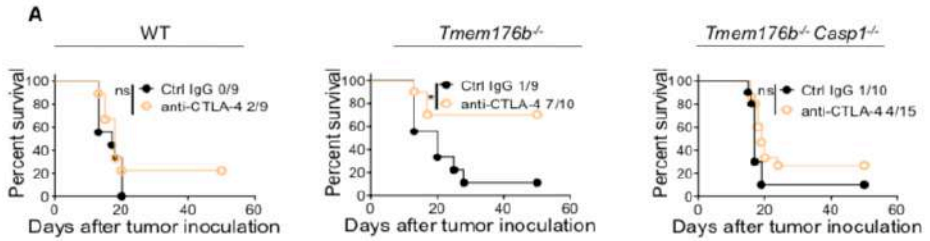
2019

CellPress

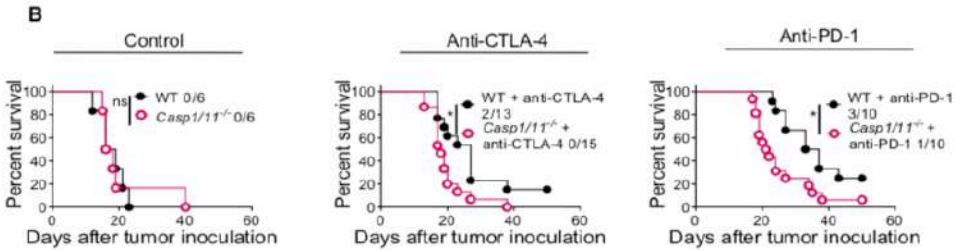
Targeting TMEM176B Enhances Antitumor Immunity and Augments the Efficacy of Immune Checkpoint Blockers by Unleashing Inflammasome Activation

Mercedes Segovia,[1,2,9] Sofia Russo,[1,2,9] Mathias Jeldres,[1] Yamil D. Mahmoud,[3] Valentina Perez,[1,2] Maite Duhalde,[1] Pierre Charnet,[4] Matthieu Rousset,[4] Sabina Victoria,[1] Florencia Veigas,[3] Cédric Louvet,[5] Bernard Vanhove,[5,6] R. Andrés Floto,[7] Ignacio Anegon,[8] Maria Cristina Cuturi,[8,10,*] M. Romina Girotti,[3,10] Gabriel A. Rabinovich,[3,8,10] and Marcelo Hill[1,2,10,11,*]

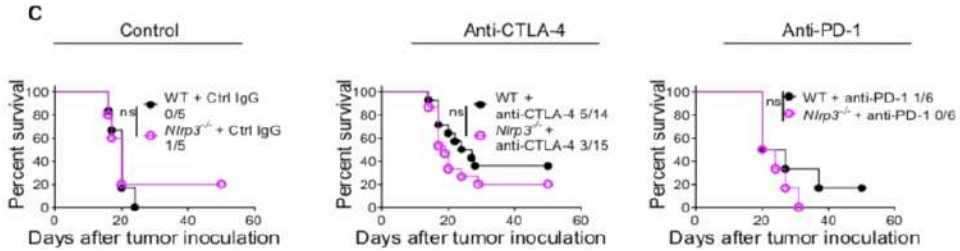




Tmem176b KO improves ICI efficacy



Casp1/11 KO decreases ICI efficacy



NLRP3 KO decreases ICI efficacy

Now (4) Inhibitors – MCC950

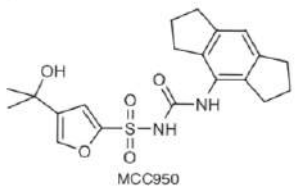
A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases 2015

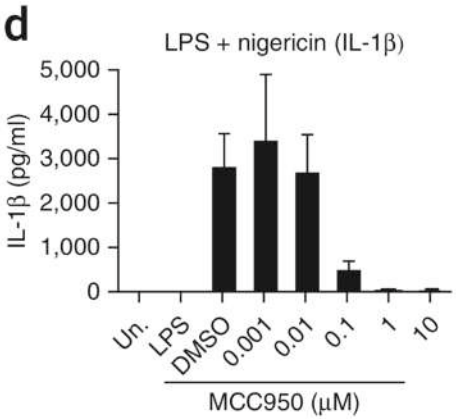
Rebecca C Coll[1,2], Avril A B Robertson[2], Jae Jin Chae[3], Sarah C Higgins[1], Raúl Muñoz-Planillo[4], Marco C Inserra[2,5], Irina Vetter[2,5], Lara S Dungan[1], Brian G Monks[6], Andrea Stutz[6], Daniel E Croker[2], Mark S Butler[2], Moritz Haneklaus[1], Caroline E Sutton[1], Gabriel Núñez[4], Eicke Latz[6–8], Daniel L Kastner[3], Kingston H G Mills[1], Seth L Masters[9], Kate Schroder[2], Matthew A Cooper[2] & Luke A J O'Neill[1]

Product Used	Cat. Code
Pam3CSK4	tlrl-pms
Nigericin	tlrl-nig
poly(dA:dT)	tlrl-patn

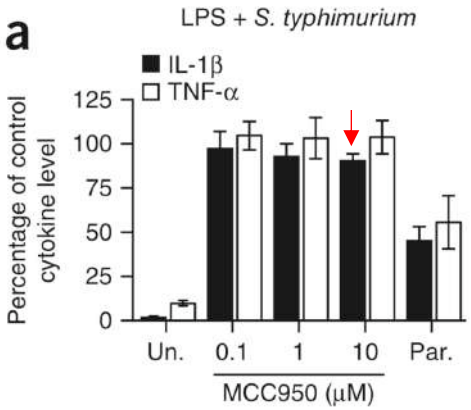
MCC950	Cat. Code	Unit Size
NLRP3 inhibitor	inh-mcc	10mg

a

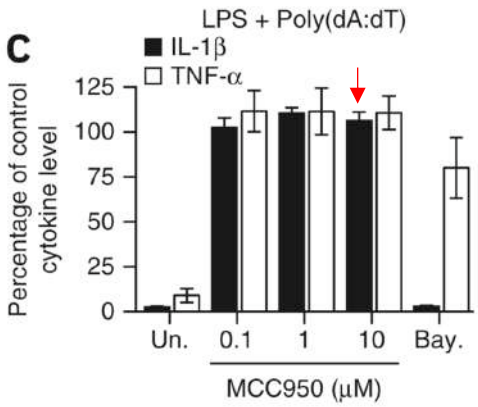




Inhibits NLRP3



No effect on NLRC4



No effect on AIM2

MCC950 specifically inhibited activation of NLRP3 but not the AIM2, NLRC4 or NLRP1

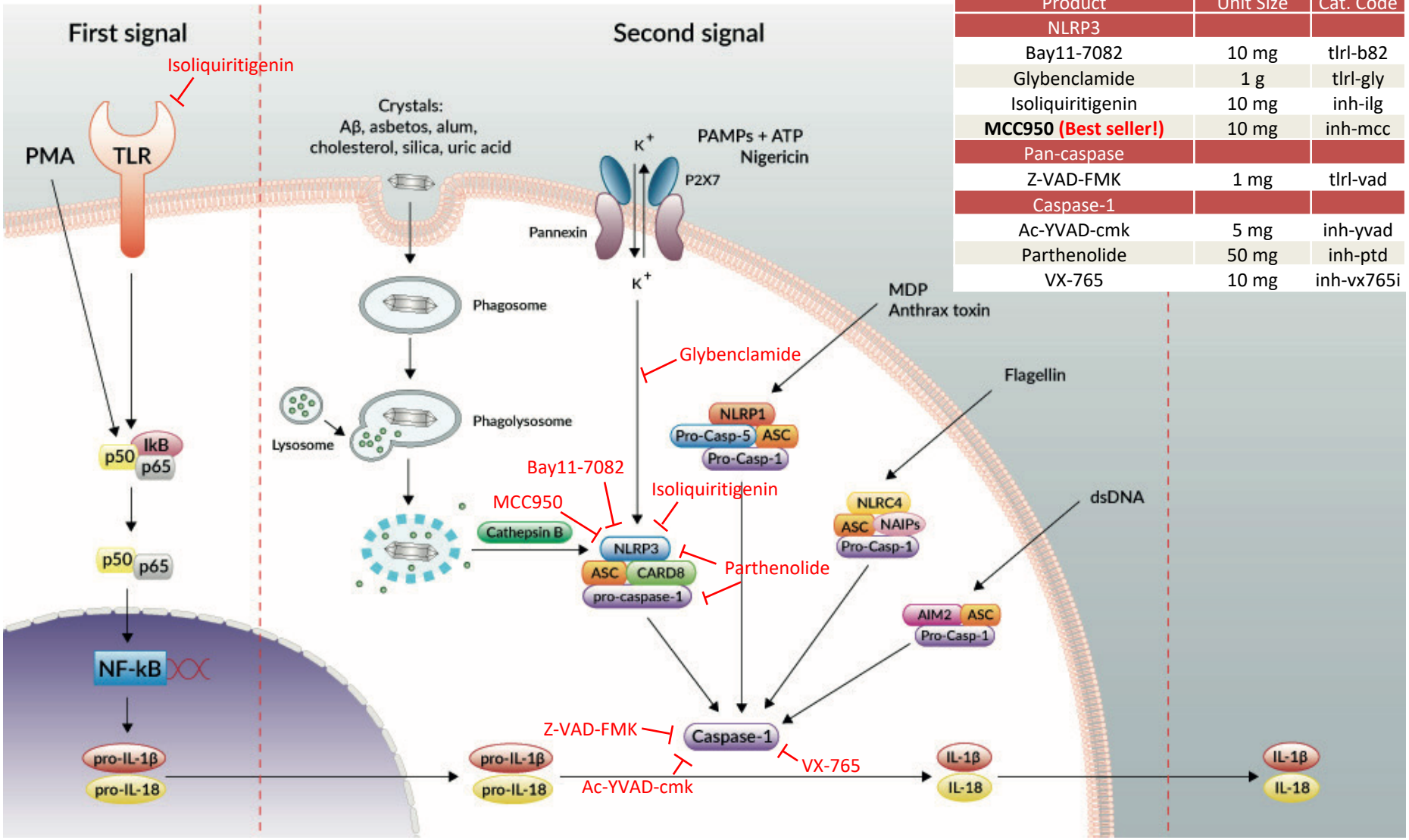
Now (4) Current NLRP3 Inhibitors

Available from InvivoGen

Agent	Target(s)	Potential mechanism
Glyburide marketed	NLRP3 (indirectly)	Inhibits ATP-sensitive K^+ channels; downstream of P2X7 resulting in inhibition of ASC aggregation
16673-34-0	NLRP3 (indirectly)	Induces NLRP3 conformational changes secondary to its activation or binding to ASC
JC124	NLRP3?	Blocks the expression of NLRP3, ASC, caspase-1, pro-IL-1β, TNFα and iNOS
FC11A-2	NLRP3 (indirectly)	Interferes with proximity induced autocleavage of pro-caspase-1, suppresses IL-1β/18 release
Parthenolide	NLRP1, NLRP3 inflammasome, Caspase-1, NF-κB, IKKβ kinase activity	Alkylates cysteine residues in caspase-1 and in ATPase domain of NLRP3, inhibits NLRP3 ATPase activity
VX-740 Phase II	Caspase-1	Covalent modification of the catalytic cysteine residue in the active site of caspase-1 resulting in caspase-1 blocking and resultant cleavage of pro-IL-1β/18
VX-765 Phase II	Caspase-1	Covalent modification of the catalytic cysteine residue in the active site of caspase-1 resulting in caspase-1 blocking and resultant cleavage of pro-IL-1β/18
Bay 11-7082 Preclinical	NLRP3, IKK, E2/3 enzymes, PTPs	Alkylates the cysteines in the ATPase domain of NLRP3, inhibits NLRP3 ATPase activity
BHB Preclinical	NLRP3 (Indirectly)	Inhibits K^+ efflux resulting in reduced oligomerization of ASC and IL-1β/18 release
MCC950 Preclinical	NLRP3	Blocks the ATPase domain of NLRP3 resulting in inhibition of canonical and non-canonical NLRP3 inflammasome activation
MNS	NLRP3	Inhibits NLRP3 ATPase activity by cysteine modification, blocks NLRP3 inflammasome activation
CY-09	NLRP3	Inhibits NLRP3 ATPase activity, blocks NLRP3 inflammasome activation
Tranilast marketed	NLRP3	Binds to NLRP3 NACHT domain to block NLRP3-NLRP3 and NLRP3-ASC interaction
OLT1177 Phase II	NLRP3	Inhibits NLRP3 ATPase activity, blocks NLRP3 inflammasome activation
Oridonin	NLRP3	Binds to cysteine 279 of NLRP3 to abolish NLRP3-NEK7 interaction, blocks NLRP3 inflammasome activation

Front Immunol. 2019 Oct 25;10:2538. doi: 10.3389/fimmu.2019.02538.

Canonical Inflammasome Inhibitors

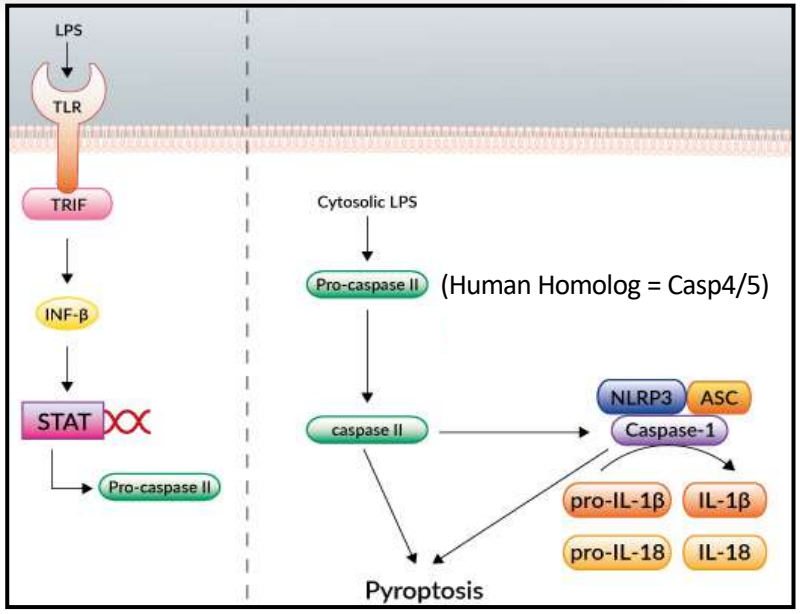


Product	Unit Size	Cat. Code
NLRP3		
Bay11-7082	10 mg	tlrl-b82
Glybenclamide	1 g	tlrl-gly
Isoliquiritigenin	10 mg	inh-ilg
MCC950 (Best seller!)	10 mg	inh-mcc
Pan-caspase		
Z-VAD-FMK	1 mg	tlrl-vad
Caspase-1		
Ac-YVAD-cmk	5 mg	inh-yvad
Parthenolide	50 mg	inh-ptd
VX-765	10 mg	inh-vx765i

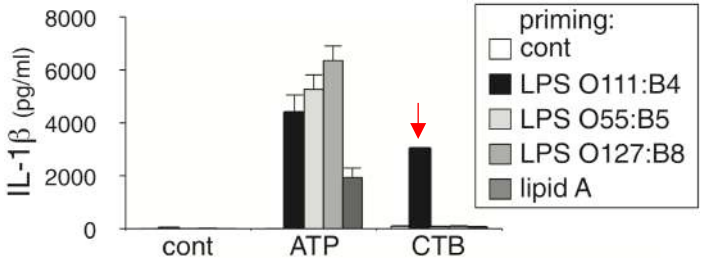
Non-canonical Inflammasome - 2011

Noncanonical Inflammasome Activation by Intracellular LPS Independent of TLR4

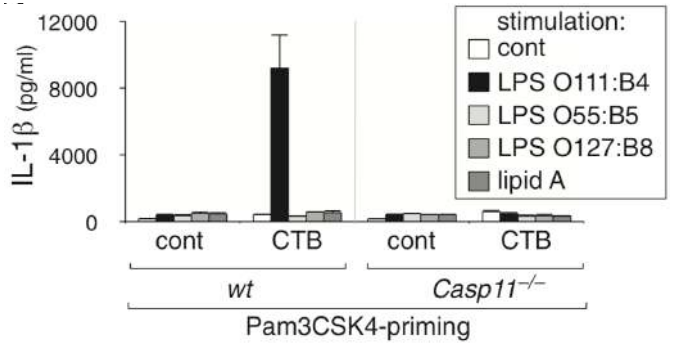
Nobuhiko Kayagaki,[1*] Michael T. Wong,[1] Irma B. Stowe,[1] Sree Ranjani Ramani,[2] Lino C. Gonzalez,[2] Sachiko Akashi-Takamura,[3] Kensuke Miyake,[3] Juan Zhang,[4] Wyne P. Lee,[4] Artur Muszyński,[5] Lennart S. Forsberg,[5] Russell W. Carlson,[5] Vishva M. Dixit[1*]



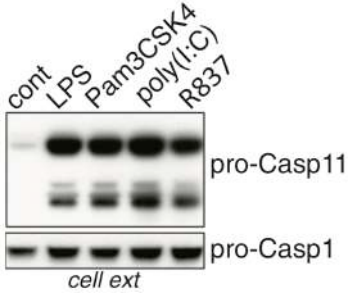
Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Pam3CSK4	tlrl-pms
Poly(I:C) LMW	tlrl-picw
R837 (Imiquimod)	tlrl-imqs



Only one serotype O111:B4 activates Casp-1 processing with CTB (presence of cytosolic LPS)



Caspase-11 is essential for IL-1β maturation



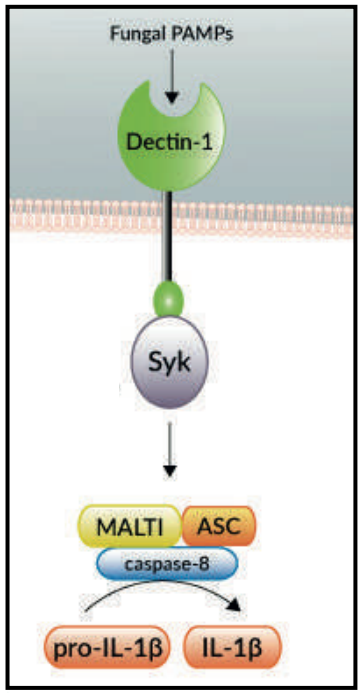
Caspase-11 expression requires priming

Caspase-8 inflammasome - 2012

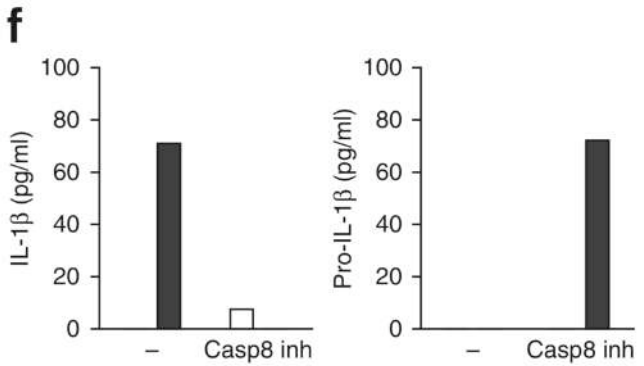
nature immunology

Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1β via a noncanonical caspase-8 inflammasome

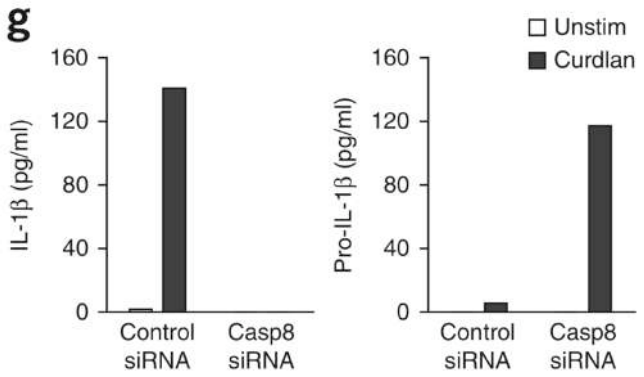
Sonja I Gringhuis[1], Tanja M Kaptein[1], Brigitte A Wevers[1], Bart Theelen[2], Michiel van der Vlist[1], Teun Boekhout[2] & Teunis B H Geijtenbeek[1]



Product Used	Cat. Code
ATP	tlrl-atpl
FLA-BS	tlrl-bsfla
Poly(I:C)	tlrl-pic



Inhibition of Caspase-8 inhibits IL-1β processing



Knockdown of Caspase-8 inhibits IL-1β processing

Caspase-8 inflammasome - 2012

Article

Caspase-8 is the molecular switch for apoptosis, necroptosis and pyroptosis

https://doi.org/10.1038/s41586-019-1770-6

Received: 18 February 2019

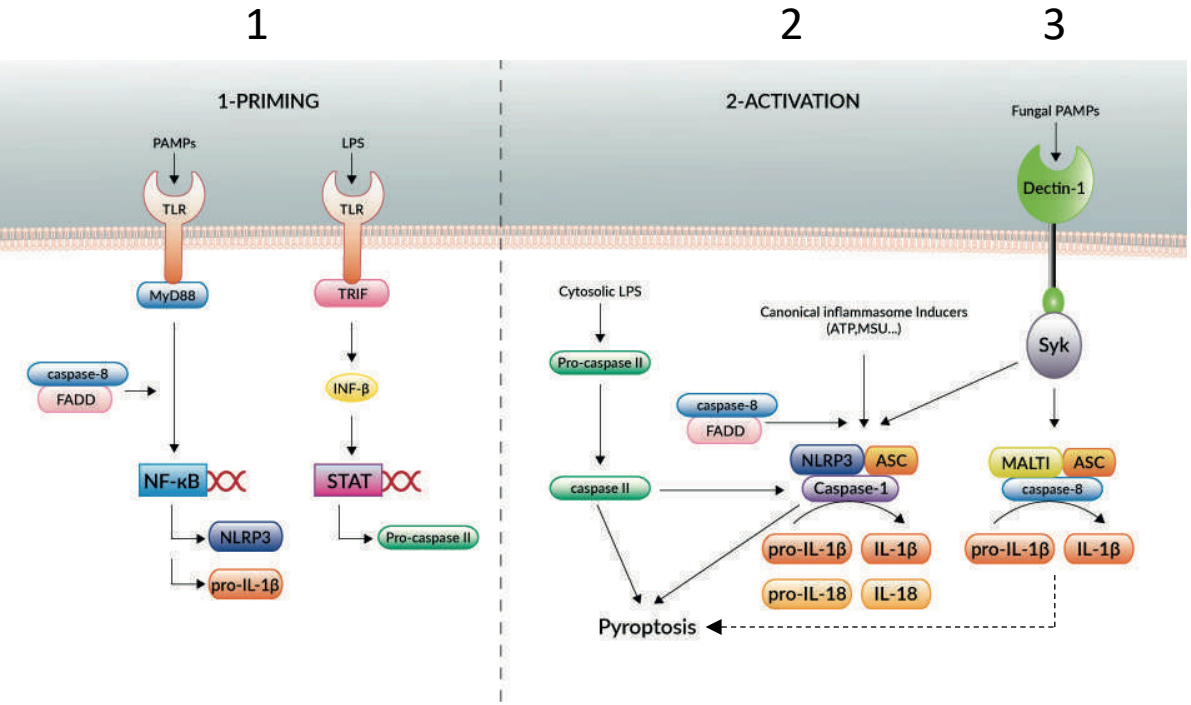
Accepted: 15 October 2019

Published online: 20 November 2019

Melanie Fritsch[1], Saskia D. Günther[1], Robin Schwarzer[2], Marie-Christine Albert[1], Fabian Schorn[1], J. Paul Werthenbach[1], Lars M. Schiffmann[1,3], Neil Stair[1,2], Hannah Stocks[1], Jens M. Seeger[1], Mohamed Lamkanfi[4,5], Martin Krönke[1], Manolis Pasparakis[2,6] & Hamid Kashkar[1,6*]

Caspase-8 is the initiator caspase of extrinsic apoptosis[1,2] and inhibits necroptosis mediated by RIPK3 and MLKL. Accordingly, caspase-8 deficiency in mice causes embryonic lethality[3], which can be rescued by deletion of either *Ripk3* or *Mlkl*[4–6]. Here we show that the expression of enzymatically inactive CASP8(C362S) causes embryonic lethality in mice by inducing necroptosis and pyroptosis. Similar to *Casp8*$^{-/-}$ mice[3,7], *Casp8*$^{C362S/C362S}$ mouse embryos died after endothelial cell necroptosis leading to cardiovascular defects. MLKL deficiency rescued the cardiovascular

Non-canonical Inflammasome



Product	
THP-1 KO-CASP4 Cells	NEW!
RAW ASC KO-CASP11 Cells	Coming Soon!

Non-canonical Inflammasome

1. Priming (e.g.TLR4) induces pro-Caspase-11

   Kagayaki, Science 2013

2. Triggering by cytosolic LPS
   - Activates Caspase-11
   - Induces Pyroptosis

   Kayagaki, Nature 2011; Kayagaki, Science 2013

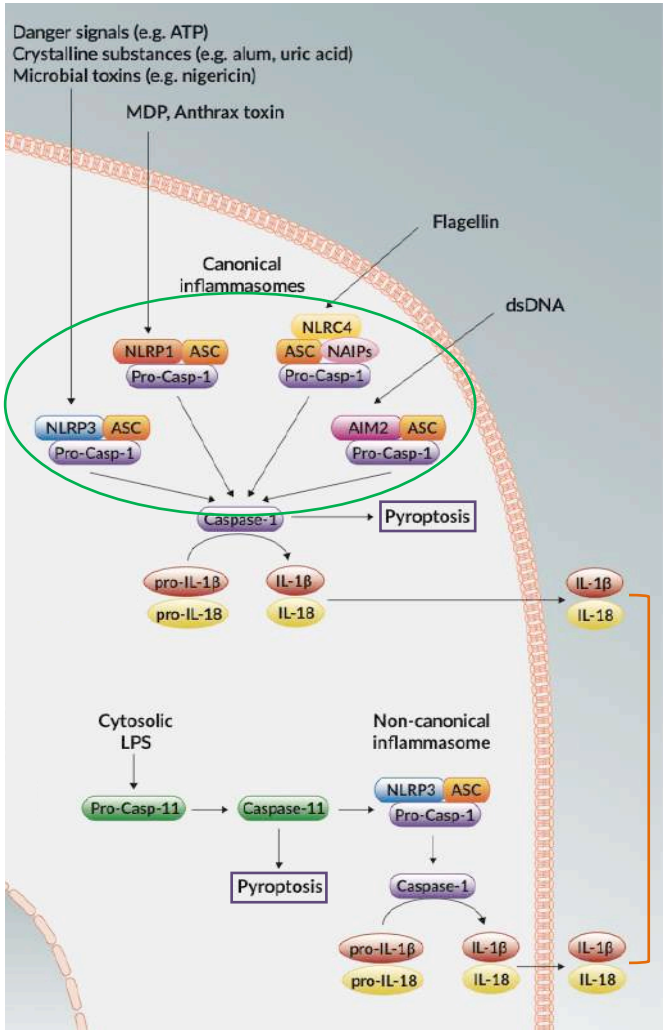
3. Caspase-8 Inflammasome
   - Through Dectin-1

   Gringhuis, Nat Immunol 2012

Canonical and Non-canonical Inflammasomes

Canonical Pathway

Non-Canonical Pathway



1. ASC Complex Formation

2. Pyroptosis

3. Interleukin Activation

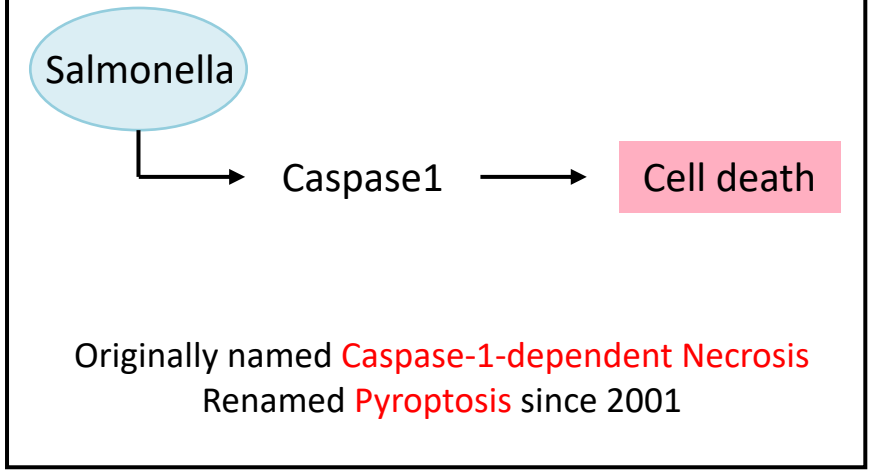
Pyroptosis - 2000

Molecular Microbiology (2000) 38(1), 31–40

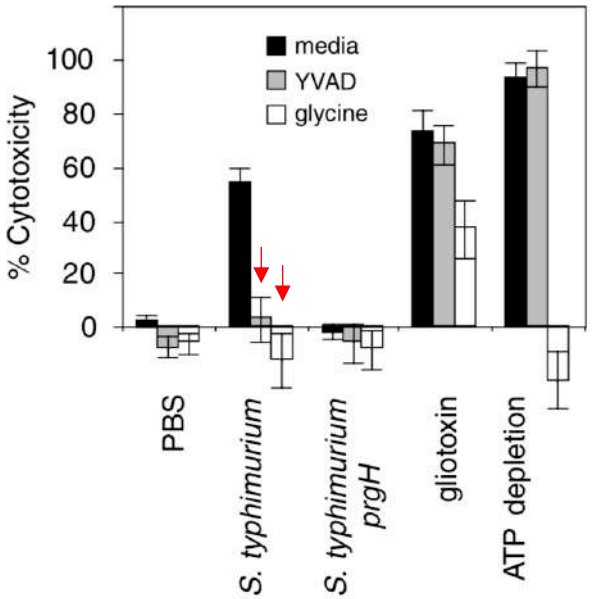
***Salmonella* induces macrophage death by caspase-1-dependent necrosis**

Molly A. Brennan[1] and Brad T. Cookson[1,2]*
Departments of [1]Microbiology and [2]Laboratory Medicine, Box 357110, University of Washington, Seattle, WA 98195, USA.

[Salmonella-induced cytotoxicity is different from caspase-3 induced apoptosis]



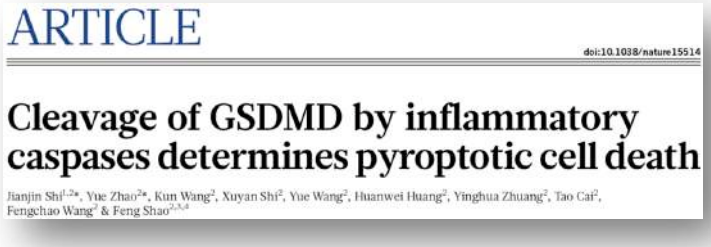
Originally named Caspase-1-dependent Necrosis
Renamed Pyroptosis since 2001



Salmonella-induced cell death inhibited by caspase-1 (YVAD) and ion flux (glycine) inhibitors

Related Product	Cat. Code
Ac-YVAD-cmk	inh-yvad
Heat Killed Salmonella typhimurium	tlrl-hkst2
FLA-ST	tlrl-epstfla-5

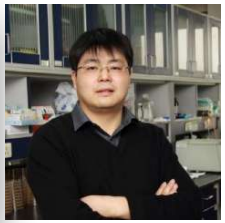
Gasdermin D - 2015


ARTICLE

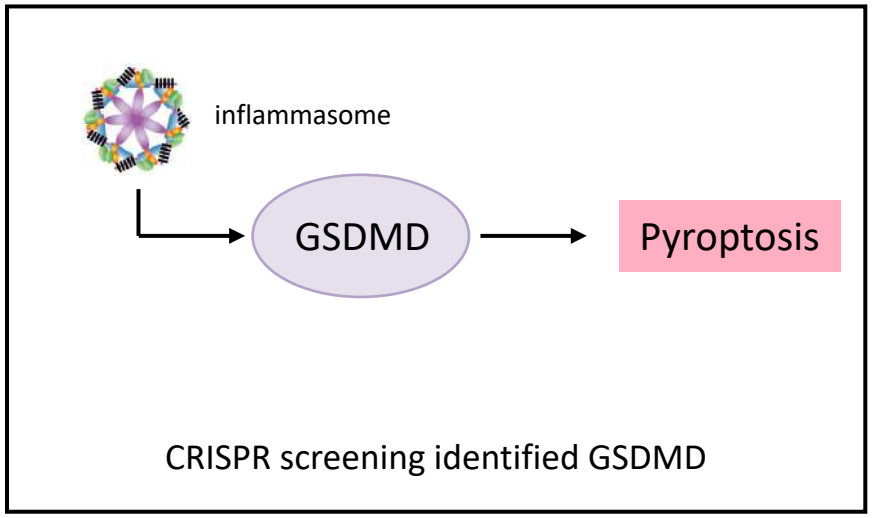
doi:10.1038/nature15514

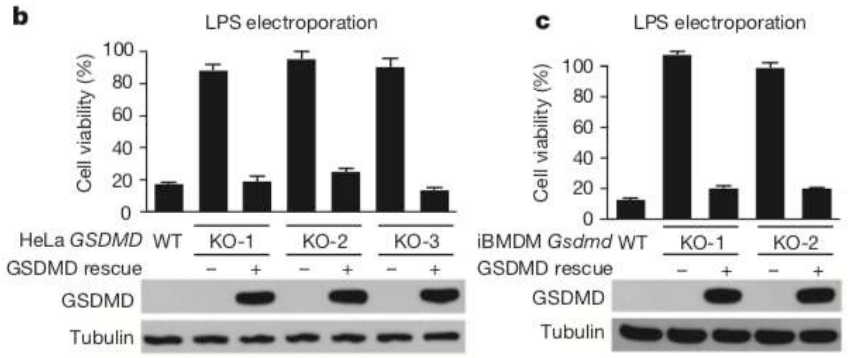
Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death

Jianjin Shi[1,2*], Yue Zhao[2*], Kun Wang[2], Xuyan Shi[2], Yue Wang[2], Huanwei Huang[2], Yinghua Zhuang[2], Tao Cai[2], Fengchao Wang[2] & Feng Shao[2,3,4]



Inflammasome → ? → Pyroptosis





Gasdermin D knockout inhibits pyroptosis

Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
poly(dA:dT)	tlrl-patn

RAW-ASC KO-GSDMD

NEW!

Gasdermin D - 2015

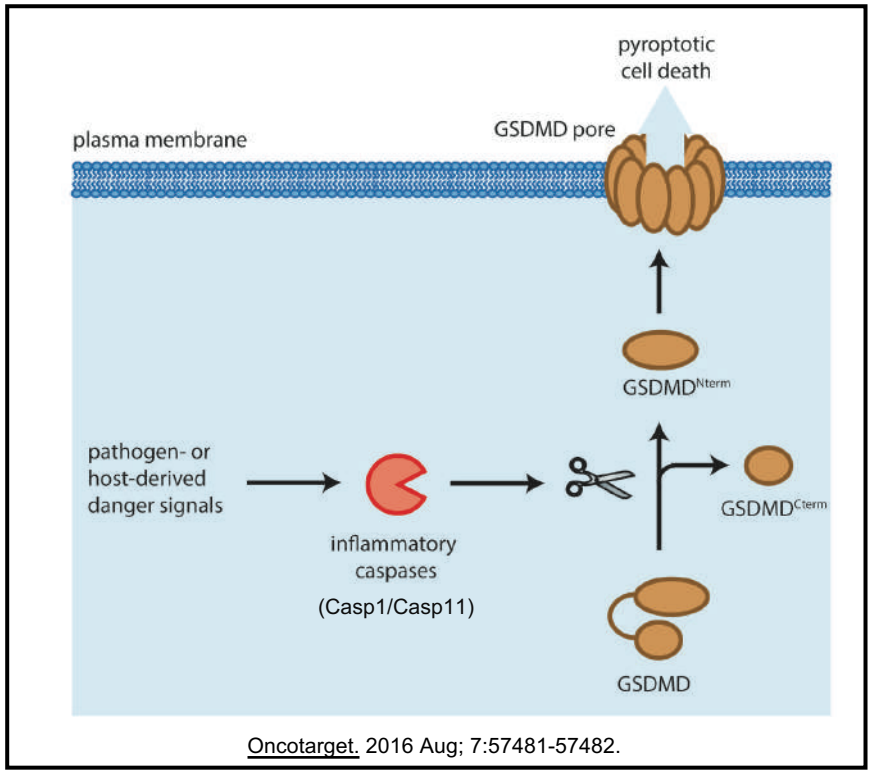
ARTICLE

doi:10.1038/nature15541

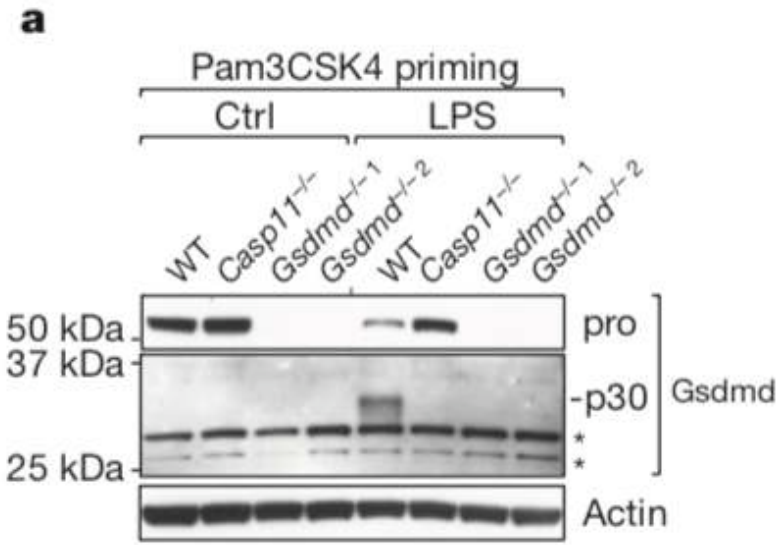
Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling

Nobuhiko Kayagaki[1], Irma B. Stowe[1], Bettina L. Lee[1], Karen O'Rourke[1], Keith Anderson[1], Søren Warming[1], Trinna Cuellar[1], Benjamin Haley[1], Merone Roose-Girma[1], Qui T. Phung[1], Peter S. Liu[1], Jennie R. Lill[1], Hong Li[1], Jiansheng Wu[1], Sarah Kummerfeld[1], Juan Zhang[2], Wyne P. Lee[1], Scott J. Snipas[3], Guy S. Salvesen[3], Lucy X. Morris[2], Linda Fitzgerald[2], Yafei Zhang[2], Edward M. Bertram[2,3], Christopher C. Goodnow[2,3,10] & Vishva M. Dixit[1]

Mechanism of GSDMD activation



Oncotarget. 2016 Aug; 7:57481-57482.



Caspase-11 cleaves Gasdermin D

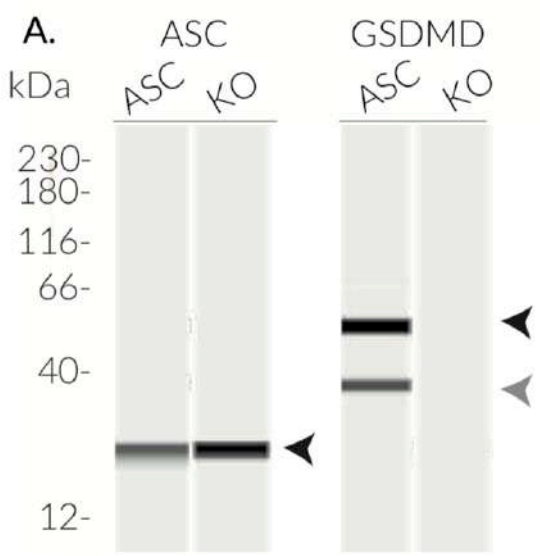
Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Pam3CSK4	tlrl-pms
Poly(I:C) LMW	tlrl-picw
R837 (Imiquimod)	tlrl-imqs
poly(dA:dT)	tlrl-patn
Flagellin-PA Ultrapure	tlrl-pafla
MSU Crystals	tlrl-msu
CPPD Crystals	tlrl-cppd
Zeocin™	ant-zn-05

GSDMD KO Cell Line

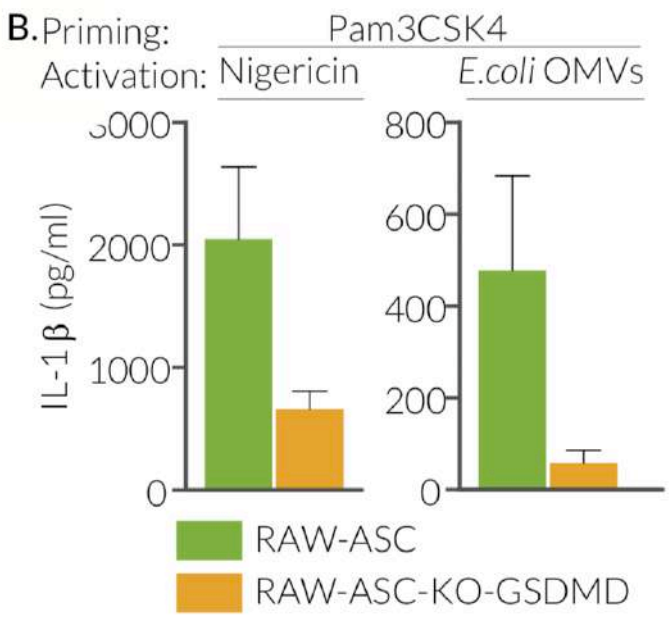
Product	Unit Size	Cat. Code
RAW-ASC	3-7 x 10^6 cells	raw-asc
RAW-ASC-KO-GSDMD	3-7 x 10^6 cells	raw-kogsdmd

NEW!

Expression

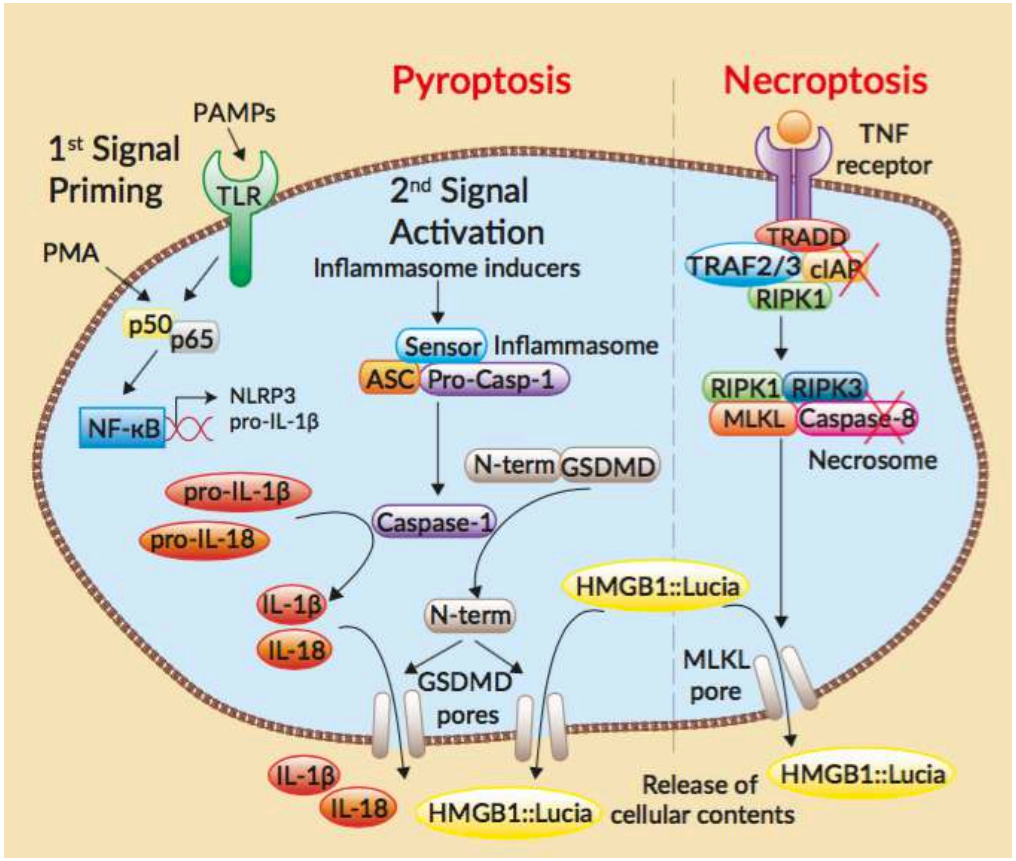


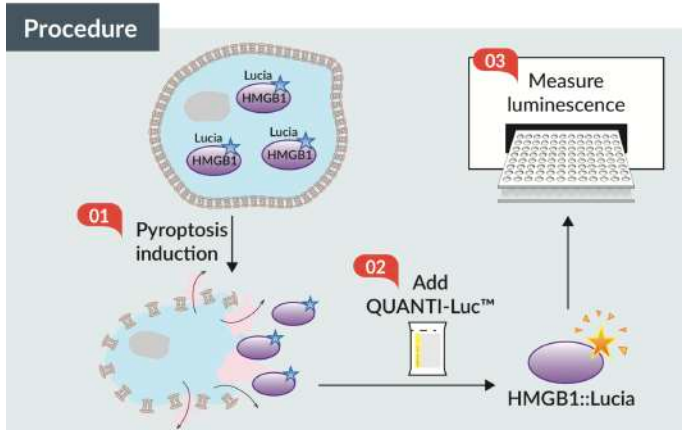
Activity



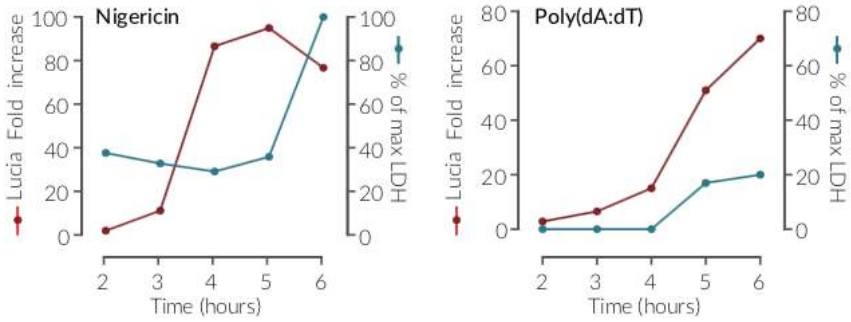
Pyroptosis Monitoring Cell Line

Product	Unit Size	Cat. Code
THP1-HMGB1-Lucia™ cells	3-7 x 10^6 cells	thp-hmgluc
QUANTI-Luc	2 x 25mL	rep-qlc1





Pyroptosis



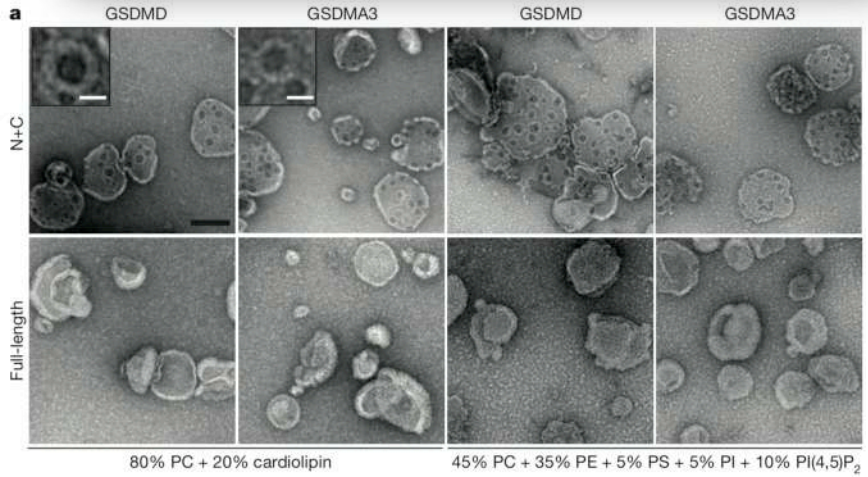
Functions of other Gasdermins

ARTICLE 2016

doi:10.1038/nature18590

Pore-forming activity and structural autoinhibition of the gasdermin family

Jingjin Ding[1,2], Kun Wang[2], Wang Liu[2], Yang She[1,2], Qi Sun[2], Jianjin Shi[2], Hanzi Sun[2], Da-Cheng Wang[1,3] & Feng Shao[1,2,4]



Related Product	Unit Size	Cat. Code
GSDMA	20 µg	puno1-hgsdma
GSDMB	20 µg	puno1-hgsdmb
GSDMC	20 µg	puno1-hgsdmc
GSDMD	20 µg	puno1-hgsdmd
GSDME	20 µg	puno1-hgsdme
GSDMF (PJVK)	20 µg	puno1-mgsdmf

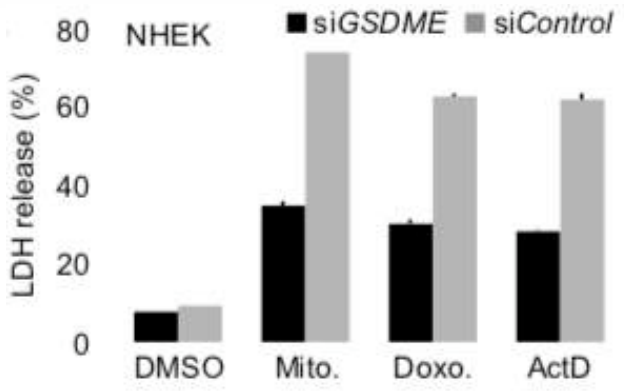
All Gasdermins form pores

LETTER 2017

doi:10.1038/nature22393

Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin

Yupeng Wang[1,2*], Wenqing Gao[1,2*], Xuyan Shi[1,2], Jingjin Ding[2,3], Wang Liu[2], Huabin He[2], Kun Wang[2] & Feng Shao[2,4]

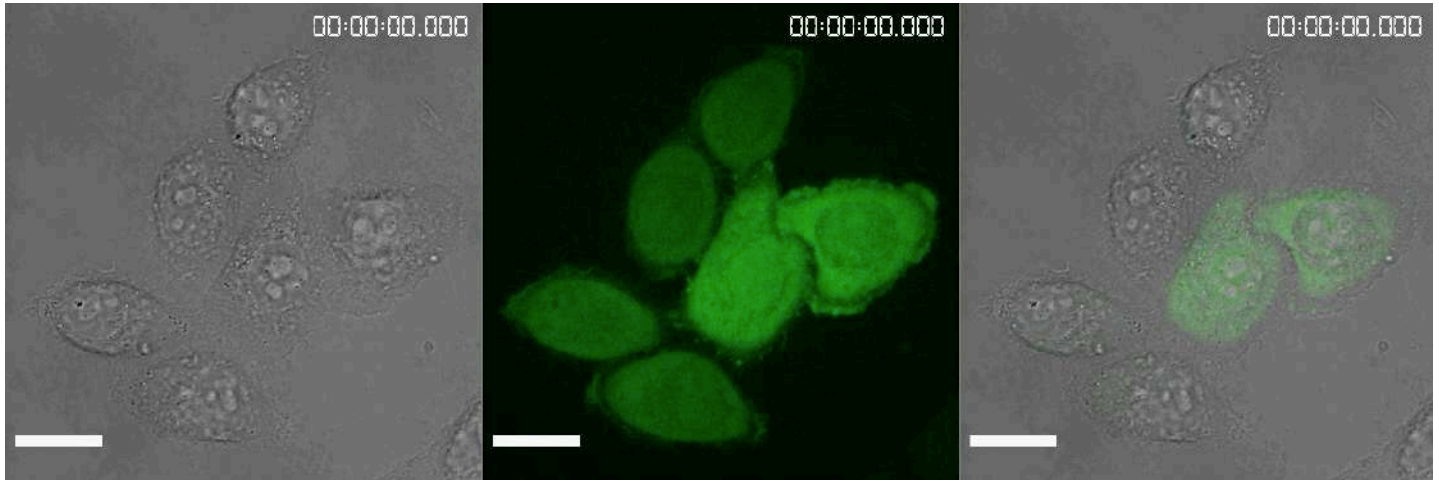


Product Used	Cat. Code
Recombinant human TNF-α	rcyc-htnfa

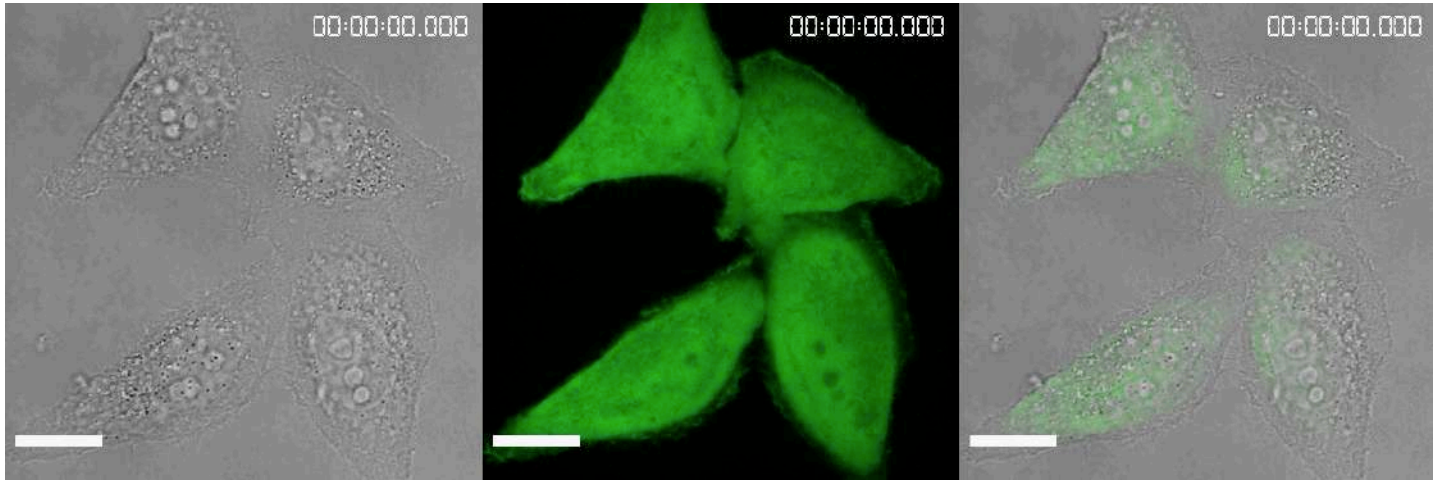
Gasdermin E causes cell death

Gasdermin E as a switch for pyroptosis

Gasdermin E -ve: Apoptosis



Gasdermin E +ve: Pyroptosis



Nature. 2017 Jul 6;547(7661):99-103. doi: 10.1038/nature22393.

Gasdermin D as a switch for pyroptosis

nature COMMUNICATIONS

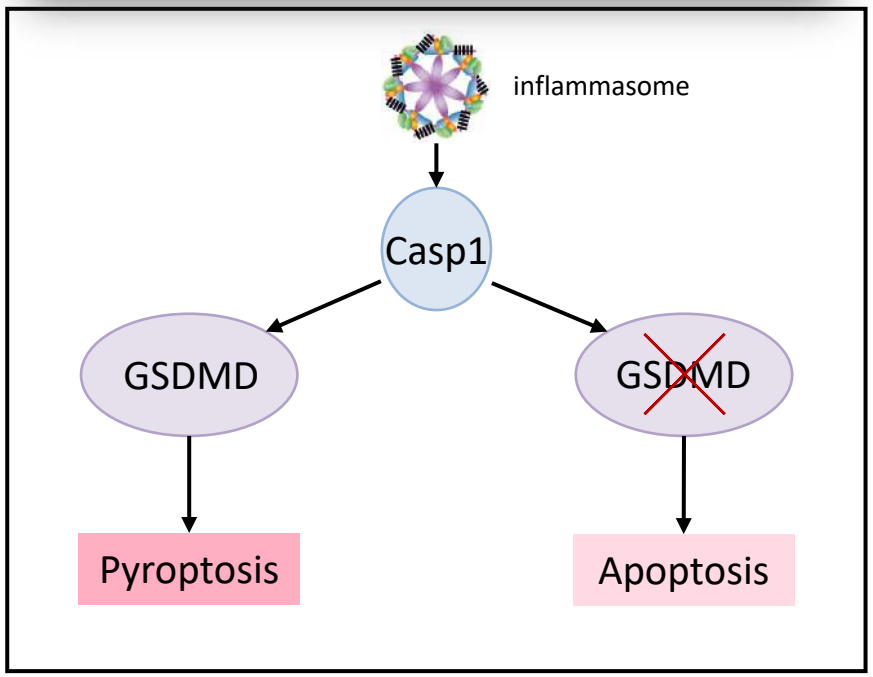
2019

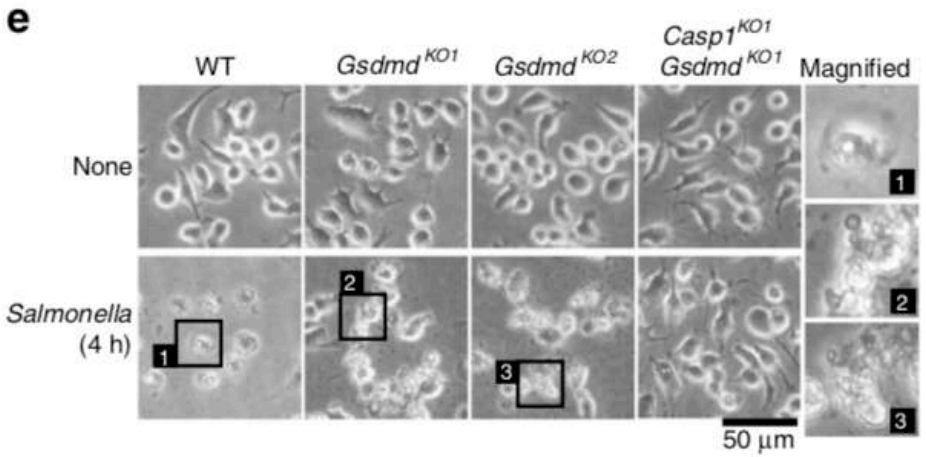
ARTICLE

https://doi.org/10.1038/s41467-019-09753-2 OPEN

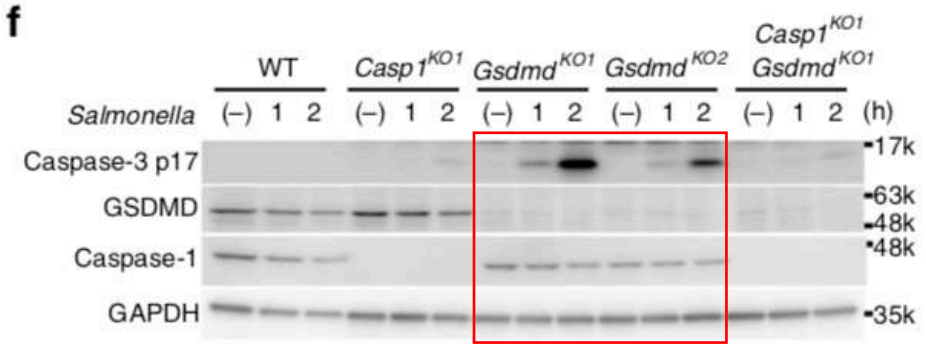
Caspase-1 initiates apoptosis in the absence of gasdermin D

Kohsuke Tsuchiya[1,2], Shinsuke Nakajima[1], Shoko Hosojima[1], Dinh Thi Nguyen[3], Tsuyoshi Hattori[3], Thuong Manh Le[3], Osamu Hori[3], Mamunur Rashid Mahib[1], Yoshifumi Yamaguchi[4], Masayuki Miura[5], Takeshi Kinoshita[1], Hiroko Kushiyama[1], Mayumi Sakurai[1], Toshihiko Shiroishi[6] & Takashi Suda[1]





Cells show apoptotic morphology when GSDMD is knocked out



Casp3 activates when GSDMD is knocked out

Gasdermin Inhibitor

Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

Unrefereed preprint

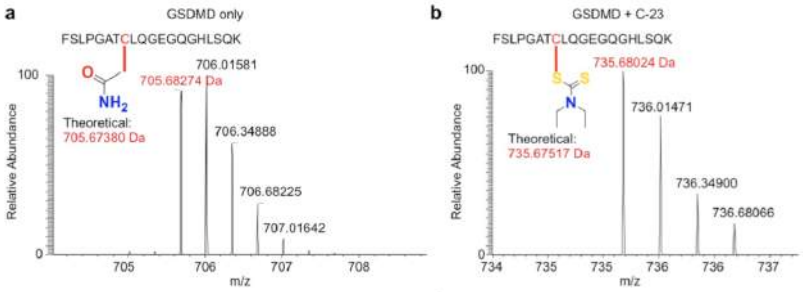
New Results

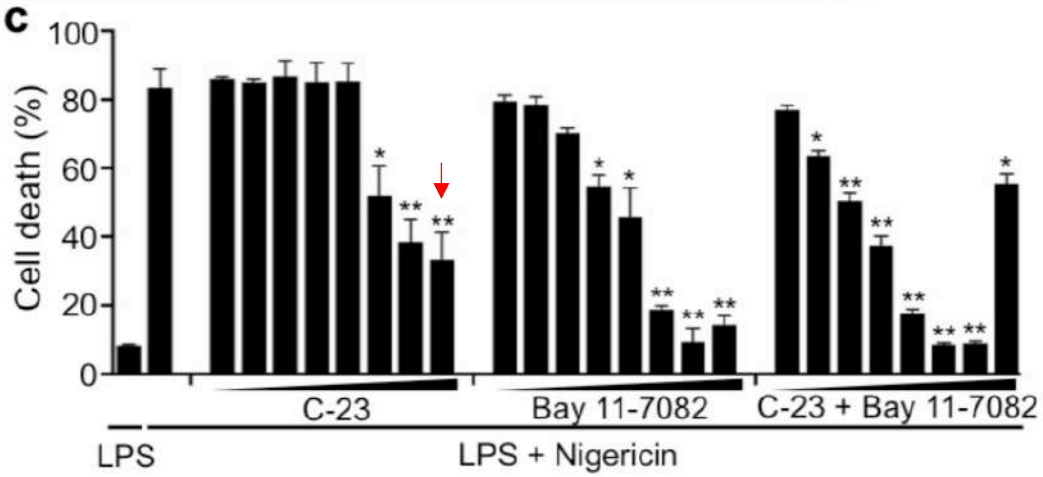
Identification of pyroptosis inhibitors that target a reactive cysteine in gasdermin D

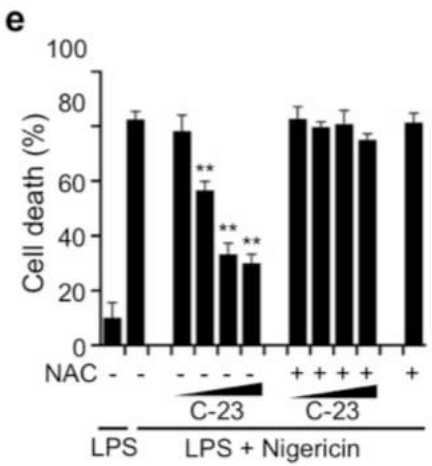
Jun Jacob Hu, Xing Liu, Jingxia Zhao, Shiyu Xia, Jianbin Ruan, Xuemei Luo, Justin Kim, Judy Lieberman, Hao Wu

doi: https://doi.org/10.1101/365908

Disulfiram inhibits GSDMD by cysteine modification







Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Nigericin	tlrl-nig

Gasdermin Inhibitor

Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis

Joseph K. Rathkey[1], **Junjie Zhao**[2], **Zhonghua Liu**[1], **Yinghua Chen**[3], **Jie Yang**[1,3], **Hannah C. Kondolf**[1], **Bryan L. Benson**[1], **Steven M. Chirieleison**[1], **Alex Y. Huang**[1,4], **George R. Dubyak**[3], **Tsan S. Xiao**[1], **Xiaoxia Li**[2], and **Derek W. Abbott**[1,*]

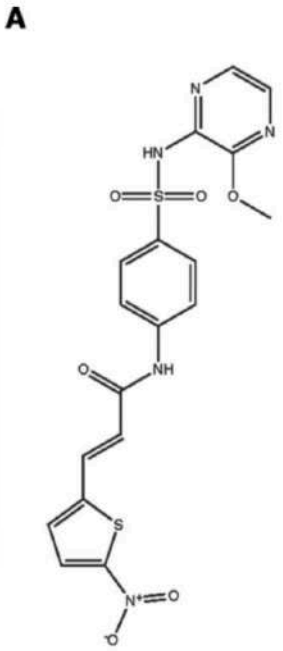
[1]Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

[2]Department of Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA.

[3]Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

[4]Division of Pediatric Hematology-Oncology, Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.

NSA inhibits pyroptosis by direct binding to GSDMD



NSA

Product Used	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
Puromycin	ant-pr

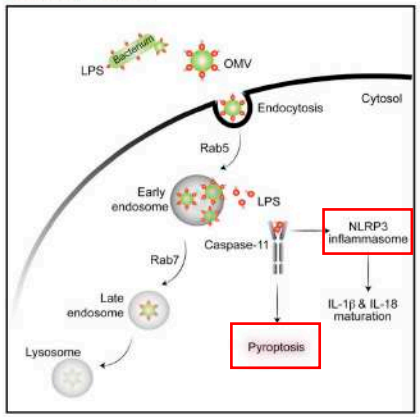
E.coli Outer Membrane Vesicles - 2016

Article

Cell

Bacterial Outer Membrane Vesicles Mediate Cytosolic Localization of LPS and Caspase-11 Activation

Graphical Abstract



Authors

Sivapriya Kailasan Vanaja, Ashley J. Russo, Bharat Behl, Ishita Banerjee, Maya Yankova, Sachin D. Deshmukh, Vijay A.K. Rathinam

Correspondence

rathinam@uchc.edu

In Brief

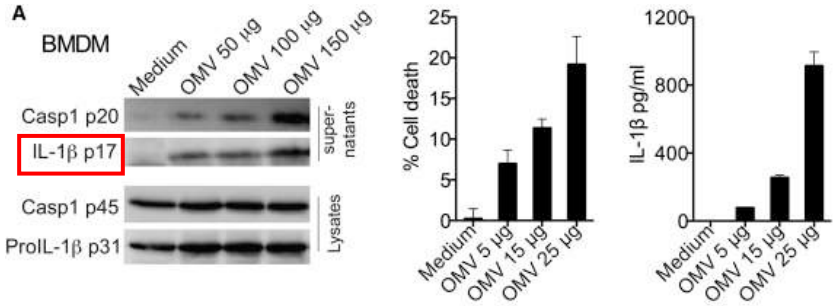
Gram-negative bacteria secrete outer membrane vesicles that deliver lipopolysaccharide (LPS) to the host cell cytosol, where it can trigger caspase-11 activation—a critical mechanism against bacterial infections and to sepsis pathogenesis.

Highlights

- OMVs secreted by bacteria enable intracellular LPS localization
- OMVs activate caspase-11-mediated cytosolic LPS sensing
- OMVs enter the cells by endocytosis, and LPS accesses cytosol from early endosomes
- OMVs are essential for the activation of caspase-11 during bacterial infections.

Vanaja et al., 2016, Cell *165*, 1–14
May 19, 2016 © 2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.cell.2016.04.015

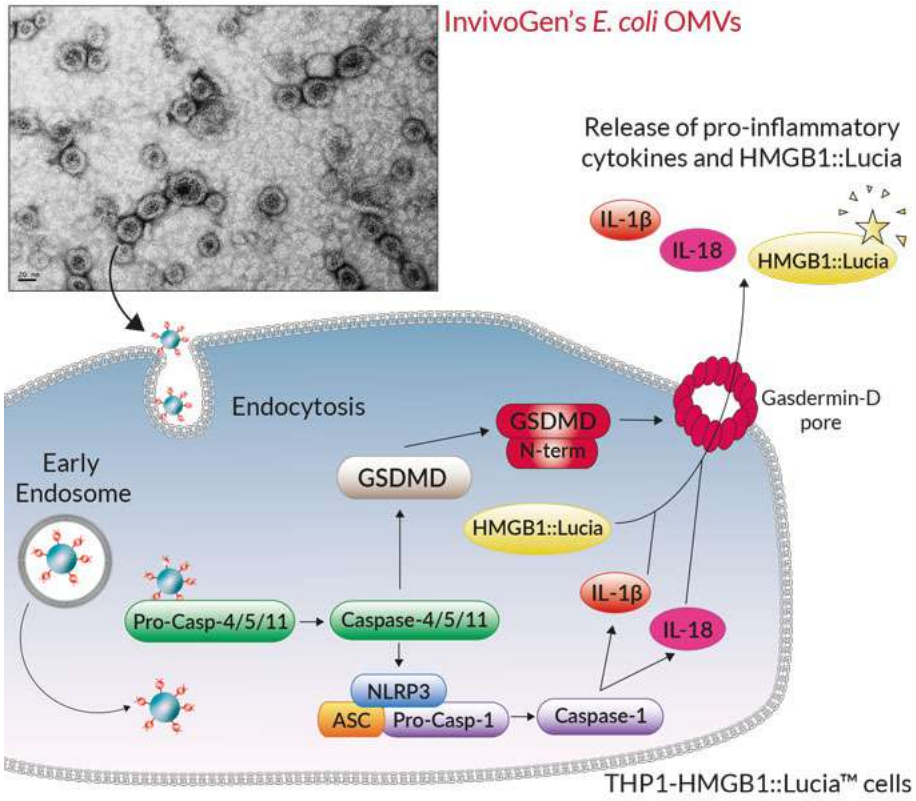
CellPress



OMVs as non-canonical inflammasome inducer

Product Used	Cat. Code
Poly(I:C) (HMW)	tlrl-pic
Pam3CSK4	tlrl-pms

E.coli Outer Membrane Vesicles

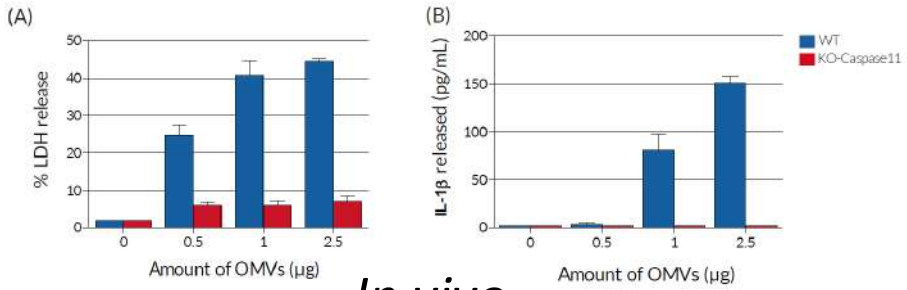


No need to transfect!!

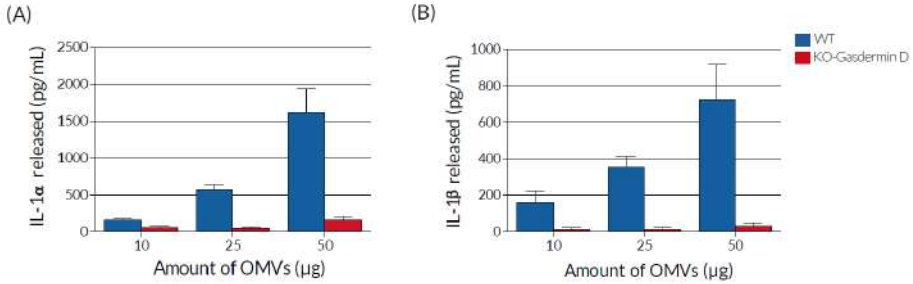
E.coli OMVs NEW

Product	Unit Size	Cat. Code
E.coli OMV (for *in vitro*)	100 ug	tlrl-omv-1
InvivoFit™ *E.coli* OMV (for *in vivo*)	500 ug	tlrl-omv

In vitro



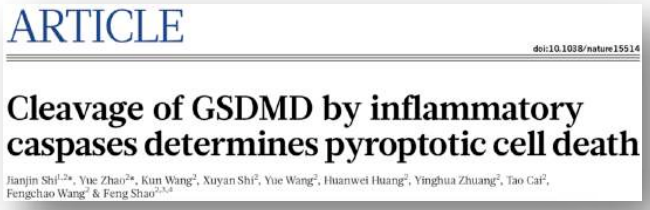
In vivo



Collaborated with Etienne Meunier, Institut de Pharmacologie et de Biologie Structurale, Toulouse

InvivoGen LPS O111:B4

TLR4 Biological Activity Test by HEK-Blue hTLR4 Reporter Cells


ARTICLE

doi:10.1038/nature15514

Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death

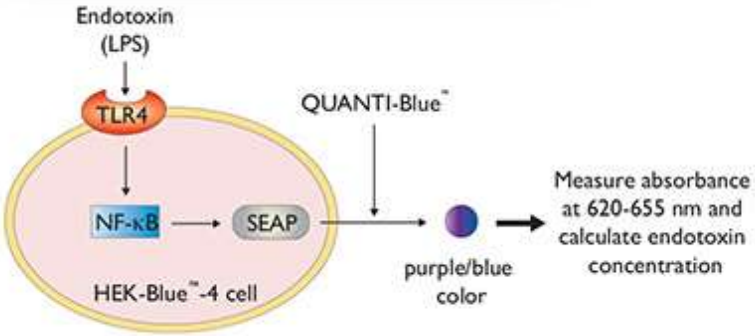
Jianjin Shi[1,2*], Yue Zhao[2*], Kun Wang[2], Xuyan Shi[2], Yue Wang[2], Huanwei Huang[2], Yinghua Zhuang[2], Tao Cai[2], Fengchao Wang[2] & Feng Shao[2,3,4]

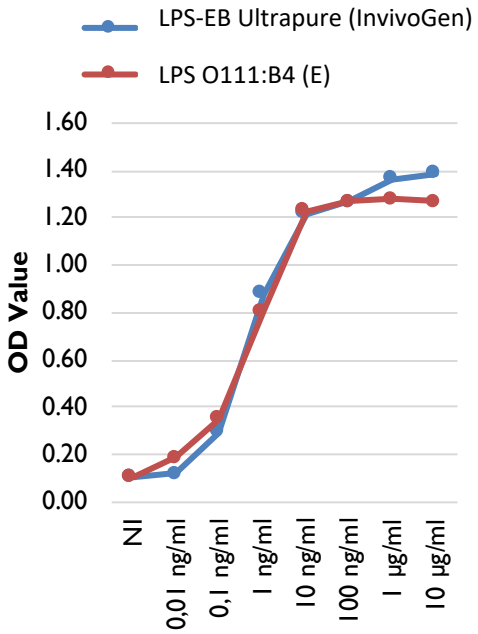

ARTICLE

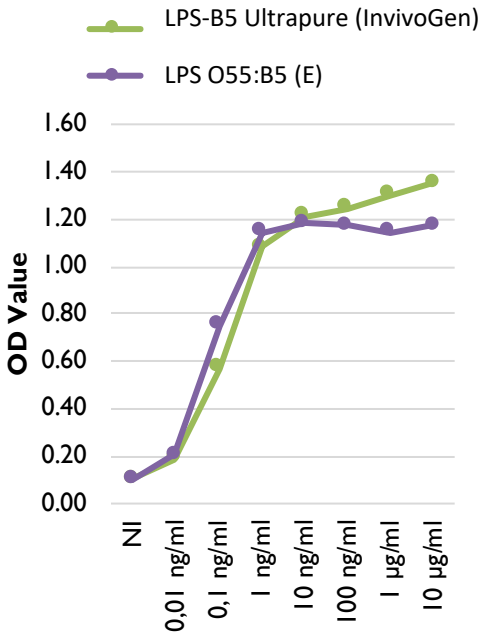
doi:10.1038/nature15541

Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling

Nobuhiko Kayagaki[1], Irma B. Stowe[1], Bettina L. Lee[1], Karen O'Rourke[1], Keith Anderson[2], Søren Warming[2], Trinna Cuellar[2], Benjamin Haley[2], Merone Roose-Girma[2], Qui T. Phung[2], Peter S. Liu[3], Jennie R. Lill[3], Hong Li[3], Jiansheng Wu[3], Sarah Kummerfeld[4], Juan Zhang[5], Wyne P. Lee[5], Scott J. Snipas[6], Guy S. Salvesen[6], Lucy X. Morris[1], Linda Fitzgerald[1], Yafei Zhang[1], Edward M. Bertram[1,4], Christopher C. Goodnow[4,7,8] & Vishva M. Dixit[1]





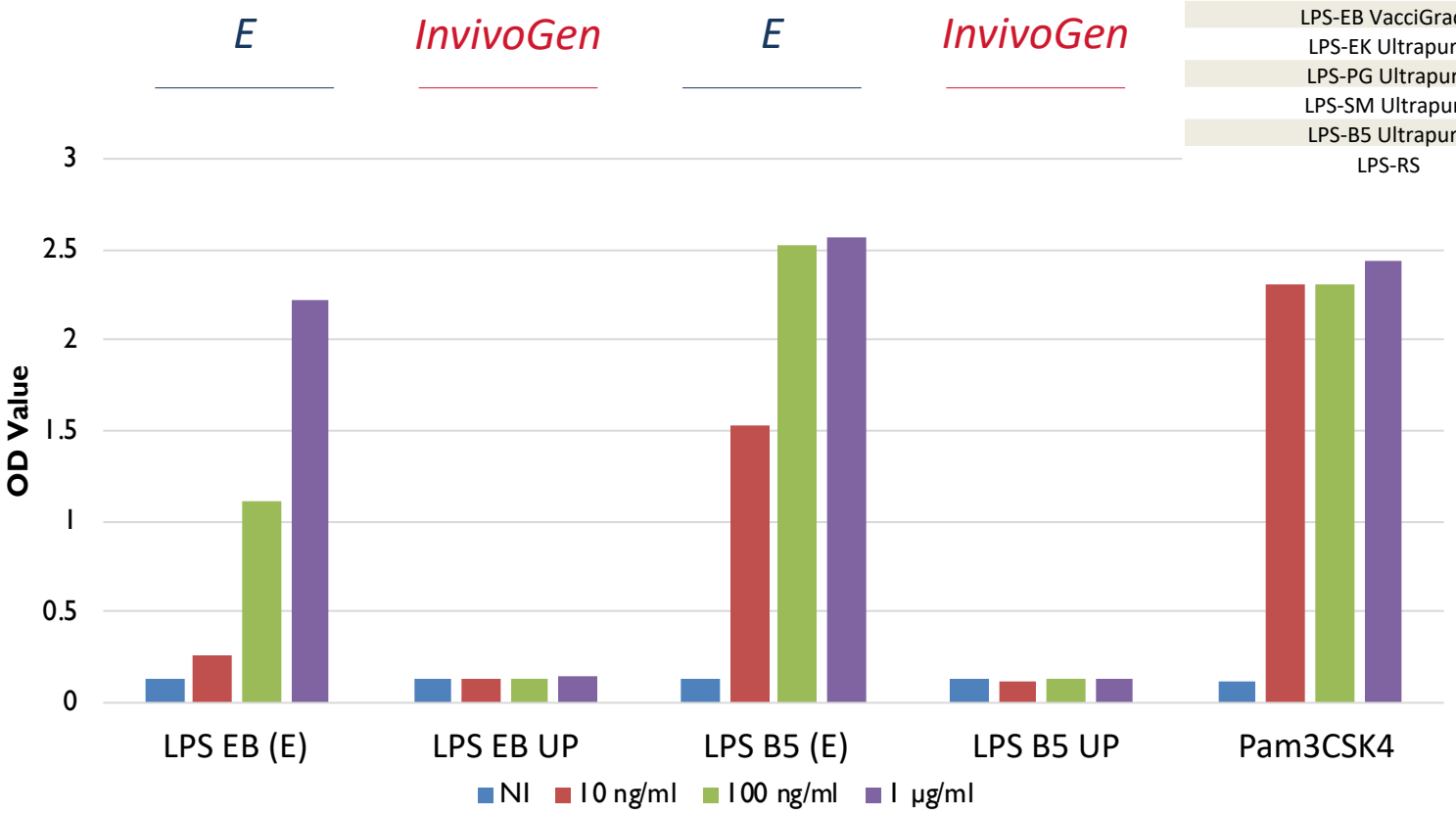


TLR4 potency is comparative

InvivoGen LPS O111:B4

TLR2 Biological Activity Test by HEK-Blue hTLR2 Reporter Cells

Related Products	Cat. Code
LPS-EB Ultrapure	tlrl-3pelps
LPS-EB VacciGrade	vac-3pplps
LPS-EK Ultrapure	tlrl-peklps
LPS-PG Ultrapure	tlrl-ppglps
LPS-SM Ultrapure	tlrl-smlps
LPS-B5 Ultrapure	tlrl-pb5lps
LPS-RS	tlrl-prslps

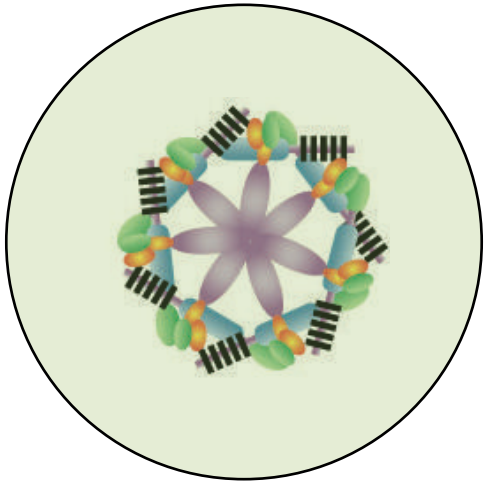


LPS from (E) is contaminated by TLR2 PAMPs

Inflammasomes and Diseases

Autoimmunity
Vitiligo, Rheumatoid Arthritis

Metabolic Disease
Type II Diabetes, Atherosclerosis



Cancer
colitis-associated colorectal cancer

Alzheimer's Disease

1. Inflammasome and Autoimmune Diseases

Opinion

Cell PRESS

Inflammasomes and autoimmunity

Patrick J. Shaw[1], Michael F. McDermott[2] and Thirumala-Devi Kanneganti[1]

[1] Department of Immunology, St. Jude Children's Research Hospital, MS #351, Suite E7004, 262 Danny Thomas Place, Memphis, TN 38105, USA
[2] NIHR-Leeds Musculoskeletal Biomedical Research Unit (NIHR-LMBRU), Leeds Institute of Molecular Medicine, Wellcome Trust Brenner Building, St. James's University Hospital, Leeds, LS9 7TF, UK

Table 1. The role of the inflammasomes in human disease

	Human disease	Cause of disease	Link to genetic mutations	Response to IL-1 blockade?	Inflammasome involved in animal model?
	Autoinflammatory diseases				
NLRP3	MWS	NLRP3 mutation	Yes [10]	Yes [12]	Yes [81]
NLRP3	FCAS	NLRP3 mutation	Yes [10]	Yes [12]	Yes [82]
NLRP3	CINCA	NLRP3 mutation	Yes [10]	Yes [12]	Not tested
	Autoimmune diseases				
NLRP3/NLRP1	Type 1 diabetes	Self destruction of pancreatic β cells	Yes [55]	Not tested	Not tested
	Multiple sclerosis	Self-destruction of oligodendrocytes	No	In progress	Yes [43]/No [44]
	Systemic lupus erythematosus	Targeting of ubiquitous, mostly intranuclear self-antigens	No	Yes60	Not tested
NLRP1	Vitiligo	Self-destruction of melanocytes	Yes [71]	Not tested	Not tested
NLRP1	Addison's disease	Destruction of the adrenal cortex resulting in impaired cortisol production	Yes [54]	Not tested	Not tested
NLRP3	Celiac disease	Destruction of the small intestine lining triggered by eating gluten	Yes [55]	Not tested	Not tested
NLRP1	Rheumatoid arthritis	Self-destruction of the synovial joints	Yes [29]	Yes [27]	No [33,34]

1. Inflammasome and Vitiligo/Rheumatoid Arthritis

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

NALP1 in Vitiligo-Associated Multiple Autoimmune Disease

Ying Jin, M.D., Ph.D., Christina M. Mailloux, B.S., Katherine Gowan, B.S., Sheri L. Riccardi, B.S., Greggory LaBerge, M.S., Dorothy C. Bennett, Ph.D., Pamela R. Fain, Ph.D., and Richard A. Spritz, M.D.

Table 2. Association of 19 *NALP1* Variants with Vitiligo and Autoimmune and Autoinflammatory Disease in 114 Multiplex Families.*

Variant/Allele	P Value			Odds Ratio (95% CI)†
	Pedigree Disequilibrium Test	Family-Based Association Test	Conditional Logistic-Regression Analysis	
Vitiligo				
rs6502867/A	0.005	0.004	<0.001	2.08 (1.37–3.15)
rs961826/A	0.002	0.003	0.007	1.67 (1.15–2.41)
rs12150220/A	0.002	0.002	0.004	1.69 (1.18–2.41)
rs11078575/C	0.005	0.005	0.02	1.55 (1.09–2.19)
rs1877658/T	0.004	0.007	0.03	1.49 (1.04–2.10)
rs925597/A	0.002	0.003	0.005	1.69 (1.18–2.44)
rs925598/A	0.003	0.003	0.005	1.66 (1.16–2.36)
rs3926687/T	0.004	0.004	0.008	1.61 (1.14–2.29)
12-bp deletion‡	0.001	0.009	0.007	1.66 (1.16–2.36)
rs2670660/C	<0.001	0.001	0.002	1.80 (1.25–2.61)
rs2733359/G	0.001	0.004	0.004	1.75 (1.21–2.53)
rs35658367/ATGA	<0.001	0.001	0.002	1.82 (1.24–2.67)
rs2716914/C	0.002	0.008	0.007	1.64 (1.15–2.35)
rs878329/G	0.002	0.003	0.002	1.75 (1.22–2.51)
rs7223628/G	<0.001	0.002	0.001	1.82 (1.26–2.63)
rs8182352/G	<0.001	<0.001	<0.001	2.01 (1.36–2.95)
rs4790796/A	0.001	0.002	0.001	1.83 (1.26–2.63)
rs4790797/T	<0.001	0.001	<0.001	2.01 (1.39–2.91)
rs8182354/A	0.001	0.002	0.001	1.83 (1.27–2.64)

Mutation of NLRP1 in Vitiligo patients

Rheumatology 2008;47:415–417
Advance Access publication 7 February 2008
doi:10.1093/rheumatology/kem372

Concise Report

Genetic variation in proteins of the cryopyrin inflammasome influences susceptibility and severity of rheumatoid arthritis (The Swedish TIRA project)

A. Kastbom[1,*], D. Verma[2,*], P. Eriksson[1], T. Skogh[1], G. Wingren[3] and P. Söderkvist[2]

TABLE 2. Number of individuals (%) carrying at least one variant allele (*CIAS1/TUCAN* −/−) compared with those carrying only wild-type alleles at both loci (*CIAS1/TUCAN* +/+)

NLRP3

CARD8

	CIAS1/TUCAN +/+	*CIAS1/TUCAN* −/−	OR (95% CI)	*P*-value
Controls ($n=360$)	130 (36)	21 (6)	Reference group	-
RA total ($n=174$)	54 (31)	19 (11)	2.2 (1.03, 4.6)	0.04

Higher mutation frequencies of NLRP3 and CARD8 in RA patients

Lead to FDA approved drug: Anakinra, IL-1R antagonist

2. Inflammasome and Cancer

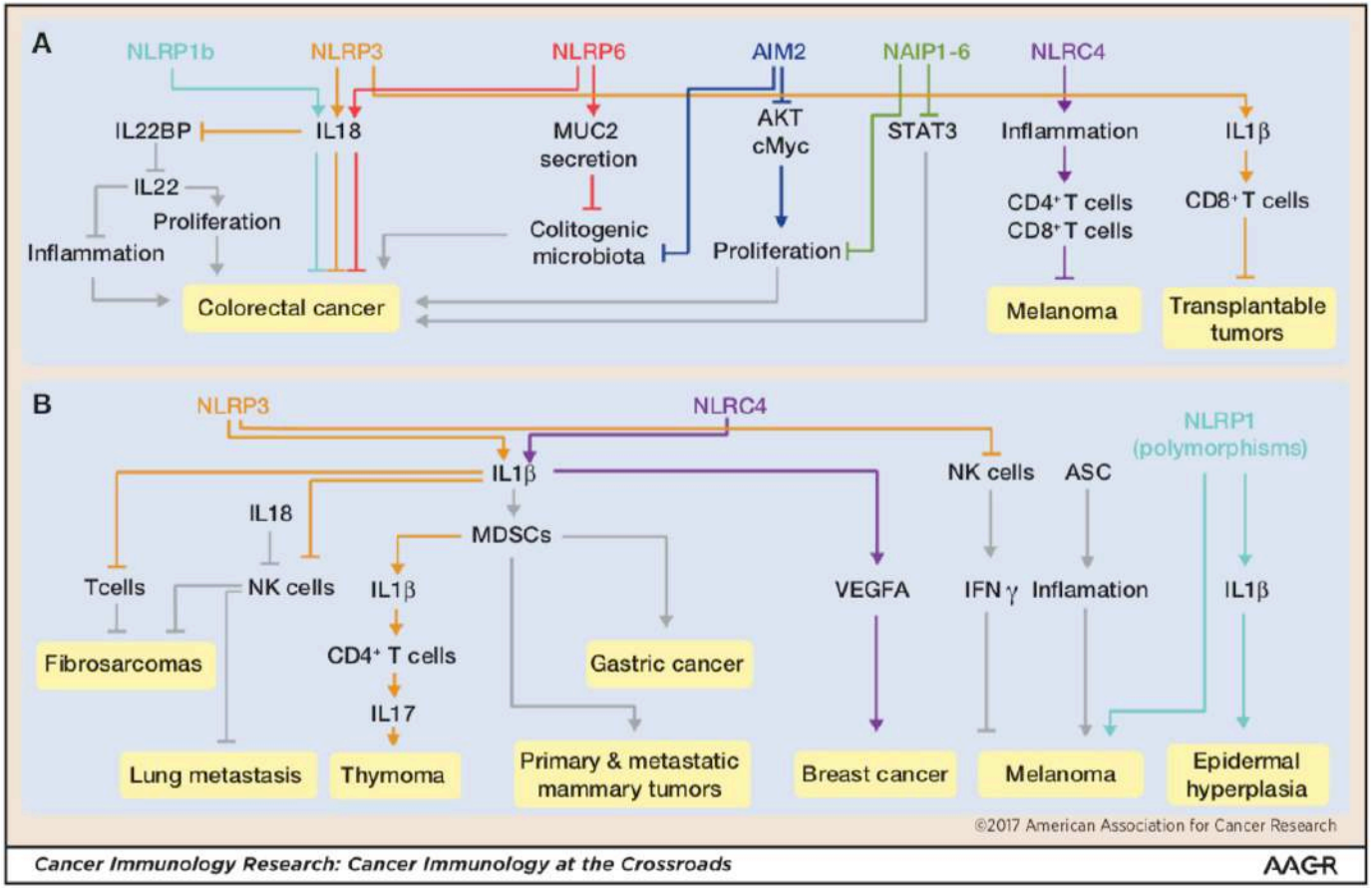
Published OnlineFirst January 16, 2017; DOI: 10.1158/2326-6066.CIR-16-0269

Cancer Immunology at the Crossroads

Cancer Immunology Research

Inflammasomes and Cancer

Rajendra Karki, Si Ming Man, and Thirumala-Devi Kanneganti



2. Inflammasome and Cancer

Oncogene (2017), 1–11
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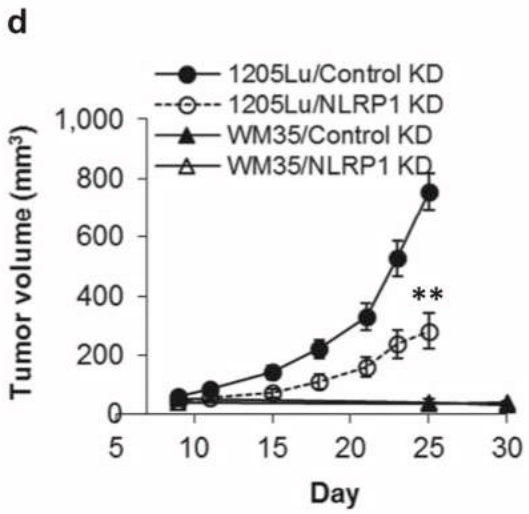
www.nature.com/onc

ORIGINAL ARTICLE

NLRP1 promotes tumor growth by enhancing inflammasome activation and suppressing apoptosis in metastatic melanoma

Z Zhai[1], W Liu[1], M Kaur[1], Y Luo[1], J Domenico[1], JM Samson[1], YG Shellman[1,2], DA Norris[1,2], CA Dinarello[3], RA Spritz[4] and M Fujita[1,2]

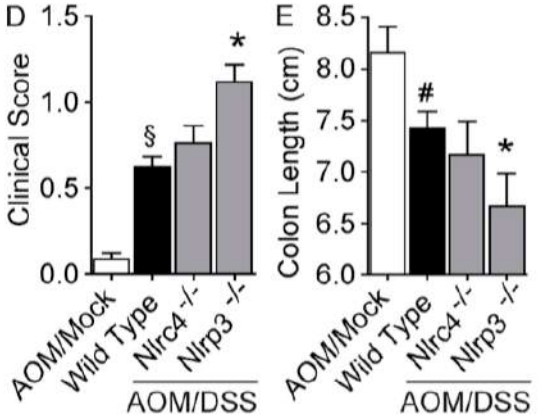


Metastatic melanoma model:

NLRP1 KD → less severe tumor

The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer

Irving C. Allen,[1] Erin McElvania TeKippe,[2] Rita-Marie T. Woodford,[3] Joshua M. Uronis,[4] Eda K. Holl,[3] Arlin B. Rogers,[5] Hans H. Herfarth,[4,6] Christian Jobin,[4,7] and Jenny P.-Y. Ting[1,2,3,4]



(AOM/DSS)-induced cancer model:

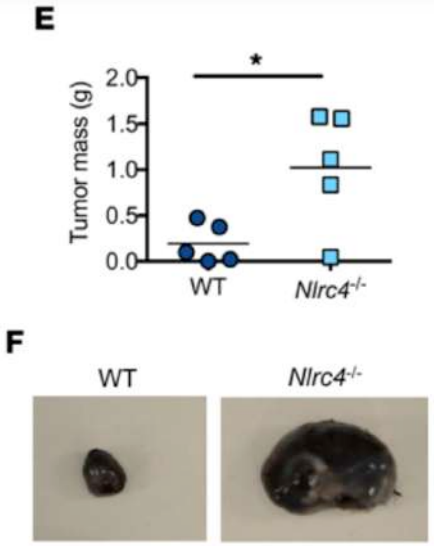
NLRP3 KO → more severe tumor

The Journal of Clinical Investigation
RESEARCH ARTICLE

NLRC4 suppresses melanoma tumor progression independently of inflammasome activation

Ann M. Janowski,[1,2] Oscar R. Colegio,[1] Emma E. Hornick,[1,2] Jennifer M. McNiff,[3] Matthew D. Martin,[2,4] Vladimir P. Badovinac,[2,4] Lyse A. Norian,[3] Weizhou Zhang,[3,4] Suzanne L. Cassel,[1,2,4,5] and Fayyaz S. Sutterwala[1,2,4,5]

[1]Inflammation Program and [2]Interdisciplinary Program in Immunology, University of Iowa, Iowa City, Iowa, USA. [3]Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA. [4]Department of Pathology, University of Iowa, Iowa City, Iowa, USA. [5]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA. [6]Department of Internal Medicine, University of Iowa, Iowa City, Iowa, USA. [7]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, California, USA.



B16F10 melanoma mode:

NLRC4 KO → more severe melanoma tumor

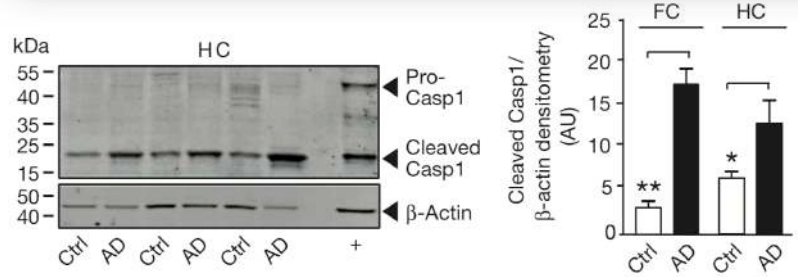
3. Inflammasome and Alzheimer's Disease

LETTER

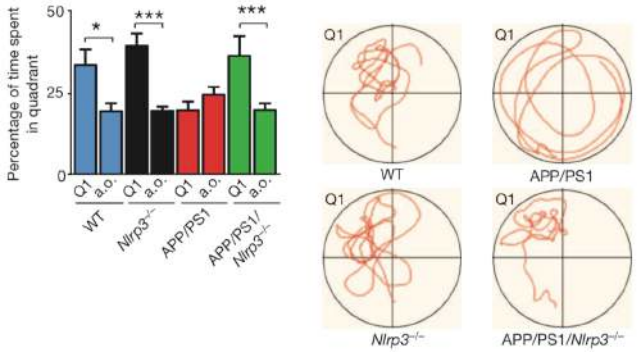
doi:10.1038/nature11729

NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice

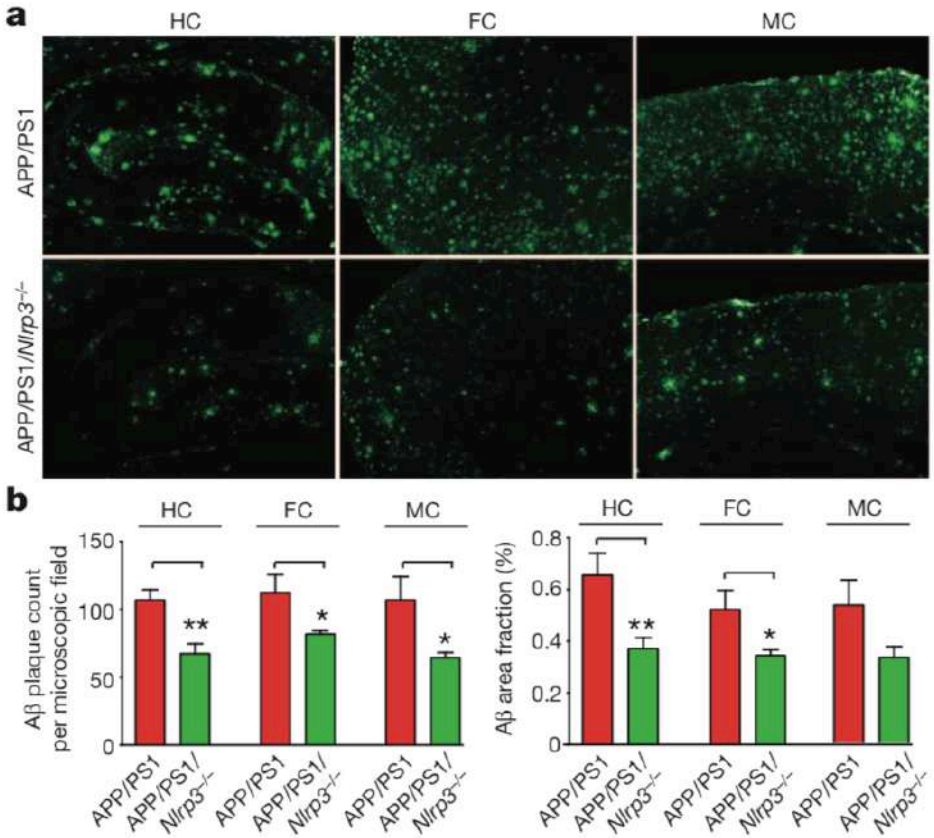
Michael T. Heneka[1,2]*, Markus P. Kummer[1], Andrea Stutz[3], Andrea Delekate[4], Stephanie Schwartz[1], Ana Vieira-Saecker[1], Angelika Griep[1], Daisy Axt[1], Anita Remus[4], Te-Chen Tzeng[5], Ellen Gelpi[6], Annett Halle[7], Martin Korte[4,8], Eicke Latz[2,3,5]* & Douglas T. Golenbock[5]*



Casp1 activated in AD patients



Protective effect of NLRP3 deficiency on mice memory



NLRP3 gene deficiency leads to decreased amyloid-b amounts and deposition

3. Inflammasome and Alzheimer's Disease

nature COMMUNICATIONS

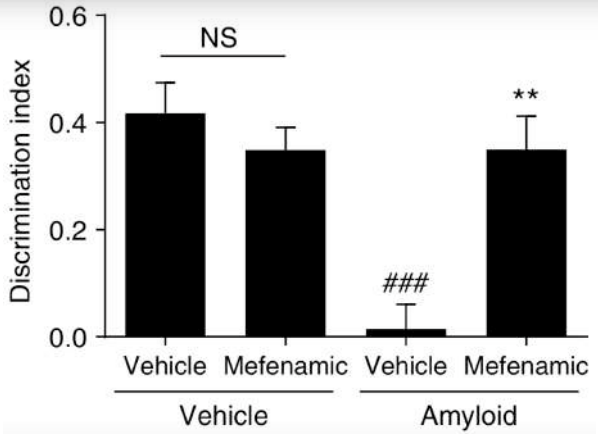
ARTICLE

Received 11 Nov 2015 | Accepted 6 Jul 2016 | Published 11 Aug 2016

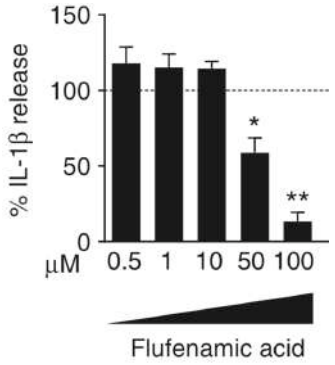
DOI: 10.1038/ncomms12504 OPEN

Fenamate NSAIDs inhibit the NLRP3 inflammasome and protect against Alzheimer's disease in rodent models

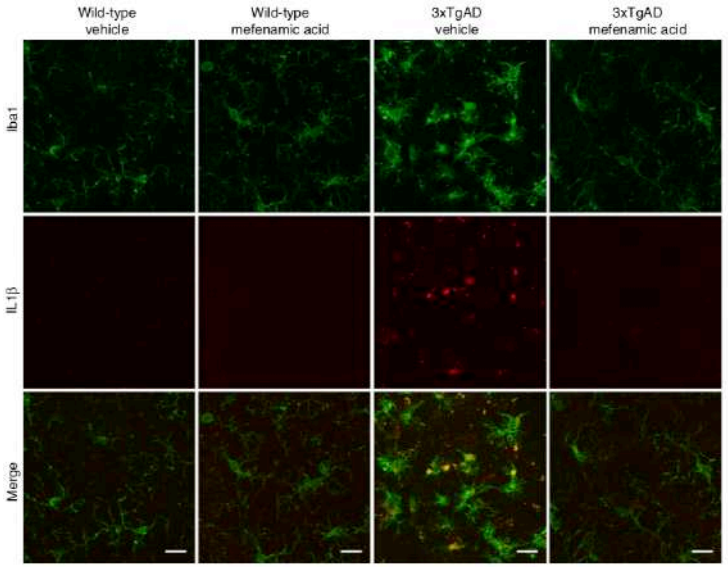
Michael J.D. Daniels[1,*], Jack Rivers-Auty[1,*], Tom Schilling[2], Nicholas G. Spencer[2], William Watremez[3], Victoria Fasolino[3], Sophie J. Booth[1], Claire S. White[1], Alex G. Baldwin[3], Sally Freeman[3], Raymond Wong[1], Clare Latta[1], Shi Yu[1], Joshua Jackson[3], Nicolas Fischer[4], Violette Koziel[4], Thierry Pillot[4], James Bagnall[1], Stuart M. Allan[1], Pawel Paszek[1], James Galea[5], Michael K. Harte[3], Claudia Eder[2], Catherine B. Lawrence[1] & David Brough[1]



Mice with Mefenamic treatment scores better in novel object recognition test



Mefenamic acid inhibits IL-1β release



Mefenamic acid reduces Alzheimer's disease-related neuroinflammation

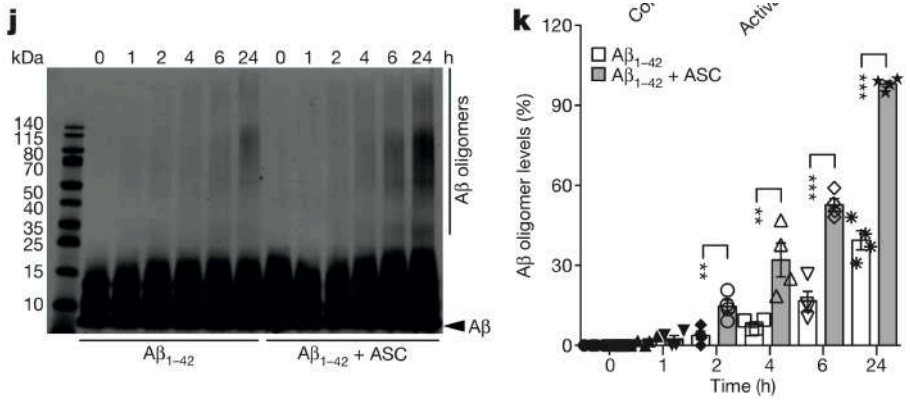
3. Inflammasome and Alzheimer's Disease

ARTICLE

doi:10.1038/nature25158

Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease

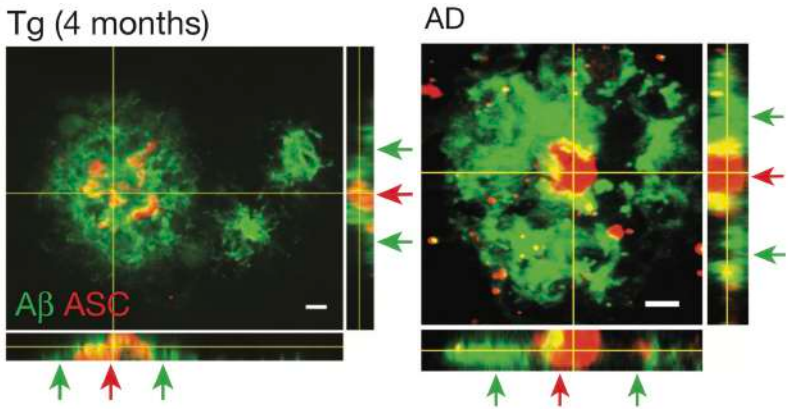
Carmen Venegas[1]*, Sathish Kumar[2]*, Bernardo S. Franklin[3], Tobias Dierkes[1,3], Rebecca Brinkschulte[3], Dario Tejera[1], Ana Vieira-Saecker[1], Stephanie Schwartz[1], Francesco Santarelli[1], Markus P. Kummer[1], Angelika Griep[1], Ellen Gelpi[4], Michael Beilharz[3], Dietmar Riedel[5], Douglas T. Golenbock[6], Matthias Geyer[3], Jochen Walter[2], Eicke Latz[3,6,7] & Michael T. Heneka[1,6,7]



ASC specks enhance Aβ oligomerization

Mouse model

AD patient



ASC specks co-sediment with Aβ and form the core of mouse and human Aβ plaques

Major Research Questions

1. What is the molecular mechanism that activates the inflammasome? What are the ligands for inflammasome?

2. How does inflammasome crosstalk with other pathways?

3. How is inflammasome involved in more complex diseases? Is there any possible therapeutic approach?

Clinical Side (1) – Therapeutic Agents

TABLE 1 | Biologics and inhibitors of IL-1, IL-18, and inflammasome activation.

Name	Trade name	Company	Class	Target	$h_{1/2}$	Status
Anakinra	Kineret®	Sobi, Inc.	recIL-Ra	IL-1α, IL-1β	4–6 hours	Marketed
Rilonacept	Arcalyst®	Regeneron	srR (IL-1Trap)	IL-1α, IL-1β, IL-Ra	~7.5 days	Marketed
Canakinumab (ACZ855)	Ilaris®	Novartis	mAb (IgG1/κ)	IL-1β	23–26 days	Marketed
Gevokizumab (XOMA 052)		XOMA	mAb (IgG2/κ)	IL-1β	22 days	Phase 3† discontinued
LY2189102		Eli Lilly and Co	mAb (IgG4)	IL-1β	16.8 days	Phase 2
P2D7KK		A*STAR	mAb (IgG1)	IL-1β	~2 weeks*	Preclinical
Pralnacasan (VX-740)		Vertex	SMI	Caspase-1	nd	Phase 2†
Belnacasan (VX-765, HMR3480)		Vertex	SMI	Caspase-1	nd	Phase 2†
MCC950			SMI	NLRP3	nd	Preclinical
BHB			SMI	NLRP3	nd	Preclinical
Glibenclamide (glyburide)	Generic		SMI	K_{ATP}	10 hours	Marketed
MABp1	Xilonix™	XBiotech	mAb (IgG1/κ)	IL-1α	8 days	Phase 3‡ Phase 2
GSK-1070806		GlaxoSmith-Kline	mAb (IgG1/κ)	IL-18	23–30 days	Phase 2

*srR, soluble recombinant receptor; SMI, small molecule inhibitor; K_{ATP}, ATP-sensitive potassium channels; $h_{1/2}$, half-life; nd, not determined. *Estimation. †Clinical trial was terminated. ‡Only for the treatment of colorectal and non-small cell lung cancers.*

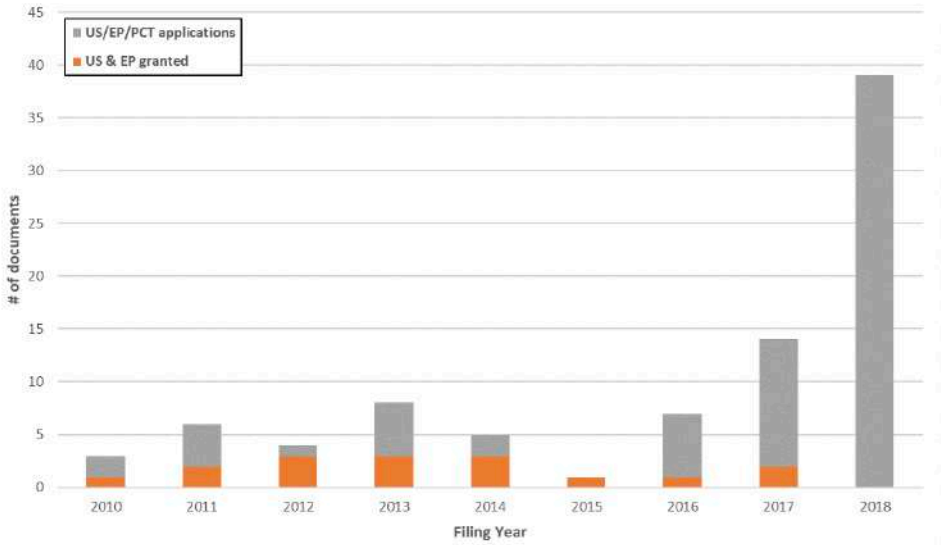
Fenini, Front. Pharmacol. 2017

InvivoGen products are for research use only

Product	Unit Size	Cat. Code
VX-765	10 mg	inh-vx765i
MCC950	10 mg	inh-mcc
Glybenclamide	1 g	tlrl-gly

Clinical Side (2) – Inflammasome Patents

Filing data for therapeutic inflammasome modulators



Therapeutic targets by INPADOC family

Target	# of INPADOC families	% of INPADOC families
NLRP3	40	51.3%
IL-1/IL-1R	25	32.1%
IL-18	11	14.1%
NLRP1	9	11.5%
caspase-1	6	7.7%
NLRP5	1	1.3%
NLRP12	1	1.3%

https://doi.org/10.1038/d41573-019-00200-x

Clinical Side (3) – Current Landscape

Assignees with the most applications or patents

Assignee	# of total docs	# of apps	# of patents	% total docs
Inflazome	13	13	0	12.5%
IFM Therapeutics	10	10	0	9.6%
University of Miami	6	4	2	5.8%
Nodthera	4	4	0	3.8%
Onxeo	4	4	0	3.8%
Trinity College Dublin	4	4	0	3.8%
University of Kentucky	4	0	4	3.8%
XOMA Technology	3	0	3	2.9%
Alligator Bioscience	2	0	2	1.9%
Apexigen	2	0	2	1.9%
Boston University	2	1	1	1.9%
Institut Gustave Roussy	2	1	1	1.9%
Regeneron Pharmaceuticals	2	0	2	1.9%
Inventor (Alan Wanderer)	2	1	1	1.9%
Kyoto University	1	0	1	1.0%
TWI Biotechnology	1	0	1	1.0%
University of Zurich	1	0	1	1.0%
Virginia Commonwealth University	1	0	1	1.0%

Nodthera
NT-0167, pre-IND testing
4/2019

IFM Therapeutics
IFM-2427, Phase I
3/2019

Inflazome
Inzomelid, Somalix,
Phase I
11/2019

Nodthera raised US$40M in series A funding 6/2018

Inflazome raised US$46M 11/2018

Genentech aquired Jecure Therapeutics 11/2018

Preclinical inhibitors

Novartis aquired IFM Tre for US$310M 4/2019

3 NLRP3 inhibitors

IFM Therapeutics raised US$31M 7/2019

Clinical Side (4) – Recent Focus of Field Leaders

1. IFM Therapeutics
   - Systemic-Peripheral NLRP3 Antagonist (IFN-2427), Phase I
   - Gut-Directed NLRP3 Antagonist, Pre-clinical
   - CNS-Penetrant NLRP3 Antagonist, Pre-clinical

2. Nodthera
   - NT-0167, Pre-clinical

3. Inflazome
   - Orally available, Brain-Penetrant Inhibitor (Inzomelid), Phase I
   - Orally available, Peripherally-Restricted Inhibitor (Somalix), Phase I

Current Challenges for Therapeutic Agents

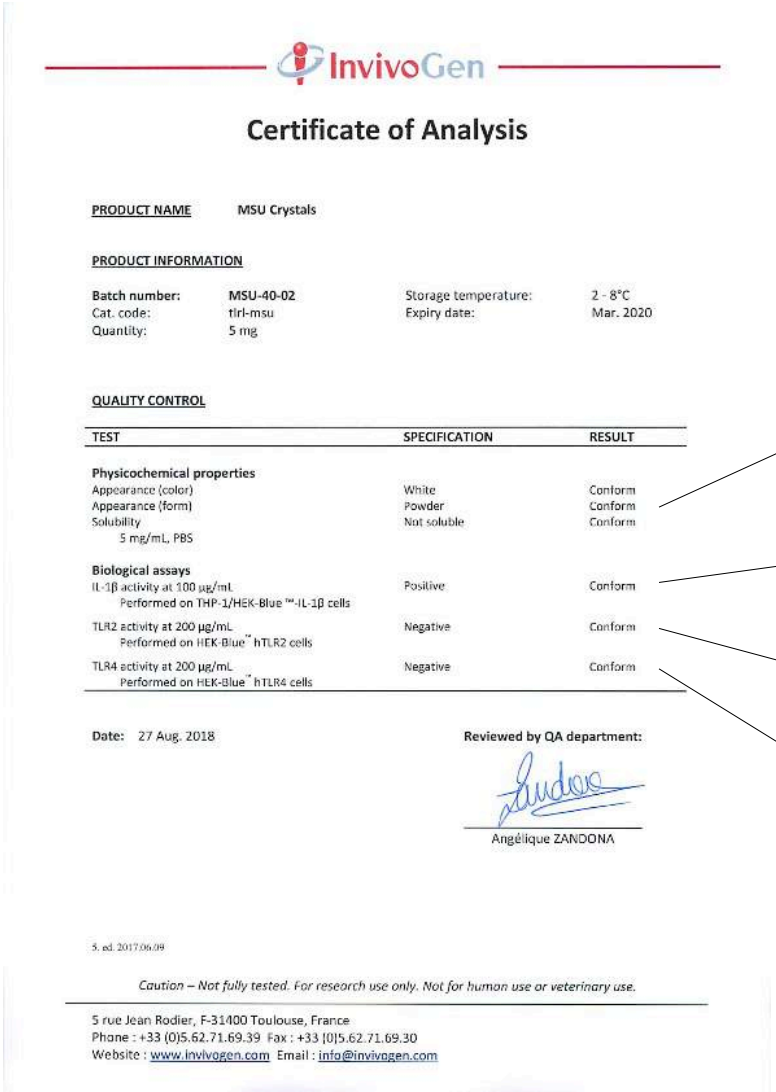
1. Specificity
   - Caspase inhibitors

2. Side Effects
   - Glyburide, MCC950, VX-740

3. Delivery
   - Bioavailability
   - Systemic vs directed
   - Blood-brain barrier

No FDA approved Small Molecules Inhibitors

InvivoGen's Ligands: High Quality



InvivoGen

Certificate of Analysis

PRODUCT NAME MSU Crystals

PRODUCT INFORMATION

Batch number:	**MSU-40-02**	Storage temperature:	2 - 8°C
Cat. code:	tlrl-msu	Expiry date:	Mar. 2020
Quantity:	5 mg		

QUALITY CONTROL

TEST	SPECIFICATION	RESULT
Physicochemical properties		
Appearance (color)	White	Conform
Appearance (form)	Powder	Conform
Solubility 5 mg/mL, PBS	Not soluble	Conform
Biological assays		
IL-1β activity at 100 µg/mL Performed on THP-1/HEK-Blue ™-IL-1β cells	Positive	Conform
TLR2 activity at 200 µg/mL Performed on HEK-Blue™ hTLR2 cells	Negative	Conform
TLR4 activity at 200 µg/mL Performed on HEK-Blue™ hTLR4 cells	Negative	Conform

Date: 27 Aug. 2018

Reviewed by QA department:

Angélique ZANDONA

5. ed. 2017.06.09

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

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Phone : +33 (0)5.62.71.69.39 Fax : +33 (0)5.62.71.69.30
Website : www.invivogen.com Email : info@invivogen.com

Product	Cat. Code	Sensor
MSU Crystals	tlrl-msu	NLRP3

Physicochemical Testing:
Appearance and Solubility

THP-1/HEK-Blue™-IL-1β Positive:
Biological Activity Confirmed

TLR2 and TLR4 Negative:
Free of Bacteria, Fungi and Mycoplasma

TLR4 Negative:
Endotoxin Free

InvivoGen's Ligands: Endotoxin-free

IMMUNOLOGY

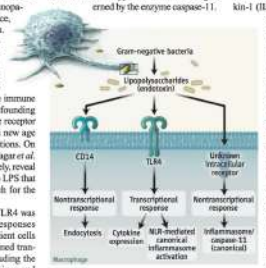
Sensing Endotoxins from Within

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 nontranscriptional 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 (3). 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

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PERSPECTIVES

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.

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

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

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

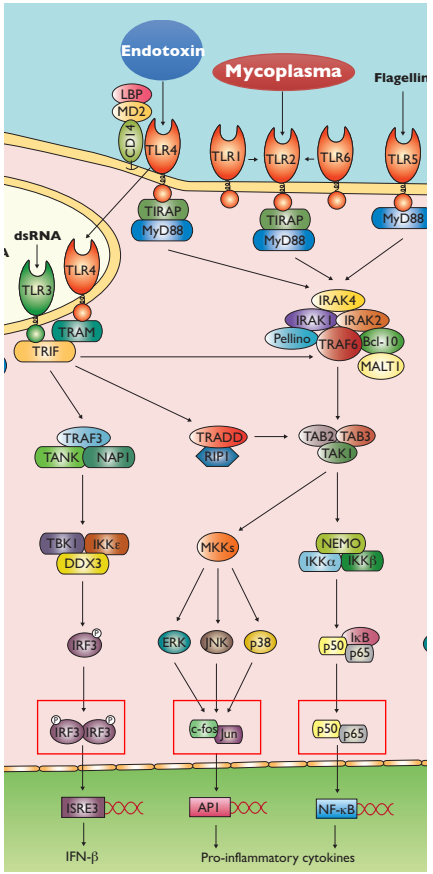
*Allison J. Greaney, Stephen H. Leppla and Mahtab Moayeri**

Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

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



InvivoGen's Ligands: Endotoxin-free

Poly(I:C) source, molecular weight and endotoxin contamination affect dam and prenatal outcomes, implications for models of maternal immune activation

H.M. Kowash[a,1], H.G. Potter[b,1], M.E. Edye[c], E.P. Prinssen[d], S. Bandinelli[d], J.C. Neill[c,*], R. Hager[b,2], J.D. Glazier[a,b,2]

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[b] Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester M13 9PT, UK
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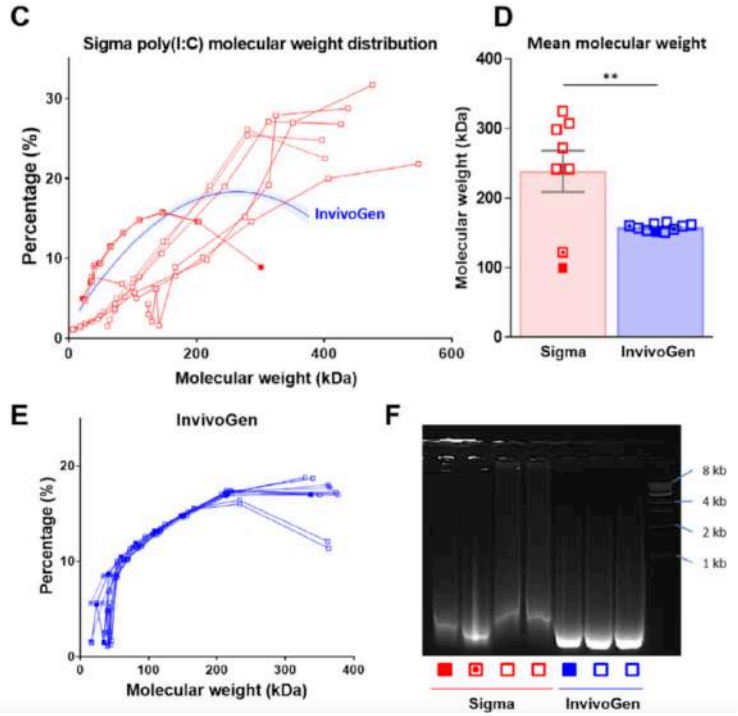
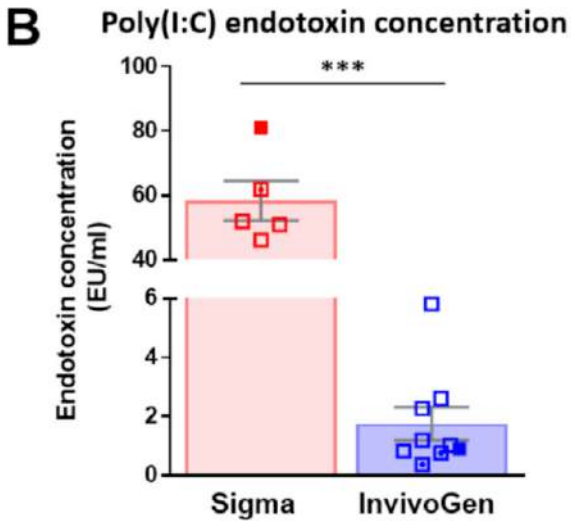
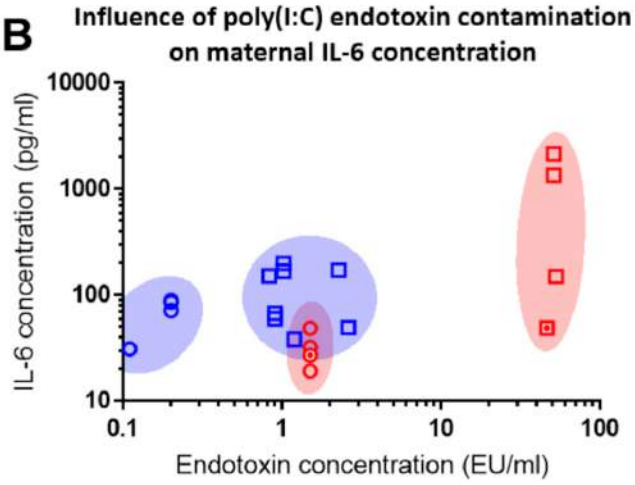
[d] Roche Innovation Centre, Basel, 124 Grenzacherstrasse, Basel, CH 4070, Switzerland



Consistent



Low endotoxin



InvivoGen insight

SUMMARY :

REVIEW

NLRP3: a sophisticated drug target

PRODUCTS

Inflammasome test cells

- THP1-KO-NLRP3 Cells
- THP1-KO-ASC Cells
- THP1-KO-CASP4 Cells
- RAW-ASC Cells
- RAW-ASC-KO-GSDMD Cells

E. coli Outer Membrane Vesicles

Lipopolysaccharides

The bad and the good of LPS

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OCTOBER 2019

NLRP3: a sophisticated drug target

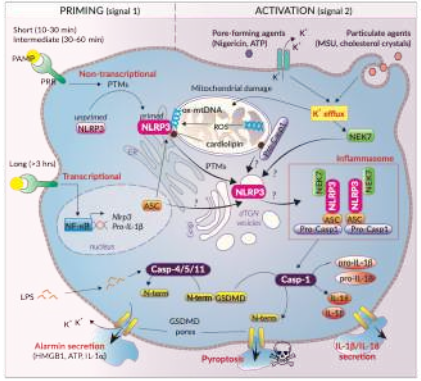
NLRP3 (NOD-like receptor pyrin domain-containing protein 3, cryopyrin or NALP3) is the best characterized inflammasome sensor and an attractive drug target. NLRP3 assembles into a multiprotein inflammasome complex to induce the secretion of IL-1β/IL-18 and pyroptosis in response to infections and cellular damages[1,2]. However, NLRP3 inflammasome functions can also be detrimental to the host, as its abberant or chronic activation is linked with pathologies such as type-2 diabetes, gouty arthritis, cardiovascular and Alzheimer's diseases, and rare genetic disorders such as CAPS (cryopyrin-associated-periodic-syndrome)[3]. Fast-paced research now aims at filling the gaps in the comprehension of NLRP3 inflammasome activation and regulation to develop novel therapeutics.

Activation of the NLRP3 inflammasome relies on a 2-signal model. Signal 1 is provided by microbial components or endogenous cytokines and primes NLRP3. Signal 2 is triggered by a plethora of stimuli and promotes NLRP3 activation and inflammasome assembly. This model is much more sophisticated than previously thought. Indeed, different priming mechanisms that depend on the stimulus' nature and duration have been described. Short and intermediate priming trigger post-translational modifications (PTMs) such as phosphorylation and (de-)ubiquitylation of NLRP3 expressed at basal level. Longer priming triggers NF-κB-mediated transcription of NLRP3, pro-caspase-1, and pro-IL-1β. Primed NLRP3 remains in an auto-repressed state until activation[2,4]. NLRP3 can be activated by a wide range of stimuli that are structurally and chemically unrelated (e.g. pore-forming toxins, activators of ion channels, uric acid crystals, β-amyloid proteins) suggesting that NLRP3 doesn't bind directly to these molecules, but rather senses downstream cytosolic stress signals[2,5]. Among those signals, cytosolic ion imbalances such as K^+ efflux, externalized mitochodrial (mt) cardiolipin and oxydized mtDNA, two proposed ligands for NLRP3. Yet, the exact mechanisms underlying NRLP3 activation remain a controversial topic[2,6].

Two major NLRP3 inflammasome responses, canonical and non-canonical, have been described and differ in their caspase (Casp) recruitment[1,2,6]. Upon activation, the canonical response is driven by aggregation of NLRP3 with the ASC adaptor and pro-Casp-1 (Fig. 2 inside). Self-cleaved active Casp-1 induces the maturation of pro-IL-1β/pro-IL-18, and cleavage of the pore-forming gasdermin D (GSDMD), leading to secretion of IL-1β/-18 and pyroptosis[1,6]. The non-canonical response relies on the activation of Casp-4/5 (human) or Casp-11 (mouse) upon sensing of cytosolic LPS (Fig. 2 inside). Casp-4/5/11 cannot cleave pro-IL-1β/-18, but they trigger stress signals through GSDMD pore formation at the plasma membrane. Thus, this ultimately induces NLRP3 inflammasome assembly and Casp-1-mediated IL-1β/-18 maturation and secretion[1,6].

Organelles play a key role in NLRP3 inflammasome assembly[2,4,6]. Coordinated priming and activation signals seem to orchestrate trafficking of NLRP3 and other proteins to mitochondria, endoplasmic reticulum (ER), and Golgi. NEK7 (NIMA-related protein kinase 7) was recently identified as an integral component of the NLRP3 inflammasome, but timing and location for its association with NLRP3 is not fully elucidated yet. While some studies suggested that the first steps of NLRP3 inflammasome assembly occurs at mitochondria-associated ER membranes (MAMs), a recent report indicates that activation and inflammasome assembly rather occur around dispersed trans-Golgi network (dTGN) vesicles without mitochondrial trafficking[7]. Whether multiple organelle-linked mechanisms can rule NLRP3 inflammasome assembly requires further work.

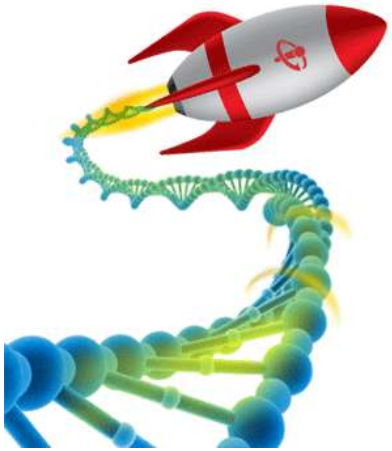
Intricate activating and inhibiting PTMs of NLRP3 inflammasome components have emerged as important regulation means at the priming and activation steps[2,4,6]. Research on the nature, timing and location of regulatory PTMs will help identifying new targets for fine-tuned therapeutics with minimal side-effects to replace existing NLRP3 inhibitors and IL-1β/-18 blockade strategies[2,4].



1. Broz P. and Dixit V.M., 2016. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 16:407. **2. Groslambert M. & Py B. 2018.** Spotlight on the NLRP3 inflammasome pathway. J. Inflamm. Res. 11:359. **3. Mangan M.S.J. et al., 2018.** Targeting the NLRP3 inflammasome in inflammatory diseases. Nat. Rev. Drug. Discov. 17:558. **4. Swanson K.V. et al., 2019.** The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat. Rev. Immunol. 19:477. **5. Hamilton C. & Anand P.K. 2019.** Right place, right time: localisation and assembly of the NLRP3 inflammasome. F1000Research. 8:676. **6. Yang Y., et al., 2019.** Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis. 10:128. **7. Chen J. & Chen Z.J., 2018.** PtdIns4P on dispersed trans-Golgi network mediates NLRP3 inflammasome activation. Nature. 564:71.

Thank you!

NATE – transfection enhancer

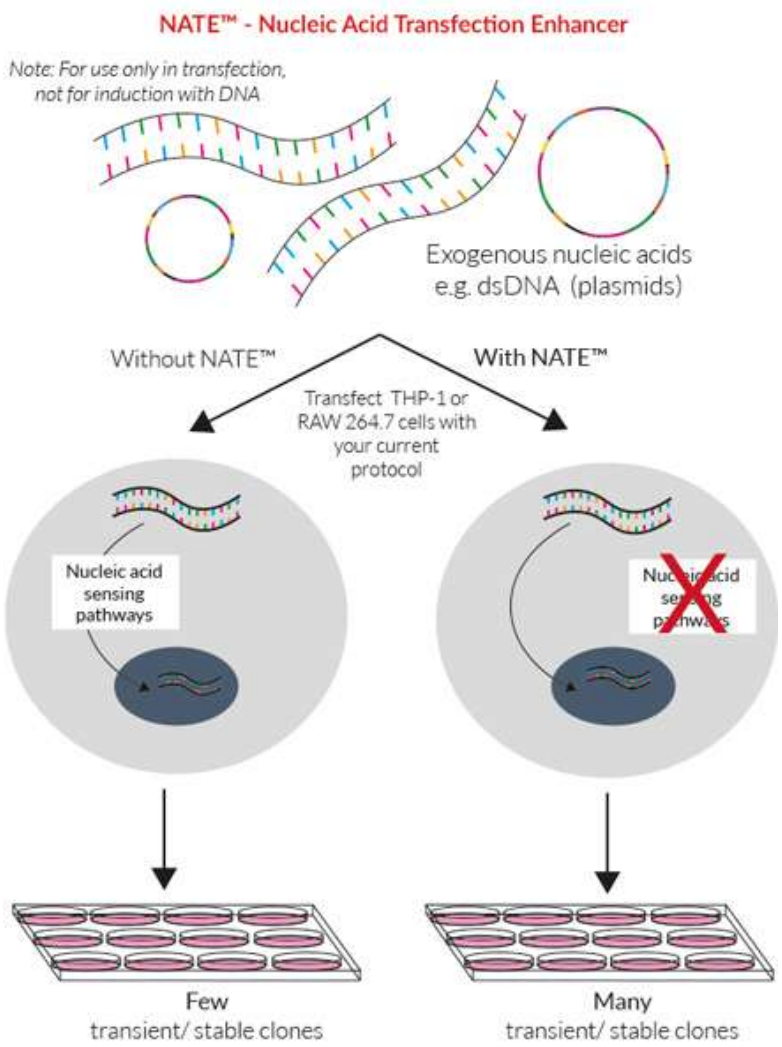


Nucleic Acid Transfection Enhancer

Boost your transfections with NATE™

Learn more

NATE (Nucleic Acid Transfection Enhancer)



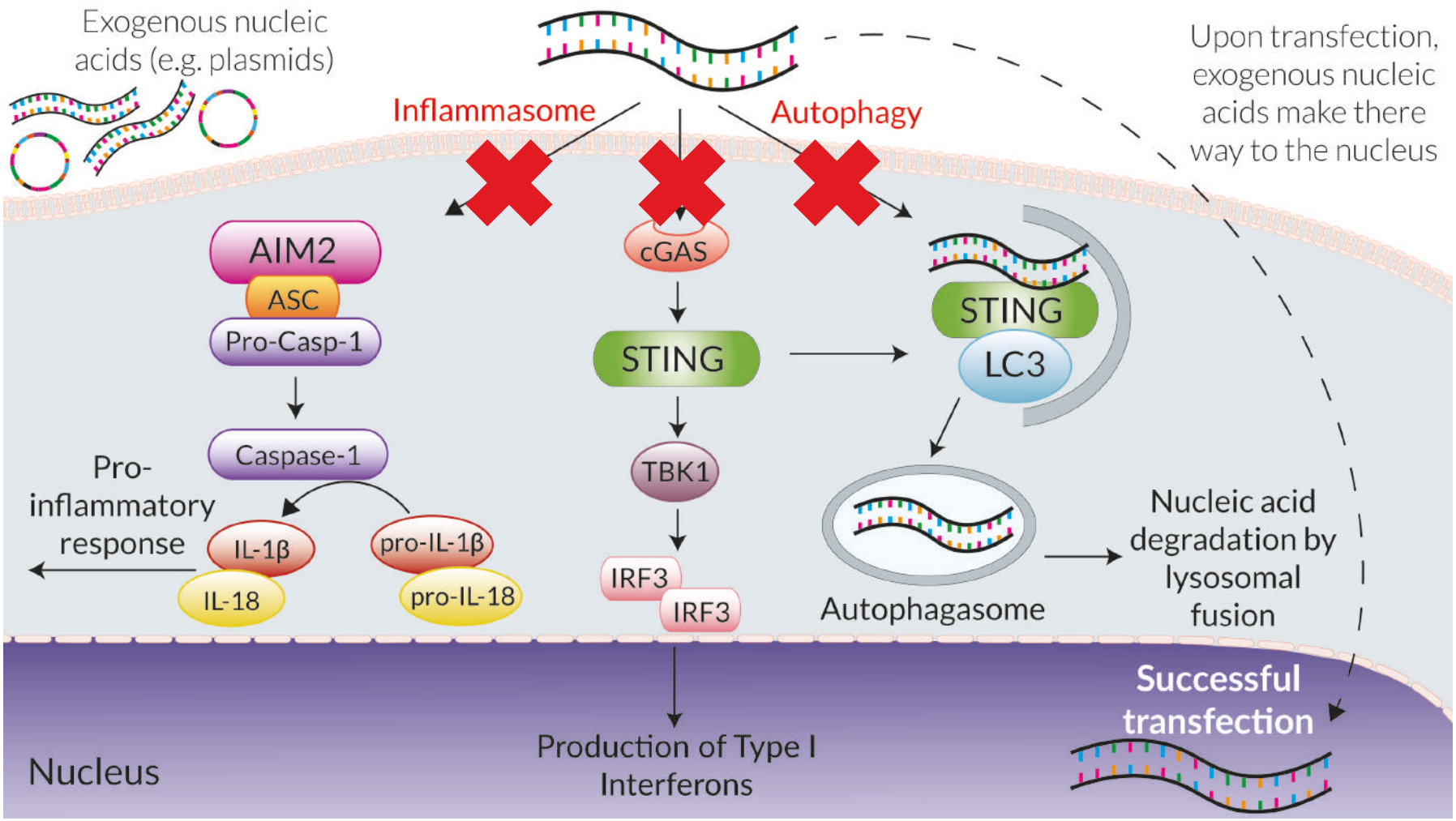
- Problem:
THP-1, RAW 264.7 hard to transfect

- Reason:
Nucleic acid sensing pathways

- Solution:
NATE

Product	Size
NATE	1ml (100 reactions)

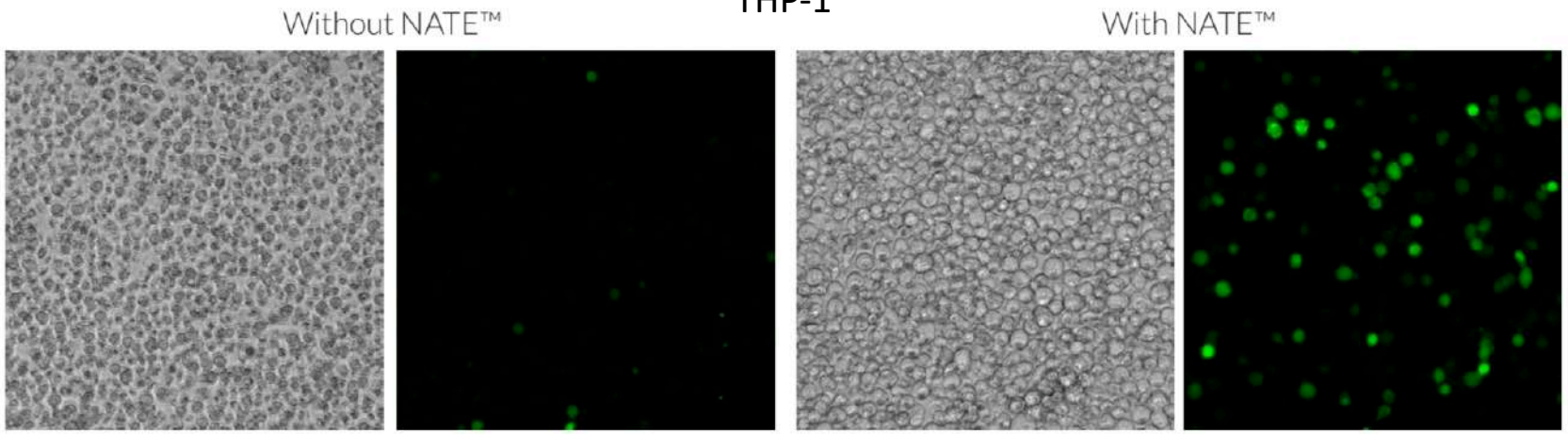
Exogenous Nucleic Acid Defences



NATE improves transfection efficiency

Simply incubate 30min before usual transfection protocol

THP-1



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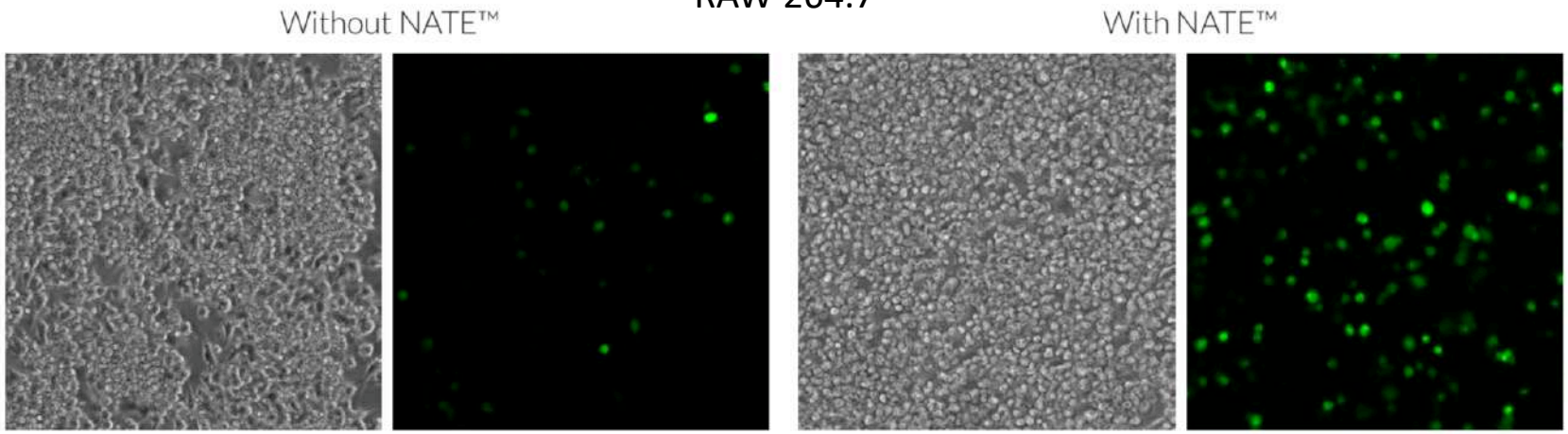
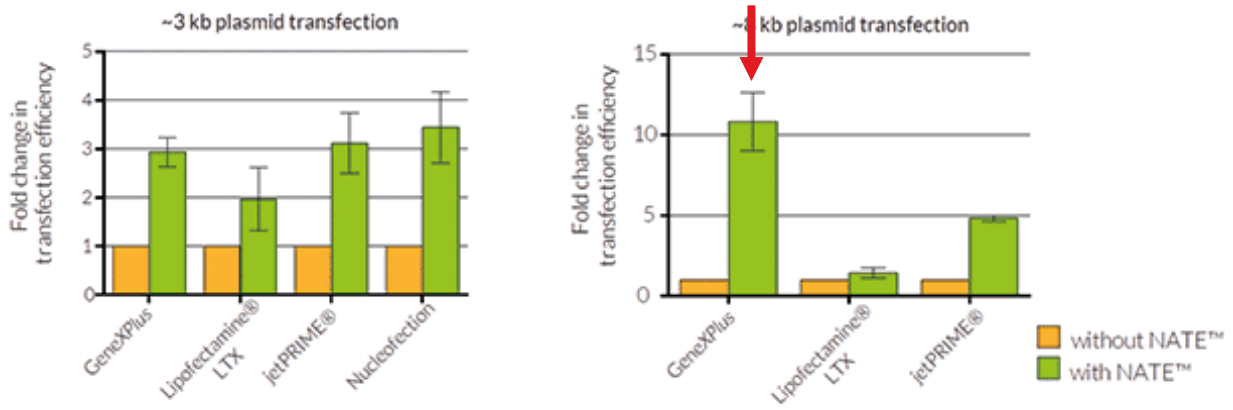


NATE supports large plasmids transfection

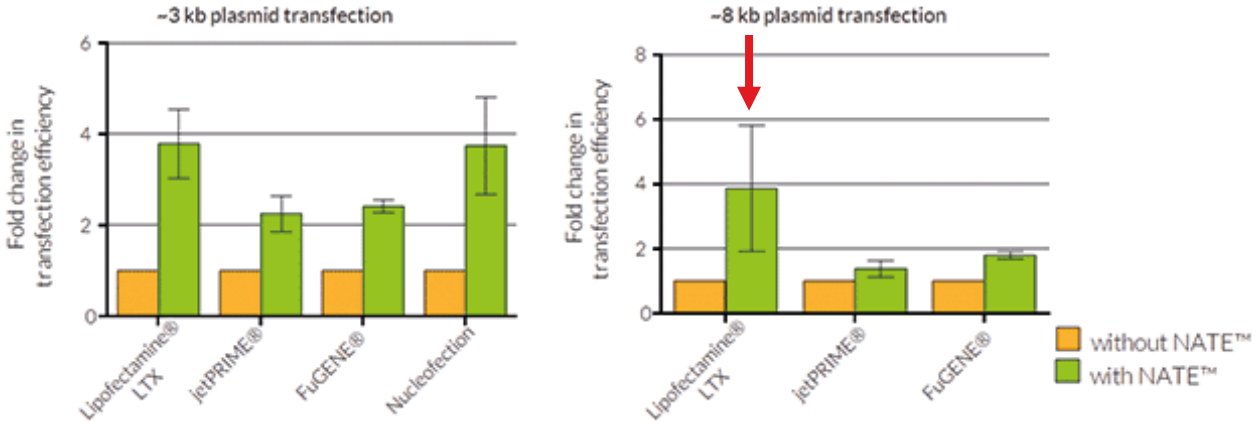
THP-1

Increased transient transfection efficiency with different sized plasmids in NATE™ treated cells



RAW 264.7

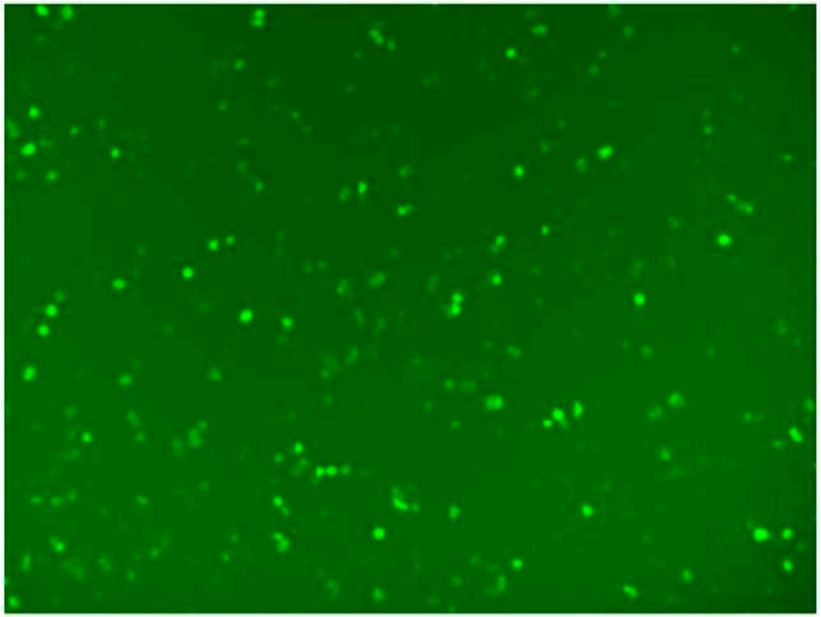
Increased transient transfection efficiency with different sized plasmids in NATE™ treated cells



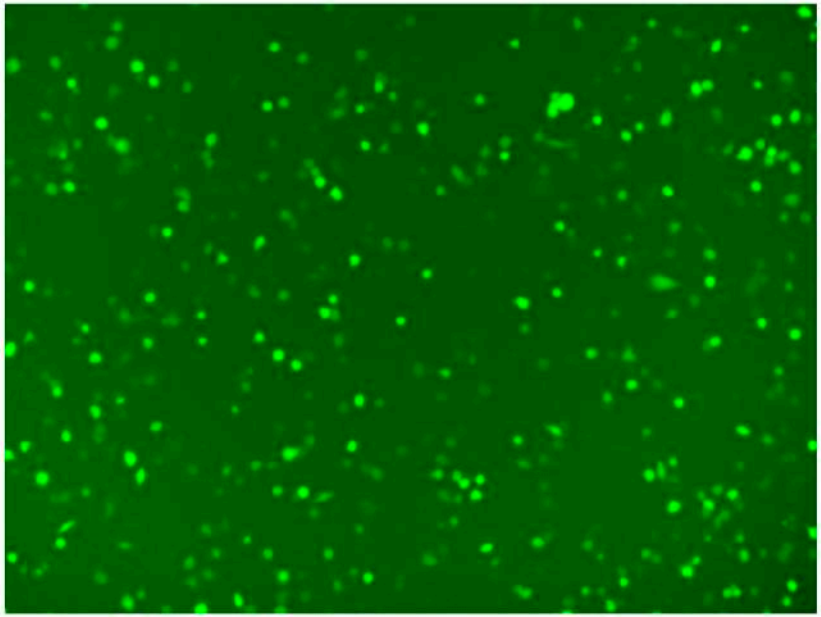
Customer Feedback

Saitama Medical University

Without NATE



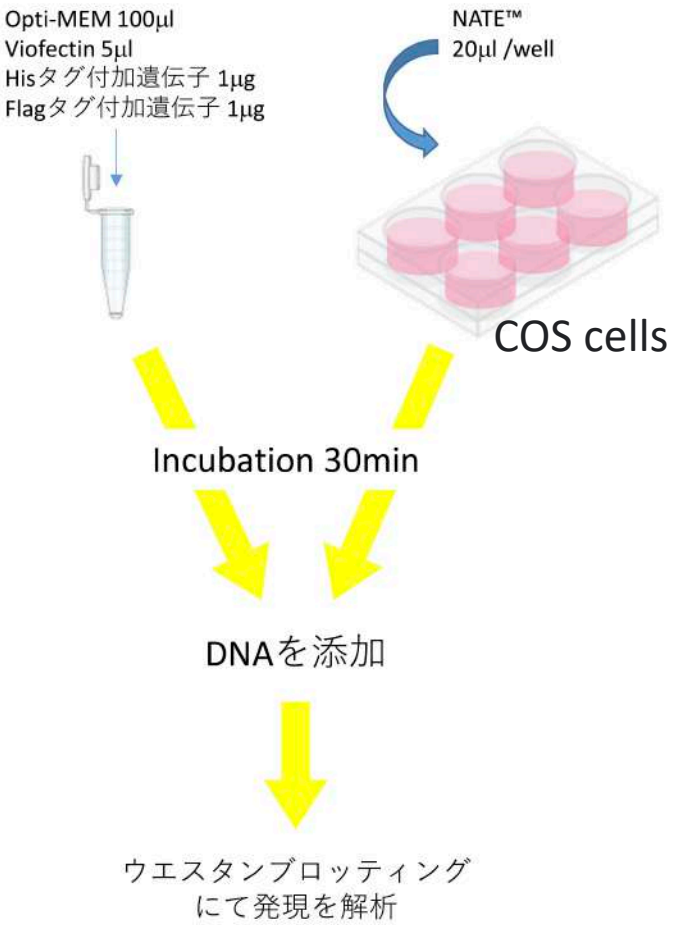
With NATE

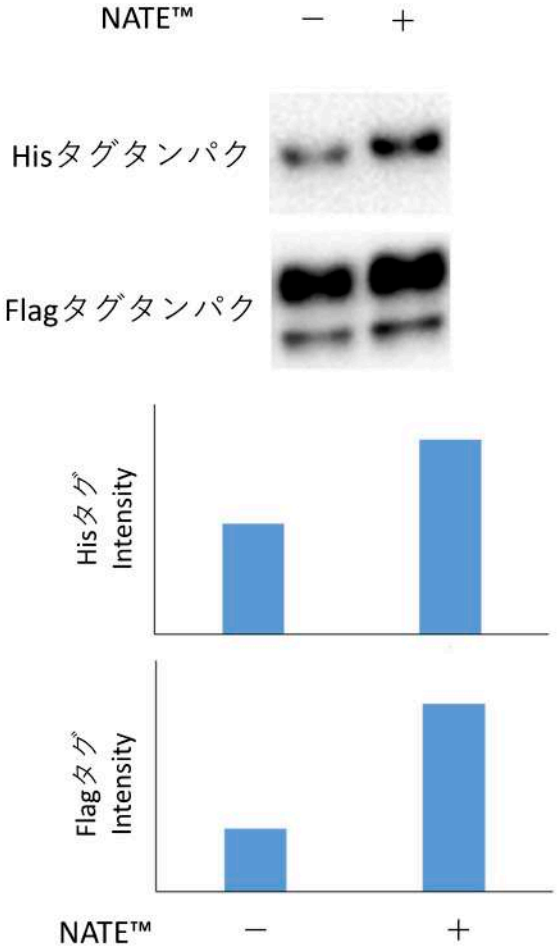


HeLa細胞 Lipofectamine 3000, GFP transfection 24時間後

Customer Feedback

YAGISHITA-KYO, Nan. Saitama Medical University





Thank you!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Zeocin™
InvivoGen
HygroGold™
InvivoGen
17-DMAG
InvivoGen
Flagellin