

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse caspase-1 (casper-1) antibody	AdipoGen	AG-20B-0042-C100; RRID:AB_2490248
Rabbit monoclonal GAPDH (14C10) antibody	CST	2118S; RRID: AB_561053
Mouse IL-1 β antibody	R&D Systems	AF-401-NA; RRID: AB_354347
Mouse NLRP3 (Cryo-2) antibody	Caltag Medsystems	AG-20B-0014-C100
Mouse NLRP3 (NALPY-3b) antibody	Enzo Life Sciences	ALX-804-819
Mouse IgG1 Isotype control	Enzo Life Sciences	ADI-SAB-600-0050
Rabbit TBK1 antibody	CST	3504; RRID: AB_2255663
Rabbit phospho-TBK1 S172 antibody	CST	5483T; RRID:AB_10693472
Rabbit AKT	CST	2938S; RRID:AB_915788
Rabbit phospho-AKT S473	CST	4060S; RRID:AB_2315049
Rabbit phospho-AKT substrate	CST	9614S; RRID: AB_331810
Mouse PP2A catalytic subunit α	BD Biosciences	610556; RRID:AB_397910
Rabbit GAPDH antibody	CST	2118; RRID:AB_561053
Mouse tubulin antibody	Sigma	T5168; RRID:AB_477579
ASC	Santa Cruz Biotechnologies	sc-22514-R
Rabbit NEK7 antibody	Abcam	ab133514
Mouse actin antibody	CST	3700; RRID: AB_2242334
Mouse HA antibody	Merck	05-904; RRID: AB_417380
Rabbit FLAG antibody	Sigma Aldrich	F7425-2MG RRID:AB_439687
Flow Cytometry Antibodies and Reagents		

TruStain fcX™ (anti-mouse CD16/32)	Biolegend	101320 RRID:AB_1574975
CD3-APC Cy7 (anti-mouse)	Biolegend	100221 RRID:AB_2057374
CD19-APCCy7(anti-mouse)	Biolegend	115530 RRID:AB_830707
CD11c-APC (anti-mouse)	Biolegend	117309 RRID:AB_313778
CD11b-BV785	Biolegend	101243 RRID:AB_2561373
LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit, for 633 or 635 nm excitation	Fisher	L10119
16% Formaldehyde (w/v), Methanol-free-10 x	Fisher	28908
Biological Samples		
Human blood	NHS Blood Bank Oxford	N/A
Chemicals, Peptides, and Recombinant Proteins		
Recombinant M-CSF	Immunotools	11343118
E.Coli LPS K12 ultrapure	Invivogen	tIrl-pekIps
E. Coli LPS 055:B5 (Figure 3 only)	Sigma Aldrich	L2637
E. Coli LPS O111:B4 (Figure 2 and 4, where indicated)	Sigma Aldrich	L4391
Zymosan	Invivogen	tIrl-zyn; CAS:58856-93-2
Pam3CSK4	Invivogen	tIrl-pms; CAS:112208-00-1
Poly(I:C)	Sigma Aldrich	P1530-25mg; CAS:42424-50-0
R837	Invivogen	tIrl-imqs; CAS: 99011-78-6
MSU	Invivogen	TIrl-msu
Nigericin	Sigma Aldrich	N7143-5MG; CAS:28643-80-3
Nigericin (Figure 3 only)	Sigma Aldrich	SML1779
Okadaic acid (Figure 3 only)	Tocris	1136; CAS: 78111-17-8
Okadaic acid (PP2A inh 1)	Cambridge Bioscience	10011490-25ug-CAY; CAS:78111-17-8
Okadaic acid (PP2A inh 2)	Sigma Aldrich	495609-25UG; CAS:78111-17-8

Okadaic acid (PP2A inh 3)	Sigma Aldrich	495604-25UG; CAS:78111-17-8
LB-100	Cambridge Bioscience	L0400-1 mg; CAS:1026680-07-8
VX-765 (Caspase-1 inhibitor)	Merck	5313720001
FuGene	Promega	E2311
UltraPure Flagellin from Salmonella typhimurium	Invivogen	tlrl-epstflfa-5
Calf-thymus DNA	Sigma Aldrich	D4522-5mg
HEPES (for transfection mix for retroviral transduction)	Fisher	15630056
Polybrene (for transfection mix for retroviral transduction)	Merck	TR-1003-G; CAS:28728-55-4
DSS (Disuccinimidyl suberate) Crosslinking reagent	Thermofisher	21655
DBK-1154	Obtained from Michael Ohlmeyer	N/A
ATP	Sigma Aldrich	A2383; CAS:34369-07-8
Hoechst 33342	Thermofisher	H3570
MCC950	Tocris	5479; CAS: 256373-96-3
MRT 68601 hydrochloride	Tocris	5067; CAS: 1190379-37-3
BX 795	Tocris	4318; CAS: 702675-74-9
MRT 67307 didhydrochloride	Tocris	5134; CAS: 1190378-57-4
Lipofectamine® RNAiMAX Reagent	ThermoFisher Scientific	13778-150
ON-TARGETplus TBK1 siRNA 1	Dharmacon/Perkin Elmer	J-063162-05-0002
ON-TARGETplus TBK1 siRNA 2	Dharmacon/Perkin Elmer	J-063162-06-0002
ON-TARGETplus TBK1 siRNA 3	Dharmacon/Perkin Elmer	J-063162-07-0002
ON-TARGETplus TBK1 siRNA 4	Dharmacon/Perkin Elmer	J-063162-08-0002
ON-TARGETplus PP2A siRNA 1	Dharmacon/Perkin Elmer	J-040657-05-0002
ON-TARGETplus PP2A siRNA 2	Dharmacon/Perkin Elmer	J-040657-07-0002
ON-TARGETplus PP2A siRNA 3	Dharmacon/Perkin Elmer	J-040657-08-0002
ON-TARGETplus Non-targeting siRNA Pool	Dharmacon/Perkin Elmer	D-001810-10-05
Dynabeads	ThermoFisher	10003D
NLRP3 commercial assays		
Cytox96 nonradioactive cytotoxicity assay	Promega	G1780
Serine/Threonine Phosphatase Assay System	Promega	V2460
Mouse IL-1 β ELISA	eBioscience	88-7013-77

Mouse TNF ELISA	eBioscience	88-7324-77
Human IL-1 β ELISA	eBioscience	88-7261-77
Mouse IL-18 ELISA	eBioscience	BMS618-3TEN
Caspase-Glo® 1 Inflammasome Assay	Promega	G9952
Microscopy & PLA reagents		
Duolink In Situ Detection Reagents - FarRed	Sigma	DUO92013-100RXN
Duolink In Situ PLA probe anti rabbit Minus	Sigma	DUO92005-100RXN
Duolink In Situ PLA probe anti mouse Plus	Sigma	DUO92001-100RXN
ProLong® Diamond Antifade Mountant with DAPI	ThermoFisher	P36971
4-well Chamber Slides	ThermoFisher	16250661
8-well Chamber Slides	ThermoFisher	16260661
Experimental models: cell lines		
Platinum-E (PlatE) retroviral packaging cell line	eBioscience	RV-101
HEK293T cells	Sigma Aldrich	12022001-1VL
WT, <i>Tnf</i> ^{-/-} <i>Tbk1</i> ^{+/-} and <i>Tnf</i> ^{-/-} <i>Tbk1</i> ^{-/-} BMDMs	Gift from Prof Cevayir Coban and Prof Michelle Lee Sue Jann University of Tokyo	
ASC-mCherry iBMDMs	Gift from Dr David Brough, University of Manchester	
WT iBMDMs	Gift from Prof Jonathan Kagan, Harvard University	
IKK ϵ KO iBMDMs	Gift from Prof Jonathan Kagan, Harvard University	
WT THP1 dual cell line	Invivogen	thpd-nfis
TBK1 KO THP1 dual cell line	Invivogen	thpd-kotbk
Experimental models: organisms/strains		
C57BL/6J mice	Charles River	RRID:IMSR_JAX:000664
B6.NLRP3 KO mice	Kevin Maloy lab, originally from Vishva Dixit (Genentech) strain B6.NLRP3 ^{tm1Vmd}	RRID:MGI:5468973
Primers used for cloning		Sequence
NLRP3 S3A Forward	TTACTCCAATTGGCCACCATGACGGCTGTCCGT TGCAAGCTG	
NLRP3 S3A Reverse	GAGTAACTCGCGGCCGCCCCAGGAAATCTCGA AGACTATAGT	

NLRP3 S3D Forward	TTACTCCAATTGGCCACCATGACGGATGTCCGT TGCAAGCTG	
NLRP3 S3D Reverse	GAGTAACTCGCGGCCGCCCCAGGAAATCTCGA AGACTATAGT	
Recombinant DNA		
pMIGRMCS retroviral packaging plasmid expressing GFP and FLAG-tag	Kate Schroder lab	N/A
pMIGRMCS retroviral packaging plasmid expressing GFP and FLAG-tagged WT NLRP3, S3A NLRP3 or S3D NLRP3	Bezbradica lab (Full sequence available on request)	N/A
pcDNA3.1 mammalian expression Vector containing FLAG-tagged mouse WT, S3A or S3D NLRP3	VectorBuilder, Michigan, USA	N/A
pcDNA3.1 mammalian expression Vector containing HA-tagged mouse TBK1	VectorBuilder, Michigan, USA	N/A
Software and Algorithms		
GraphPad Prism	http://www.graphpad.com/	RRID: SCR_002798
FlowJo	https://www.flowjo.com/	RRID: SCR_008520
Fiji	https://fiji.sc/	RRID: SCR_002285