Subversion of Macrophage Functions by Bacterial Protein Toxins and Effectors

Muyang Wan, Yan Zhou* and Yongqun Zhu*

Life Sciences Institute and Innovation Center for Cell Signaling Network, Zhejiang University, Hangzhou, China.

*Correspondence: zhuyongqun@zju.edu.cn and zhouyanlsi@zju.edu.cn

https://doi.org/10.21775/cimb.025.061

Abstract

Macrophages represent one of the first lines of host immune defences against the invasion of pathogenic bacteria. Many receptors, immune signalling pathways and cellular processes in macrophages, including Toll-like receptors, Nod-like receptors, phagocytosis, autophagy and programmed cell death, are involved in combating the infection of bacterial pathogens. For efficient colonization in the host, bacterial pathogens have evolved diverse mechanisms to interfere with macrophage functions to evade host defences. The major weapons utilized by bacterial pathogens are protein toxins and effectors secreted via specific bacterial secretion systems, including type I–VII secretion apparatuses. In recent years, great advances have been achieved in understanding how bacterial toxins and effectors subvert immune signalling and cellular processes of macrophages. In this review, we focus on the toxins and effectors that modulate the phagocytosis, intracellular immune signalling pathways, autophagy and programmed cell death processes of macrophages from the bacterium *Legionella pneumophila, Shigella flexneri, Listeria monocytogenes, Salmonella* spp., *Yersinia* spp., enteropathogenic *E. coli* and *Mycobacterium tuberculosis*.

Introduction

As professional phagocytes, macrophages engulf invading bacteria as particles for degradation and counteract the bacterial infection as the first line of host immune defences. Macrophages recognize the invading bacteria via pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), or indirectly by Opsonins, including complement components and immunoglobulin G (Flannagan *et al.*, 2009; Mosser, 1994). Among PRRs, Toll-like receptor (TLR) 1, TLR2, TLR4, TLR-5, TLR6 and TLR10 embed in the cell membrane and are responsible for the recognition of microorganisms on the cell surface, while TLR-3, TLR-7, TLR-8 and TLR-9 are present in endosomes (Bryant *et*

al., 2015; Medzhitov and Janeway, 2002). The extracellular N-terminal leucine-rich repeat (LRR) domains of TLRs form homo- or heterodimers to directly recognize various PAMPs, such as lipopolysaccharide (LPS), flagellin and CpG DNAs. For instance, TLR-4 senses the LPS of Gram-negative bacteria, while TLR-5 recognizes bacterial flagellin (Bryant *et al.*, 2015). The C-terminal Toll-interleukin-1 receptor (TIR) domain of TLRs exists in the intracellular cytoplasm and recruits adaptor molecules to activate downstream immune signalling pathways, including mitogen-activated protein kinase (MAPK), NF-κB and type I interferon pathways. Different from TLRs, NOD-like receptors (NLRs) are cytoplasmic receptors, which sense intracellular bacterial PAMPs and subsequently form large protein complexes known as inflammasomes (Bryant and Fitzgerald, 2009; Zhao and Shao, 2016). The human gene encodes more than 20 NLRs, including NALPs, IPAF and NAIPs. Similarly to TLRs, different NLRs sense distinct stimuli from bacterial pathogens to form specific inflammasomes, which activate caspase-1 and induce downstream inflammatory responses, such as the secretion of the pro-inflammatory cytokines, including IL-1β and IL-18, and pyroptosis. For instance, Naip2 and Naip5 serve as specific inflammasome receptors for T3SS rod proteins and bacterial flagellin, respectively (Zhao *et al.*, 2011). Recognition of T3SS rod proteins or flagellin promotes the formation of Naip2/5-NLRC4 inflammasome complexes and activation of caspase-1 to produce IL-1β and induce cell pyrotosis. Caspase-4 and caspase-11 function as specific intracellular LPS receptors to sense invading bacteria in the cytoplasm and activate downstream Gasdermin D (GSDMD) to induce pyroptosis (Shi *et al.*, 2015; Shi *et al.*, 2014). In addition, STING is a specific receptor in the endoplasmic reticulum (ER) for bacterial cyclic di-GMP (Burdette *et al.*, 2011). cGAS is a newly identified receptor for bacterial or viral DNAs, which are released into the cytoplasm (Sun *et al.*, 2013).

Phagocytosis is a major defence mechanism of macrophages, which is initiated by opsonin receptors, such as Fcγ receptors (FcγRs) and complement receptor 3 (CR3) (Flannagan *et al.*, 2009). These receptors recognize Opsonins, including immunoglobulin G (IgG) and complement component are attached to the bacterial surface, and start an intracellular signalling cascade that activates remodelling of the actin cytoskeleton and initiates phagocytosis. For example, for FcγRs, phagocytosis is initiated by Src-family kinases, which catalyse tyrosine phosphorylation on the immunoreceptor tyrosine-based activation motif (ITAM) within the receptor's cytoplasmic domain (Flannagan *et al.*, 2009). Phosphorylated tyrosines in ITAM act as docking sites for the SH2 domain of the tyrosine kinase SyK. SyK in turn recruits and activates downstream signalling molecules, including phosphatidylinositol 3-kinase (PI3K), phospholipase C-gamma (PLCγ) and the RhoGEF Vav, which ultimately activates actin cytoskeleton signalling to remodel the cell membrane and engulf pathogens (Crowley *et al.*, 1997; Mócsai *et al.*, 2010). Phosphatidylinositols (PtdIns), which are abundant components of the cell membrane, play important roles in actin cytoskeleton remodelling during phagocytosis (Pizarro-Cerdá *et al.*, 2015). PtdIns-kinases and phospholipases cooperate to produce different types of phosphoinositides. Phosphoinositides act as second messengers or directly recruit downstream proteins to change membrane morphology and remodel the actin cytoskeleton for phagosome formation and maturation. For example, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) and phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3) accumulate at sites of particle engagement and are essential for phagosome formation (Flannagan *et al.*, 2009). After internalization of a pathogen and sealing of the membrane, phagosome maturation

starts immediately and is accomplished via sequential fusion with early endosomes and late endosomes of the endocytic pathway, and finally fuses with lysosomes to form phagolysosomes. Proteins and protein complexes in the endocytic pathway including Rab GTPases, Arf GTPases and endosomal-sorting complex required for transport (ESCRT), play important roles in the phagosome maturation process. There is an accumulation of PtdIns3P on the cytosolic leaflet of the limiting membrane of the early phagosome (Flannagan *et al.*, 2012). The v-ATPases inserted into the phagosome membrane translocates H^+ across the bilayer and acidify the phagosomal lumen. Phagolysosomes finally become extremely acidic and oxidative organelles, which eliminate and degrade bacterial pathogens. In addition to phagocytosis, the cellular process of autophagy and programed cell death also plays key roles in clearing invading bacteria in macrophages. Many chemokines, cytokines, anti-microbial effectors are released by macrophages to initiate pro-inflammatory responses upon recognition of invading pathogens. Through presenting pathogen-derived antigens to T cells, macrophages also promote host adaptive immunity responses to counteract the bacterial invasion.

Despite the essential roles in host innate immune defences, macrophages are also specific host cells for many intracellular bacterial pathogens, such as *Mycobacterium tuberculosis*, *Legionella pneumophila* and *Coxiella burnetii* for survival and replication. Some intracellular bacterial pathogens, such as *Listeria monocytogenes*, *Shigella* spp., *Salmonella* spp. and *Yersinia* spp., invade both epithelial cells and macrophages. These intracellular bacteria have evolved distinct strategies to counteract the defence pathways of macrophages for survival. For extracellular bacteria, such as enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC), they do not directly invade macrophages. To avoid degradation by macrophages, extracellular bacterial pathogens mainly inhibit phagocytosis and cytokine secretion of macrophages. Both extracellular and intracellular bacterial pathogens escape macrophage defences predominantly through protein toxins and effectors secreted via specifically evolved bacterial secretion systems, including type I to type VII secretion systems. In this review, we discuss how bacterial toxins or effector proteins from *L. pneumophila*, *S. flexneri*, *L. monocytogenes*, *Salmonella* spp., *Yersinia* spp., EHEC/EPEC and *M. tuberculosis* subvert various macrophage functions during infection (Tables 3.1 and 3.2).

Evasion of phagocytosis by macrophages

Because phagocytosis is a major defence mechanism of macrophages, many bacterial pathogens inhibit the macrophage phagocytosis. The extracellular pathogens, EPEC and EHEC, inject the type III effector EspJ to target Src kinase (Fig. 3.1 and Table 3.1). EspJ adopts a similar structural fold as the ADP-ribosyltransferase domain of the *Pseudomonas syringae* effector AvrPphF-ORF2 (Young *et al.*, 2014). Via a unique ADP-ribosyltransferase activity, EspJ catalyse the ADP-ribosylation and amidation on the residue E310 of Src. The novel modification on the residue E310 by EspJ blocks the kinase activity of Src, which transinhibits macrophage opsono-phagocytosis. SeoC, a homologue of EspJ from *S. enterica* subspecies salamae and arizonae, has the same ADP-ribosylation and amidation activity to inactivate Src and inhibits IgG-mediated phagocytosis of macrophage (Pollard *et al.*, 2016). The effector YopH from *Yersinia* spp. dephosphorylates the Crk-associated substrate $p130^{Cas}$ and Fyn-binding protein Fyb via protein tyrosine phosphatase activity (Black and Bliska, 1997; Deleuil *et al.*, 2003). Dephosphorylation of $p130^{Cas}$ and Fyn-binding protein Fyb

Table 3.1 Summary of the host targets, enzymatic activities and mechanisms of bacterial toxins and effectors that subvert macrophage phagocytosis and cytoplasmic immune signalling pathways

Effector	Bacteria	Host target	Enzymatic activity
Evasion of phagocytosis by macrophages			
EspJ	EPEC/EHEC	Src kinase	ADP-ribosyltransferase
YopH	*Yersinia* spp.	p130Cas, Fyb	Tyrosine phosphatas
LLO	*L. monocytogenes*	phagosome membrane	Pore-forming
IpaC	*S. flexneri*	phagosomal membrane	Lyse the phagosomal membrane
SifA	*S.* Typhimurium	PLEKHM1	Scaffold protein
SopD2	*S.* Typhimurium	Rab32	GAP
GtgE	*S.* Typhimurium	Rab32	Cysteine protease
SapM	*M. tuberculosis*	PtdIns3P	PtdIns3P phosphatase
EsxA	*M. tuberculosis*	Phagosomal membrane	Membrane-permeabilizing
EsxH	*M. tuberculosis*	Hrs	PROTEIN binding
DrrA/SidM	*L. pneumophila*	Rab1	Nucleotidyl transferase, GDF, GEF
LepB	*L. pneumophila*	Rab1	GAP
SidD	*L. pneumophila*	Rab1	DeAMPylase
AnkX	*L. pneumophila*	Rab1	Phosphocholine transferase
Lem3	*L. pneumophila*	Rab1	Phosphocholine hydrolase
CvpB	*C. burnetii*	PtdIns3P, phosphatidylserine	Lipid binding
Modulation of cytoplasmic immune signalling pathways			
OspI	*S. flexneri*	Ubc13	Deamidase
OspG	*S. flexneri*	Ubiquitin-conjugated E2	Half-kinase
IpaH9.8	*S. flexneri*	NEMO	Ubiquitin E3 ligase
IpaH1.4/ IpaH2.5	*S. flexneri*	HOIP	Ubiquitin E3 ligase
OspF	*S. flexneri*	MAPK	Phosphothreonine lyase
NleE	EPEC/EHEC	TAB2/3	Cysteine methyltransferase
NleB	EPEC/EHEC	GAPDH	N-acetyl-D-gulcosamine transferase
NleC	EPEC/EHEC	p65	Zinc metalloprotease
NleD	EPEC/EHEC	JNK	Zinc metalloprotease
Tir	EPEC/EHEC	TRAF, SHP-2	Protein binding
SpvC	*S.* Typhimurium	MAPK	Phosphothreonine lyase
PipA family	*S.* Typhimurium	p65, RelB	Protease
GogB	*S.* Typhimurium	SCF E3 ligase	Protein binding
YopJ	*Yersinia* spp.	MKK6, IKKβ	Acetyltransferase
PtpA	*M. tuberculosis*	JNK, p38, TAB3	Tyrosine phosphatase
LegK1	*L. pneumophila*	IκBα, p100	Serine/threonine kinase

GEF, Guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDF, GDI-displacement factor.

Table 3.2 Summary of the host targets, enzymatic activities and mechanisms of bacterial toxins and effectors that interfere with autophagy and cell death signalling

Effector	Bacteria	Host target	Enzymatic activity
Interference with autophagy			
VirA	*S. flexneri*	Rab1	TBC-like GAP
IcsB	*S. flexneri*	Atg5	Protein binding
RavZ	*L. pneumophila*	LC3	Cysteine protease
LpSpl	*L. pneumophila*	Sphingosine-1 phosphate	Sphingosine-1 phosphate lyase
SapM	*M. tuberculosis*	PtdIns3P	PtdIns3P phosphatase
PE_PGRS47	*M. tuberculosis*	Unknown	Unknown
SseL	*S.* Typhimurium	ALIS	Deubiquitinase
Interference with cell death signalling			
SidF	*L. pneumophila*	BNIP3, Bcl-rambo, PtdIns(3,4)P2, PtdIns(3,4,5)P3	Phosphatidylinositol phosphatase
SdhA	*L. pneumophila*	LCV	Protect the LCV integrity
IpaB	*S. flexneri*	Cell membrane	Pore-forming
IpaD	*S. flexneri*	Mitochondrial	Membrane-permeabilizing
IpaH7	*S. flexneri*	GLMN	Ubiquitin E3 ligase
OspC3	*S. flexneri*	Caspase-4	Protein binding
SipB	*S.* Typhimurium	Caspase-1	Protein binding
YopM	*Yersinia* spp.	PRK1, PRK2	Protein binding
EsxA	*M. tuberculosis*	Cell membrane	Membrane-permeabilizing
CpnT	*M. tuberculosis*	NAD^+	NAD^+ glycohydrolase

ALIS, aggresome-like induced structures; GLMN, the glomulin/flagellar-associated protein 68; LCV, *Legionella*-containing vacuole.

destabilizes focal adhesion and inhibits bacterial internalization by macrophages (Deleuil *et al.*, 2003). *L. monocytogenes*, a facultative intracellular pathogen, escapes the phagosomes of macrophages by releasing the cholesterol-dependent cytolysin listeriolysin O (LLO). LLO is an essential virulence factor of *L. monocytogenes* with multiple functions. LLO oligomerizes and produces pores in the phagosome membrane, which induce the loss of H^+ and Ca^{2+} in phagosomes and inhibits the fusion of phagosome with endosomes and lysosomes (Birmingham *et al.*, 2008; Shaughnessy *et al.*, 2006). Destruction of the phagosome membrane enables *L. monocytogenes* to cross phagosomes and enter the cytoplasm for proliferation (Birmingham *et al.*, 2008).

The intracellular pathogen *S. flexneri* escapes from the phagosomes into the cytoplasm of infected cells using its type III secretion apparatus (Du *et al.*, 2016). The *Shigella* type III secretion apparatus is sufficient to promote efficient vacuole lysis and evasion of phagocytosis. IpaC, a translocator component and effector of the *Shigella* type III secretion apparatus mediates phagosome escape efficiency (Du *et al.*, 2016) (Fig. 3.1). However, the homologue

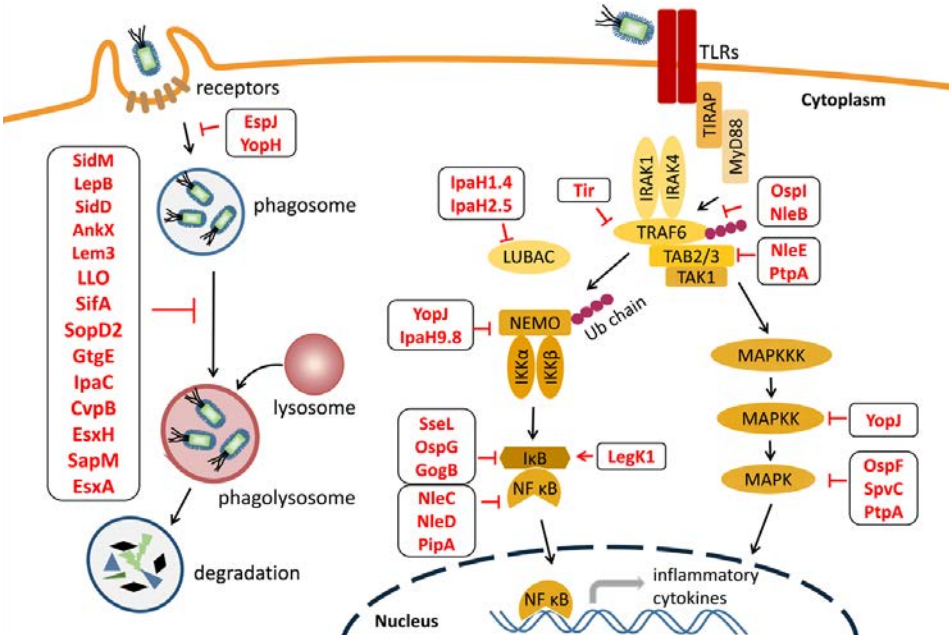

Figure 3.1 Bacterial evasion of phagocytosis by macrophages and modulation of cytoplasmic immune signalling pathways. The effectors SidM, LepB, SidD, AnkX, Lem3, IpaC, SifA, SopD2, GtgE, EsxH, EsxA, SapM, CvpB and the toxin LLO inhibit phagocytosis of macrophages. OspF, SpvC and PtpA inactivate MAPK. YopJ inactivates MAPKK and IKK. OspI, IpaH9.8, IpaH1.4, IpaH2.5, OspG, NleE, NleB, Tir and GogB inhibit NF-κB signalling, while LegK1 activates NF-κB signalling. LUBAC, linear ubiquitin chain assembly complex; MAPK, mitogen-activated protein kinase; TLR, Toll-like receptor; TIRAP, TIR domain containing adaptor protein.

of IpaC in *Salmonella*, SipC, cannot promote phagosome escape, suggesting a functional difference between IpaC and SipC (Osiecki *et al.*, 2001). In contrast to *Shigella*, *Salmonella* survive within both epithelial cells and macrophages by forming the *Salmonella*-containing vacuole (SCV) after entry into host cells. The formation of the SCV promotes the phagosome escape of *Salmonella*. Several type III effectors of *Salmonella* are involved in promoting the biogenesis of the SCV and inhibiting phagosome maturation. The *Salmonella* effector SifA directly interacts with Pleckstrin homology domain-containing protein family member 1 (PLEKHM1), a lysosome adaptor, to recruit Rab7 and the HOPS tethering complex, which transports phagolysosomal membranes to the SCV (McEwan *et al.*, 2015). The effectors, SopD2 and GtgE of *S.* Typhimurium, are a specific GAP and cysteine protease for Rab32, respectively (Spanò *et al.*, 2016). Rab32 mediates traffic to lysosome-related organelles in conjunction with components of the biogenesis of lysosome-related organelle complexes (BLOCs) (Spanò and Galán, 2012). SopD2 inactivates Rab32 by mimicking eukaryotic GAPs to catalyse GTP hydrolysis. Via the cysteine protease activity of the papain subfamily, GtgE cleaves Rab32 to disrupt the switch I region, which is required for interactions with downstream Rab effectors. SopD2 and GtgE redundantly inactivate Rab32 and prevent the recruitment of Rab32 to the SCV (Spano *et al.*, 2016).

M. tuberculosis secrets several effectors to arrest phagosomal maturation at different steps and to form the *Mycobacterium*-containing phagosomes (MCVs) in macrophages via type

VII secretion systems (also known as ESX systems). SapM, a secreted PtdIns3P phosphatase of *M. tuberculosis*, hydrolyses PtdIns3P to PtdIns, which arrests phagosomal maturation at an early stage (Puri *et al.*, 2013; Saleh and Belisle, 2000). EsxA (also known as ESAT-6), a key virulence factor of *M. tuberculosis*, is a membrane-permeabilizing protein and is essential for phagosome rupture and bacterial cytosolic translocation in macrophages (Ma *et al.*, 2015). EsxA lyses the membrane to arrest phagosomal maturation and to promote bacterial cytosolic escape and spread. An additional secreted protein EsxH of *M. tuberculosis* interacts with human hepatocyte growth factor-regulated tyrosine kinase substrate (Hgs/Hrs), a component of ESCRT (Mehra *et al.*, 2013). ESCRT is required to transport the MCVs to the lysosome and to restrict intracellular bacterial growth. The interaction between EsxH with Hrs disrupts ESCRT function and blocks the fusion of the MCV with the lysosome (Mehra *et al.*, 2013).

L. pneumophila reprogrammes the phagosomal maturation processes of macrophages to generate the *Legionella*-containing vacuoles (LCV) for intracellular survival and proliferation. *L. pneumophila* translocates nearly 300 effectors into macrophages via the Dot/Icm type IVb secretion system (T4BSS) during infection (Zhu *et al.*, 2011). Multiple effectors participate in the reprogramming of phagosome maturation and biogenesis of the LCV. The *Legionella* effector DrrA/SidM is a multifunctional protein possessing an N-terminal nucleotidyl transferase domain, a central dual-function domain with GDI-displacement factor (GDF) and RabGEF activities, and a C-terminal PtdIns4P-binding domain (Ingmundson *et al.*, 2007; Machner and Isberg, 2007). The C-terminal PtdIns4P-binding domain of SidM mediates the localization of the effector to the LCV. The central dual-function domain displaces GDI from the Rab1–GDI complex to release GDP-bound inactive Rab1. The dual-function domain subsequently catalyses the exchange of GDP in Rab1 to GTP for activation. The N-terminal nucleotidyl transferase domain of SidM AMPylates the GTP-bound active Rab1 for constitutive activation (Müller *et al.*, 2010). The effector SidD reverses the AMPylation modification of Rab1 via its deAMPylase activity (Neunuebel *et al.*, 2011; Tan and Luo, 2011). The deAMPylated Rab1 is further inactivated by the effector LepB, a GTPase-activating protein (GAP). An additional effector AnkX is a phosphocholine transferase that phosphocholinates Rab1 (Mukherjee *et al.*, 2011), while Lem3 removes the phosphocholination modification (Tan *et al.*, 2011). Via the collaboration of these effectors, *L. pneumophila* manipulates the membrane trafficking of ER-derived vesicles to the LCV and evades phagosome maturation. Similar to *L. pneumophila*, the Q fever bacterium *C. burnetii* exhibits a Dot/Icm type IVb secretion system to deliver effectors. *C. burnetii* renovates a lysosome into a mature *Coxiella*-containing vacuole (CCV), which makes it permissive to intracellular replication (van Schaik *et al.*, 2013). It has been reported that the *C. burnetii* effector Coxiella vacuolar protein B (CvpB) binds PtdIns3P and phosphatidylserine (PS) on the CCVs to perturb the activity of the phosphatidylinositol 5-kinase PIKfyve and manipulate PtdIns3P metabolism for optimal vacuole development (Martinez *et al.*, 2016). However, it is still largely unknown how the effectors of *C. burnetii* antagonize elimination by lysosomes.

Modulation of cytoplasmic immune signalling pathways

Cytoplasmic immune signalling pathways, including NF-κB, MAPK and type I interferon signalling pathways are activated upon bacterial recognition by immune receptors

in macrophages. The NF-κB signalling pathway plays a pivotal role in the innate immune response by regulating the transcription of inflammation-related genes (Li and Verma, 2002). NF-κB signalling is initiated by cell surface receptors, including TLRs, and is transduced by cytosolic death-domain-containing adaptors to activate TRAF-mediated polyubiquitination, which sequentially activates the downstream IKK complex. The IKK complex phosphorylates IκB and induces its degradation by the proteasome. Upon IκB degradation, NF-κB translocates from the cytoplasm to the nucleus to initiate the gene expression of inflammation-related cytokines (Li and Verma, 2002). MAPK pathways consist of a three-tiered cascade of kinases. MAPK family kinases, including ERK1/2, c-Jun N-terminal kinase (JNK and p38, are phosphorylated and activated by MAPK kinase (MAPKK), which is activated by upstream MAPKK kinase kinase (MAPKKK) (Zheng and Guan, 1994). MAPK pathways regulate the expression of many inflammation-related cytokines. With the exception of the roles in immune defence, MAPK pathways regulate cell proliferation.

To inhibit the secretion of pro-inflammatory cytokines, bacterial effectors usually cooperate to target key signalling molecules of cytoplasmic immune signalling pathways in macrophages (Fig. 3.1 and Table 3.1). *S. flexneri* secretes several type III effectors to interfere with the NF-κB and MAPK signalling pathways. The *Shigella* effector OspI harbours a unique deamidase activity with a papain-like fold. OspI catalyses a specific deamidation reaction on the glutamine residue Q100 of Ubc13, a key ubiquitin-conjugating E2 enzyme in the NF-κB signalling pathway, which converts Q100 to a glutamic acid residue E100 (Fu *et al.*, 2013; Sanada *et al.*, 2012). The unique deamidation modification disrupts the interactions between Ubc13 and the ubiquitin E3 ligase TRAF6, and impairs the formation of Lys63-linked polyubiquitin chains, resulting in inhibition of the TRAF6-NF-κB signalling pathway. The *Shigella* effector OspG is a half-kinase and binds to ubiquitin-conjugated E2 enzymes (UbcH5 and UbcH7) to interfere with the ubiquitination-mediated degradation of IκB-α via the proteasome (Kim *et al.*, 2005). The ubiquitin E3 ligase effector IpaH9.8 of *Shigella* inhibits NF-κB signalling by inducing polyubiquitination and degradation of NEMO (Ashida *et al.*, 2010). In addition, the *Shigella* effector IpaH1.4 and IpaH2.5 are two E3 ligase effectors. Both effectors directly interact with the HOIL-1L subunit of the linear ubiquitin chain assembly complex (LUBAC) to ubiquitinate and degrade the subunit HOIP, which irreversibly inactivates linear ubiquitination and blocks NF-κB nuclear translocation (de Jong *et al.*, 2016). Moreover, MAPK pathways are inhibited by *Shigella* during infection. The *Shigella* effector OspF harbours a classic D-motif at the N-terminus to specifically target dual-phosphorylated active MAPKs. Via a novel β-elimination catalytic mechanism, OspF functions as a phosphothreonine lyase to dephosphorylate the phosphothreonine residue in the activation loop of active MAPKs to β-methyldehydroalanine. This 'dephosphorylation' irreversibly inactivates MAPKs and inhibits MAPK signalling pathways during *Shigella* infection (Li *et al.*, 2007; Zhu *et al.*, 2007).

EPEC/EHEC suppresses the NF-κB and MAPK signalling pathways via multiple effectors, including NleE, NleB, NleC, NleH, and Tir. NleE is a unique cysteine methyltransferase, which methylates the cysteine residue in the zinc finger domain of TAB2/3 (Zhang *et al.*, 2011). The methylation modification on the cysteine residue impairs the sensing and binding capability of TAB2/3 for the Lys63-linked polyubiquitin chains, which completely disable TRAF6-TAK1-NF-κB signalling. NleB is an N-acetyl-D-gulcosamine transferase and interacts with the host GAPDH. GAPDH regulates the NF-κB pathway by interacting with

the ubiquitin E3 ligase TRAF2 (Gao *et al.*, 2013). NleB transfers N-acetylglucosamine from UDP-GlcNAc to modify GAPDH at multiple sites, which disrupts GAPDH–TRAF2 interactions, resulting in NF-κB inactivation. Both NleC and NleD are zinc metalloproteases, and specifically cleave p65 and JNK, respectively, to block NF-κB and AP-1 activation (Baruch *et al.*, 2011). The effector Tir plays an important role in regulating pedestal formation for EPEC/EHEC adhesion to epithelium cells by activating actin polymerization. In addition, Tir participates in the modulation of NF-κB signalling pathways. Tir inhibits NF-κB signalling by interacting with cytoplasm-located TRAF adaptor proteins to induce their proteasomal-independent degradation and the recruitment of SHP-1 to decrease TRAF6 ubiquitination (Ruchaud-Sparagano *et al.*, 2011; Yan *et al.*, 2012).

Salmonella spp., *Yersinia* spp., *M. tuberculosis* and *L. pneumophila* also interfere with NF-κB and MAPK signalling pathways. The *Salmonella* effector SpvC, a homologue of OspF, inhibits the p38 MAPK signalling pathway via phosphothreonine lyase activity during *Salmonella* infection (Haneda *et al.*, 2012; Li *et al.*, 2007). The PipA family effectors of *S.* Typhimurium, including PipA, GogA and GtgA, cleave the p65 and RelB subunits to inhibit NF-κB response (Sun *et al.*, 2016). SpvD specifically interacts with the exportin Xpo2 to block the nuclear translocation of p65, which subsequently blocks NF-κB activation (Rolhion *et al.*, 2016). The *Salmonella* effector GogB targets the SCF E3 ligase via interactions with Skp1 and FBXO22 to inhibit IκB degradation and NF-κB activation (Pilar *et al.*, 2012). The *Yersinia* effector YopJ is an acetyltransferase that blocks the activation of both MAPK and NF-κB pathways by acetylating serine and threonine residues on MAPKK6 and IKKβ (Mukherjee *et al.*, 2006). *M. tuberculosis* PtpA is a secreted tyrosine phosphatase and binds to the host ubiquitin. Binding to ubiquitin activates PtpA phosphatase activity, which dephosphorylates active JNK and p38 (Wang *et al.*, 2015). PtpA also inhibits NF-κB signalling in macrophages by competitively binding to the ubiquitin-interacting domain of TAB3 (Wang *et al.*, 2015). Unlike most bacterial secreted effectors inhibiting NF-κB signalling, the *L. pneumophila* effectors LnaB and LegK1 function as activators of the NF-κB pathway (Ge *et al.*, 2009; Losick *et al.*, 2010). LegK1 mimics the host IKK to phosphorylate IκBα and other inhibitors of the IκB family, including p100 via the serine/threonine kinase activity to activate NF-κB signalling (Ge *et al.*, 2009). However, how LnaB activates NF-κB signalling remains completely unknown.

Interference with autophagy

Autophagy is an intracellular degradation process that targets unfolded proteins, lipids and damaged organelles for cellular homeostasis. It also combats the invasion of various bacterial pathogens, thereby playing an important role in host immune defences (Huang and Brumell, 2014). Autophagy undergoes the following steps: signal induction, membrane nucleation, cargo targeting, autophagosome formation, lysosomal fusion, and cargo degradation. Induction of autophagy is triggered by a series of signals, including nutrient limitation, damaged organelles or invading bacteria (Parzych and Klionsky, 2014). Formation of the double-membrane autophagosome is a hallmark of autophagy. The cytoplasmic sequestering membrane (also known as a phagophore) at the early stage of autophagy is initiated in the endoplasmic reticulum (ER) and mitochondria. An autophagosome is formed by the subsequent expansion and closure of the phagophore. The autophagosome finally fuses with the lysosome to form an autolysosome, which degrades autophagic cargo (Parzych

and Klionsky, 2014). Autophagy-related (Atg) genes mainly regulate the formation of the autophagosome. Several proteins involved in vesicle trafficking and protein ubiquitination, such as Rab GTPases, SNARE complexes, ubiquitin E3 ligases and deubiquitinases, are also involved in autophagosome formation and maturation. Two ubiquitination-like conjugation systems of Atg proteins are essential for autophagosome maturation (Fujita *et al.*, 2008; Mizushima *et al.*, 1998; Tanida *et al.*, 2004). Atg12 and Atg8 (also known as LC3) are two ubiquitin-like proteins in autophagy. Atg12 is conjugated to Atg5 in the presence of the E1-like enzyme Atg7 and the E2-like enzyme Atg10, which promotes phagophore expansion. Atg12–Atg5 forms a complex with Atg16L1 and associates with the expanding membrane of the phagophore. The Atg12–Atg5–Atg16 complex is then released from the autophagosome when the phagophore is closed. The Atg12–Atg5–Atg16L1 complex directs Atg8 to the phagosome membrane. In the presence of the E1-like enzyme Atg7 and the E2-like enzyme Atg3, Atg12-Atg5 functions as an E3-like enzyme and conjugates phosphatidylethanolamine to Atg8, the second ubiquitin-like conjugate (Atg8-PE). The cysteine protease Atg4 cleaves the carboxyl terminus of Atg8 and exposes a glycine residue for the covalent attachment of PE, and is thus essential for LC3-PE formation. Atg4 also deconjugates and releases a portion of the LC3–PE conjugates from the autophagosome membrane to recycle LC3 for the formation of new autophagosomes. Cargo receptors and adaptor proteins play important roles in cargo targeting of autophagy. Cargo receptors usually harbour an LC3-interacting region (LIR) that recruits LC3-decorated autophagosomes. There are several adaptor proteins, including p62, NDP52, NBR1 and optineurin, which have been identified (Fujita *et al.*, 2008; Kirkin *et al.*, 2009; Wild *et al.*, 2011). They consist of both a ubiquitin-binding domain and LIR to recognize ubiquitin-associated specific cargo for autophagosomes.

To counteract autophagic degradation, bacteria have developed diverse strategies to avoid or exploit autophagy by interfering with autophagy signalling via effector proteins (Fig. 3.2 and Table 3.2). The *Shigella* effector VirA is a RabGAP protein that harbours a TBC-like dual finger motif with a novel structural fold. Via a dual-figure catalytic mechanism, VirA specifically inactivates the host Rab1 GTPase. Inactivation of Rab1 by VirA blocks the autophagic defence of host cells to facilitate bacterial cytoplasmic survival (Dong *et al.*, 2012). IcsB, an effector of *S. flexneri*, also promotes the bacteria to evade host cell autophagy. It has been proposed that IcsB blocks the autophagic protein Atg5 binding to VirG, a surface protein of *S. flexneri* important for bacterial actin-based motility, to abolish autophagic recognition (Ogawa *et al.*, 2005). The *Legionella* effector RavZ is a cysteine protease and consists of an N-terminal catalytic domain with an Ulp family deubiquitinase-like fold and a C-terminal PtdIns3P-binding module (Choy *et al.*, 2012; Horenkamp *et al.*, 2015). RavZ binds to the autophagosome via the PtdIns3P-binding module. Through its cysteine protease activity, RavZ cleaves the amide bond between the carboxyl-terminal glycine residue and an adjacent aromatic residue in Atg8 proteins. The cleavage deconjugates Atg8 from PE and releases Atg8 from the phospholipid membranes (Choy *et al.*, 2012). The irreversible inactivation of Atg8 by RavZ inhibits host autophagy during *Legionella* infection. The *Legionella* effector sphingosine-1 phosphate lyase (LpSpl) also modulates autophagy (Rolando *et al.*, 2016). LpSp1 exhibits a similar structure as human sphingosine-1 phosphate lyase. LpSp1 degrades sphingosine-1 phosphate and modulates the host sphingosine biosynthesis in infected macrophages to inhibit autophagy during *Legionella* infection. The described lipid phosphatase SapM of *M. tuberculosis* hydrolyses PtdIns3P to maintain the MCVs PtdIns3P-free, which

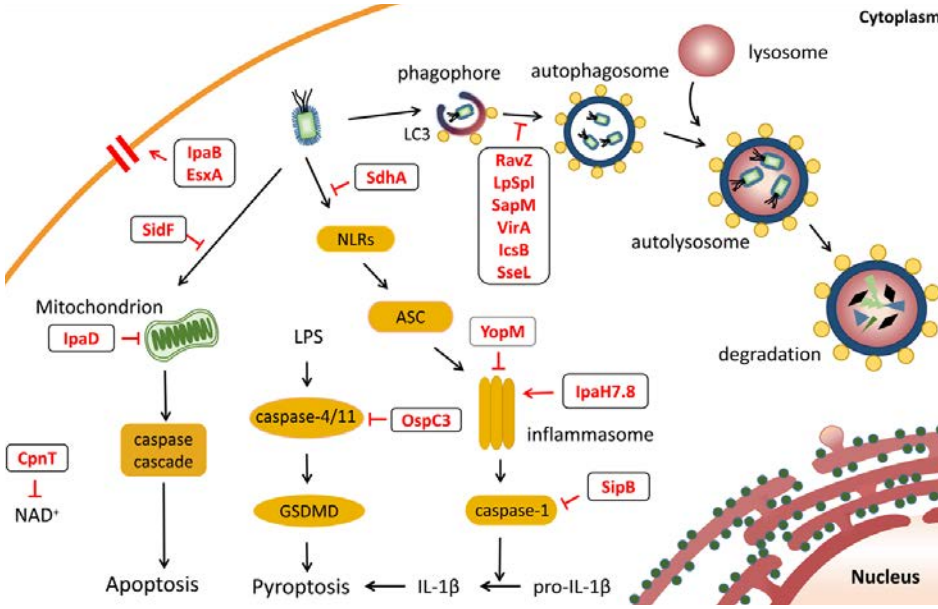

Figure 3.2 Interference with autophagy and cell death signalling by bacterial protein toxins and effectors. VirA, IcsB, RavZ, LpSp1, SapM and SseL inhibit host autophagy. SdhA, IpaH7.8, OspC3 and YopM inhibit pyroptosis, while IpaB induces pyroptosis. *Legionella* SidF antagonizes apoptosis, while *Shigella* IpaD activates apoptosis. EsxA and CpnT of *M. tuberculosis* induce macrophage necrosis. NLRs, NOD-like receptors; GSDMD, Gasdermin D.

inhibits phagocytosis and autophagosome formation in macrophages (Puri *et al.*, 2013; Saleh and Belisle, 2000). It was also reported that SapM blocks the autophagosome–lysosome fusion via binding Rab7 (Hu *et al.*, 2015). PE_PGRS47 (Rv2741), the other secreted effector of *M. tuberculosis*, has also been reported to inhibit macrophage autophagy, although its molecular mechanism remains unclear (Saini *et al.*, 2016). The *Salmonella* effector SseL functions as a deubiquitinase to clear the ubiquitinated structures, including SPI-2 T3SS-dependent aggregates and aggresome-like induced structures (ALIS) around the SCVs. Deubiquitination of ALIS prevents the recognition by the autophagy receptor p62 to recruit LC3 for autophagic degradation. The deubiquitinase activity of SseL lowers autophagic flux and favours intracellular *Salmonella* replication in macrophages (Mesquita *et al.*, 2012).

Interference with cell death signalling

The induction of host cell death is a conserved immune defence strategy throughout the animal and plant kingdoms. Apoptosis, necrosis and pyroptosis are three key cell death pathways in macrophages. Apoptosis is initiated by either intrinsic mitochondria/ER signalling or extrinsic death receptors. Both signalling pathways activate the downstream caspase cascade and induce cell shrinkage, membrane blebbing and the formation of apoptotic bodies. Necrosis is tightly regulated by RIP3, which phosphorylates the downstream pseudokinase MLKL. The phosphorylation modification relieves the autoinhibitory state of MLKL. The released N-terminal domain of MLKL oligomerizes in the cell membrane and disrupts the

cellular ionic homeostasis via its pore-forming activity, which induces necrotic cell death (Sun and Wang, 2014). Pyroptosis is induced upon pathogen recognition by NLR receptors and the formation of corresponding inflammasomes (Zhao and Shao, 2016).

Bacterial pathogens modulate cell death pathways for intracellular survival and replication (Fig. 3.2 and Table 3.2). As previously described, the *L. pneumophila* effector LegK1 functions as a Ser/Thr kinase that phosphorylates IκBα and other inhibitors of the IκB family, which activates the NF-κB pathway (Ge *et al.*, 2009). Activation of NF-κB signalling prevents *Legionella*-infected macrophages from apoptotic cell death. The *Legionella* effector SidF, a phosphatidylinositol phosphatase, also blocks the apoptotic pathway by interacting with the Bcl2 family proapoptotic proteins, BNIP3 and Bcl-rambo (Banga *et al.*, 2007). The *Legionella* effector SdhA forms a unique ring-shaped membrane structure to protect the integrity of the LCV, which blocks DNA release from the LCV to the cytoplasm and prevents the DNA sensing by the AIM2 inflammasome (Creasey and Isberg, 2012; Ge *et al.*, 2012). Deletion of SdhA of *L. pneumophila* induces cell death of macrophages. In contrast to *L. pneumophila*, *S. flexneri* multiplies in the cytoplasm after entry into macrophages and induces rapid pyroptotic cell death. The bacteria released from dying macrophages then enter the intestinal epithelium via the basolateral surface of polarized enterocytes. The T3SS effector IpaB is a component of T3SS of *S. flexneri.* IpaB undergoes oligomerization on the cell membrane to form ion channels, which permeabilizes the host cell membrane and induces potassium influx. The potassium influx in turn activates the NLRC4/ASC/caspase-1 inflammasome and induces pyroptosis of macrophages (Senerovic *et al.*, 2012). In contrast to IpaB, *Salmonella* SipB, a homologous effector of IpaB, was reported to trigger macrophage cell death by directly binding to caspase-1 (Hernandez *et al.*, 2003). However, the real form of SipB-induced cell death of macrophages requires further validation and investigation. The translocator protein IpaD is an additional tip component of the T3SS apparatus needle of *Shigella*. IpaD activates a classical apoptotic pathway via the activation of host caspases and disruption of mitochondrial morphology to trigger apoptosis (Arizmendi *et al.*, 2016). The N-terminal domain of IpaD has been proposed to be required for apoptosis activation. Similarly, SipD, a structural homologue of IpaD from *Salmonella*, also triggers cell death in macrophages and exhibits the same cytotoxicity. However, the *Yersinia* counterpart LcrV, which lacks the N-terminal structures present in IpaD and SipD, cannot promote macrophage cell death (Arizmendi *et al.*, 2016). In addition, the effector IpaH7.8 of *Shigella* target the glomulin/flagellar-associated protein 68 (GLMN) for degradation via its ubiquitin E3 ligase activity. GLMN is a negative regulator of NLRP3 and NLRC4 inflammasomes. The IpaH7.8-mediated GLMN degradation activates NLRP3 and NLRC4 inflammasomes and caspase-1, resulting in macrophage pyroptotic cell death (Suzuki *et al.*, 2014). The *Shigella* effector OspC3 specifically interacts with the p19 subunit of caspase-4. The interaction inhibits the heterodimerization of the p19 subunit with the p10 subunit, which disrupts the activation of caspase-4 by LPS and antagonizes the detection of invading bacteria in the cytoplasm (Kobayashi *et al.*, 2013). *Y. pestis* and *Y. pseudotuberculosis* secrete the type III effector YopM to recruit and activate the host kinases PRK1 and PRK2, which inhibit activation of the pyrin inflammasome that is triggered by the RhoA-inactivating enzymatic activities of the *Yersinia* effectors, YopE and YopT (Chung *et al.*, 2016). *M. tuberculosis* activates macrophage necrosis during infection. EsxA (ESAT-6) secreted by the ESX-1 secretion system of *M. tuberculosis* produces pores in the MCV membranes via its pore-forming activity, facilitating bacterial escape from the vacuole. EsxA also induces necrosis

of the macrophage and promotes bacterial cell-to-cell spread (Aguilo *et al.*, 2013; Simeone *et al.*, 2012). *M. tuberculosis* also secretes another endotoxin CpnT to induce necrotic cell death. The N-terminal domain of CpnT forms a channel to take up nutrients across the outer membrane of the bacteria. The C-terminal domain of CpnT harbours NAD^+ glycohydrolase activity to hydrolyse NAD^+, which causes NAD^+ depletion and necrosis of macrophages (Danilchanka *et al.*, 2014; Sun *et al.*, 2015).

Conclusion and future perspective

Infection of bacterial pathogens causes many diseases and elicits severe threats to global human health. As an important component of human innate immune system, macrophages are professional phagocytic cells that are present in diverse tissues to clear the invading bacteria. Following the long-term co-evolution between bacterial pathogens and host, bacterial pathogens have evolved diverse strategies to modulate macrophage functions to evade host defence. In this review, we discussed how bacterial pathogens, including *Legionella*, *Shigella*, *Salmonella*, *Listeria*, EPEC, *Yersinia* and *M. tuberculosis* subvert macrophage functions via their secreted toxins and effector proteins (Tables 3.1 and 3.2). On the basis of the distinct infection processes of these bacterial pathogens, the secreted toxins and effector proteins specifically target key signalling molecules of defence pathways to subtly interfere with macrophage functions, thereby promoting the establishment of safe niches for bacterial survival and proliferation. These toxins and effector proteins harbour unique biochemical activities or mimic eukaryotic proteins to target host substrates. The uncovered functions and mechanisms of these effectors enhance our understanding of bacterial pathogenesis and related eukaryotic signalling pathways.

The characterized effector proteins or toxins are also important for clinical therapies and biotechnological application. For example, the *Shigella* effector OspF harbours the phosphothreonine lyase activity and contains a classic 'D motif' to irreversibly inactivate the host MAPK (Li *et al.*, 2007; Zhu *et al.*, 2007). The unique biochemical activity and the specific targeting motif enable OspF to be applied in rewire kinase pathways in immune cells and the precise control of T-cell activation (Wei *et al.*, 2012). In addition, the *Shigella* effector VirA is a TBC-like RabGAP with a new structural fold (Dong *et al.*, 2012). VirA is essential for *Shigella* invasion and intracellular survival (Uchiya *et al.*, 1995). The gene deletion of VirA severely inhibits the *Shigella* infection. The novel structural fold of VirA and its essential role in infection indicate that VirA is a good candidate for designing new antibiotics to counteract the drug-resistant *Shigella*. As many effectors and toxins modulate the host immune pathways via novel biochemical activities, it will be very interesting to develop these bacterial virulence proteins as tools for clinical therapies and biomedical research.

Despite emerging progress, many questions still remain and require further investigation. For example, it has been revealed that *L. pneumophila* secrete LegK1 to activate NF-κB signalling and inhibit macrophage apoptosis (Ge *et al.*, 2009). Given that NF-κB signalling regulates the expression of pro-inflammation cytokines, it would be interesting to determine whether *Legionella* effectors inhibit the NF-κB pathway during infection. In addition, whether MAPK signalling pathways are involved in *Legionella* pathogenesis remains an open question. Intriguingly, *M. tuberculosis* interferes with macrophage cell death by EsxA-mediated and CpnT-induced necrosis (Danilchanka *et al.*, 2014; Ma *et al.*, 2015; Sun *et al.*, 2015). However, the roles of other types of cell death in *M. tuberculosis* modulation of

macrophages are unknown. The bacterium *C. burnetii* invade and survival in macrophages, and contains a similar type IVb secretion system as *L. pneumophila*. However, the life cycle of *C. burnetii* is completely different from that of *L. pneumophila*. How *C. burnetii* modulates intracellular immune signalling pathways, autophagy and cell death of macrophages is only beginning to be illustrated. In addition, there are many functionally unknown toxins and effectors in *L. pneumophila*, *C. burnetii* and *M. tuberculosis*. Further efforts are required to investigate the mechanisms of these effectors in counteracting macrophage defence.

Acknowledgements

We apologize to colleagues whose work could not be cited due to space limitation. This work was supported by grants from National Natural Science Foundation of China (81561130162, 81322024, 81530068 and 31370722 to Y. Zhu, 81501717 to Y. Zhou), the Natural Science Foundation of Zhejiang province (LR13C050001 to Y. Zhu) and the Royal Society (NA140239 to Y. Zhu). M.W prepared the figures. M. W., Y. Zhou and Y. Zhu wrote the manuscript.

References

Aguilo, J.I., Alonso, H., Uranga, S., Marinova, D., Arbués, A., de Martino, A., Anel, A., Monzon, M., Badiola, J., Pardo, J., *et al.* (2013). ESX-1-induced apoptosis is involved in cell-to-cell spread of *Mycobacterium tuberculosis*. Cell. Microbiol. *15*, 1994–2005. https://doi.org/10.1111/cmi.12169.

Arizmendi, O., Picking, W.D., and Picking, W.L. (2016). Macrophage apoptosis triggered by IpaD from *Shigella flexneri*. Infect. Immun. *84*, 1857–1865. https://doi.org/10.1128/IAI.01483-15.

Ashida, H., Kim, M., Schmidt-Supprian, M., Ma, A., Ogawa, M., and Sasakawa, C. (2010). A bacterial E3 ubiquitin ligase IpaH9.8 targets NEMO/IKKgamma to dampen the host NF-kappaB-mediated inflammatory response. Nat. Cell Biol. *12*, 66-73. https://doi.org/10.1038/ncb2006.

Banga, S., Gao, P., Shen, X., Fiscus, V., Zong, W.X., Chen, L., and Luo, Z.Q. (2007). *Legionella pneumophila* inhibits macrophage apoptosis by targeting pro-death members of the Bcl2 protein family. Proc Natl Acad Sci U S A *104*, 5121-5126. https://doi.org/10.1073/pnas.0611030104.

Baruch, K., Gur-Arie, L., Nadler, C., Koby, S., Yerushalmi, G., Ben-Neriah, Y., Yogev, O., Shaulian, E., Guttman, C., Zarivach, R., *et al.* (2011). Metalloprotease type III effectors that specifically cleave JNK and NF-κB. EMBO J. *30*, 221–231. https://doi.org/10.1038/emboj.2010.297.

Birmingham, C.L., Canadien, V., Kaniuk, N.A., Steinberg, B.E., Higgins, D.E., and Brumell, J.H. (2008). Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. Nature *451*, 350–354. https://doi.org/10.1038/nature06479.

Black, D.S., and Bliska, J.B. (1997). Identification of p130Cas as a substrate of Yersinia YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. EMBO J. *16*, 2730–2744. https://doi.org/10.1093/emboj/16.10.2730.

Bryant, C., and Fitzgerald, K.A. (2009). Molecular mechanisms involved in inflammasome activation. Trends Cell Biol. *19*, 455–464. https://doi.org/10.1016/j.tcb.2009.06.002.

Bryant, C.E., Gay, N.J., Heymans, S., Sacre, S., Schaefer, L., and Midwood, K.S. (2015). Advances in Toll-like receptor biology: Modes of activation by diverse stimuli. Crit. Rev. Biochem. Mol. Biol. *50*, 359–379. https://doi.org/10.3109/10409238.2015.1033511.

Burdette, D.L., Monroe, K.M., Sotelo-Troha, K., Iwig, J.S., Eckert, B., Hyodo, M., Hayakawa, Y., and Vance, R.E. (2011). STING is a direct innate immune sensor of cyclic di-GMP. Nature *478*, 515–518. https://doi.org/10.1038/nature10429.

Choy, A., Dancourt, J., Mugo, B., O'Connor, T.J., Isberg, R.R., Melia, T.J., and Roy, C.R. (2012). The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. Science *338*, 1072–1076. https://doi.org/10.1126/science.1227026.

Chung, L.K., Park, Y.H., Zheng, Y., Brodsky, I.E., Hearing, P., Kastner, D.L., Chae, J.J., and Bliska, J.B. (2016). The *Yersinia* virulence factor YopM hijacks host kinases to inhibit Type III effector-triggered activation of the pyrin inflammasome. Cell Host Microbe *20*, 296–306. https://doi.org/10.1016/j.chom.2016.07.018.

Creasey, E.A., and Isberg, R.R. (2012). The protein SdhA maintains the integrity of the Legionella-containing vacuole. Proc. Natl. Acad. Sci. U.S.A. *109*, 3481–3486. https://doi.org/10.1073/pnas.1121286109.

Crowley, M.T., Costello, P.S., Fitzer-Attas, C.J., Turner, M., Meng, F., Lowell, C., Tybulewicz, V.L., and DeFranco, A.L. (1997). A critical role for Syk in signal transduction and phagocytosis mediated by Fcgamma receptors on macrophages. J. Exp. Med. *186*, 1027–1039. https://doi.org/10.1084/jem.186.7.1027.

Danilchanka, O., Sun, J., Pavlenok, M., Maueröder, C., Speer, A., Siroy, A., Marrero, J., Trujillo, C., Mayhew, D.L., Doornbos, K.S., *et al.* (2014). An outer membrane channel protein of *Mycobacterium tuberculosis* with exotoxin activity. Proc. Natl. Acad. Sci. U.S.A. *111*, 6750–6755. https://doi.org/10.1073/pnas.1400136111.

de Jong, M.F., Liu, Z., Chen, D., and Alto, N.M. (2016). *Shigella flexneri* suppresses NF-κB activation by inhibiting linear ubiquitin chain ligation. Nat. Microbiol. *1*, 16084. https://doi.org/10.1038/nmicrobiol.2016.84.

Deleuil, F., Mogemark, L., Francis, M.S., Wolf-Watz, H., and Fällman, M. (2003). Interaction between the Yersinia protein tyrosine phosphatase YopH and eukaryotic Cas/Fyb is an important virulence mechanism. Cell. Microbiol. *5*, 53–64. https://doi.org/10.1046/j.1462-5822.2003.00236.x.

Dong, N., Zhu, Y., Lu, Q., Hu, L., Zheng, Y., and Shao, F. (2012). Structurally distinct bacterial TBC-like GAPs link Arf GTPase to Rab1 inactivation to counteract host defenses. Cell *150*, 1029–1041. https://doi.org/10.1016/j.cell.2012.06.050.

Du, J., Reeves, A.Z., Klein, J.A., Twedt, D.J., Knodler, L.A., and Lesser, C.F. (2016). The type III secretion system apparatus determines the intracellular niche of bacterial pathogens. Proc. Natl. Acad. Sci. U.S.A. *113*, 4794–4799. https://doi.org/10.1073/pnas.1520699113.

Flannagan, R.S., Cosío, G., and Grinstein, S. (2009). Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. Nat. Rev. Microbiol. *7*, 355–366. https://doi.org/10.1038/nrmicro2128.

Flannagan, R.S., Jaumouillé, V., and Grinstein, S. (2012). The cell biology of phagocytosis. Annu. Rev. Pathol. *7*, 61–98. https://doi.org/10.1146/annurev-pathol-011811-132445.

Fu, P., Zhang, X., Jin, M., Xu, L., Wang, C., Xia, Z., and Zhu, Y. (2013). Complex structure of OspI and Ubc13: the molecular basis of Ubc13 deamidation and convergence of bacterial and host E2 recognition. PLOS Pathog. *9*, e1003322. https://doi.org/10.1371/journal.ppat.1003322.

Fujita, N., Itoh, T., Omori, H., Fukuda, M., Noda, T., and Yoshimori, T. (2008). The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol. Biol. Cell *19*, 2092–2100. https://doi.org/10.1091/mbc.E07-12-1257.

Gao, X., Wang, X., Pham, T.H., Feuerbacher, L.A., Lubos, M.L., Huang, M., Olsen, R., Mushegian, A., Slawson, C., and Hardwidge, P.R. (2013). NleB, a bacterial effector with glycosyltransferase activity, targets GAPDH function to inhibit NF-κB activation. Cell Host Microbe *13*, 87–99. https://doi.org/10.1016/j.chom.2012.11.010.

Ge, J., Gong, Y.N., Xu, Y., and Shao, F. (2012). Preventing bacterial DNA release and absent in melanoma 2 inflammasome activation by a Legionella effector functioning in membrane trafficking. Proc. Natl. Acad. Sci. U.S.A. *109*, 6193–6198. https://doi.org/10.1073/pnas.1117490109.

Ge, J., Xu, H., Li, T., Zhou, Y., Zhang, Z., Li, S., Liu, L., and Shao, F. (2009). A Legionella type IV effector activates the NF-kappaB pathway by phosphorylating the IkappaB family of inhibitors. Proc. Natl. Acad. Sci. U.S.A. *106*, 13725–13730. https://doi.org/10.1073/pnas.0907200106.

Haneda, T., Ishii, Y., Shimizu, H., Ohshima, K., Iida, N., Danbara, H., and Okada, N. (2012). Salmonella type III effector SpvC, a phosphothreonine lyase, contributes to reduction in inflammatory response during intestinal phase of infection. Cell. Microbiol. *14*, 485–499. https://doi.org/10.1111/j.1462-5822.2011.01733.x.

Hernandez, L.D., Pypaert, M., Flavell, R.A., and Galán, J.E. (2003). A Salmonella protein causes macrophage cell death by inducing autophagy. J. Cell Biol. *163*, 1123–1131. https://doi.org/10.1083/jcb.200309161.

Horenkamp, F.A., Kauffman, K.J., Kohler, L.J., Sherwood, R.K., Krueger, K.P., Shteyn, V., Roy, C.R., Melia, T.J., and Reinisch, K.M. (2015). The Legionella anti-autophagy effector RavZ targets the autophagosome via PI3P- and curvature-sensing motifs. Dev. Cell *34*, 569–576. https://doi.org/10.1016/j.devcel.2015.08.010.

Hu, D., Wu, J., Wang, W., Mu, M., Zhao, R., Xu, X., Chen, Z., Xiao, J., Hu, F., Yang, Y., *et al.* (2015). Autophagy regulation revealed by SapM-induced block of autophagosome-lysosome fusion via binding RAB7. Biochem. Biophys. Res. Commun. *461*, 401–407. https://doi.org/10.1016/j.bbrc.2015.04.051.

Huang, J., and Brumell, J.H. (2014). Bacteria-autophagy interplay: a battle for survival. Nat. Rev. Microbiol. *12*, 101–114. https://doi.org/10.1038/nrmicro3160.

Ingmundson, A., Delprato, A., Lambright, D.G., and Roy, C.R. (2007). *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. Nature *450*, 365–369. https://doi.org/10.1038/nature06336.

Kim, D.W., Lenzen, G., Page, A.L., Legrain, P., Sansonetti, P.J., and Parsot, C. (2005). The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. Proc. Natl. Acad. Sci. U.S.A. *102*, 14046–14051. https://doi.org/10.1073/pnas.0504466102.

Kirkin, V., Lamark, T., Sou, Y.S., Bjørkøy, G., Nunn, J.L., Bruun, J.A., Shvets, E., McEwan, D.G., Clausen, T.H., Wild, P., *et al.* (2009). A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol. Cell *33*, 505–516. https://doi.org/10.1016/j.molcel.2009.01.020.

Kobayashi, T., Ogawa, M., Sanada, T., Mimuro, H., Kim, M., Ashida, H., Akakura, R., Yoshida, M., Kawalec, M., Reichhart, J.M., *et al.* (2013). The Shigella OspC3 effector inhibits caspase-4, antagonizes inflammatory cell death, and promotes epithelial infection. Cell Host Microbe *13*, 570-583. https://doi.org/10.1016/j.chom.2013.04.012.

Li, H., Xu, H., Zhou, Y., Zhang, J., Long, C., Li, S., Chen, S., Zhou, J.M., and Shao, F. (2007). The phosphothreonine lyase activity of a bacterial type III effector family. Science *315*, 1000–1003.

Li, Q., and Verma, I.M. (2002). NF-kappaB regulation in the immune system. Nat. Rev. Immunol. *2*, 725–734. https://doi.org/10.1038/nri910.

Losick, V.P., Haenssler, E., Moy, M.Y., and Isberg, R.R. (2010). LnaB: a *Legionella pneumophila* activator of NF-kappaB. Cell. Microbiol. *12*, 1083–1097. https://doi.org/10.1111/j.1462-5822.2010.01452.x.

Ma, Y., Keil, V., and Sun, J. (2015). Characterization of *Mycobacterium tuberculosis* EsxA membrane insertion: roles of N- and C-terminal flexible arms and central helix-turn-helix motif. J Biol Chem *290*, 7314-7322. https://doi.org/10.1074/jbc.M114.622076.

Machner, M.P., and Isberg, R.R. (2007). A bifunctional bacterial protein links GDI displacement to Rab1 activation. Science *318*, 974–977. https://doi.org/10.1126/science.1149121.

Martinez, E., Allombert, J., Cantet, F., Lakhani, A., Yandrapalli, N., Neyret, A., Norville, I.H., Favard, C., Muriaux, D., and Bonazzi, M. (2016). *Coxiella burnetii* effector CvpB modulates phosphoinositide metabolism for optimal vacuole development. Proc Natl Acad Sci U S A *113*, E3260-3269. https://doi.org/10.1073/pnas.1522811113.

McEwan, D.G., Richter, B., Claudi, B., Wigge, C., Wild, P., Farhan, H., McGourty, K., Coxon, F.P., Franz-Wachtel, M., Perdu, B., *et al.* (2015). PLEKHM1 regulates Salmonella-containing vacuole biogenesis and infection. Cell Host Microbe *17*, 58–71. https://doi.org/10.1016/j.chom.2014.11.011.

Medzhitov, R., and Janeway, C.A., Jr. (2002). Decoding the patterns of self and nonself by the innate immune system. Science *296*, 298-300. https://doi.org/10.1126/science.1068883.

Mehra, A., Zahra, A., Thompson, V., Sirisaengtaksin, N., Wells, A., Porto, M., Köster, S., Penberthy, K., Kubota, Y., Dricot, A., *et al.* (2013). *Mycobacterium tuberculosis* type VII secreted effector EsxH targets host ESCRT to impair trafficking. PLOS Pathog. *9*, e1003734. https://doi.org/10.1371/journal.ppat.1003734.

Mesquita, F.S., Thomas, M., Sachse, M., Santos, A.J., Figueira, R., and Holden, D.W. (2012). The Salmonella deubiquitinase SseL inhibits selective autophagy of cytosolic aggregates. PLOS Pathog. *8*, e1002743. https://doi.org/10.1371/journal.ppat.1002743.

Mizushima, N., Noda, T., Yoshimori, T., Tanaka, Y., Ishii, T., George, M.D., Klionsky, D.J., Ohsumi, M., and Ohsumi, Y. (1998). A protein conjugation system essential for autophagy. Nature *395*, 395-398. https://doi.org/10.1038/26506.

Mócsai, A., Ruland, J., and Tybulewicz, V.L. (2010). The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat. Rev. Immunol. *10*, 387–402. https://doi.org/10.1038/nri2765.

Mosser, D.M. (1994). Receptors on phagocytic cells involved in microbial recognition. Immunol. Ser. *60*, 99–114.

Mukherjee, S., Keitany, G., Li, Y., Wang, Y., Ball, H.L., Goldsmith, E.J., and Orth, K. (2006). *Yersinia* YopJ acetylates and inhibits kinase activation by blocking phosphorylation. Science *312*, 1211–1214. https://doi.org/10.1126/science.1126867.

Mukherjee, S., Liu, X., Arasaki, K., McDonough, J., Galán, J.E., and Roy, C.R. (2011). Modulation of Rab GTPase function by a protein phosphocholine transferase. Nature *477*, 103–106. https://doi.org/10.1038/nature10335.

Müller, M.P., Peters, H., Blümer, J., Blankenfeldt, W., Goody, R.S., and Itzen, A. (2010). The Legionella effector protein DrrA AMPylates the membrane traffic regulator Rab1b. Science *329*, 946–949. https://doi.org/10.1126/science.1192276.

Neunuebel, M.R., Chen, Y., Gaspar, A.H., Backlund, P.S., Yergey, A., and Machner, M.P. (2011). De-AMPylation of the small GTPase Rab1 by the pathogen *Legionella pneumophila*. Science *333*, 453–456. https://doi.org/10.1126/science.1207193.

Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., and Sasakawa, C. (2005). Escape of intracellular Shigella from autophagy. Science *307*, 727–731. https://doi.org/10.1126/science.1106036.

Osiecki, J.C., Barker, J., Picking, W.L., Serfis, A.B., Berring, E., Shah, S., Harrington, A., and Picking, W.D. (2001). IpaC from Shigella and SipC from Salmonella possess similar biochemical properties but are functionally distinct. Mol. Microbiol. *42*, 469–481. https://doi.org/10.1046/j.1365-2958.2001.02654.x.

Parzych, K.R., and Klionsky, D.J. (2014). An overview of autophagy: morphology, mechanism, and regulation. Antioxid. Redox Signal. *20*, 460–473. https://doi.org/10.1089/ars.2013.5371.

Pilar, A.V., Reid-Yu, S.A., Cooper, C.A., Mulder, D.T., and Coombes, B.K. (2012). GogB is an anti-inflammatory effector that limits tissue damage during Salmonella infection through interaction with human FBXO22 and Skp1. PLOS Pathog. *8*, e1002773. https://doi.org/10.1371/journal.ppat.1002773.

Pizarro-Cerdá, J., Kühbacher, A., and Cossart, P. (2015). Phosphoinositides and host-pathogen interactions. Biochim. Biophys. Acta *1851*, 911–918. https://doi.org/10.1016/j.bbalip.2014.09.011.

Pollard, D.J., Young, J.C., Covarelli, V., Herrera-Leon, S., Connor, T.R., Fookes, M., Walker, D., Echeita, A., Thomson, N.R., Berger, C.N., *et al.* (2016). The type III secretion system effector SeoC of *Salmonella enterica* subspecies salamae and arizonae ADP-ribosylates Src and inhibits opsono-phagocytosis. Infect Immun. https://doi.org/10.1128/IAI.00704-16.

Puri, R.V., Reddy, P.V., and Tyagi, A.K. (2013). Secreted acid phosphatase (SapM) of *Mycobacterium tuberculosis* is indispensable for arresting phagosomal maturation and growth of the pathogen in guinea pig tissues. PLOS ONE *8*, e70514. https://doi.org/10.1371/journal.pone.0070514.

Rolando, M., Escoll, P., Nora, T., Botti, J., Boitez, V., Bedia, C., Daniels, C., Abraham, G., Stogios, P.J., Skarina, T., *et al.* (2016). *Legionella pneumophila* S1P-lyase targets host sphingolipid metabolism and restrains autophagy. Proc. Natl. Acad. Sci. U.S.A. *113*, 1901–1906. https://doi.org/10.1073/pnas.1522067113.

Rolhion, N., Furniss, R.C., Grabe, G., Ryan, A., Liu, M., Matthews, S.A., and Holden, D.W. (2016). Inhibition of Nuclear Transport of NF-κB p65 by the Salmonella Type III Secretion System Effector SpvD. PLOS Pathog. *12*, e1005653. https://doi.org/10.1371/journal.ppat.1005653.

Ruchaud-Sparagano, M.H., Mühlen, S., Dean, P., and Kenny, B. (2011). The enteropathogenic E. coli (EPEC) Tir effector inhibits NF-κB activity by targeting TNFα receptor-associated factors. PLOS Pathog. *7*, e1002414. https://doi.org/10.1371/journal.ppat.1002414.

Saini, N.K., Baena, A., Ng, T.W., Venkataswamy, M.M., Kennedy, S.C., Kunnath-Velayudhan, S., Carreño, L.J., Xu, J., Chan, J., Larsen, M.H., *et al.* (2016). Suppression of autophagy and antigen presentation by *Mycobacterium tuberculosis* PE_PGRS47. Nat. Microbiol. *1*, 16133. https://doi.org/10.1038/nmicrobiol.2016.133.

Saleh, M.T., and Belisle, J.T. (2000). Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases. J. Bacteriol. *182*, 6850–6853. https://doi.org/10.1128/JB.182.23.6850-6853.2000.

Sanada, T., Kim, M., Mimuro, H., Suzuki, M., Ogawa, M., Oyama, A., Ashida, H., Kobayashi, T., Koyama, T., Nagai, S., *et al.* (2012). The *Shigella flexneri* effector OspI deamidates UBC13 to dampen the inflammatory response. Nature *483*, 623–626. https://doi.org/10.1038/nature10894.

Senerovic, L., Tsunoda, S.P., Goosmann, C., Brinkmann, V., Zychlinsky, A., Meissner, F., and Kolbe, M. (2012). Spontaneous formation of IpaB ion channels in host cell membranes reveals how Shigella induces pyroptosis in macrophages. Cell Death Dis. *3*, e384. https://doi.org/10.1038/cddis.2012.124.

Shaughnessy, L.M., Hoppe, A.D., Christensen, K.A., and Swanson, J.A. (2006). Membrane perforations inhibit lysosome fusion by altering pH and calcium in *Listeria monocytogenes* vacuoles. Cell. Microbiol. *8*, 781–792.

Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., Zhuang, Y., Cai, T., Wang, F., and Shao, F. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature *526*, 660–665. https://doi.org/10.1038/nature15514.

Shi, J., Zhao, Y., Wang, Y., Gao, W., Ding, J., Li, P., Hu, L., and Shao, F. (2014). Inflammatory caspases are innate immune receptors for intracellular LPS. Nature *514*, 187–192. https://doi.org/10.1038/nature13683.

Simeone, R., Bobard, A., Lippmann, J., Bitter, W., Majlessi, L., Brosch, R., and Enninga, J. (2012). Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. PLOS Pathog. *8*, e1002507. https://doi.org/10.1371/journal.ppat.1002507.

Spanò, S., and Galán, J.E. (2012). A Rab32-dependent pathway contributes to Salmonella typhi host restriction. Science *338*, 960–963. https://doi.org/10.1126/science.1229224.

Spanò, S., Gao, X., Hannemann, S., Lara-Tejero, M., and Galán, J.E. (2016). A bacterial pathogen targets a host Rab-Family GTPase defense pathway with a GAP. Cell Host Microbe *19*, 216–226. https://doi.org/10.1016/j.chom.2016.01.004.

Sun, H., Kamanova, J., Lara-Tejero, M., and Galán, J.E. (2016). A Family of Salmonella Type III Secretion Effector Proteins Selectively Targets the NF-κB Signaling Pathway to Preserve Host Homeostasis. PLOS Pathog. *12*, e1005484. https://doi.org/10.1371/journal.ppat.1005484.

Sun, J., Siroy, A., Lokareddy, R.K., Speer, A., Doornbos, K.S., Cingolani, G., and Niederweis, M. (2015). The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD. Nat. Struct. Mol. Biol. *22*, 672–678. https://doi.org/10.1038/nsmb.3064.

Sun, L., and Wang, X. (2014). A new kind of cell suicide: mechanisms and functions of programmed necrosis. Trends Biochem. Sci. *39*, 587–593. https://doi.org/10.1016/j.tibs.2014.10.003.

Sun, L., Wu, J., Du, F., Chen, X., and Chen, Z.J. (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science *339*, 786–791. https://doi.org/10.1126/science.1232458.

Suzuki, S., Mimuro, H., Kim, M., Ogawa, M., Ashida, H., Toyotome, T., Franchi, L., Suzuki, M., Sanada, T., Suzuki, T., *et al.* (2014). Shigella IpaH7.8 E3 ubiquitin ligase targets glomulin and activates inflammasomes to demolish macrophages. Proc. Natl. Acad. Sci. U.S.A. *111*, E4254–63. https://doi.org/10.1073/pnas.1324021111.

Tan, Y., Arnold, R.J., and Luo, Z.Q. (2011). *Legionella pneumophila* regulates the small GTPase Rab1 activity by reversible phosphorylcholination. Proc. Natl. Acad. Sci. U.S.A. *108*, 21212–21217. https://doi.org/10.1073/pnas.1114023109.

Tan, Y., and Luo, Z.Q. (2011). *Legionella pneumophila* SidD is a deAMPylase that modifies Rab1. Nature *475*, 506–509. https://doi.org/10.1038/nature10307.

Tanida, I., Ueno, T., and Kominami, E. (2004). LC3 conjugation system in mammalian autophagy. Int. J. Biochem. Cell Biol. *36*, 2503–2518. https://doi.org/10.1016/j.biocel.2004.05.009.

Uchiya, K., Tobe, T., Komatsu, K., Suzuki, T., Watarai, M., Fukuda, I., Yoshikawa, M., and Sasakawa, C. (1995). Identification of a novel virulence gene, virA, on the large plasmid of Shigella, involved in invasion and intercellular spreading. Mol. Microbiol. *17*, 241–250. https://doi.org/10.1111/j.1365-2958.1995.mmi_17020241.x.

van Schaik, E.J., Chen, C., Mertens, K., Weber, M.M., and Samuel, J.E. (2013). Molecular pathogenesis of the obligate intracellular bacterium *Coxiella burnetii*. Nat. Rev. Microbiol. *11*, 561–573. https://doi.org/10.1038/nrmicro3049.

Wang, J., Li, B.X., Ge, P.P., Li, J., Wang, Q., Gao, G.F., Qiu, X.B., and Liu, C.H. (2015). *Mycobacterium tuberculosis* suppresses innate immunity by coopting the host ubiquitin system. Nat. Immunol. *16*, 237–245. https://doi.org/10.1038/ni.3096.

Wei, P., Wong, W.W., Park, J.S., Corcoran, E.E., Peisajovich, S.G., Onuffer, J.J., Weiss, A., and Lim, W.A. (2012). Bacterial virulence proteins as tools to rewire kinase pathways in yeast and immune cells. Nature *488*, 384–388. https://doi.org/10.1038/nature11259.

Wild, P., Farhan, H., McEwan, D.G., Wagner, S., Rogov, V.V., Brady, N.R., Richter, B., Korac, J., Waidmann, O., Choudhary, C., *et al.* (2011). Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science *333*, 228–233. https://doi.org/10.1126/science.1205405.

Yan, D., Wang, X., Luo, L., Cao, X., and Ge, B. (2012). Inhibition of TLR signaling by a bacterial protein containing immunoreceptor tyrosine-based inhibitory motifs. Nat Immunol *13*, 1063-1071. https://doi.org/10.1038/ni.2417.

Young, J.C., Clements, A., Lang, A.E., Garnett, J.A., Munera, D., Arbeloa, A., Pearson, J., Hartland, E.L., Matthews, S.J., Mousnier, A., *et al.* (2014). The *Escherichia coli* effector EspJ blocks Src kinase activity via amidation and ADP ribosylation. Nat. Commun. *5*, 5887. https://doi.org/10.1038/ncomms6887.

Zhang, L., Ding, X., Cui, J., Xu, H., Chen, J., Gong, Y.N., Hu, L., Zhou, Y., Ge, J., Lu, Q., *et al.* (2011). Cysteine methylation disrupts ubiquitin-chain sensing in NF-κB activation. Nature *481*, 204–208. https://doi.org/10.1038/nature10690.

Zhao, Y., and Shao, F. (2016). Diverse mechanisms for inflammasome sensing of cytosolic bacteria and bacterial virulence. Curr. Opin. Microbiol. *29*, 37–42. https://doi.org/10.1016/j.mib.2015.10.003.

Zhao, Y., Yang, J., Shi, J., Gong, Y.N., Lu, Q., Xu, H., Liu, L., and Shao, F. (2011). The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature *477*, 596–600. https://doi.org/10.1038/nature10510.

Zheng, C.F., and Guan, K.L. (1994). Cytoplasmic localization of the mitogen-activated protein kinase activator MEK. J. Biol. Chem. *269*, 19947–19952.

Zhu, W., Banga, S., Tan, Y., Zheng, C., Stephenson, R., Gately, J., and Luo, Z.Q. (2011). Comprehensive identification of protein substrates of the Dot/Icm type IV transporter of *Legionella pneumophila*. PLOS ONE *6*, e17638. https://doi.org/10.1371/journal.pone.0017638.

Zhu, Y., Li, H., Long, C., Hu, L., Xu, H., Liu, L., Chen, S., Wang, D.C., and Shao, F. (2007). Structural insights into the enzymatic mechanism of the pathogenic MAPK phosphothreonine lyase. Mol. Cell *28*, 899–913. https://doi.org/10.1016/j.molcel.2007.11.011