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Mechanisms of Immunomodulation in human macrophages by *Yersinia enterocolitica*

Dissertation

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I Introduction

1. The genus *Yersinia*

Yersinia are Gram negative bacteria belonging to the family of Enterobacterales including a total of 18 different apathogenic and pathogenic species to humans and animals [1]. Human pathogenic species include enteropathogenic *Yersinia* (*Yersinia enterocolitica* and *Yersinia pseudotuberculosis*) and *Yersinia pestis*. *Yersinia* can grow in a range of temperatures from 4-42°C, with optimal growth at 20-28°C [2].

Y. pestis, the causative agent of plague, was first described by Alexandre Emile Jean Yersin in 1984 [3] and is known to be transmitted to humans via the flea [4] or from human-to-human via droplet infection [5].

1.1. Enteropathogenic *Yersinia*

Enteropathogenic *Yersinia* cause gastrointestinal infections and are foodborne pathogens transmitted primarily via water, soil and contaminated food [6-10]. In contrast to the most common pathogens of bacterial enteritis caused by Campylobacter or Salmonella, yersiniosis is comparatively rare [11]. In 2019, 2800 infections with *Y.enterocolitica* were reported in Germany [11]. Once ingested via the gastrointestinal tract, the bacteria enter the terminal ileum, where they adhere to the mucosa and enter Peyer's patches via epithelial M cells, highly specialized cells of the epithelial layer [12, 13]. Extracellular proliferation begins in Peyer's patches followed by immigration of polymorphonuclear lymphocytes into the infected tissue. Bacterial dissemination occurs via lymphatic vessels and mesenteric lymph nodes [14].

1.1.1. *Yersinia enterocolitica*

Y. enterocoltica can be classified into six different biogroups with varying pathogenicity, where group 1A is apathogenic, 1b is highly pathogenic, and groups 2-5 are moderately pathogenic [15]. Serological classification is based on variable flagella (H) and surface (O) antigens [6]. Of these, 11 serogroups are associated with human infections, with serotypes O:3, O:9, O:5, and O:8 being the most common, while highly infectious strains include serotypes O:8, O:13, O:20, and O:21 of biogroup 1B [6]. The *Y.enterocolitica* strain WA314 used in this study is assigned to biovar 1B with serotype O:8 [16].

1.1.2. Yersiniosis

Yersiniosis manifests as an extensive clinical picture of gastrointestinal symptoms [17, 18] with acute enteritis (especially in young children), enterocolitis, fever, acute mesenteric lymphadenitis and terminal ileitis [19, 20]. The symptoms appear within 24-48 hours after ingestion of contaminated food and persist for up to 14 days or as long as 28 days in the case of children [21]. Systemic spread in the late phase of infection can lead to microabscesses in the liver and spleen [21]. Secondary immunologically complications may develop after 1-2 weeks including reactive arthritis and glomerulonephritis [21, 22].

Yersiniosis is mostly self-limiting and does not require antibiotic treatment. However, if antibiotic therapy is necessary, such as in severe infections with sepsis that may occur in immunocompromised individuals [23], it can be administered using ciprofloxacin for example.

2. *Yersinia* virulence factors

Yersinia spp. sustain infection via a repertoire of chromosomal and virulence plasmid (plasmid *Yersinia* Virulence; pYV) encoded proteins. The virulence plasmid pYV (70kb) encodes for the Type Three Secretion System (TTSS), seven secreted effector proteins called Yops (*Yersinia* Outer Proteins) and the adhesion factor *Yersinia* adhesin A (YadA) [24]. Chromosomally encoded virulence determinants are necessary to achieve full virulence, i.e. adhesins Invasin and Attachment and Invasion Locus (Ail) [25-30].

Invasin is essential for effective effector translocation through the TTSS across the epithelial barrier by binding β1-integrins on M cells [28, 31-38], while plasmid-encoded adhesin YadA binds extracellular matrix (ECM) to interfere with b1 integrins indirectly and furthermore binds to different target cells mediating serum and phagocytosis resistance [39]. Adhesion Ail is able to bind collagen to ensure the survival of intracellular bacteria [40], contributing to establish systemic infections [41]. Besides adhesins and their regulators the *Yersinia* chromosome harbors genes encoding the Yst A toxin, a heat-stable enterotoxin causing secretory diarrhea [42], and a gene set encoding flagellum-proteins [43]. HPI encodes an iron- uptake system essential for systemic *Yersinia* infection [44, 45].

Invasin shows the highest expression at 26 °C [46] and is regulated by transcriptional regulator rovA [47]. The expression of pYV TTSS, Yops, YadA and chromosomally encoded factors is induced at 37 °C [40, 48].

2.1. *Yersinia* type III secretion system (TTSS)

Human pathogenic *Yersinia* harbor a type three secretion system (TTSS) by which bacterial effector proteins (Yersinia outer proteins; Yops) are injected into host target cells [49, 50], thereby evading the host organism's adaptive immune defenses [51]. The TTSS is a multiprotein complex consisting of a basal body, needle and tip complex encoded by numerous Gram-negative bacterial pathogens [52]. The formation of the TTSS, secretion and translocation of Yops is regulated by environmental conditions [53], like temperature and Ca2+ concentrations [49].

2.2. *Yersinia* effector proteins

Seven different secreted bacterial effector proteins that are injected into the host cell via the TTSS system have been described to date. They counteract several important innate defense mechanisms of phagocytes in order to suppress immune response signaling [53, 54].

2.2.1. Prevention of phagocytosis by YopE, YopT, YopE and YopO

The effector proteins YopE, YopT, YopH and YopO inhibit the host cell actin cytoskeleton in different ways to prevent phagocytosis [55, 56]. Of these, YopT and YopE have overlapping functions [57, 58]. YopE is essential [59, 60], while YopT did not play a major role in virulence *in vivo* [61]. YopO/YpkA has a contradictory role for virulence *in vivo* [55], given that mouse infection models revealed that YpkA deletion mutants possessed attenuated virulence [62], whereas no reduced virulence was observed upon infection in other models [61]. YopH-deficient strains fail to colonize mesenteric lymph nodes [63] and activate neutrophil recruitment to Peyer's patches [64] during infection *in vivo.*

YopE is a GTPase activating protein (GAP) that inactivates Rho-GTPases Rac1, RhoA and Cdc42 leading to rupture of actin filaments, rounding and detachment of cells [24, 60]. Furthermore, YopE inhibits Rac-1 mediated activation of caspase-1 and release of IL-1β [65-67] and also impairs the inflammatory response by inhibiting MAPK and NF-κB signaling pathways [57].

YopT is a cysteine protease disrupt the actin cytoskeleton and inhibit phagocytosis [68] targeting small Rho GTPases, mainly RhoA, to cleave the C-terminal isoprenylated cysteine, which mediates localization Rho GTPase to the membrane [69].

YopO (*Yersinia* protein kinase A (YpkA) in *Y. pseudotuberculosis* and *Y. pestis*) has several functional domains. As a serine-threonine kinase, YopO causes rounding of infected cells with formation of peripheral retraction fibers [62, 70-72]. YopO binds and inactivates

RhoA and Rac1 via its GDP dissociation inhibitor (GDI)-like domain and causes depolymerization of the actin cytoskeleton [73-75].

YopH is a protein tyrosine phosphatase [76], that targets focal adhesion proteins, such as p130Cas and kinase FAK to inhibit phagocytosis [77-79]. Moreover, YopH inhibits adaptor proteins involved in T- and B-cell signaling [80] and was also shown to suppress interleukin-10 (IL10) expression in neutrophils [81, 82].

2.2.2. YopQ/YopK: a regulator of pore protein translocation

YopQ (YopK in *Y. pseudotuberculosis* and *Y. pestis*) shows no homology to other known proteins and is involved in the control of Yop translocation [83-85]. Furthermore, YopQ/YopK prevents inflammasome activation [84-86] and is essential for *Yersinia* virulence, as mutants are highly attenuated *in vivo* [61, 86].

2.2.3. YopP: a potent suppressor of pro-inflammatory signaling

YopP (YopJ in *Y. pseudotuberculosis* and *Y. pestis*) is an acetylate transferase that acetylates critical serine and threonine residues of MAPK family kinases (MAPKK), IκB kinase (IKKβ) and transforming growth factor beta-activated kinase 1 (TAK1), thereby preventing NF-kB dependent transcription and the release of proinflammatory cytokines, such as TNF-α, IL8 or IL6 [87-91]. This renders YopP/J an effective suppressor of pro-inflammatory signaling and cytokine expression [92-96].

Furthermore, YopP induces cell death in antigen-presenting cells (APC), such as dendritic cells or macrophages [88, 90, 97, 98] by interfering with receptor-interacting serine/threonine protein kinase 1 (RIPK1)-mediated cleavage of caspase-8 [99-101], which in turn triggers formation of the gasdermin D pore (GSDM) in the plasma membrane [99], K+ efflux and inflammasome activation [102]. YopP cleaves caspase-1 to inhibit the immunoregulatory effect of IL1ß [103], an interplay of YopP with the effector protein YopM in the inhibition of IL1ß production has been postulated [66, 104]. In infections with corresponding YopM deletion mutants, YopP was shown to inhibit IL1ß production [104]. In this context, YopM together with the effector protein YopP modulates inflammasome formation and the process of pyroptosis [66, 104]. The effector proteins YopM and YopP can take over each other's function in caspase-1 activation (Figure 1.1). In mouse infection models, YopP alone was shown to be essential for the *in vivo* virulence of enteropathogenic *Yersinia* [101, 105-107]. Of note, *Y.pestis* double mutant strains, that express neither YopM nor YopP can no longer establish infection [66].

2.2.4. YopM: a scaffold protein

YopM is a leucine-rich-repeat (LRR) protein, for which no enzymatic activity is known to date [108, 109]. YopM is a horseshoe-shaped protein composed of three structural element including two N-terminal alpha helices, a set of LRRs and a conserved C-terminus [98, 109, 110]. Isoforms of YopM in distinct *Yersinia* strains differ in size, caused by the variable number of leucine-rich repeats [53]. YopM of *Yersinia pseudotuberculosis* YPIII pIB1 harbors 15 LRRs (~46 kDa; [111]) similar to YopM of *Y. pestis* KIM5 (46.2-kDa; [112]), whereas YopM from *Y.enterocolitica* WA314 (type 0:8) possesses 5 additional LRRs resulting in a higher molecular weight (~57 kDa; [113]). Given that LRR domains provide an important structural framework required for molecular interactions [114], numerous interaction partners of the different YopM isoforms have been described [55, 115] and include the extracellular proteases thrombin and trypsin, isoforms of ribosomal S6 kinase (RSK) and protein kinase N (PKN), DEAD box RNA helicase (DDX3), scaffold protein IQ Motif Containing GTPase Activating Protein (IQGAP) and caspase-1 [65, 115-118]. Although few of these interactions have been reconstituted with purified proteins *in vitro*, the available data indicate that most interaction partners bind to the LRR region of YopM [115], except for RSK, where the C-terminus of YopM is responsible for interaction [111].

YopM has been hypothesized to be secreted extracellularly to prevent platelet aggregation in the extracellular compartment via an interaction with thrombin [119]. However, YopM is also translocated into target cells via the TTSS [120], where it is the only effector protein that traffics into the host cell nucleus via a vesicle-associated pathway [110, 119, 121, 122]. In this context, neither a nuclear localization sequence of YopM, nor a cellular importin that enables active nuclear transport of YopM could be identified. So far, only the C-terminal sequence of YopM, with some similarity to nuclear localization sequence structures already described, seems to play a crucial role for nuclear import [110, 123]. The DEAD box RNA helicase DDX3 is functionally involved in shuttling YopM between the nucleus and cytoplasm and regulates the amount of nuclear YopM [115]. This DDX3-mediated nucleo-cytoplasmic fine-tuning of nuclear YopM has beneficial effects on the transcriptional regulation of cytokines [115].

Previous work has already shown that YopM mediates increased activation of RSK by preventing dephosphorylation of the kinase [117]. YopM interacts with RSK to increase the phosphorylation of RSK in the nucleus and cytoplasm of infected cells. By regulating the level of phosphorylated RSK in the nucleus, YopM governs the transcriptional control of immunosuppressive cytokines [115]. Phosphorylated RSK is presumed to phosphorylate PKN [116] in the formed ternary complex of YopM, RSK, and PKN in the cytoplasm. Since PKN is not a physiological RSK substrate, it can be concluded that YopM brings the two

eukaryotic kinases together [116, 117]. By recruiting and activating PKN, YopM facilitates phosphorylation and inhibition of pyrin [124]. Given that both YopE and YopT downregulate RhoA activity to prevent PKN1 activation, pyrin inflammasome activation is efficiently hijacked by YopM [66, 125-128].

YopM plays a critical role in the establishment of infection [129, 130]. In mouse infection models using appropriate *Yersinia* YopM deletion mutants, YopM was shown to be an essential virulence factor [61, 111, 131-133]. Here, YopM has been suggested to play a role in the reduction of natural killer cells in the spleen [112, 132]. Beyond that, polymorphonuclear leucocytes and inflammatory monocytes are considered target cells of YopM-mediated virulence [132, 133].

3. The ribosomal S6 Kinase: a known interaction partner of YopM

YopM interacts with all isoforms of RSK [117]. In humans, four isoforms of RSK are described, of which isoforms 1-3 are ubiquitous and expressed in various human tissues [134]. In contrast, RSK4 is lower expressed and restricted to some specific tissue types [135]. The similar structure of all RSK isoforms consists of two conserved kinase domains. The N-terminal kinase domain is responsible for substrate phosphorylation, whereas the C-terminal kinase domain mediates auto-phosphorylation of RSK [136].

Phosphorylation and thus activation of RSK is initiated within the MAP kinase signaling pathway by the association of extracellular signal-regulated kinase (ERK). ERK binding to RSK initiates autophosphorylation of RSK. Six conserved phosphorylation sites have been identified throughout the molecule, of which four (Ser-221, Ser-363, Ser-380, and Thr-573) are critical for RSK activation [137-140]. Autophosphorylation is mandatory to provide a docking site for phosphoinositide-dependent kinase-1 (PDK1), a constitutively active Ser/Thr kinase, which induces phosphorylation of RSK Ser-221 completing RSK activation [140].

Activated RSK phosphorylates multiple nuclear and cytoplasmic substrates in the cell, regulating various cellular processes such as cell growth, proliferation, survival and motility, [136, 141]. RSK translocates to the nucleus [141], where it is thought to control gene expression by interfering with transcription factors [142]. For example, the cAMP response element binding protein (CREB), that controls early gene transcription, is regulated by RSK [143, 144]. Furthermore, RSK enhances the function of the transcriptional co-activators CREB-binding protein (CBP) and p300, thereby indirectly regulating FOS, JUN, STAT and NF-κB dependent-transcription [141, 145, 146]. Genes involved in inflammatory processes

such as proliferation and apoptosis are regulated by RSK through control of NF-κB [147-149].

4. Host immune regulation

4.1. Innate immune receptor- signaling

The innate immune system uses a variety of pattern recognition receptors (PRRs) for recognition of microbes. PRRs recognize microbe-specific molecular signatures known as pathogen-associated molecular patterns (PAMPs) and in addition damage-associated molecular patterns (DAMPs) derived from damaged cells [150]. PRRs activate downstream signaling pathways that effect induction of innate immune responses and produce inflammatory cytokines, type I interferon (IFN) and other mediators. Several established classes of PRRs comprise most importantly the Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and Nod-like receptors (NLRs) [150, 151]. The family of NLR proteins and pyrin are both essential cytosolic sensors for recognition of pathogens and DAMPs, that trigger inflammasome formation [124].

Recognition of PAMPs and DAMPs, recruit TIR domain-containing adaptor proteins such as MyD88 and TRIF to TLRs, which initiate signal transduction pathways of the immune response leading to the activation of NF-κB, IRFs, or MAP kinases [150, 151]. Bacterial lipopolysaccharide (LPS), a well-characterized PAMP found in the outer membrane of Gram-negative bacteria, is recognized by TLR4 in complex with myeloid differentiation factor MD-2 [152, 153]. The major signaling cassettes of the MAP kinase signaling pathway include kinases c-Jun N-terminal kinase (JNK), p38 and ERK, which induce inflammatory gene expression via various transcriptionfactors [154]. These signaling pathways are modulated by various pathogens to overcome the innate immune response.

4.2. Transcriptional regulation of anti-inflammatory cytokine IL10

IL10 is an anti-inflammatory cytokine produced by T cells, B cells, thymocytes, keratinocytes and macrophages with the ability to inhibit production of pro-inflammatory cytokines, such as TNF, IL1, and IL12 [155, 156]. Induction of IL10 expression [115] and secretion was demonstrated to be a crucial function of YopM [131, 157]. IL10 is an essential modulator of the response to infection wthin the JAK-STAT signaling pathway (JAK, Janus kinase; STAT, signal transducer and activator of transcription) [158-160]. Recognitition of pathogens by the JAK-STAT circuitry link IL10 with diverse microbial survival strategies dependent on specific downstream effector mechanism to establish an effective host defense [161]. JAK-STAT

signaling is activated through a canonical signaling cascade initiated by binding of IL10 or IL6 on the JAK-receptor [162]. IL10 effects the activation of receptor-associated Janus tyrosine kinases, JAK1 and Tyk2 [158], which directly enables the recruitment of Stat3, initiates tyrosine phosphorylation and subsequent dimerization of Stat3 [156, 159, 162, 163]. Stat3 phosphorylated at tyrosine 705 translocates to the nucleus and triggers expression of IL10-regulated genes via binding to STAT binding elements (SBE) of the promoter region [156, 159, 164]. On the other hand Stat3 serine phosphorylation at position 727 enhances transcriptional activity [165-168]. Stat3 is the key activator of IL10 transcription [169] required for the anti-inflammatory effects of IL10, including inhibition of pro-inflammatory gene expression [163, 169, 170]. Emerging evidence indicates that Stat3 does indirectly inhibit inflammatory gene expression by induction of genes and transcriptional repressors [163, 171]. IL10-responsive genes include IL7, IL10 and Suppressor of Cytokine Signaling-3 (SOCS3) [172, 173]. SOCS3 signaling induces a negative feedback loop regulating the JAK-STAT signaling cascade activity and interferes with the TLR signaling cascade to suppress NF-κB regulated genes including TNF, IL6 and IL1β [163, 169, 174, 175]. This negative feedback loop is targeted by various pathogens to regulate JAK-STAT signaling during infection.

4.3. *Yersinia* virulence factor modulation of gene expression

The eminent role of the Yop effector proteins for virulence of *Yersinia* in infected animals has been related to the subversion of cytokine gene expression and –production [176]. YopP/J is known to inhibit NF-κB dependent cytokine expression by modulating MAPK signaling [95, 97, 98, 177]. However, also YopM is presumed to influence host gene expression programs. YopM was shown to inhibit the expression of cytokines, such as interleukin IL12, IL18, IL1β, IL15 [112, 178, 179] and IFN-γ [131] in macrophages. Recombinant YopM of *Y. enterocolitica* 8081, acting as a cell penetrating peptide (CPP) is able to downregulate the transcription of pro-inflammatory cytokine TNF-α and interleukins 12, 15, 18 [180, 181]. Other studies with *Y. pestis* showed that YopM has no effect on the expression of cytokines such as IL4 and IL10 [112]. These findings are in contrast to mouse infection experiments in which YopM caused an increase in IL10 and IL18 in sera from infected mice [131, 157]. Anti-inflammatory cytokine IL10 is upregulated by YopM on the transcriptional and translational level in *Y. pseudotuberculosis* 32777 and *Y. enterocolitica* WA314 infected mice or macrophages [115, 131, 157]. Unlike YopM, YopJ inhibits IL-10 expression during *Y. pseudotuberculosis* infection of BMDM macrophages [182].

Although a plethora of different microarray and RNA-seq studies were performed such as in BMDMs [183, 184], HeLa cells [185], NK cells [186] and neutrophils [187, 188], a comprehensive analysis investigating the effect of IL10 in YopM regulated host gene expression in primary human macrophages is lacking.

Microarray analysis stated an impact of YopM on several genes implicated in cellular growth and cell cycle control including i.e. genes Trio and P52rIpk and transcription factor B-myb [189], whereas another study could not identify any genes regulated by YopM in bovine macrophages [190].

Recent studies demonstrate the interplay of YopM and YopP/J in caspase-1 activation and secretion of the interleukins 18 and IL1ß [66, 104]. Both, YopM and YopJ of *Y. pseudotuberculosis* counteract on inhibition of caspase-1 activation [104]. YopM inhibits caspase-1 activity by interaction with IQGAP in the host cell [65, 118], while YopP cleaves caspase-1. Both effectors block the release of cytokine IL1β [65, 100, 103] and IL18 and therefore the induction of pyroptosis in mouse infection [191, 192]. In the absence of YopM, YopJ minimally affects caspase-1 cleavage but suppresses IL1β, IL18 and other cytokines, i.e. IL10 and IL6 *in vivo* and *in vitro*. Strikingly, YopM primarily inhibits IL1ß and IL18 maturation, mainly in neutrophils and macrophages, but has minor influence on the expression of immune-modulatory genes *in vivo* [104].

Thus, key questions about the interplay of YopM and YopP on cytokine expression despite IL1ß and IL18 remained unanswered. Taken into account, that *Y. pestis* strains lacking both effectors highly attenuated infection in a mouse model, whereas strains lacking one of the effectors either YopM or YopP are still able to induce lethality [66] emphasizes the importance of the role of both effector proteins to establish full Yersinia virulence [65, 100, 103, 118].

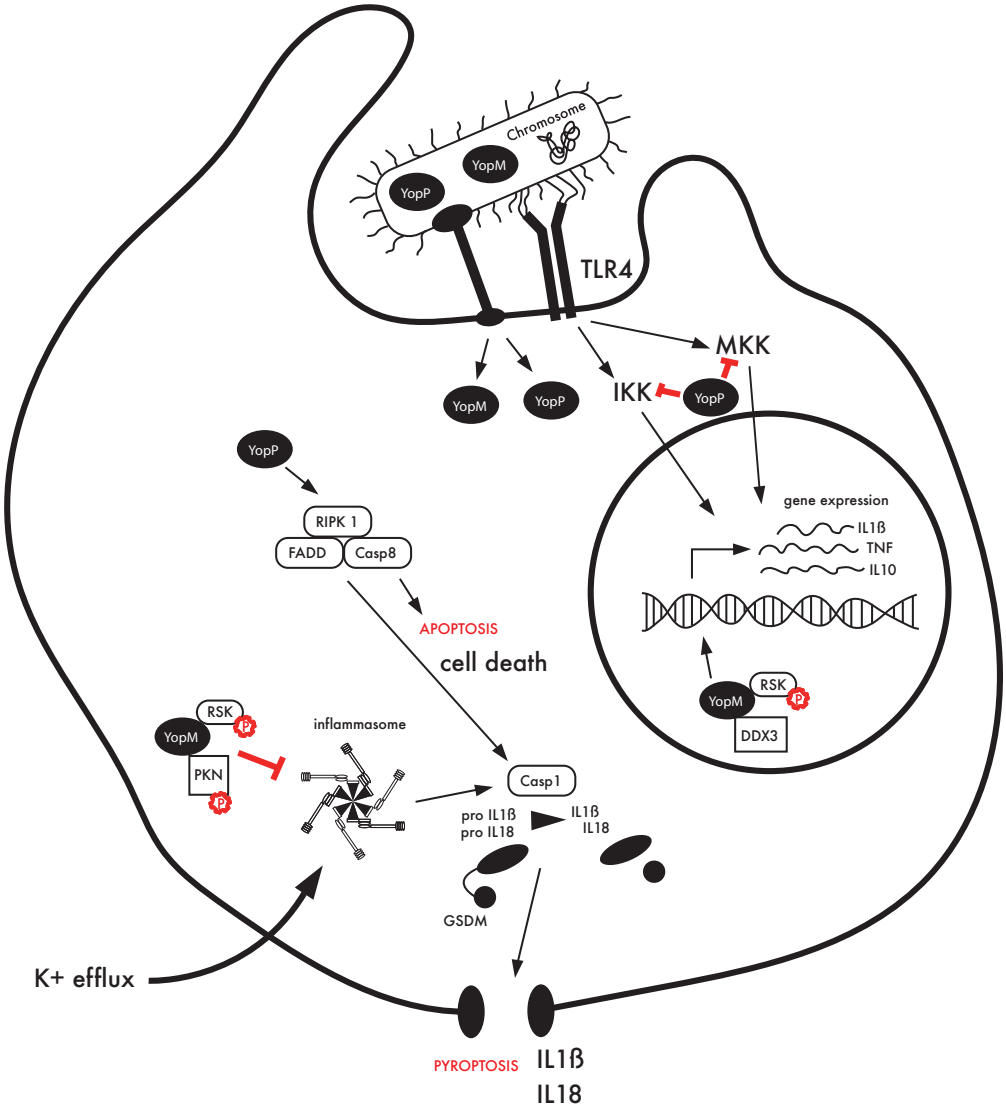

Figure 1.1: Overview diagram of Yersinia virulence factor activities YopM and YopP in the host cell.
Yersinia virulence factors modulate host-signaling pathways to counteract immune response in macrophages. Activities of the effector proteins YopM and YopP targeting inflammatory gene expression, inflammasome activation and cell death are represented. Modified figure from the dissertaton of Indra Bekere [193].

5. Aim of the study

The main virulence mechanisms of the pathogenic *Yersinia* is, next to prevention of phagocytosis, the modulation of gene expression in target host cells [99]. Until now a systematic analysis of how gene expression in human macrophages is modulated by *Yersinia* virulence factor YopM is missing. Performed gene expression analysis using microarrays in mouse macrophage cell lines could not provide an accurate picture of the role of YopM in gene expression modulation [189, 190].

In this study, we wish to understand how *Yersinia* selectively mediates expression of a component of IL10-induced genes to manipulate macrophages during infection by performing a comprehensive transcriptomic analysis using primary human macrophages. The aim of this research project is to characterize the immunomodulatory effect of YopM in primary human macrophages and therefore to determine through which of its interaction partners and which distal signaling pathways YopM mediates modulation of gene.

YopM is known to increase activation of RSK by causing hyperphosphorylation of the kinase [117]. However, it remains completely unclear whether and if so how the strong immunosuppressive effect of YopM is related to the phosphorylation status of its target protein RSK and/or its localization in the nucleus. It is hypothesized that increased nuclear hyperphosphorylation of RSK mediated by YopM results in altered gene activity that *Yersinia* use to prevent release of anti-inflammatory cytokines in macrophages.

Specifically, the following questions will be answered:

- How does effector protein YopM regulate gene expression of specific genes in the target immune cell during *Yersinia* infection?
- What is the role of the cellular kinase RSK in this process?
- Which pathways does YopM modulate at gene expression level?
- Through which proteins or transcription factors does YopM regulate gene expression in host immune cells during *Yersinia* infection?

II Material and Methods

1. Material

1.1. Equipment

Table 1: Equipment

Device	Typ, Manufacturer
Agarose gel electrophoresis	Agarose gel chamber: Roth, Karlsruhe; Germany
Accu -Jet	Accu-jet pro, Brand, Wertheim, Germany
Blotting chamber	OWL Hep-1,Thermo scientifc, Rockford, Ilinois, USA
Centrifuge	5417R and 5810R, Eppendorf, Hamburg, Germany; Sigma 3-18K, Sigma-Aldrich, St. Louis, Missouri,USA
Cell counting chamber	Neubauer-cell counting chamber, Hartenstein, Würzburg, Germany
Cell culture incubators	CB Series, Binder, Tuttlingen, Germany
Clean bench	Herasafe, Thermo Scientific, Rockford,USA
Developer for X-ray films	Curix 60, Agfa, Mortsel, Beligum
Film Cassette	Hartenstein, Würzburg, Germany
Freezing Container	5100-0001 Cryo 1°C “Mr. Frosty“, Nalgene Scientific, Rockford, USA
Freezer	-80°C: HERA freeze, Heraeus, Kendro Laboratory, Hanau, Germany; -20°C: comfort, Liebherr-International AG, Bulle, Swiss
Fridge	4 -8 °C, Liebherr Premium, Liebherr-International AG, Bulle, Swiss
Microscope	Microscop SZX12 with Kamera DP10 , Olympus, Japan
Microwave	900W, Panasonic, Kadoma/Osaka, Japan
NanoDrop® ND-1000	PeqLab, Erlangen, Germany
pH-Meter	Seven easy, Mettler-Toledo, Giessen, Germany
Photometer	Ultrospec 3100 pro, Amersham/GE healthcare Europe, Munich, Germany
Pipettes	2, 10, 100, 200, 1000 µl, Research Plus, Eppendorf, Hamburg, Germany
Power supplies	Biorad power pac universal, Biorad power PC 200, Biorad, Hercules, Californien, USA
Real Time PCR System	Lightcycler 480, Roche Diagnostics, Risch, Swiss
Scanner	CanoScan 4400F, Canon, Amsterdam, Netherland
SDS-PAGE electrophoresis cell	SDS-PAGE: Mini-Protean II Biorad, Munich, Germany
Sonifier	Digital Sonifier 250-D, Branson, Danbury, USA
Thermoblock	DRI-Block DB3 Techne, Bibby Scientific Limited, Staffordshire, UK
Thermocycler	Eppendorf MasterCycler EP S system, Eppendorf, Hamburg, Germany
Transilluminator	Vilber Lourmat, ETX, Eberhardzell, Germany
UV-Transilluminator and Detector	ChemiDoc XRS, Biorad, Hercules,Californien, USA
Vortex	REAX Topo, Heidolph Instruments, Schwabach, Germany
Weighing scale	440-47N, Kern, Balingen-Frommern, Germany

1.2. Disposables

Table 2: Disposables

Device	Type, Manufacturer
A/G Plus-Agarose beads	A/G Plus-Agarose ;Santa Cruz biotechnology, Dallas, Texas, USA
Bottle-top sterile filter units	Vacuum filtration system, capacity 500 ml, pore size 0.2 µm, Nalgene**,** Rochester, NY, USA or Stericup™ filtration system, capacity 1000 ml, pore size 0.22 µl, Merck Millipore, Massachusetts, USA
Coverslips	12 mm diameter, Hartenstein, Würzburg, Germany
Cell culture dishes	100mm, 150mm, Sarstedt, Nümbrecht, Germany
Disposable needles	0.40x 20 mm ,0.55x 25mm, 0.6x 25 mm STERICAN disposable needles, B.Braun, Melsungen Germany
Disposable cuvettes	1.5 ml, 12.5x 12.5x 45mm, BRAND GmbH + CO KG, Wertheim, Germany
Disposable inoculation loop	Sarstedt, Nürnbrecht , Germany
Flag-sepharose	Anti-Flag® M2 Affinity Gel, Sigma Aldrich,St Louis, Missouri, USA
Multiwell plates	6- / 12-well, Sarstedt, Nümbrecht, Germany LightCycler 480 Multiwell Plate 96 for RT-PCR, white, Roche Diagnostics, Risch, Swiss
Object slides	76x 26 mm, Karl Hecht, Sondheim, Germany
Parafilm	Bemis®, Pechiney Plastic Packaging, Neenah, Wisconsin, USA
Pipette tips	Sterile Biosphere filter tips and non-sterile 10, 200, 1000 µl, Sarstedt, Nümbrecht, Germany
Plastic syringe	2 ml, 5 ml, 10 ml, 20 ml, B. Braun, Melsungen, Germany
PVDF Membrane	Immobilion-P, 0,45 µm pore size, Millipore, Billerica, Massachusetts, USA
Reaction tubes	0.2 ml, Biozym Scientific, Hessisch Odendorf, Germany 0.5 ml, 1.5 ml, 2 ml, Sarstedt, Nürnbrecht , Germany 15 ml, 50 ml Centrifuge Tubes, CELLSTAR, Greiner Bio-One, Kremsmuenster, Austria
Scalpels	B. Braun, Melsungen, Germany
Serological pipettes	2, 5, 10, 25 ml, Sarstedt, Nümbrecht, Germany
Streptavidin binding protein	Streptavidin Sepharose High Performance, GE Healthcare Life Sciences, Little Chalfront, UK
Syringe sterile filters	SFCA 0.2 µm, Thermo Scientific/Nalgene, Rockford, Illinois, USA
Whatman filter paper	190 g/m^2, Biorad, Hercules, Californien; USA
X-ray film	Super RX, Fuji medical X-ray film, Fujifilm, Tokyo, Japan
µ MACS Protein A/G beads µ MACS Columns µ MACS Columns separation column 25LE CD 14 Microbeads, human	MACS, Milteny Biotec GmbH, Bergisch Gladbach, Germany

1.3. Kits, enzymes, agents and inhibitors

Table 3: Kits, enzymes, agents and inhibitors

Kit, enzyme, agent	Provider, Manufacturer
Accutase, Enzyme Cell Detachement Medium	eBioscience, San Diego; Califiornia, USA
BioRad Protein Assay	BioRad, Munich, Germany
Complete (Protease inhibitor)	Roche Diagnostics, Risch, Swiss
Digitionin	Sigma-Aldrich, St Louis, USA
DNase, RNase-Free DNase Set	Qiagen, Hilden, Germany
FastAP™ (Alkalische Phosphatase)	Fermentas, St. Leon-Rot, Germany
FastDigest® restriction enzymes	Fermentas, St. Leon-Rot, Germany
Nucleo Spin Extract II Kit	Macherey-Nagel, Düren, Germany
Gibson® Assembly Kit	New England Biolabs, Frankfurt, Germany
iScript cDNA Synthesis Kit	Bio-Rad Hercules, California, USA
Nucleospin Extract II Kit	Macherey-Nagel, Düren, Germany
5 Prime PCR Extender System	5 Prime, Hamburg, Germany
Phosstop (Phosphatase Inhibitor)	Roche Diagnostics, Risch, Swiss
Plasmid Midi Kit	Qiagen, Hilden, Germany
RNeasy Mini Kit	Qiagen, Hilden, Germany
SuperSignal West Femto/ Pico detection	Thermo Scientific, Rockford ,USA
TaqMan Fast Advanced Master mix	Applied Biosystems, Carlsbad, California, USA
TaqMan Primer and Probe mix	Life Technologies, Carlsbad, California; USA
Transfection reagent polyethylenimine (pei)	Polysience Inc, Pennsylvania, USA
Trypsin	Invitrogen/Life Technologies
ZR Plasmid Miniprep	Zymo Research, Irvine, USA

1.4. Growth media, additives, antibiotics

Media were autoclaved for 20 min at 121 °C and 1.4 bar or sterile filtered.

Table 4: Bacterial growth medium

Media	**Composition**	
LB-Medium (*lysogenic broth*), pH 7.5		
	10 g/l	Tryptone
	5 g/l	Yeast extract
	5 g/l	NaCl
		ad 1000 ml H_2O

Product	**Additives**		**Provider, Manufacturer**
Dulbeco's Modified Eagle Medium (DMEM) +Glutamax			Gibco, Carlsbad, Californien, USA
	10 % (v/v)	Fetal calf serum (FCS)	Gibco, Carlsbad, USA
RPMI Medium 1640 + Glutamax for cultivation of Human peripheral blood monocytes			Gibco, Carlsbad,USA
	20 % (v/v)	autologous serum	
	1 % (v/v)	Penicillin/Streptomycin	Gibco, Carlsbad,USA

Table 5: Antibiotics

Antibiotics	Dissolvent	Working concentration	Provider, Manufacturer
Ampicillin	ddH_2O	100 µg/ml	Sigma-Aldrich, St. Louis, USA
Kanamycin	ddH_2O	50 µg/ml	Sigma-Aldrich, St. Louis, USA
Chloramphenicol	EtOH	20 µg/ml	Roth, Karlsruhe, Germany
Nalidixic acid	1M NaOH	100 µg/ml	Sigma-Aldrich, St. Louis, USA

1.5. Chemicals and buffers

Buffers were autoclaved for 20 min, 121 °C, and 1.4 bar for sterilization. Certain supplements were sterile filtered.

Chemicals were obtained from Amersham/GE Healthcare, Munich (Germany), BD Biosciences, Heidelberg (Germany), Invitrogen/Life Technologies, Carlsbad (USA), Roche, Mannheim (Germany), Biozyme, Oldendorf (Germany), Dianova, Hamburg (Germany), Fermentas, St. Leon-Rot (Germany), Merck, Darmstadt (Germany), PAA, Pasching (Austria), PromoCell, Heidelberg (Germany), Roth, Karlsruhe (Germany) and Sigma-Aldrich, St. Louis (USA).

Table 6: Buffer

Buffer	Concentration	Composition
PBS (10x)	137 mM	NaCl
	2.7 mM	KCl
	14.4 g	Na_2HPO_4
	2.3 mM	KH_2PO_4
	ddH_2O	adjust to pH 7.4 with NaOH
SDS-PAGE		
Resolving buffer	1.5 M	Tris-HCl
	0.1 % (w/v)	SDS
	ddH_2O	adjust to pH 8.8
Stacking buffer	0.5 M	Tris-HCl
	0.1 % (w/v)	SDS
	ddH_2O	adjust to pH 6.8
SDS-PAGE sample buffer (4x)	1 M	Tris
	250 mM	SDS
	20 % (w/v)	Glycerol
	4 %	β-Mercaptoethanol
	0.2 %	Bromphenolblau
	ddH_2O	adjust to pH 6.8
SDS-PAGE running buffer	25 mM	Tris
	192 mM	Glycine
	0.1 % (w/v)	SDS
	ddH_2O	
Western blot		
Blotting buffer	150 mM	Tris
	25 mM	Glycine
	20 % (v/v)	Methanol
	ddH_2O	
TBS (10x)	20 mM	Tris
	150 mM	NaCl
	ddH_2O	adjust to pH 7.4
TBS-Tween (TBS-T)	1x	TBS, pH 7.4
	0.3 % (v/v)	Tween20
Electrophorese		
TAE (50x)	40 mM	Tris-Acetat pH 8.3
	10 mM	EDTA
	ddH_2O	adjust to pH 7.4
Cell Lysis Buffer	1x	TBS, pH 7.4
	1.3%	Digitonin
	1x	Complete & Phosstop
Nuclear Lysis Buffer	1x	TBS, pH 7.4
	0.5 %	NP-40
	1x	Complete & Phosstop

1.6. siRNA

Table 7: siRNA sequence

Target	Name	Sequence (5' → 3')	Manufacturer
control	siRNA/siCtrl	Pool of 4 unspecific siRNAs without target gene	Dharmacon, Lafayette, Louisiana, USA
Stat3	siStat3_1	Pool of 4 siRNAs GGAGAAGCAUCGUGAGUGA CCACUUUGGUGUUUCAUAA UCAGGUUGCUGGUCAAAUU CGUUAUAUAGGAACCGUAA	Dharmacon, Lafayette, Louisiana, USA

1.7. Bacterial strains and eukaryotic cells

1.7.1. *Yersinia enterocolitica* strains

The *Yersinia enterocolitica* strains used were cultured on LB agar plates at 27°C according to their antibiotic resistance.

Table 8: *Y. enterocolitica* strains

Strains	Characteristics	Resistence	Reference
WA314	*Y. enterocolitica* serotype O:8, clinical isolate, pYVO8+,	Nal	[16]
WAC	Plasmidless derivate of WA314	Nal	[16]
WA314ΔYopM	Derivative of the *Yersinia* WA314 harbouring the virulence plasmid pYVO8 , in which the YopM gene had been replaced by a kanamycin resistance cassette from pUC4k	Kana	[61]
WA314ΔYopP	Derivative of the *Yersinia* WA314 in which the YopP gene was replaced by a chloramphenicol resistance	Chlor	[194]
WA314ΔYopM+YopM	WA314ΔYopM complemented with YopM in pACYC184	Kana, Chlor	[61]
WA314ΔYopM+YopMΔC	WA314ΔYopM complemented with YopM_1-481 in pACYC184	Kana, Chlor	[115]

1.7.2. Eukaryotic cells

Table 9: Eukaryotic cells

Cells	Characteristics	Reference
HeLa	HeLa cells were cultured in DMEM-GlutaMAX-I (Invitrogen, GIBCO Darmstadt, Germany) supplemented with 10% FCS and 5% non-essential amino acids (Sigma-Aldrich)	ACC# 57, DSMZ-German Collection of Microorganisms and Cell Cultures
Human peripheral blood monocytes	Isolated monocytes were cultured for 6-9 days until differentiated into macrophages	Self-made isolation [195] from buffy coats, which were provided by Frank Bentzien, UKE, Hamburg, Germany

1.8. Eukaryontic expression constructs

For the expression in eukaryotic cells either the pCS2+MT (XB-VEC-12442480) or the pCDNA3.1 + vector (Invitrogen/Life Technologies, Thermo Fisher Scientific, Waltham, USA) was used. Primers used for amplification are listed in Table 11.

Table 10: Eukaryotic expression constructs

Insert	Vector	Description	Origin
myc-YopM	pCS2+MT	YopM was amplified from virulence plasmid pYVa127 with YopM-NCOI-for and YopM-XHOI-rev and cloned into pCS2+MT	[115]
Stat3-flag	pCDNA3.1 +	Stat3 was derived from the pLEGFP-Stat3-WT [196] construct via restriction site EcoRI and ligated into the pCDNA3.1 (Invitrogen)	this study
Stat3 Y705F-flag	pCDNA3.1 +	Stat3-Y705F was derived from the pLEGFP-Stat3-Y705F [196] construct via restriction site EcoRI and ligated into the pCDNA3.1 (Invitrogen)	this study
GFP	pEGFP-C3	GFP expression vector, used as a transfection control	Clontech/Takara, California, USA

1.9. Primer

Table 11: Primer name and sequences

Name	Sequence 5´-3´
Stat3 EcoRI F	CCACTAGTCCAGTGTGGTGGATGGCCCAATGGAATCAG
Stat3 EcoRI R	ACTGTGCTGGATATCTGCAGTCACTTGTCATCGTCGTC

1.10. Antibodies

Antibodies used in this study were: (Diluted 1:1000) Monoclonal mouse anti-STAT3 (F-2X) antibody (Santa Cruz Biotechnology), mouse anti-STAT3 antibody Stat3 (Cell signaling), rabbit anti-STAT3 antibody Stat3 (Cell signaling), rabbit anti-phospho-STAT3(Tyr705) antibody Stat3 (Cell signaling), rabbit anti-phospho-STAT3(Ser727) antibody Stat3 (Cell signaling), rabbit anti-acetyl-STAT3(K685) antibody Stat3 (Cell signaling), rabbit anti-STAT1 antibody Stat1 (Cell signaling), rabbit anti-phospho-STAT1(Tyr701) antibody Stat1 (Cell signaling), rabbit anti-phospho-STAT2(Tyr690) antibody Stat2 (Cell signaling), rabbit anti-phospho-STAT5(Tyr694) antibody Stat5 (Cell signaling), rabbit anti-phospho-STAT6(Tyr641) antibody Stat6 (Cell signaling), monoclonal rabbit anti-RSK1(C-21) antibody (Santa Cruz Biotechnology), monoclonal rabbit Lamin A/C (Cell Signaling), monoclonal mouse anti-FLAG (Sigma-Aldrich), monoclonal mouse anti-myc 9B11 antibody (Cell Signaling), polyclonal rabbit myc-tag antibody (Cell Signaling), monoclonal mouse anti-Histon3 (H3, Cell signaling),

monoclonal rabbit anti-phospho-H3S10 (Abcam), anti-phospho-S380RSK (Cell Signaling); (Diluted 1:2000) anti-phospho-S221RSK (R&D systems), polyclonal anti-GAPDH (Sigma-Aldrich), monoclonal anti-actin (Millipore, Schwalbach, Germany); (Diluted 1:2500). Secondary antibodies for immunofluorescence were AlexaFluor-488- or AlexaFluor-568-labeled goat anti-mouse, -anti-rabbit IgG and -anti-rat IgG (Molecular Probes, Karlsruhe, Germany). All used in a dilution of 1:200. Secondary antibodies for Western blot were horseradish peroxidase linked sheep anti-mouse IgG (GE Healthcare), donkey anti-rabbit IgG F(ab´)2 (Amersham Biosiences), -anti-goat IgG (Santa Cruz Biotechnology) and -anti-rabbit IgG (Cell signaling) and goat anti-rat IgG (GE Healthcare). All used in a dilution of 1:10000.

1.11. Software and databases

Table 12: Software and databases

Software	Provider
Adobe Photoshop CS5	Adobe Systems GmbH, Munich, Germany
ImageJ analysis software Version 1.43u	National Institute of Health, NIH. https://imagej.nih.gov/ij/
GraphPad Prism 5	GraphPad Software, San Diego, California USA, www.graphpad.com
Roche LightCycler® 480 software; Software release 1.5.1.62	Roche Diagnostics, Risch, Swiss
CLC Genomics Workbench	CLC bio, QIAGEN Company, Aarhus, Denmark

2. Methods

2.1. Microbiological methods

2.1.1. Cultivation of bacteria

For infection experiments *Yersinia spp.* were grown in LB-media. The culture was prepared with 4 ml media containing antibiotics, inoculated with bacteria freshly plated or from glycerol stocks and grown overnight in a shaking incubator at 27 °C whereas *E.coli* cultures were grown overnight at 37 °C.

2.1.2. Conservation of bacteria

For long-term storage of bacteria glycerol stocks were prepared. LB medium containing 40 % (w/v) glycerol was mixed with the same amount of liquid bacterial culture and transferred to -80 °C for long time storage. Ideally, used bacteria should be in their exponential growth phase (optical density OD_{600} = 0.3-0.6).

2.1.3. *Yersinia* Infection

Prior to infection, cells were maintained in antibiotic-free medium for 24 hours. Bacterial cultures were cultured overnight at 27°C and then diluted 1:20 in fresh LB medium and incubated for 1.5 hours at 37°C. The bacteria were centrifuged and resuspended in ice-cold PBS. To adjust the bacterial suspension to the same concentration, the OD_{600} of the bacterial suspension was determined photometrically and adjusted to an equal density of 3.6. Human macrophages were infected at a bacterial to cell ratio (multiplicity of infection, MOI) of 100.

2.2. Molecular biology techniques

2.2.1. Isolation of plasmid DNA

Plasmid DNA was isolated from 5 ml bacterial cultures using the ZR Plasmid Miniprep-Classic Kit (Zymo Research, USA) following the manufacturer's instructions. For transfection of mammalian cells the DNA the Plasmid Midi Kit (Qiagen, USA) was used according to manufacturer's instructions. To determine purity and concentration the NanoDrop® ND-1000 spectrophotometer (Peqlab, Germany) was employed.

2.2.2. Polymerase chain reaction

For generation of PCR-products the *5 Prime Extender Systems* (5 PRIME GmbH, Germany), suitable amplification of long PCR products, was used. PCR reaction mix and PCR amplification program performed in gradient thermocyclers (Eppendorf, Germany) are shown in Table 13 and Table 14.

Table 13: PCR reaction mix

Reaction composition	Concentration	Amount
DNA template	50-100 ng/µl	1 µl
PCR buffer	10x	5 µl
forward primer	10 pmol/µl	1 µl
reverse primer	10 pmol/µl	1 µl
dNTPs	10 mM	1 µl
Taq polymerase	10 units/µl	0.3 µl
ddH_2O		*ad* 50 µl

Table 14: PCR program

PCR program	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	30 s	30-35
Annealing	40-72 °C	30 s	
Extension	72 °C	1 kb DNA/45-60 s	
Final elongation	72 °C	10 min	1
Storage	8 °C	forever	1

2.2.3. Agarose-gel-electrophoresis

The agarose gel-electrophoresis was used to separate DNA by fragment length. Agarose (1-2 %) was solved in adequate volume of 1xTAE buffer and heated in the microwave (800 W). Nuclear acid staining solution RedSAFE (Intron Biotechnology, Korea) was added according to the manufacturer's instructions. Prepared gels were transferred to an electrophoresis chamber containing 1xTAE buffer and electrophoresis was performed with voltage 8 V/cm. DNA was mixed with Orange DNA loading dye (Fermentas, USA) and the 1 kb DNA-Ladder GeneRuler marker (Thermo Fisher Scientific, USA) was used. DNA was visualized by UV light on a Transilluminator (BioRad, Germany).

2.2.4. Restriction digest of DNA

For molecular cloning vector and PCR fragments were digested with FastDigest® according to the manufacturer's recommendations. DNA fragments were separated on agarose gels, the desired bands were purified using the Nucleo Spin Extract II kit (Macherey-Nagel, Germany) and purified fragments were subjected to ligation.

2.2.5. Ligation

Ligation reactions with Gibson Assembly® Master Mix (New England Biolabs, Frankfurt, Germany) were performed for 15 min at 50°C. The reaction was performed according to the manufacturer's recommendations using a molar ratio of 1:3 (vector: insert) calculated with the NEBioCalculator version 1.13.1.

2.2.6. Generation of expression vectors used in this study

For expression in eukaryotic cells, YopM constructs were amplified via PCR from virulence plasmid pYVa127/90 (accession NC_004564.1) and cloned into pCS2+MT (XB-VEC-12442480) via the restriction sites *NcoI* and *XbaI* resulting in N-terminally myc-tagged YopM (myc-YopM) [194]. For the expression of Flag-Stat3 constructs Stat3 was derived amplified the pLEGFP-Stat3-WT or pLEGFP-Stat3-Y705F [196] construct and subsequently cloned into the pCDNA3.1 + vector (Invitrogen/Life technologies, Thermo Fisher Scientific, USA) using the restriction site EcoRI resulting in the constructs Stat3-WT-flag or Stat3-Y705F-flag. Flag-tags where introduced by employing suitable reverse primers (Table 11).

2.2.7. DNA sequencing

To revise cloning, probes were given to Seqlab (Göttingen, Germany) for sequencing with suitable primers. Provided sequences were analyzed with the *CLC Genomics Workbench* (CLC bio; Denmark).

2.2.8. Real Time (RT)-PCR

Total RNA was extracted from primary human macrophages using RNeasy extraction kit (Qiagen) according to the manufacturer's instructions. A total of 2 µg of RNA from each cell lysate was transcribed into complementary DNA (cDNA) using the iScript cDNA Synthesis Kit (Bio-Rad). Subsequent quantitative RT-PCR was performed using the TaqMan® Fast Advanced Master mix (Applied Biosystems).

The specific primers used to analyze the expression of IL10 (Hs00961622_m1) were purchased from ThermoFisher Scientific. As a reference, expression of the "housekeeper" genes Glyceraldehyde-3-phosphate-Dehydrogenase (GAPDH; Hs02758991_g1), TATA-binding protein (TBP; Hs00427620_m1) and Beta-2 microglobulin (B2M; Hs00187842_m1) was examined. The RT-PCR was performed on the LightCycler® 480 instrument (Roche Life Science) and data were analyzed using Roche LightCycler® 480 software version 1.5.1.62 according to manufacturer's instruction. The program run on the Light cycler 480 is shown in table 15.

Table 15: RT-PCR program

PCR program	temperature	time	cycles
Initial denaturation	95 °C	10 min	1
Polymerase activation and Denaturation	95 °C	15 s	40-45
Annealing and Extension	60 °C	1 min	
Cooling of the instrument	37 °C	10 min	1

2.3. Biochemical methods

2.3.1. Immunoprecpitation

FLAG-tagged Stat3 was immunoprecipitated using the MultiMACS Protein G Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Transfected HeLa cells (6–8 x 10^6) were harvested and lysed in 1 ml of µMACS Lysis-Buffer (150 mM NaCl, 1% Triton X-100, 50 mM Tris-HCl, pH 8) according to the manufacturer's instruction. Lysates were centrifuged at 13.000 x g, 4°C for 10 min and incubated with 20 µl Anti-FLAG-sepharose (Anti-FLAG M2 Affinity Gel, Sigma) at 4°C for 30 min followed by incubation with 50 µl magnetic µMACS Protein G MicroBeads (Miltenyi Biotec GmbH) for 2 h. Immunoisolation was performed using the µColumns and µMACS Separator according to the manufacturer´s instruction. To elute proteins beads columns were incubated with 70 µl hot SDS-buffer and eluates were analyzed by immunoblot using the indicated antibodies.

2.3.2. Determination of protein concentration

Bradford spectroscopic analytical method was used to determine concentration of solubilized proteins. Bradford reagent was mixed in a 5:1 ratio with water and 1 µl of sample was added to 1 ml Bradford mix. The absorbance at 595 nm was measured against a blank value in a photometer (Ultrospec 3100 pro, GE Healthcare).

2.3.3. Western blot analysis

Cells were harvested in TBS containing protease inhibitor (Complete®, Roche) and phosphatase inhibitor (PhosStop®, Roche) to obtain cell lysates. Protein concentration was determined using the Bradford protein assay kit (BioRad). Equal amounts of protein were separated according to their sizes in a 10% SDS gel (Tris-HCl pH 8.8). A pre-stained protein marker (PageRuler Pretstained Protein Ladder, Thermo Fisher Scientific, Waltham, USA) was used as a standard for molecular weight. Transfer of proteins onto a polyvinylidene difluoride (PVDF) membrane (Immobilion-P, Millipore) was performed using the semidry protein blotting system. For blocking, the membrane was incubated in 5% milk in PBS-T for 30 minutes at room temperature (RT). Incubation of the primary antibody was performed overnight at 4°C. After three subsequent washing steps with TBS-T, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 2 hours at RT. Membranes were incubated with Supersignal West Femto reagent (Pierce Chemical) and developed on X-ray films (Fujifilm) in Curix 60 (Agfa, Belgium) system. The X-ray films were scanned on a CanonScan 4400F (Canon, Tokyo, Japan) for signal quantification using ImageJ analysis software version 1.43u (National Institute of Health, NIH).

2.4. Cell culture and cell biological methods

2.4.1. Cultivation of eukaryotic cells

HeLa cells (ACC# 57, DSMZ-German Collection of Microorganisms and Cell Cultures) were cultured in DMEM-GlutaMAX-I (Invitrogen, GIBCO Darmstadt, Germany) supplemented with 10% FCS and 5% non-essential amino acids (Sigma-Aldrich, Steinheim, Germany).

Human peripheral blood monocytes were isolated from buffy coats via CD14+-specific isolation (provided by Frank Bentzien, University Medical Center Eppendorf, Hamburg, Germany) as described previously [195]. Approval for the analysis of anonymized blood donations (WF-015/12) was obtained by the Ethical Committee of the Ärztekammer Hamburg (Germany). Isolated CD14+ cells were differentiated into human macrophages in the presence of human serum for seven days. Cells were cultured in RPMI1640 containing 20% autologous serum with medium changes every three days and used for *Yersinia* infection after 1 week. All cells were cultured in a humidified 5 % CO_2 atmosphere at 37 °C

2.4.2. Preparing cyro-stocks for long time storage of cells

Cryo-stock for storage of eukaryotic cells were prepared with 4 x 10^6 cells suspended in 1.5 ml FCS with 10 % (v/v) Dimethyl sulfoxid (DMSO), transferred to a cryo-vial and frozen over night at -80 °C in an isopropanol filled cryo-container. After 24h cells were transferred in liquid nitrogen at 196 °C for storage.

2.4.3. Incubation with inhibitors

Primary human macrophages were treated with DMSO (Sigma) or RSK inhibitor LJI308 (LJI; 10μM, Sigma) for 1h (LJI308, [197, 198] before the infection for 3 h.

2.4.4. Transfection of HeLa cells

HeLa cells were transfected with indicated plasmids employing polyethylenimine (PEI; Polysience Inc, Pennsylvania, USA) following the manufacturer's instructions.

2.4.5. SiRNA based knockdown

The siRNA used to silence Stat3 expression and an unspecific siRNA collection used as negative control was purchased from Dharmacon. siRNA transfection of human primary macrophages were performed using the Neon Transfection System (Invitrogen, Darmstadt, Germany) with standard settings (1000 V, 40 ms, 2 pulses) and 1.5 μg of siRNA. The Stat3 knockdown efficiency was controlled by analyzing lysates of siNT and siStat3 transfected cells by Western blotting with corresponding actin signals as loading control.

2.4.6. Preperation of nuclear and cytocolic cell fractions

Cells were harvested by scraping after two washing steps with ice-cold PBS, resuspended in tris-buffered saline (TBS) containing digitonin (130 μg/ml; Sigma; [199]), PhosSTOP

phosphatase inhibitor (Roche Diagnostic, Mannheim, Germany) and Complete protease inhibitors (Roche Diagnostics) and incubated on ice for 5- 15 min until the majority of the cells (> 98%) appeared to be lysed according to microscopic analysis (Microscop SZX12, Olympus). Cell extracts were centrifuged at 2000 × g at 4°C for 10 min. The supernatant was saved as cytosolic fraction and pellets were resuspended in PBS containing 0.5% NP-40 and protease inhibitors followed by sonication to yield a homogenous nuclear fraction. Purity of nuclear and cytosolic fractions was assessed by detection of cytosolic GAPDH and nuclear Lamin A/C using immunoblot.

2.5. Confocal laser microscopy

To visualize the intracellular distribution of YopM and Stat3, infected primary human macrophages, washed with PBS and fixed in 4% PFA, were permeabilized with 0.1% Triton X100 for 5-10 min and then fluorescently stained. Alternatively, to detect phosphorylation, cells were permeabilized in ice-cold methanole for 6 min at -20°C. Nonspecific binding sites were saturated by blocking with 5% BSA for at least 15 min. Cells were then incubated with the respective primary antibody in 5% BSA for one hour and, after washing three times, with the fluorophore-coupled secondary antibody for an additional 45 min. DAPI was added to the secondary antibodies to visualize cell nuclei and bacterial DNA. Finally, the coverslips were embedded in Prolong Diamond after washing three times with PBS. Fixed slides were analyzed on a confocal high resolution laser scanning microscope (Leica TCS SP8) with a 63x oil immersion objective (NA 1.4). Images were taken using Leica LAS X SP8 software (Leica Microsystems, Wetzlar, Germany).

2.6. ELISA

Secreted IL10 was determined in the supernatant of infected macrophages by commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems) according to the manufacturer's instructions. The amount of secreted IL10 was normalized to the amount of IL10 detected in the supernatant of noninfected macrophages.

2.7. RNA-Sequencing (RNA-Seq)

Human macrophages from two to four different donors per infection condition were analyzed. RNA was isolated from the human macrophages using RNeasy extraction kit (Qiagen), mRNA was extracted using the Next Poly(A) mRNA Magnetic Isolation Module (New England Biolabs; NEB), and a library was prepared for high-throughput sequencing as previously described [115] using the Next Ultra RNA Library Prep Kit Illumina (NEB).

The quality of the libraries was analyzed using the BioAnalyzer High Sensitivity Chip (Agilent). Sequencing was performed on the Illumina HiSeq 2500 instrument at 39.1- 55.8 million reads per condition.

The reads were assembled using STAR to the human genome as a reference [200]. FeatureCounts was used to assign the reads to a gene [201]. Statistical analysis of differential expression of genes was performed with DESeq2 and normalized with a log transformation (rlog) [202]. The rlog transformation accounts for the variance of the means, thereby improving the linear modeling of the data. DESeq2 was then used to identify differentially expressed genes (DEGs) between infection conditions and subsequently plotted in principal component analysis (PCA) and heatmap analyses.

2.7.1. Clustering analysis and heatmaps

Clustering analysis of all DEGs from comparisons between mock, WAC, WA314 was done with rlog counts in R with heatmap package. Clustering was performed with clustering distance based on Pearson correlation and Ward.D2 clustering method. Clustering distance, clustering method and number of clusters were selected so that all meaningful clusters were identified by the analysis. For RNA-seq heatmaps rlog counts from DESeq2 analysis were scaled by row (row Z-score) and low to high expression levels are indicated by blue-white-red color gradient. 2 representative replicates were shown for each sample

2.7.2. Pathway analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms were determined for RNA-seq by using the Webgestalt webtool [203].

2.7.3. Transcription factor motif enrichment analysis

TF binding motifs were identified with HOMER (http://homer.ucsd.edu/homer/motif/).

III Results

1. Increased IL10 production is a major function of YopM during human macrophage infection

1.1 YopM regulates the expression and secretion of the anti-inflammatory cytokine IL10

To date, the effect of YopM on IL10 gene expression in human macrophages is not understood in detail. IL10 secretion is increased in the sera of *Yersinia pseudotuberculosis* infected C57BL/6J mice [131, 157], but analysis of IL10 gene expression shows contradictory data in different cell types [115, 180]. Therefore, we aimed to characterize in detail the YopM effect on IL10 expression in macrophages and performed quantitative RT-PCR of primary macrophages mock-infected or infected with *Y.enterocolitica* wildtyp strain WA314, its avirulent derivative WAC or YopM deletion strain WA314ΔYopM for 1.5 or 6 h (Figure 3.1A). WAC with an absent TTSS was used to distinguish the effects of *Yersinia* PAMPs from the effects of WA314 TTSS effectors.

The expression of IL10 is highly increased in infection with WAC. This PAMPs-mediated immune response is massively inhibited by wild-type *Yersinia*, as shown in WA314 infected cells demonstrating a 30-fold decrease in IL10 expression compared to WAC-infected cells (Figure 3.1B). Therefore, WA314 counteract transcriptional change of IL10 induced by the PAMPs of *Yersinia*. Compared to WA314-infected cells, the expression of IL10 is further decreased in WA314ΔYopM-infected cells, indicating that IL10-expression is maintained at a certain level by TTSS effector YopM. This effect is even more significant after 6h infection, where cells infected with WA314ΔYopM show a 1.5 fold decrease in IL10 expression compared to WA314 infected cells (Figure 3.1B; right panel).

The *Y. enterocolitica* TTSS effectors YopP blocks inflammatory gene expression by inhibiting NF-κB and MAPK signaling initially triggered by the *Yersinia* PAMPs [99]. Therefore, we investigated whether YopP contributes to inhibition of IL10 expression in wildtype *Yersinia*-infected cells. To this end, IL10 expression in WA314ΔYopP-infected cells was compared to other used infection conditions as described before (Figure 3.1C). The amount of expressed IL10 was 8-fold increased in WA314ΔYopP-infected cells compared to WA314-infected cells (Figure 3.1C). Furthermore, the level of IL10 mRNA in WA314ΔYopP-infected cells was comparable to WAC- infected cells. These data suggest that YopP is the major effector inhibiting the expression of IL10 by it's known activity of MAPK inhibition.

Next, we analyzed whether the altered IL10 expression during *Yersinia* infection had an impact on the production of the cytokine. For this purpose, we determined the amount of

secreted IL10 in the supernatant of mock-infected human macrophages compared to WA314-infected, WAC-infected or WA314ΔYopM-infected cells (Figure 3.1D). Infection with WA314 resulted in increased secretion of IL10 compared with uninfected cells and WA314ΔYopM-infected cells. This suggests that YopM is critical for the secretion of IL10. WAC-infected cells showed 10-fold higher IL10 protein levels in the supernatant compared to WA314-infected macrophages. Accordingly, the results suggest that *Yersinia* WA314 can effectively prevent the secretion of IL10 by inhibiting RNA expression.

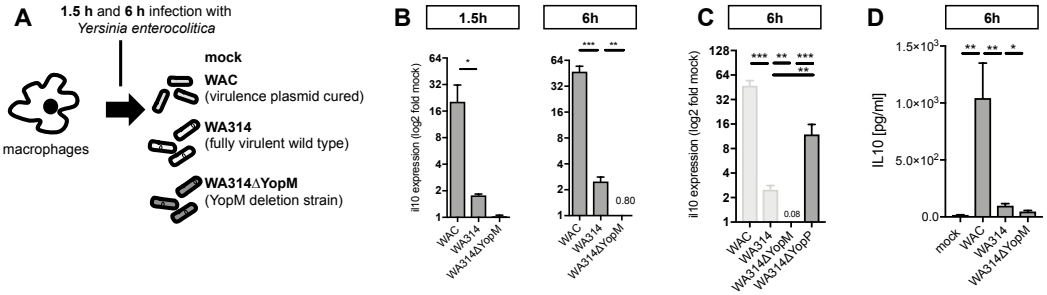

Figure 3.1: YopM induces the expression and production of IL10.
A) Experimental design. Monocytes were differentiated to human macrophages in the presence of human serum and infected after 7 days for 1.5 or 6 hours with *Yersinia* strains WAC, wildtype WA314, WA314ΔYopM or treated with PBS (mock). **B)** Total RNA was isolated from primary human macrophages infected for 1.5 and 6 h with indicated *Yersinia* strains and subjected to RT-qPCR using human IL10 specific primers. Expression was normalized to expression of three different housekeeping genes (GAPDH, TBP, B2M). For each condition triplicate samples of macrophages derived from different donors were investigated. Each bar in graph represents mean ± SEM of values from all donors normalized to mock. **C)** Macrophages infected for 6 h with WA314ΔYopP were analyzed for IL10 expression by RT-qPCR. **D)** Macrophages from four donors were infected and supernatants were harvested at 6 h for analysis of IL10 by ELISA. Results shown are the mean of four independent experiments with error bars representing standard deviation. Analysis was performed with a linear mixed modell taking into account random intercept. Data was transformed to ln-Score to ensure normal distribution; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Overall, YopP is responsible for most of the inhibitory effect of *Yersinia* on PAMP-induced IL10 gene expression, but YopM significantly contributes to modulation of gene expression in maintaining the level of the anti-inflammatory cytokine IL10 at a certain level. This virulence activity of YopM-mediated IL10 expression leads to increased production of IL10.

1.2. Regulation of IL10 cytokine expression by YopM relies on RSK activity.

The kinase RSK is known to play an important role in regulating the expression of diverse genes [141, 146, 204]. RSK1 is phosphorylated at multiple Ser- and Thr- residues by

different kinases, including its own C-terminal kinase domain [141]. The ERK/MAPK cascade directly or indirectly regulates these phosphorylation events [138]. One of the crucial phosphorylation sites in RSK is Ser-380, which is a docking site for PDK1 and is thought to activate RSK [141]. Previous studies have shown that phosphorylation of RSK is increased by YopM in infected cells, thereby increasing the expression of IL10 [115, 117]. Based on the assumption that YopM increases the activity of the nuclear kinase to target host gene transcription for the benefit of *Yersinia* infection, we investigated whether hyperphosphorylation of RSK induced by YopM accounts for increased expression of IL10.

To this end, primary macrophages were infected with WA314, WAC, WA314ΔYopM, WA314ΔYopP and WA314ΔYopM+YopM, a YopM deletion strain complemented with native YopM that translocates up to 5-fold increased levels of YopM via the TTSS [194]. Cells were lysed and subsequently analysed by Westernblot for phosphorylation of RSK using anti-pRSK-Ser-380 and anti-RSK antibodies (Figure 3.2A).

RSK phosphorylated on Ser-380 could neither be found in mock-infected nor in WA314ΔYopM-infected cells (Figure 3.2A, B), while in WA314-infected cells RSK phosphorylated on Ser-380 became detectable (Figure 3.2A, B). Upon infection with WA314ΔYopM+YopM a strong 5-fold increase in RSK phosphorylated on Ser-380 occurred (Figure 3.2A, B). In contrast, infection with avirulent *Yersinia* strain WAC showed a 6-fold increase in phosphorylation of RSK compared with wild-type infected cells (Figure 3.2B), suggesting a partly induction of RSK phosphorylation by PAMPs and not by a specific activity of the TTSS effectors. Furthermore, phosphorylation of RSK was not significantly increased in WA314ΔYopP compared with WA314-infected macrophages (Figure 3.2B). Notably, infection with different *Yersinia* strains did not alter the total amount of endogenous RSK (Figure 3.2A). Altogether these data suggest that YopM increases the phosphorylation of RSK by it's known direct interaction.

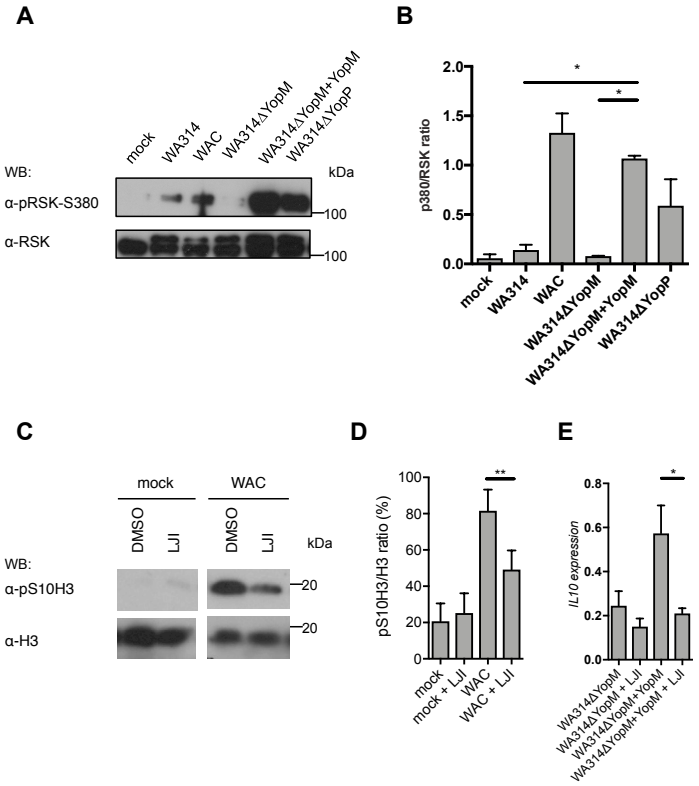

Figure 3.2: YopM induces the expression of IL10 via activation of the cellular kinase RSK.
A) Primary human macrophages were infected with *Yersinia* strains WA314, WAC, WA314ΔYopM, WA314ΔYopM+YopM and WA314ΔYopP for 1.5h or left untreated. Cell lysates were then analyzed by Western blot for the presence of phosphorylation p380 of RSK. The amount of total RSK served as a cellular loading control. Shown is a representative Western blot analysis for n=2 independent experiments. **B)** Band intensity of p380 and RSK signals was quantified in Image-J and the ratio of phosphorylated RSK to total RSK was calculated. **C)** Primary human macrophages were treated with DMSO (Sigma) or RSK inhibitor LJI (10μM, Sigma), infected with *Yersinia* WAC for 5h and subjected to Westernblot-analysis. **D)** Phosphorylation of Histone 3 at Serine 10 residue was evaluated by quantification of band intensities to determine ratio of pS103/H3. **E)** Primary human macrophages were treated with DMSO or inhibitor LJI (10μM) and then infected with *Yersinia* strains WA314ΔYopM, WA314ΔYopM+YopM for 5h.The isolated RNA was then analyzed by rtPCR using IL10 specific primers The expression of IL10 was normalized to the expression of the housekeeper genes GAPDH, TBP and B2M. For each condition, triplicates of cells from three different donors were analyzed. Shown are the mean values with standard deviation SEM for all 3 donors.*p<0.05

Next, the role of activated RSK in YopM-induced IL10 expression was investigated. For this purpose, cells were treated with an RSK inhibitor (LJI; [197]) 60 min, subsequently infected with WA314, WA314ΔYopM+YopM, and WA314ΔYopP strains for 3h, subjected to total RNA isolation and analyzed for transcription of IL10 by RT-PCR. RSK has been reported to be a H3 kinase activated by the Ras-MAPK signal transduction pathway [205]. Therefore, efficacy of the LJI inhibitor on the activity of RSK was assessed by Western blot analysis of RSK substrate histone 3 phosphorylation on the Ser-10 residue (Figure 3.2 C, D). Treatment with LJI significantly decreased the level of Histone 3 phosphorylated on Ser-10 in WAC-infected cells, confirming the effective inhibition of RSK (Figure 3.2 C, D).

Finally, to test whether hyperphosphorylation of RSK by YopM is required for the induction of IL10 expression we infected macrophages with WA314ΔYopM or WA314ΔYopM complemented with native YopM (WA314ΔYopM+YopM). When compared to IL10 expression in WA314ΔYopM infected macrophages, IL10 RNA levels were significantly higher in macrophages infected with strain WA314ΔYopM+YopM. The increased amount of IL10 transcripts detected in WA314ΔYopM+YopM-infected cells compared with wild-type *Yersinia*-infected macrophages was significantly lower in LJI-treated cells (Figure 3.2 E). These data suggest that YopM requires activation of all RSK isoforms to induce IL10 expression in macrophages.

In conclusion, the YopM-mediated increase in IL10 expression is caused by phosphorylated RSK kinase.

2. YopM modulates IL10-dependent gene expression induced in *Yersinia* infected primary human macrophages

Modulation of the amount of IL10 in infected cells is an essential function of YopM in infection. Due to the fact that IL10 is an important anti-inflammatory cytokine that induces specific gene expression, but also inhibits the gene expression of NF-κB-regulated cells, we aimed to investigate whether YopM has an effect on the transcription of infected cells through acting on IL10. So far, an analysis of how gene expression in human macrophages is modulated by the *Yersinia* virulence factor YopM and which role IL10 plays to mediate effects of YopM on gene transcription is lacking. Therefore, we performed a systematic global transcriptome analysis on *in vitro* differentiated M1 type primary human macrophages from 2-4 donors that were mock-infected or infected with strains WA314, WAC or WA314ΔYopM for 6 h (Figure 3.3A). Total RNA of macrophages was isolated and subjected to RNA-seq. At 6 hours, the stimulatory activity of YopM on IL10 expression was the more pronounced (see Figure 3.1.B). Therefore we focused our analysis on this later timepoint to investigate the effect of YopM on IL10 downstream genes. High reproducibility between replicates was confirmed by PCA analysis (Figure 3.3A). In addition, we obtained clustering of mock-infected and WA314ΔYopM samples, while samples infected for 6 hours with WA314 and WAC were not clearly clustered indicating main changes in gene expression (Figure 3.3B). Of note, WA314ΔYopM and WA314 replicates clustered separately (Figure 3.3B), indicating modulation of transcription by YopM. Clustering of mock-infected and WA314ΔYopM samples reveals that YopM is essential for modulating gene expression in Yersinia-infected macrophages.

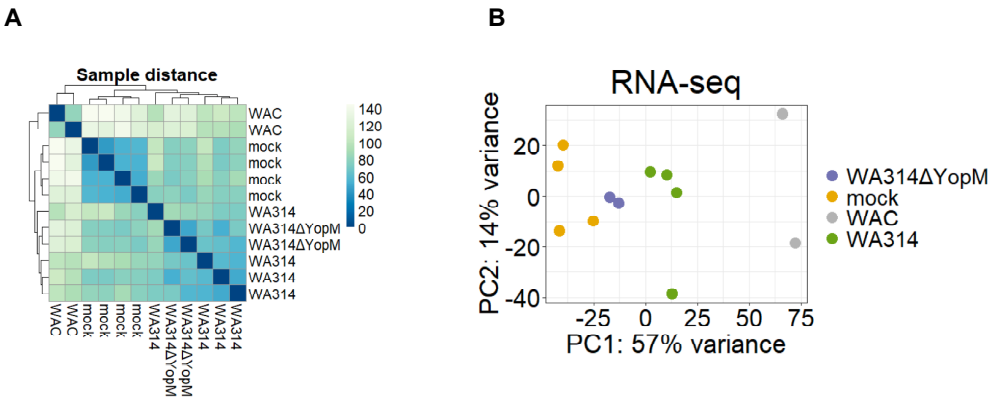

Figure 3.3: Transcriptional regulation by *Yersinia enterocolitica*.
A) Heatmap of principal component analysis (PCA) of RNA-seq rlog gene counts for each sample replicate used in the analysis. For plotting 1000 genes with the highest row variance were used. **B)** PCA of mean centered and scaled *rlog* transformed read count values of RNA-seq data of uninfected and *Yersinia*-infected macrophages. Figures were created by Indra Bekere.

We next examined significantly differentially expressed genes (DEGs) with a log2fold change higher than 2 and adjusted p-value <0.05 in WAC, WA314 and WA314ΔYopM -infected cells to mock-infected cells. We aimed to investigate whether IL10 contributes in *Yersinia* triggering gene expression changes in macrophages. For this purpose, a publicly available RNA-seq dataset from IL10- treated monocytes (GSE43700; [206]) was compared to the data from this study. Hereupon, approximately 5% of identified the 5698 DEGs in WAC, WA314 and WA314ΔYopM infected cells (FDR < 0.05, > 2-fold change; 189/2972; 108/1800, 40/926, respectively) compared to non infected cells correspond to IL10-inducible genes (Figure 3.4A). Of note, the number of upregulated IL10-inducible genes complies with detectable RNA levels of IL10 (see Figure 3.1C).

WAC upregulated 189 genes vs mock, mainly reflecting inflammatory response induced by the PAMPs of *Y. enterocolitica* (Figure 3.4A). In comparison, 108 genes were induced by WA314 vs mock (Figure 3.4A), of which 67 were also upregulated by WAC vs mock (Figure 3.4A), indicating partially PAMP-induced transcription in wild-type infection. In contrast only 40 genes were upregulated in WA314ΔYopM infected cells compared to mock-infected cells (Figure 3.4A).

Closer examination of these genes by Venn diagram analysis showed that 136 genes are upregulated by IL10 only in the WAC group and WA314ΔYopM group and therefore are regulated in the absence of YopM (Figure 3.4B). More importantly, in total 78 IL10-inducible genes (67 in the WAC/WA314 overlap and 11 in the WA314 group) were specifically upregulated by YopM (Figure 3.4B). Kyoto Encyclopedia of Genes and Genomes (KEGG)

analysis revealed that each group showed enrichment in genes associated with JAK-STAT signaling (Figure 3.4B) and included several regulators of this pathway (Table 6.1), such as SOCS1/3 [163, 207], PTPN2 [208] and PIM1 [209]. Furthermore genes were enriched for transcription, FoxO signaling and osteoclast differentiation, involving pro-inflammatory cytokines, chemokines and feedback regulators (Figure 3.4C and Table 6.1) and included several regulators of macrophage activation and T cell population development, such as members AP-1 transcription factor family Fos-like 2 (Fosl2 [210]) or Basic Leucine Zipper ATF-Like Transcription Factor (BATF [211]).
Overall, these data suggest that YopM tightly controls transcription within the JAK-STAT signaling pathway.

In order to identify profiles of gene expression a heatmap of all IL10-induced DEGs between mock-, WAC-, WA314- and WA314ΔYopM-infected cells was created (Figure 3.4D), by which 214 DEGs were identified when taking all comparisons together (WAC, WA314 and WA314ΔYopM versus mock; Figure 3.4D Table 6.2). Clustering analysis of DEGs revealed three main sets with distinct patterns of gene expression referred to as classes R1, R2 and R3 (Figure 3.4D). Class R1 genes (n=17) showed mostly higher expression levels in WA314- and WA314ΔYopM-infected cells compared to WAC-infected cells (Figure 3.4D). In class R2 (n=161) WAC upregulated gene expression, which was abolished by WA314. In a certain extent, YopM controls this suppression at a very specific level by upregulating these R2 class genes (Figure 3.4D). Therefore, this class was assigned to the biological function profile “Upregulation” (Table 6.2). Genes of class R3 (n=36) showed generally higher expression levels in WAC-, WA314- and WA314ΔYopM-infected cells (Figure 3.4D). This pattern of transcriptional response in class R3 thus widely reflects genes that are upregulated during infection, suggesting induction by PAMPs unrelated to any YopM specific activity.
Of the three distinct gene classes identified, only genes of the upregulation profile were enriched in unique Gene Ontology (GO) and KEGG terms (Table 6.3). The specifically YopM upregulated IL10-induced genes (class R2, orange color code, Figure 3.4D) were enriched in immune response, cell-cell-adhesion processes and JAK-STAT signaling pathway (Table 6.3). These genes include several members of JAK-STAT signaling: JAK3, SOCS1/3 and IL10 itself (Figure 3.4D; Table 6.2), suggesting a complex control of expression of this transcriptional regulator network involved in IL10-signaling by *Yersinia*. Upregulation profile contained several transcriptionfactor family members, e.g. SOCS (SOCS1/3), E26 transformation-specific (ETS), and C2H2 domain ZNFs KLF4, KLF6 [212]. Especially the family of ETS are known to interact with several other TFs [213], Therefore, we performed next a TF motif analysis of promoter and enhancer regions associated with these genes (see

Figure 3.4D, Table 6.2) to identify transcriptional regulators. Motifs were analysed in promotor regions 400bp upstream and 100 bp downstream from the TSS against the default geneset as the background. Only motifs with length of 8, 10 and 12 bp were included in the analysis. TF motif analysis revealed binding sites for the rel homology domain- (RHD; NFκB-p65-Rel), STAT- (Stat3/4) and bZIP- (C/EBP, AP-1 [214]) families (Figures 3.4E). Interestingly, Stat3 was the most significant enriched TF, implicating that *Yersinia* modulates JAK-STAT signaling specifically by transcriptionfactor Stat3 to upregulate expression of certain target genes, i.e. IL10 and SOCS3 (Figures 3.4F).

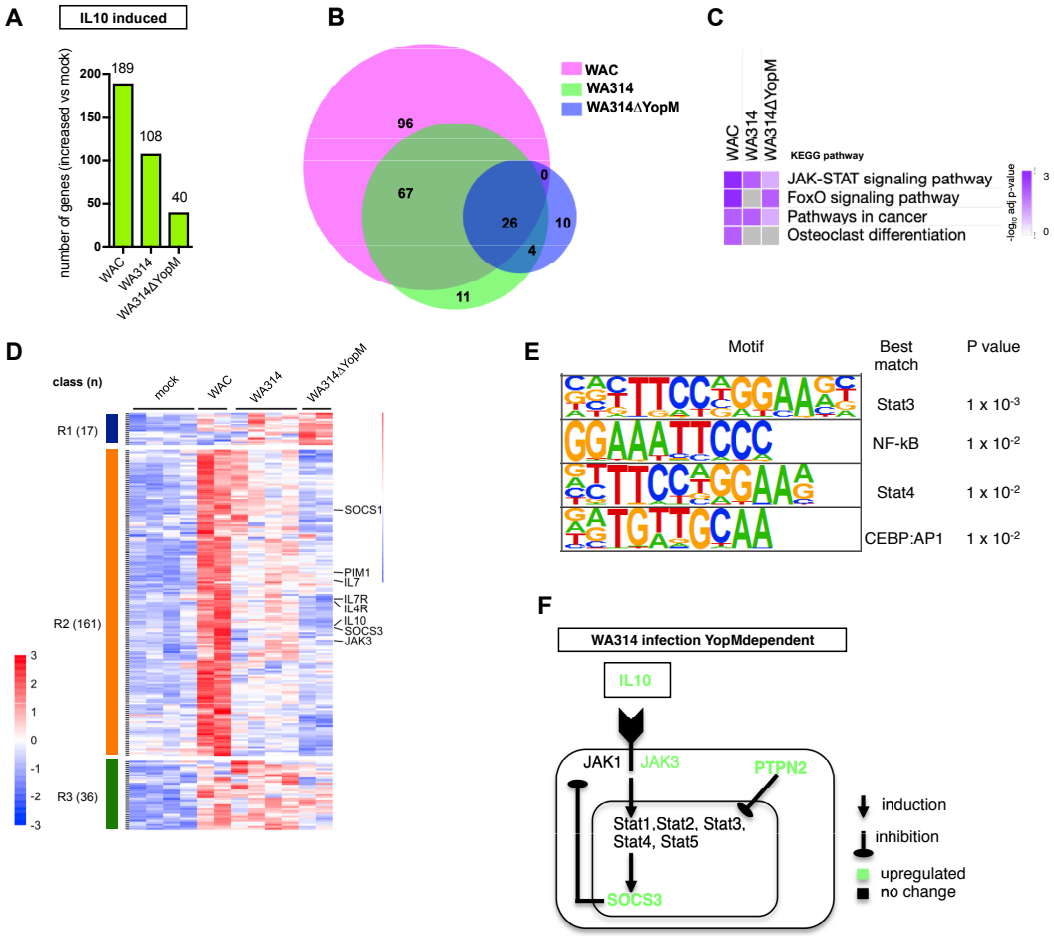

Figure 3.4: IL10 induced transcriptional regulation by TTSS effector YopM.
A) Differential expressed genes (DEGs; log2fold>2; adjusted p-value>0.01) in indicated infection-condition compared to mock that are IL10-inducible as obtained from GSE43700 [206]. **B)** Venn Diagram of DEGs from mock, WAC, WA314 and WA314ΔYopM; the sum of the numbers in each circle represents total number of DEGs between combinations; the overlapping part of the circles represents common DEGs. **C)** Heatmap showing the *P*-value significance of distinct KEGG pathway enrichment for genes in each condition in (A). **D)** Heatmap showing the relative expression of significant DEGs for IL10-induction. Heatmap from clustering of all IL10-induced DEGs from mock, WAC, WA314 and WA314ΔYopM comparisons at 6 h. Clustering identified 3 major classes. Rlog counts of DEGs were row-scaled (row Z-score). **E)** TF motif enrichment associated with DEGs in (D). **F)** Modulation of the JAK-STAT signaling pathway during *Yersinia* infection. Figures 3.4 D and E were created by Indra Bekere.

As an anti-inflammatory cytokine, IL10 is an important repressor of gene expression [215]. Consequently we next analyzed which genes were downregulated in an IL10-dependent manner in *Yersinia*-infected cells. Therefore genes that showed lower expression levels in WAC, WA314 and WA314ΔYopM-infected cells compared to mock-infected cells were compared to the RNA-seq dataset from IL10- treated monocytes (GSE43700; [206]). In general less IL10-suppressed DEGs (n=112) were identified compared to IL10-induced genes (n=337) during *Yersinia* infection. WAC infected cells showed IL10-dependent suppression of 75 genes compared to mock, while in WA314 and WA314ΔYopM infected cells less genes, 26 and 11 genes, respectively, were found (Figure 3.5A). Of the 75 genes downregulated by WAC vs mock, 19 were also down- regulated by WA314 vs mock (Figure 3.5B). Genes specifically downregulated by YopM were found both, in the WA314 and WAC group (19 and 3 genes), but not in the WA314ΔYopM group (Figure 3.5B).

We aimed to visualize gene expression profiles of all IL10-suppressed DEGs between mock-WAC-, WA314- and WA314ΔYopM- infected cells and relate them to biological pathways. Therefore a heatmap of IL10-suppressed genes was created (Figure 3.5C). Clustering analysis grouped DEGs in three main classes named R1-R3 with distinct patterns of gene expression (Figure 3.5C). In class R2 and R3 WA314 prevented the downregulation of genes by WAC, as genes were upregulated in WA314- compared to WAC- infected cells (Figure 3.5C). Therefore this gene classes were assembled to a profile termed “Inhibition” (R2-R3). This downregulation was clearly dependent on *Yersinia* effector YopM in class R2 in this cluster and some genes were specifically downregulated by YopM as WA314ΔYopM-infected cells showed higher expression levels compared to mock-infected cells (Figure 3.5C). Genes of the inhibition profile were enriched for GO term regulation of cytokine secretion (Table 6.5), including for example leucine-rich repeat kinase 2 (LRRK2). This kinase is a key regulator of immune function during infection and involved in the induction of phagocytosis [216], suggesting that innate immune response is tightly controlled by *Yersinia* effector YopM.

Finally, some specific genes in class R1 and R2 belong to mechanisms in degradation of extracellular matrix (ECM), i.e. MMP9 (Matrix metallopeptidase 9) and SPARC (Secreted Protein Acidic And Cysteine Rich; Table 6.4). This suggests a complex control of expression of ECM degradation regulator networks by *Yersinia* to utilize host ECM proteins for adhesion and invasion ([217]; (Figure 3.5D). We conclude from RNA-Seq data analysis that YopM downregulates a subset of genes specifically regulated by anti-inflammatory cytokine IL10.

Overall, theses findings suggest that YopM of *Yersinia* selectively modulates JAK-STAT signaling activation to boost expression of different subsets of IL10-regulated genes.

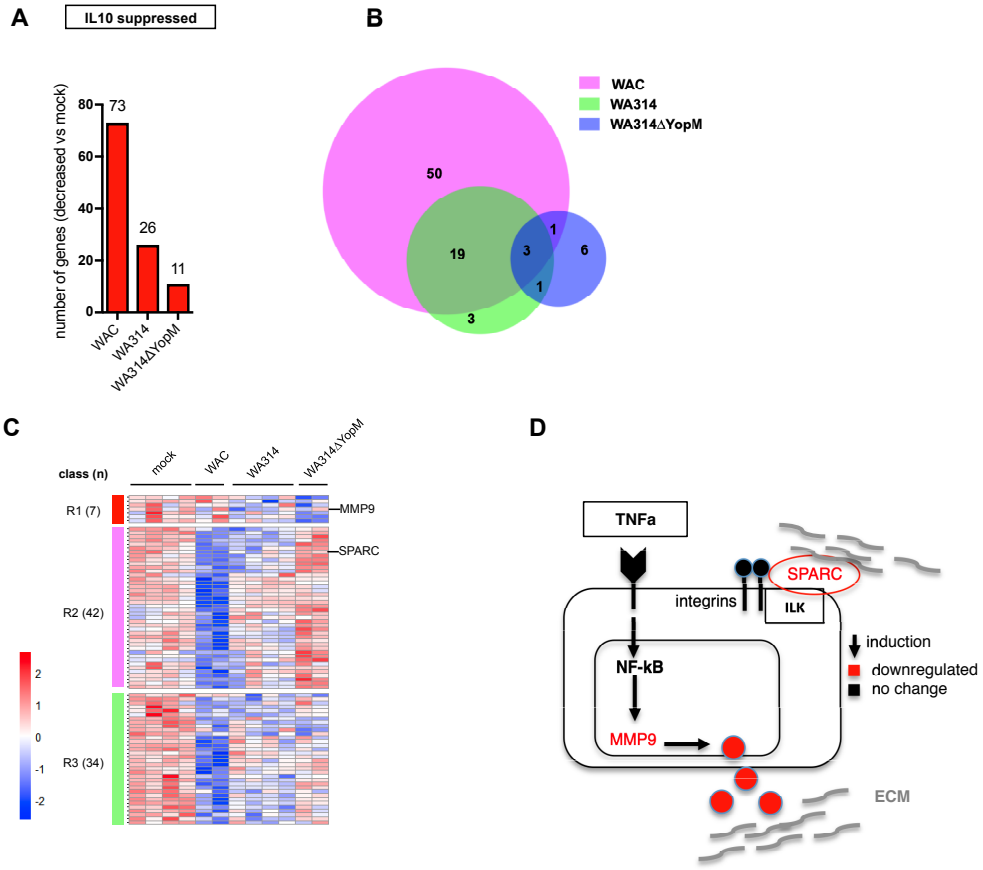

Figure 3.5: Anti-inflammatory effects of IL10 in transcriptional regulation by the effectorprotein YopM. **A)** The proportion of DEGs downregulated versus mock-infected cells that are suppressed by IL10 as obtained from GSE43700 for indicated infection condition and **B)** corresponding Venn diagram. **C)** Heatmap from clustering of all IL10-suppressed DEGs from mock, WAC, WA314 and WA314ΔYopM comparisons at 6 h. Clustering identified 3 major classes. Rlog counts of DEGs were row-scaled (row Z-score). **D)** Modulation of IL10 suppressed genes in Extracellular Matrix (ECM) degradation during *Yersinia* infection. Figures 3.5C was created by Indra Bekere.

3. JAK-STAT signaling pathway regulation during human macrophage infection

3.1. YopM induces nuclear translocation of transcriptionfactor Stat3

The expression of IL10 is in part regulated by the transcription factor Stat3 through initiation of Stat3 binding via the two STAT binding elements (SBE) in the promoter region of IL10. In general, phosphorylated Stat3 translocates as a dimer into the nucleus and binding to the SBE of the IL10 promoter region induces the expression of IL10 [218]. Therefore, our next objective was to investigate whether altered localization of Stat3 occurs in *Yersinia* infection

and whether increased nuclear translocation of Stat3 possibly induces the expression of IL10.

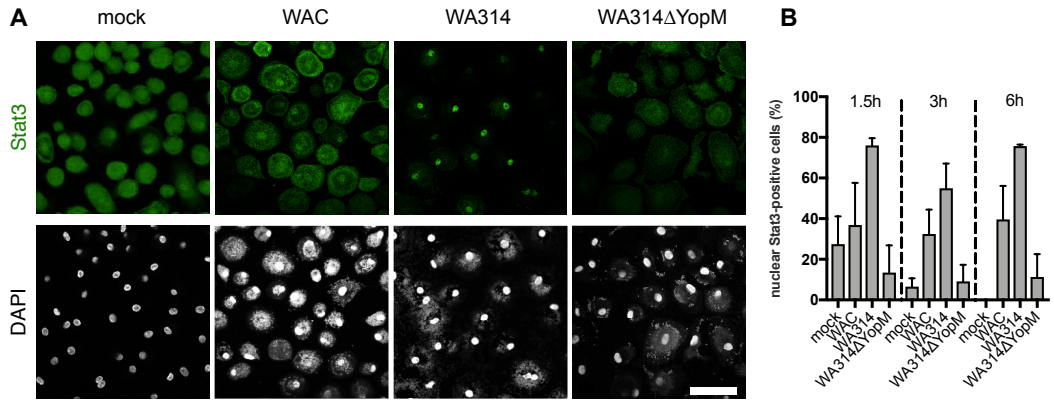

Figure 3.6: YopM induces nuclear translocation of Stat3.
A) Primary human macrophages were infected with *Yersinia* strains WA314, WAC, WA314ΔYopM for 3h or left untreated (mock), permeabilized and immunostained with Stat3 antibody (green) or DAPI. Scale bar, 20µm. **B)** Quantification of cells with nuclear Stat3 signal from three independent experiments with n=100 cells infected for 1.5h, 3h and 6h.

The cellular distribution of Stat3 was analyzed in untreated, as well as WAC, WA314 and WA314ΔYopM infected human macrophages by immunofluorescence staining using Stat3 antibody and DAPI to visualize the cellular nuclei and bacteria (Figure 3.6A).

In untreated cells, it is evident that Stat3 is normally found in the cytoplasm and nucleus in a homogeneous distribution. Interestingly, in wild-type *Yersinia*-infected cells Stat3 became detactable almost exclusively in the nucleus (Figure 3.6A). Nuclear accumulation of Stat3 is already visible after 1.5h hours and is robust for longer infection periods up to 6h (Figure 3.6A). After 3h infection, the proportion of cells with a primary nuclear Stat3 signal relative to all cells increased significantly from approximately 10% to 60% in wild-type *Yersinia*-infected cells compared to untreated cells (Figure 3.6B). WA314ΔYopM infected macrophages have a comparable distribution of Stat3 to untreated cells (Figure 3.6A, B) therefore nuclear translocation appears to be dependent on YopM. To test whether this effect is possibly mediated via LPS, cells infected with the avirulent strain WAC were examined. WAC infected cells continued to show a homogeneous distribution between nucleus and cytoplasm, yet the proportion of cells with a nuclear Stat3 signal increased from approximately 10% to 30% (Figure 3.6B). Accordingly, a supporting role of LPS in YopM-mediated nuclear translocation of Stat3 could be excluded.

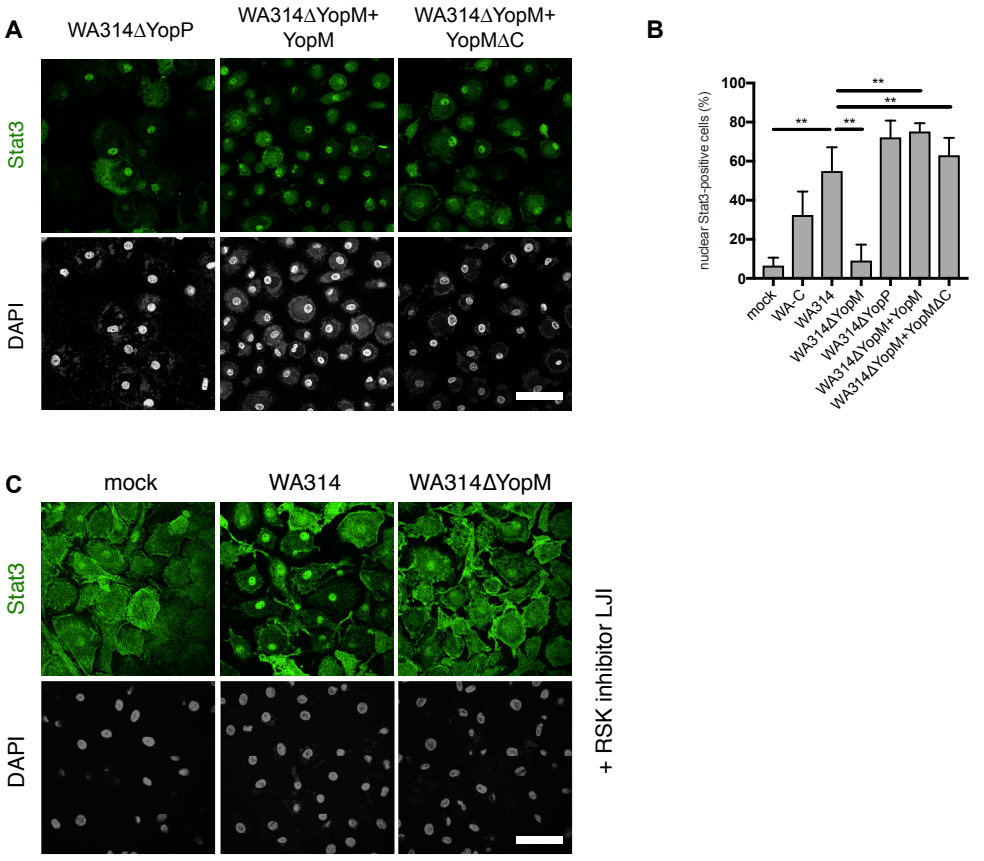

Figure 3.7: YopM induces nuclear translocation of Stat3 independent of other TTSS effectors or direct interaction with RSK.
A) Primary human macrophages were infected with *Yersinia* strains WA314ΔYopP, WA314ΔYopM+YopM and WA314ΔYopM+YopMΔC for 3h and immunostained with Stat3 antibody (green) or DAPI. Scale bar, 20µm. **B)** Quantification of cells with nuclear Stat3 signal from three independent experiments with n=100 cells infected for 3h. **C)** Primary human macrophages were treated with RSK inhibitor LJI (10µM, Sigma), infected with *Yersinia* WA314, WA314ΔYopM or left mock-infected and immunostained with Stat3 antibody (green) or DAPI. Scale bar, 20µm.

To investigate whether other TTSS effectors are involved in the YopM-mediated nuclear translocation of Stat3, localization of Stat3 in WA314ΔYopP infected cells was investigated. In infection with YopP-deficient bacteria, increased accumulation of Stat3 in the nucleus comparable to WA314-infected cells was observed. Thus, YopP has no effect on YopM-dependent nuclear translocation of Stat3.

To clarify whether direct binding of YopM to RSK is required for the transport of Stat3 into the nucleus, we infected macrophages with WA314ΔYopM complemented with YopMΔC (WA314ΔYopM+YopMΔC; Table 1) or with native YopM (WA314ΔYopM+YopM) and analyzed intracellular distribution of Stat3. When compared to nuclear Stat3 in WA314- and WA314ΔYopM+YopM infected macrophages, levels of nuclear Stat3 were similar in macrophages infected with strain WA314ΔYopM+YopMΔC translocating a YopM mutant that

does not interact with RSK [115]; Figure 3.7A). Accordingly, the interaction of RSK and YopM showed no effect on the nuclear translocation of Stat3. Similarly, in the nucleus of WA314-infected cells pretreated with LJI, Stat3 increased compared to WA314ΔYopM-infected cells (Figure 3.7C). Thus, we conclude that YopM increases levels of Stat3 in the nuclear cell compartment independent of RSK interaction or hyperphosphorylation.

3.2. YopM promotes Stat3 nuclear localization in the absence of phosphorylation

Next, we investigated expression and signaling activation of JAK-STAT pathway members during *Yersinia* infection. To this end, human macrophages were infected with WAC, WA314, WA314ΔYopM and WA314ΔYopP and lysed after 3h for subsequent Western blot analysis. First, phosphorylation of Stat3 on Tyr-705 became only detactable in whole cell lysates of WAC-infected and IL6-treated cells, while no phosphorylation of Stat3 and other Stat isoforms (Stat2, Stat5 and Stat6) could be observed in *Yersinia*-infected cells (Figure 3.8 A). Of note, Stat3 phosphorylated on Tyr-705 became detectable in WA314ΔYopM, indicating a potential inhibition of Stat3 phosphorylation due to YopM.

In contrast, Stat1 phosphorylated on Tyr-701 became detectable in *Yersinia*-infected cells; in particular a strong increase in Stat1 phosphorylated on Tyr-701 occurred in WAC-, as well as WA314ΔYopP -infected cells (Figure 3.8 A). Here, the level of Tyr-701 phosphorylated Stat1 was comparable to IFNy-stimulated cells (Figure 3.8 A). These results indicate that indirect activation of Stat1 by LPS-induced type I IFN is triggered by *Yersinia* PAMPs and suggests that Stat1 plays a critical role in innate immunity to *Yersinia* infection, but is not regulated by YopM [219, 220]. Next, we investigated the impact of YopM on Stat3 phosphorylated on Ser-727 in *Yersinia* infected macrophages, because transcriptional activation of Stat3 is regulated by phosphorylation at Ser727 through the MAPK or mTOR pathways ([168, 221], (Figure 3.8 B)). The level of cellular Stat3 and its phosphorylation on Ser-727 were not altered by bacterially injected YopM and showed comparable high levels in *Yersinia*-infected cells as in IL6-stimulated cells (Figure 3.8 B), indicating that Stat3-inducible transcription dependent on Ser-727 is not affected by YopM.

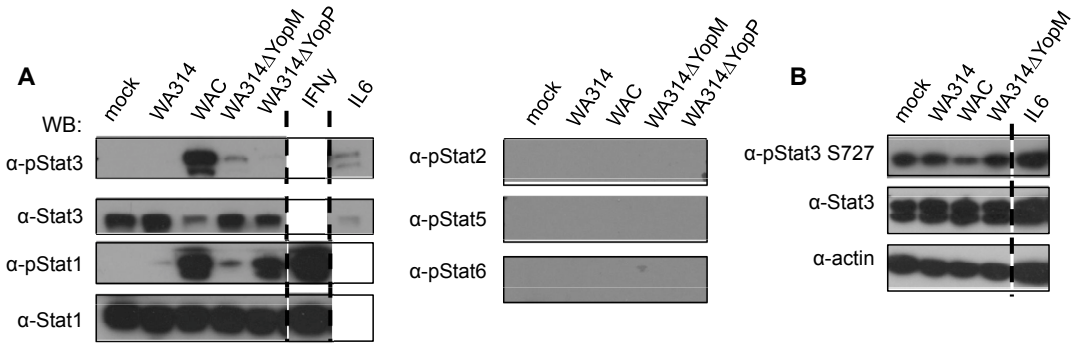

Figure 3.8: JAK-STAT signaling in *Yersinia*-infected cells
A) Primary human macrophages were infected with *Yersinia* strains WA314, WAC, WA314ΔYopM or WA314ΔYopP for 3h, stimulated with interferon gamma (IFNγ) or interleukin 6 (IL6) for 30 min, and subsequently analyzed by Western blot with indicated phospho-antibodies Stat1, Stat2, Stat3 (Tyr-705), Stat5 and Stat6 or Stat1 and Stat3 antibody or **B)** phospho-antibody Stat3 (Ser-727) and Stat3. Actin served as cytoplasmic loading control.

Stat3 is primarily activated by phosphorylation at Tyr-705, which induces dimerization in the cytosol, nuclear translocation, and DNA binding [164, 222]. YopM affects cellular distribution of transcriptionfactor Stat3 according to immunofluorescence staining. In order to evaluate the role of Stat3 phosphorylation at Tyr-705 in the process of nuclear import of Stat3 mediated by bacterially injected YopM, we next analyzed the cellular distribution of phosphorylated Stat3 in *Yersinia* infected macrophages.

Digitonin was used for cell lysis to specifically disrupt the cell wall of eukaryotic cells at a low concentration (0.5% in PBS) without damaging the nuclear membrane allowing the cells to be separated into a cytoplasmic and nuclear fraction. Adequate seperation into cytoplasmic and nuclear fractions was ensured using lamin as a loading control for the nuclear fraction and GAPDH for the cytoplasmic fraction. As a positive control of Stat3 phosphorylation, cells were stimulated with IL6 for 15 min. Phosphorylation of Stat3 was analyzed using specific antibodies against phosphorylation at tyrosine 705 residue. In IL6-stimulated cells, an increase of phospho-Stat3 in both the cytoplasmic and nuclear fractions became detectable (Figure 3.9A). In contrast, no phosphorylation of Stat3 was found in *Yersinia*-infected cells, even after prolonged exposure of the film compared to IL6 stimulated cells (Figure 3.9A). Only in WAC infected cells phosphorylated Stat3 could be detected in the cytoplasmic fraction. Under infection conditions with wild-type *Yersinia*, an increased amount of Stat3 was detected in the nuclear fraction compared with untreated and WA314ΔYopM infected cells.

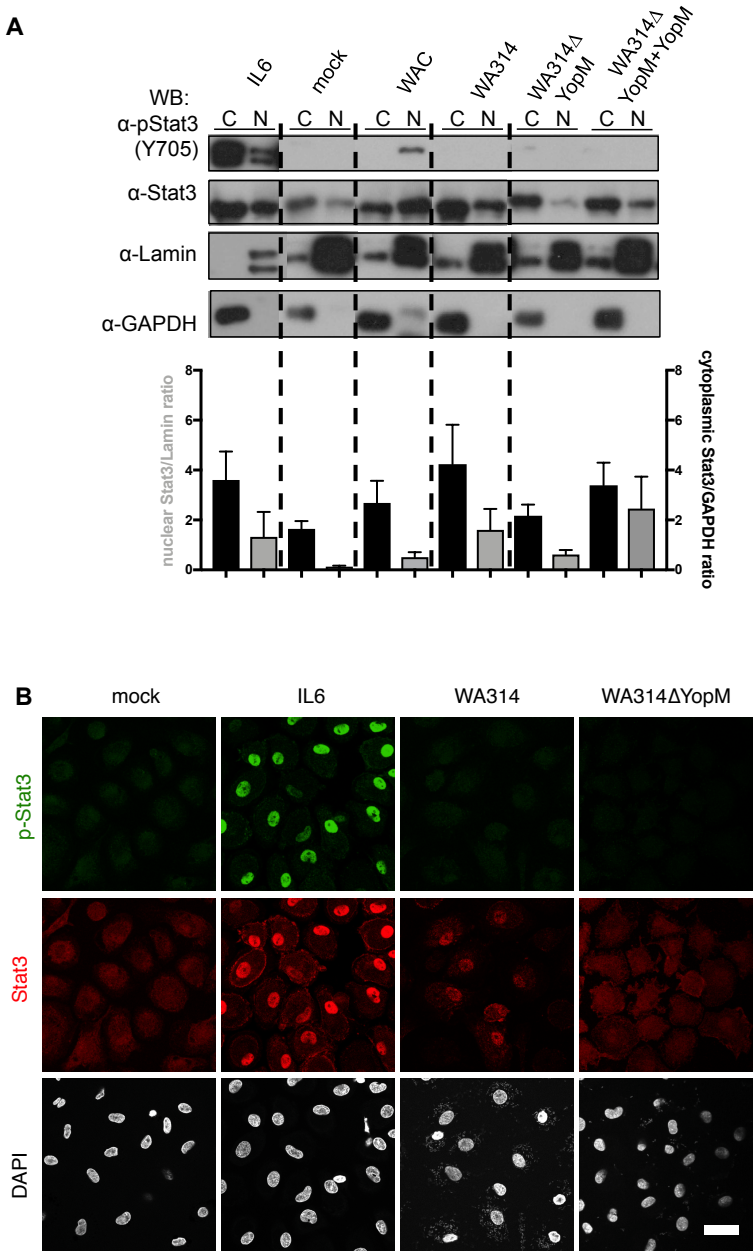

Figure 3.9: Bacterially injected YopM controls nuclear translocation of unphosphorylated Stat3.
A) Primary human macrophages were infected with *Yersinia* strains WAC, WA314, WA314ΔYopM or WA314ΔYopM+YopM for 3h, stimulated with IL6 or left untreated (mock), permeabilized and immunostained with Stat3 antibody (green), phospho-Stat3 antibody (red) or DAPI. **B)** Nuclear (N) and cytoplasmic (C) fractions were subsequently analyzed by Western blot. GAPDH served as cytoplasmic and lamin as nuclear loading control. Band intensity was quantified and the ratio of nuclear Stat3 to lamin (grey colored bars) and cytoplasmic Stat3 to GAPDH (black colored bars) was calculated. The mean values +/- SEM of 6 independent experiments are shown. Scale bar, 20µm.

This increase in Stat3 in the nuclear fraction was also evident in WA314ΔYopM+YopM and thus always correlated with the presence of YopM. Cells stimulated with IL6 also showed an

increased Stat3 amount in the nuclear fraction (Figure 3.9A). Furthermore, the total amount of Stat3 in the cytoplasmic and nuclear fractions was increased in both WA314 and WA314ΔYopM+YopM infected macrophages compared to mock-treated macrophages (Figure 3.9A). Immunofluorescence staining with phospho-Stat3 antibodies of WA314-infected cells revealed in accordance with the immunoblot data that overall a larger amount of unphosphorylated cellular Stat3 is located in the nucleus than in the cytosol in WA314-infected cells (Figure 3.9B). Of note, Stat3 phosphorylated on Tyr-705 became detectable only in the nucleus of IL6-treated cells (Figure 3.9B).
Overall, this data suggests that Stat3 translocates to the nucleus independent of phosphorylation at Tyr-705 in *Yersinia*-infected human macrophages.

3.3. YopM forms a complex with nuclear Stat3 to increase IL10-expression in *Yersinia*-infected macrophages

To investigate whether direct interaction of YopM with Stat3 might promote nuclear translocation of Stat3, an interaction analysis was carried out in HeLa cells. We first considered that the nuclear levels of Stat3 might vary considerably between individual cell types. For this purpose, we first investigated whether the nuclear translocation of Stat3 by YopM could also be observed in other cell types aside human macrophages. HeLa cells were mock-infected or infected with WA314 and WA314ΔYopM and immunofluorescence stained with anti-Stat3 and DAPI. While Stat3 in untreated HeLa cells is mainly found exclusively in the cytoplasm, WA314-infected cells showed exclusive nuclear Stat3, whereas in WA314ΔYopM-infected cells Stat3 was evenly distributed between cytoplasm and nucleus (Figure 3.10A).
When Stat3-flag was co-expressed with myc-YopM in HeLa cells and visualized by immunofluorescence, cells displayed a predominant nuclear localization of Stat3-flag whereas cells expressing Stat3-flag alone showed a predominant cytoplasmic localization (Figure 3.10B), indicating that YopM directly promotes nuclear translocation of Stat3.
Phosphorylation of Stat3 at Tyr-705 activates Stat3 and induces dimerization, nuclear translocation and DNA binding [164, 222]. To investigate whether phosphorylation of Stat3 is required for the YopM-induced nuclear import of Stat3 we cotransfected HeLa cells with myc-YopM and vectors expressing a Stat3 Y705F mutant (Stat3Y705F-flag; Figure 3.10B). The Tyr (Tyrosine; T) to Phe (Phenylalanine; F) substitution at position 705, causes the disruption of the canonical tyrosine phosphorylation site preventing nuclear translocation and the transcriptional activity of Stat3 in IL6-stimulated cells [223].

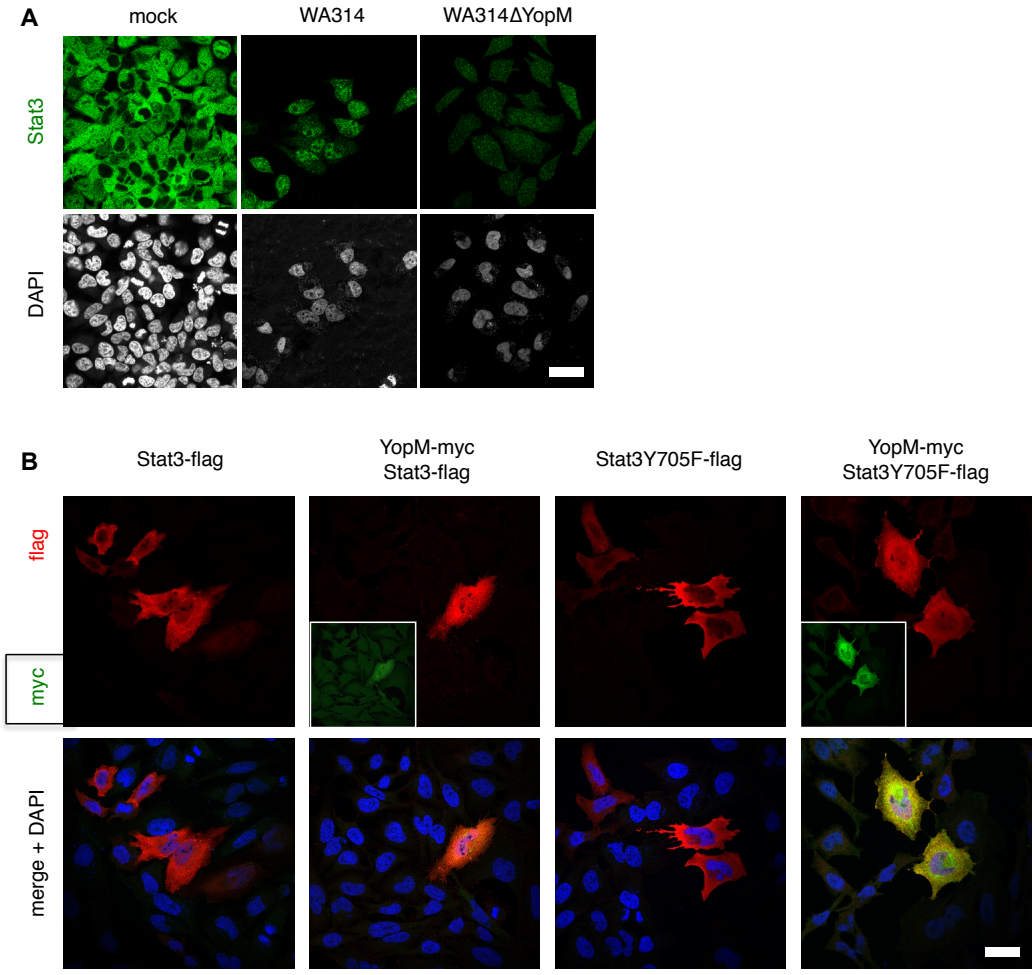

Figure 3.10: Nuclear levels of Stat3 are increased by bacterially injected and overexpressed YopM in HeLa cells.

A) HeLa cells were infected with *Yersinia* strains WA314, WA314ΔYopM for 3h, or left untreated (mock), permeabilized and immunostained with Stat3 antibody (green), phospho-Stat3 antibody (red) or DAPI. Scale bar, 50µm. **B)** HeLa cells were transfected with myc-YopM, Stat3-flag or Stat3Y705F-flag, permeabilized and immunostained with myc-antibody (green), flag- antibody (red) or DAPI. Scale bar, 50µm.

Stat3Y705F-flag expressed in HeLa cells is predominantly cytoplasmically localized (Figure 3.10B). Co-expression of dominant negative Stat3Y705F-flag and myc-YopM almost completely reversed the high level of nuclear localized Stat3 observed in control Stat3-flag transfected cells (Stat3-flag; Figure 3.10B). Thus, by expressing tagged YopM and Stat3 constructs in cells we can demonstrate that YopM increases Stat3 in the nucleus independent of phosphorylation at thyrosine residue 705.

In order to evaluate the interaction of YopM with unphosphorylated Stat3 in the nucleus we transfected HeLa cells with myc-YopM and Stat3-flag, fractionated cells and subjected lysates of the transfected cells to flag-immunoprecipitation using the MultiMACS Protein G Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Precipitates of the cytoplasmic

and nuclear fractions were tested for the presence of YopM, (phospho-)Stat3 and RSK by immunoblotting. In previous work it was demonstrated that YopM could form a ternary complex with RSK1 and PKN2 [116]. We demonstrate here, that in Stat3-flag immunoprecipitates, myc-YopM and Stat3 but not pStat3 or RSK were detected in the nuclear cell compartment, while in the cytoplasmic compartment phospho-Stat3 but no myc-YopM was detected (Figure 3.10). We conclude from these data that YopM can form a complex with unphosphorylated Stat3 but that YopM does not bring RSK and Stat3 together in the same complex. Thus, YopM directly interacts with unphosphorylated Stat3 in the nucleus of *Yersinia* infected host cells.

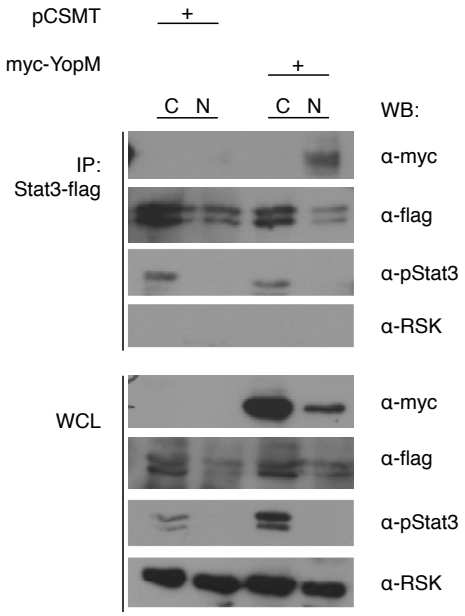

Figure 3.11: Myc-YopM co-immunoprecipitates with Stat3-flag.
Stat3-flag was immunoprecipitated in HEK293T cells expressing myc-YopM. HeLa cells expressing either empty vector pCS-MT (left panel) or myc-YopM (right panel) and Stat3-flag were lysed and subjected to anti-FLAG immunoprecipitation. Nuclear (N) and cytoplasmic (C) fractions of precipitates (IP) and whole cell lysates (WCL) were analyzed by Western blot using indicated antibodies.

Finally, the question arises which nuclear processes the nuclear localized YopM-Stat3 complex might regulate. While Stat3 is a master transcriptional factor involved in a broad spectrum of adaptive and innate immune functions, in the context of *Yersinia* infection we found it particularly compelling that Stat3 is a indispensable transcriptionfactor required for expression of IL10 [164]. Therefore we investigated whether the increased nuclear translocation of Stat3 is accompanied by an increased YopM-induced IL10 expression. For this, human macrophages from three different human donors were treated with siRNA

directed against Stat3 (siStat3) or a non-target siRNA directed against GAPDH (siNT, control) and were mock-infected or infected with WA314 for 6 h and investigated by quantitative RT-PCR (Figure 3.12). Increased transcription of IL10 in wild-type *Yersinia* infected cells was significantly decreased in cells treated with a pool of four different Stat3 specific siRNAs (Figure 3.12). In comparison, there was no significant difference between siNT- and siStat3-treated cells in mock-infected cells. These results suggest that YopM mediates nuclear translocation of Stat3 leads to expression of IL10.

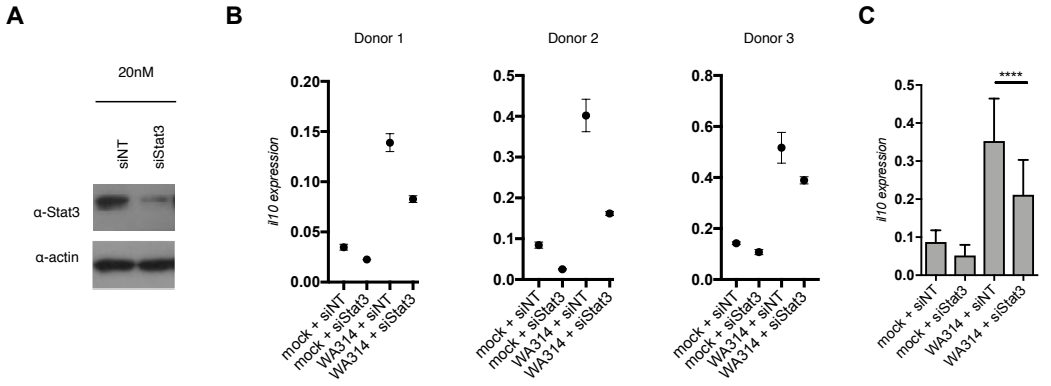

Figure 3.12: Transcription factor Stat3 mediates IL10 expression in wild-type infected macrophages.
A) Primary human macrophages were treated with siNT or siSTAT3 (20nM) and **B)** infected with *Yersinia* wild type WA314 for 6h after 48h or left untreated (mock), RNA isolated and analyzed by rtPCR with IL10 specific primers. **C)** The expression of IL10 from triplicates from 3 different donors was normalized to the expression of the housekeeper genes GAPDH, TBP and B2M. Shown are the mean values with SEM of n=3 independent experiments. ****p<0.0001.

In summary, examination of the cellular localization of Stat3 revealed that YopM induces translocation of unphosphorylated Stat3 to the nucleus, whilst IL10 transcriptional analysis suggests that Stat3 plays an important role in YopM-mediated expression of IL10 (Figure 3.12). We conclude that YopM mediates nuclear import independent of the canocical JAK-STAT signaling pathway. Altogether these results are consistent with the notion that nuclear levels of unphosphorylated Stat3 are controlled by YopM. Nuclear YopM directly interacts with Stat3 and thereby upregulates IL10 expression (Figure 3.12).

IV Discussion

The role of virulence factor YopM in *Yersinia*-induced gene expression changes is not fully understood, although YopM is the only virulence factor present in the host cell nucleus. Previously, the effect of YopM on host gene expression in mouse macrophage cell lines was analyzed using microarrays [189, 190].

The main objective of this study was to analyze in detail the effect of YopM on gene expression in primary human macrophages using RNA-seq and then specifying the involvement of cytokine IL10 and intracellular interaction partners of YopM in modulating gene expression.

1. *Yersinia* virulence factor YopM induces gene expression and secretion of IL10

Previous studies showed that IL10 was already upregulated by YopM after 1.5h of infection [115], which we could confirm with RT-PCR analysis. We could as well confirm the upregulation of IL10 expression by YopM after 6h of infection in RT-PCR (Figure 3.1) and RNAseq data generated from mRNA isolated from macrophages uninfected or infected with WA314 and WA314ΔYopM for 6h [115]. Studies on transcriptional regulation in HS60 cells treated with recombinant purified YopM state a minor effect of YopM on IL10 transcription [181]. This is contradictory to our results (Figure 3.1), as we observe a 3-fold upregulation of IL10 mRNA resulting also in elevated IL10 protein levels after 6 hours of infection (Figure 3.1). This upregulation reflects well the 4-fold increase of IL10 protein induced by YopM in *Y. pseudotuberculosis* infected mice [131, 157]. Thus, YopM stimulates gene expression, which is in clear contradiction to the general virulence strategy of *Yersinia*, which involves extensive suppression of immune response genes by the MAPK and NF-κB inhibitor YopP [189, 190]. RT-PCR analysis demonstrated that YopP is primarily responsible for inhibition of IL10 transcription in primary human macrophages (Figure 3.1C). These results were surprising as IL10 suppresses important immunoregulatory processes in the host cell during bacterial infection [224] and inhibition of anti-inflammatory IL10 would not benefit to promote virulence of *Yersinia enterocolitica*.

YopM and YopP counteract the expression of the immunoregulatory cytokine IL10 in an opposing way. However, it can be assumed that in the sum YopM and YopP more effectively manipulate host immune response contributing to virulence of *Yersinia*. Recently, an interplay of YopM and YopP to inhibit the inflammasome and IL1ß production in different ways was described [66]. Therefore, *Yersinia* must balance suppression of inflammation and

induction of specific genes including anti-inflammatory IL10. Further studies are needed to determine the details of the mode of action of the two Yops on the transcription of specific genes in the host cell.

2. YopM regulates RSK phosphorylation in macrophages

YopM induces sustained activation of RSK by blocking dephosphorylation of its activatory phosphorylation sites [117]. We re-evaluated RSK activation in primary human macrophages by determining phosphorylation on serine residue 380 (Figure 3.2A). Although the induction of RSK phosphorylation seems to be one common feature of YopM from different *Y. enterocolitica* [116, 117, 180], no direct downstream targets of phosphorylated RSK had been identified so far. RSKs are principally involved in the immunosuppressive action of YopM as demonstrated by a recent study showing that *Y. pseudotuberculosis* mutants expressing YopM proteins unable to interact with RSK1 are strongly reduced in virulence and suppression of IL-1β secretion by macrophages [65].

The well-accepted role in phosphorylating transcription factors and the position of the RSK-kinases in the nucleus could qualify RSK as a YopM target responsible for stimulation of transcription [115]. Consistent with this, inhibition of RSK activation by a pan-Rsk inhibitor that shuts down all four RSK isoforms at once attenuated the induction of IL10 expression of YopM ([197, 198]; (Figure 3.2E)). However, the exact mechanism behind the regulation of IL10 by RSK remains unclear. Recently, a RSK- and CREB dependent regulation of IL10 was described in BMDM exposed to products released by the parasite *S. mansoni* [215]. The parasite induces phosphorylation of RSK by activation of TLR- and the MEK/ERK/RSK-cascade and thereby was proposed to enhance CREB phosphorylation. This mode of activation as a consequence induced IL10 transcription [215]. Interestingly also the HIV-1 transactivator protein Tat has been shown to induce IL10 transcription macrophages by activating CREB, Sp1, and Ets-1 through MAPKs ERK1/2 and p38 [225].

A similar mechanism would be plausible for the YopM- and RSK dependent upregulation of IL10 expression in *Y. enterocolitica* WA314 infected macrophages [131], although hyperphosphorylation of RSK by YopM occurs in the absence of signaling from its upstream kinase ERK1/2 [117].

RSK phosphorylation interferes with transcription of genes encoding inflammatory mediators through activation of NF-κB and c-fos [226, 227], but exerts additional effects on transcription by phosphorylation of the chromosomal proteins histone H3 and histone acetyltransferase CBP (CREB-binding protein, [154]). For example, c-fos implicated in cell proliferation, differentiation, and development [228] is regulated by CREB targeted by RSK [227, 229].

Notably, microarray studies could not detect c-fos, but identified genes involved in cell-cycle regulation and cell to be regulated by YopM.
Future work should determine which exact RSK substrates are recruited to the IL10 promoter to mediate IL10 expression induced by YopM.

3. *Yersinia* modulates expression of IL10-inducible genes

We performed RNA-seq with *Yersinia* strains lacking YopM to determine whether this effector modulates *Yersinia*-induced gene expression. YopM is the only TTSS effector that translocates to the nucleus [121]. However, the nuclear function of YopM is not clear and we envisioned that YopM regulates gene expression via induction IL10. To elucidate the modulation of gene expression by the *Yersinia* virulence factor YopM primary human macrophages were mock-infected or infected with *Y. enterocolitica* strains WA314 (wild type), WAC (virulence plasmid-cured), or WA314ΔYopM (YopM deletion strain) for 6 h and subjected to RNA-Seq (Figures 3.4, 3.5). IL10 is significantly induced and produced by YopM after 6 h of infection (Figure 3.1). Thus, it can be concluded that by impling longer infection duration we were able to show expression changes dependent of IL10-modulation. Additionally the amount and activity of YopM is opposed to be sufficient after 6h of infection to comprehensively modulate signaling.
With this we were able to investigate whether IL10 contributes to gene expression changes induced by *Yersinia* in macrophages by comparing publicly available RNA-seq datasets from IL10-treated mononuclear cells [206] with the data from this study. About 5% of the 5698 DEGs up- or down-regulated by the different *Yersinia* strains compared to mock (Figure 3.4A, 3.5A) were correspondingly induced or suppressed by IL10 compared to naive adherent peripheral blood mononuclear cells [206]. This implies that YopM regulates gene expression in large part via IL10, but that other mechanisms are also involved that require further investigation.
It should be emphasized that we identified a much larger number of DEGs compared with previously performed microarray gene expression studies in murine macrophage lines J774A.1 or PU5–1.8, which identified only below 100 and 1000 DEGs, respectively [189, 190]. This difference can be due to a variety of factors. Firstly, the cell types employed show great differences in how they respond to stimulation [230]. In addition, RNA-seq is a highly sensitive unbiased method that allows studying the modulation of gene expression in detail. In contrast, microarray studies are dependent on the given sample number of the array [231]. Furthermore the infection time in the microarray studies was limited to 1-2.5h [189, 190], as mouse macrophages experience *Yersinia*-induced cell death early from 4h post-infection

[232, 233]. In contrast, cell death occurs much later after 18h in *Yersinia*-infected primary human macrophages [232, 233]. Therefore, we assume that cell death-induced expression changes do not play a role in our examination at the 6 h infection time used. However, the prolonged infection of physiological target cells also allowed us to identify the differential expression of genes modulated in IL10 signaling during Yersinia infection due to complex autocrine mechanisms and feedback loops [234].

4. *Yersinia* regulates IL10 related gene expression important for macrophage immune response

A clustering analysis of all IL10-induced DEGs (n=214) revealed specific profiles that determine gene expression in WA314 in relation to mock, WAC and WA314ΔYopM (Figure 3.4, 3.5). YopM clearly induced changes in gene expression compared to WA314 (Figure 3.3B). WA314 suppressed induction of gene expression by WAC in the upregulation profile, while YopM functions to counteract strong suppression and mantains expression of specific genes including IL10 at a certain level (Figure 3.4D). Genes from the upregulation profile were enriched for immune response (Figure 3.4D, Table 6.3), known to be induced by LPS and consistent with the known immunosuppression by *Yersinia* [99, 189, 190, 235]. With this in line, the upregulation profile was associated with other known inflammatory mediators from the RHD, STAT and AP-1 families [236] including inflammatory regulators NF-κB and CEBP:AP1 (Figure 3.4E), which promote inflammatory response and furthermore Stat4, which is essential for mediating responses to IL12 in lymphocytes and regulating the differentiation of T helper cells [237].

Interestingly by focusing on IL10-dependent gene expression we could provide evidence, that YopM from *Y. enterocolitica* systematically regulates transcription of genes involved in the JAK-STAT signaling pathway (Figure 3.4D; Table 6.3). Genes directly associated with the JAK-STAT cascade contained Jak3, IL10 and SOCS3. SOCS3 plays a major role in modulating the outcome of infections and autoimmune diseases by hampering JAK activation [238]. For example, *Salmonella* effector protein AvrA was identified to induce SOCS3 expression in vivo [239]. Furthermore, *Salmonella* induced SOCS3 expression in J774 cells through *Salmonella* pathogenicity island (SPI-2)-dependent activation of ERK1/2 to inhibit cytokine signaling via the JAK/STAT pathway [240]. So far only YopP was shown to be involved in modulation of the JAK-STAT pathway by inhibition of phosphorylation of key components of the MAPK and Tyk2–STAT4 pathways resulting in IL-12 and IL-18 induced IFN- γ production in NK cells [186]. But others questioned the impact of YopP on IFN-γ-

induced gene transcription downstream of the MAP kinase and/or JAK-STAT pathway in macrophages [190].

Other identified genes included PTPN2, PTX3 and IL7 that counteract to the regulation of the immunoregulatory JAK-STAT pathway. Negative regulators that determine the initiation, duration and termination of the JAK-STAT signaling cascade consist of SOCS proteins, protein inhibitors of activated STATs (PIAS) and Protein Tyrosine Phosphatases (PTPs) [162]. PTPN2 reduces the phosphorylation level of Stat3 at Tyr-705 [241, 242]. Constitutively activated Stat3 induces the production of PTX3 [243], which plays a critical role in inflammation and promotes disease pathogenesis in leishmaniasis [244]. IL7 induces activation of the JAK-STAT pathway [245] and YopM appears to promote IL7 signaling, as the IL7 receptor antagonist IL7RA is suppressed in WA314-infected cells (Table 6.4), whereas IL7 and the IL7 receptor (IL7R) are associated with the upregulation profile and induced by YopM (Table 6.3). Overall, these data suggest a very complex control of JAK-STAT signaling during Yersinia infection.

Upregulation profile was enriched for motif of TF Stat3, a key regulator of the JAK-STAT signaling cascade, which mediates the canonical activation by direct transcriptional induction of Stat3 target genes. IL10 requires Stat3 activation for its anti-inflammatory properties on macrophages, i.e. Stat3 and Jak1 are essential for IL10-dependent inhibition of TNF production by macrophages [156]. Stat3 promotes a potent anti-inflammatory response that is exploited by various pathogens. Various viral proteins interact with Stat3 to promote its phosphorylation in infected cells [246-249]. For example, *Toxoplasma gondii* targets Stat3 to prevent LPS-triggered proinflammatory cytokine production in infected mouse macrophages [250].

In summary this data suggests that virulent *Yersinia* specifically target TF Stat3 to modulate expression of Stat3 inducible genes.

On the other hand much less genes were found specifically downregulated by the anti-inflammatory function of IL10 (Figure 3.5A). By inhibiting the activity of Th1 cells, NK cells, and macrophages during infection IL10 maintains the balance of the immune response, allowing the clearance of infection while minimizing damage to the host [156]. Interestingly, the IL10 action is mostly indirect and requires the synthesis of intermediary proteins [251]. Stat3 has been shown to be essential for all known aspects of the IL10 anti-inflammatory pathway [169, 170, 252]. Thus, Stat3 activated by IL10 must act to target genes activated after TLR stimulation [251]. Several groups have searched for IL10-dependent genes that can execute the anti-inflammatory response [252, 253]. At the mRNA level, IL10 inhibited expression of a subset of inflammatory genes including COX2, IL1, IL8, IL6, IL12p40 and

TNF. Notably, most LPS-induced genes are unaffected by IL10 treatment, suggesting that IL10 cannot affect a basic process of gene expression [251].

Rather, IL10 is thought to act multifactorially via I) induction of transcriptional repressor expression II) posttranslation modification of the activity of transcription factors at specific promoters (e.g., NF-κB) or III) modification of chromatin environments to silence active inflammatory promoters [251]. Previous studies have suggested that IL10 can inhibit NF-κB activation resulting in the anti-inflammatory effects [254-256]. In contrast, others have proposed that IL10 has negligible effects on NF-κB activity [169, 172, 252]. Elevated IL10 levels in WA314-infected macrophages could affect TNF expression through complex autocrine mechanisms and feedback regulation leading to attenuation of TNF transcripts in the presence of YopM. Indeed, YopM-dependent downregulation of TNF expression has been reported in HS60 cells treated with recombinant YopM [181].

Nevertheless, we found IL10-related DEGs that were specifically downregulated during *Yersinia* infection in a YopM dependent manner assigned to the inhibition profile. Inhibition profile associated with GO term regulation of cytokine secretion included genes PTPN22 and LRRK2. The leucine-rich repeat kinase 2 (LRRK2) is important for the control of growing Gram-negative pathogens in the gut [216]. In macrophages lacking LRRK2 the increased susceptibility to *Salmonella* Typhimurium infection is associated with the reduced activation of the NLR family CARD domain-containing protein 4 (NLRC4) inflammasome and failure to establish an appropriate inflammatory response [216, 257]. Instead tyrosine phosphatase PTPN22 is multifunctional regulator of immune signaling and innate immune cell-mediated host defense that promotes host antiviral responses by interfering with TLR agonist-induced, type 1 IFN-dependent suppression of inflammation [258]. Thus YopM seems to balance suppression of inflammation by interfering with IL10 dependent immunoregulation.

Finally, some genes repressed by IL10 and regulated by YopM were found to be related to extracellular matrix degradation (ECM), i.e. MMP9 (Figure 3.5D; Table 6.4). Microbial pathogens can modify the ECM to evade immune responses and prevent pathogen recognition [259]. MMPs degrade collagen networks in extracellular matrices by cleaving collagen and thus enhance migration of mammalian cells [260]. MMP9 expression is dependent on activation of both transcription factors NF-κB and AP-1, therefore the observed downregulation of MMP9 by YopM could result from the previously described IL10-dependent inhibition of NF-κB activation [254-256]. Future studies will be necessary to confirm that transcriptional changes of ECM regulator genes correlate with a complex control of ECM degradation networks by *Yersinia* ([217]; (Figure 3.5D).

In summary, the *Yersinia* effector YopM targets genes from different biological pathways and comprehensively regulates macrophage gene expression to affect key signaling pathways of

the immune response. The upregulation profile includes transcriptional regulators that both promote and reduce inflammation, likely to ensure an effective response against infection, but also to limit the damage of excessive inflammation. Potential future studies should address how these signaling pathways play a role in *Yersinia* infection to modulate the immune response of macrophages.

5. YopM interacts with transcriptionfactor Stat3

We identified Stat3 as a new interaction partner of YopM by immunoprecipitation experiments with overexpressed Stat3 and YopM (Figure 3.10). However, we could not identify a triple complex containing Stat3, YopM and RSK. Infection with *Yersinia* strain WA314ΔYopM+YopMΔC showed that C-terminally truncated YopM is able to induce nuclear translocation of Stat3. On this account we conclude that the C-terminus is dispensable for the interaction of YopM and Stat3. Further studies should address the exact binding region of Stat that conveys the interaction with YopM from *Y.enterocolitica*.

We provide evidence that YopM mediates nuclear import of Stat3 (Figure 3.6). Phosphorylation of Stat3 on a single tyrosine residue in response to growth factors, cytokines and interferons activates its dimerization, translocation to the nucleus, binding to the INF-γ -activated sequence (GAS) DNA-binding site and activation of transcription of target genes [159, 164]. It remains uncertain how the nucler import of unphosphorylated Stat3 is facilitated in *Yersinia*-infected cells (Figure 3.9). Nuclear import of Stat3 is mediated by various importins (alpha 5, alpha 7, alpha 3) via Ran nuclear import pathway and importin-β1 [261, 262]. Interestingly, Stat3 nuclear import mediated by importin-α3 is independent of tyrosine phosphorylation [263, 264], while phosphorylated STATs are translocated to the nucleus in importin α-5 dependent manner [162]. Until now, neither a classical nuclear localization sequence (NLS) nor any importin had been identified that mediates the mechanisms behind YopMs nuclear entry, but the first three LRRs (LRR1-3) and the 32 C-terminal residues of YopM are described to be crucial for nuclear targeting [110, 119, 121, 122]. Thus, the molecular basis of YopM-induced Stat3 nuclear import is an interesting question that should be addressed in the future.

YopM has multiple interaction partners in cells including kinases of the RSK- and PKN/PRK families, the scaffolding protein IQGAP1, DDX3 and recently also caspase-1 [115, 116, 118]. Even though we excluded a role of YopM interaction partner RSK in the nuclear translocation of Stat3 by YopM (Figure 3.7), it remains a task for future work to decipher, which interaction partners may contribute. Taking into account that Rho GTPases are able to induce Stat3

activation independently of the IL6 autocrine pathway [265, 266], also modulation of Rho GTPase might contribute to mediate YopM induced translocation of nuclear Stat3.

In this study we show to our knowledge for the first time that a bacterial effectorprotein induces nuclear translocation of Stat3 independent of phosphorylation (Figure 3.8; 3.9 and 3.10) and suggest that unphosphorylated Stat3 (uStat3) has an important role in transcriptional regulation during *Yersinia* infection. Immunoprecipitation experiments of overexpressed Stat3 and YopM showed that YopM interacts with uStat3 only in the nucleus and not in the cytoplasm and does not form a complex together with the kinase RSK (Figure 3.11). UStat3 is targeted by several pathogens, i.e human cytomegalovirus (HCMV) infection inhibits Stat3 phosphorylation but promotes nuclear localization of uStat3 to disrupt IL6-induced gene expression [267]. UStat3 is believed to be dormant and reside in the cytoplasm [268]. However, several studies have shown that uStats can be found in the nucleus [268, 269] and are required for the constitutive expression of certain genes [270-272]. The mechanisms underlying uStat3 binding to DNA has not been fully investigated, but it is known that uStat3 binds to unphosphorylated NFκB (uNFκB) in competition with IκB. The resulting uStat3/uNFκB complex accumulates in the nucleus with guidance from the nuclear localization signal of Stat3 and activates a subset of κB-dependent genes [272]. UStat3, through direct interaction with p65, serves as a dominant-negative inhibitor to suppress the ability of pNFκB to induce cytokine-dependent activation of the iNOS promoter [273]. Stat3 and p65 physically interact *in vivo* and p65 homodimers can cooperate with uStat3 when bound to a specific type of κB motif [274].
In addition to its interactions with uNFκB, uStat3 has been described to bind to other transcription factors, including CRE-binding protein on the JunB promoter [275] and c-Jun on the α2-macrogloblin APRE (Acute Phase Response Element; [276]). Furthermore a Stat3 effect on CRE-like sites in the C/EBPβ promoter was described [277]. Less than half of the genes that respond to high-level expression of uStat3 respond also to TNF. The uStat3-responsive genes that do not respond to TNF probably have no functional κB elements, and do not require p65 to respond to uStat3 [271]. Therefore, it is extremely likely that uStat3 interacts with other transcription factors besides NF-κB to control expression [271].
Because siRNA-mediated knockdown of Stat3 reduces levels of IL10 expression in *Yersinia enterocolitca* infected macrophages, we conclude that YopM adjusts the level of nuclear uStat3 to regulate gene expression (Figure 3.12). A potential mode of Stat3 activation by YopM might be lysine acetylation as an important regulatory modification of Stat3 [196] or improvement of chromatin accessibility [278]. Stat3 possesses noncanonical function of JAK-STAT signaling in promoting heterochromatin formation and directly controls heterochromatin

stability by interacting with heterochromatin protein 1 (HP1) [268, 269], therefore uStat3 regulates gene expression by influencing chromatin organization [218]. This indicates that the JAK-STAT pathway also controls the epigenetic cell status, which affects expression of genes beyond those under direct Stat3 transcriptional control, which might be targeted by *Yersinia* effector YopM. Of note, uStat3 regulated genes, i.e. BATF were upregulated in WA314-infected cells. In line with our results, published reports have shown that binding of BATF-IRF4 complexes to DNA mediate chromatin accessibility to facilate binding of Stat3 to distinct promoter regions [278].

In the noncanonical model, uStat3 is consistently found as a result of constant nuclear import and export [279, 280]. Furthermore the expression of uStat3 is greatly increased in response to Stat3 activation, since the Stat3 gene expression is strongly activated by pStat3 dimers [271]. With this in line, transcriptom analysis revealed elevated expression levels of Stat3 in WA314 infected cells dependent on YopM [115], resulting in eminent endogenous Stat3 levels in nuclear and cytoplasmic fractions of WA314 infected cells (Figure 3.9A). Full biological relevance of the ability of pStat3 to increase the intracellular concentration of uStat3 remains to be established [270, 271]. Future experiments are clearly necessary to elucidate in detail if upregulation of Stat3 by YopM in infected cells might be a consequence of sequestering uStat3 in the nucleus.

V Summary

Injection of Yersinia outer proteins (Yops) into macrophages via the bacterial type-III-secretion system is essential for virulence of pathogenic *Yersinia* species including *Y. pestis, Y. enterocolitica* and *Y. pseudotuberculosis*. Yops effectively suppress innate immune responses in infected macrophages to promote pathogenesis. Although the eminent role of the effector YopM for *Yersinia* virulence has been related to the subversion of cytokine gene expression and –production, the molecular and cellular mechanisms underlying these YopM activities are not well understood.

The aim of this study was to investigate the effect of YopM on the global transcriptional response of primary human macrophages. YopM increased the level of Interleukin-10 (IL10) mRNA dependent on activation of cellular kinase RSK by YopM. With RNA-seq analysis, we found a stringed pattern of IL0-dependent gene expression regulated by YopM. JAK-STAT signaling was among the most highly activated pathways triggered by wild type *Yersinia* after 6h of infection. Interestingly, we found Stat3-target genes (i.e. IL10, SOCS3) upregulated by YopM. Furthermore we could demonstrate that YopM promotes nuclear translocation of Stat3 in the absence of robust phosphorylation on tyrosine residue 705 to induce IL10 expression. Thus, our study points to an unanticipated mechanism involving transcriptionfactor Stat3, anti-inflammatory cytokine IL10 and related JAK-STAT signaling by which *Yersinia* affects the function of macrophages.

We provide evidence that *Y. enterocolitica* tightly controls transcriptional regulation belonging to JAK-STAT signaling pathway, in that YopM upregulates expression of IL10. This regulation is dependent on the phosphorylation of the host interaction partner RSK and nuclear translocation of transcriptionfactor Stat3 (Figure 5.1).

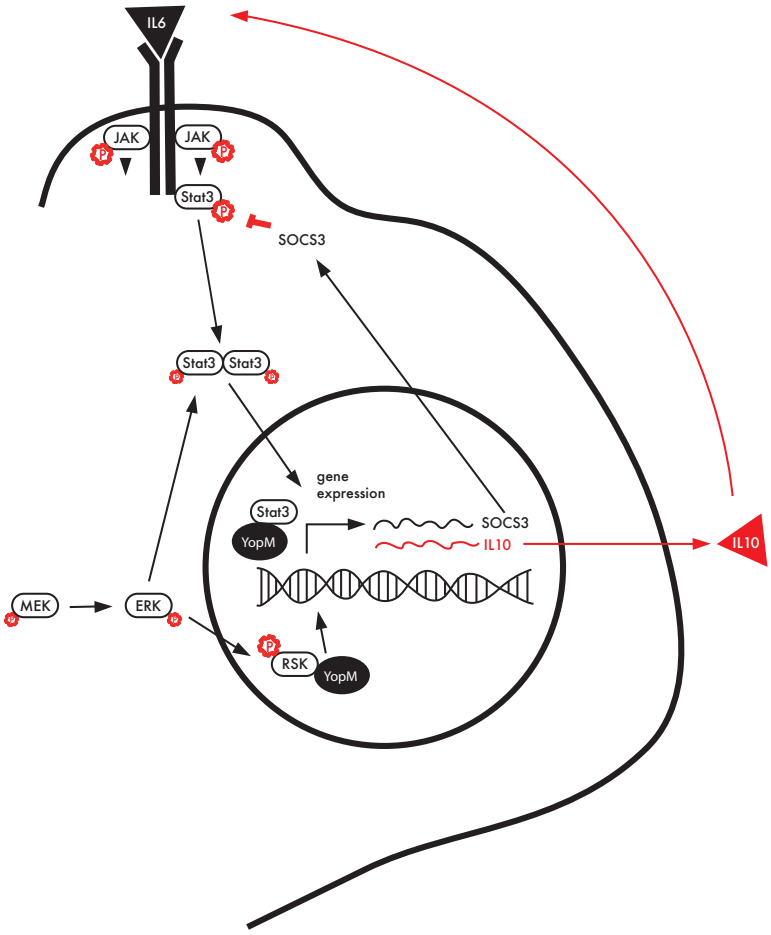

Figure 5.1: Model of YopMs nuclear-cytoplasmatic shuttling and transcriptional IL10 regulation
Cytokines like IL6 and IL10 activate JAK-STAT pathway and trigger phosphorylation, dimerization and nuclear translocation of Stat3. YopM induces nuclear translocation of Stat3 independent of phosphorylation at Tyr-705 to modulate Stat3-dependent gene expression and subsequent production of IL10. The activity of YopM on RSK phosphorylation counteracts signaling to induce expression of IL10.

VI Zusammenfassung

Enteropathogene Yersinien verfügen über ein Typ-III-Sekretionssystem mit dem sie *Yersinia* outer proteins (Yops) in Zielzellen, wie dendritische Zellen oder Makrophagen, translozieren. Yops sind essentiell zur Vermittlung der Virulenz bei humanpathogen Yersinien (*Y. pestis, Y. enterocolitica, Y. pseudotuberculosis*) und beeinflussen Signalwege in der Wirtszelle um die initiale Immunantwort des Wirtes zu unterdrücken. Obwohl die eminente Rolle des Effektors YopM für die Virulenz von Yersinien mit der Regulation der Genexpression und Produktion von Zytokinen in Verbindung gebracht wurde, sind die molekularen und zellulären Mechanismen, die diesen YopM-Aktivitäten zugrunde liegen unzureichend verstanden.

In der vorliegenden Arbeit sollte der Einfluss von YopM auf die globale transkriptionelle Antwort von *Yersinia*-infizierten Makrophagen untersucht werden. Es wurde gezeigt, dass die Kinase RSK eine entscheidende Rolle in der YopM-vermittelten Induktion der Interleukin-10-Expression spielt. Mittels RNA-seq-Analyse wurde ein stringentes Muster der IL0-abhängigen Expression von Genen indentifiziert, das mit dem JAK-STAT Signalweg assoziiert ist und durch YopM reguliert wird. Interessanterweise sind Stat3-responsive Gene, einschließlich IL10 und SOCS3 durch YopM spezifisch hochreguliert. In diesem Zusammenhang konnte gezeigt werden, dass YopM die Translokation von Stat3 in den Zellkern unabhängig von einer robusten Phosphorylierung vermittelt, um die Expression von IL10 zu induzieren.

Zusammenfassend zeigt die vorliegende Studie, dass Y. enterocolitica die zum JAK-STAT-Signalweg gehörende Transkription wirksam reguliert. YopM bewirkt eine erhöhte Expression von IL10 über eine gesteigerte Aktivierung der Kinase RSK und über die nukleäre Translokation des Transkriptionsfaktors Stat3 (Figure 5.1).

VII Supplementary material

Table 6.1: Selected pathways associated with IL10-induced genes

KEGG Term	P-Value	Genes
JAK-STAT signaling	0.009	**WAC:** IL10, IL4R, IL7, IL7R, JAK3, PDGFA, PIM1, PTPN2, SOCS1, SOCS3
	0.009	**WA314:** IL10, IL12RB2, IL7, JAK3, PIM1, PTPN2, SOCS1, SOCS3
	0.10	**WA314ΔYopM:** IL7, PIM1, SOCS1, SOS1
FoxO signaling	0.009	**WAC:** BCL2L11, BCL6, CDK2, CDKN2D, GADD45B, IL10, IL7R, PLK3, S1PR1
	0.096	**WA314ΔYopM:** BCL2L11, CDKN2D, GADD45B, SOS1
Pathways in cancer	0.017	**WAC:** BCL2L11, BRCA2, CDK2, DLL1, ETS1, GADD45B, GNB4, GNG12, IL4R, IL7, IL7R, JAK3, LAMB3, PDGFA, PIM1, RALA,
	0.02	RASGRP2, VEGFA
	0.17	**WA314:** BCL2L11, BRCA2, DLL1, ETS1, GADD45B, GNG12, IL12RB2, IL7, JAK3, PIM1
		WA314ΔYopM: BCL2L11, GADD45B, IL7, PIM1, RASGRP2, SOS1
Osteoclast differentiation	0.02	**WAC:** FOSL2, LCP2, LILRB1/2/3/4, SOCS1, SOCS3

Table 6.2: Summary of IL10-induced genes with gene expression changes

Class R1 Genes (17): BLK, MS4A1, RARRES1, CDC42EP5, NOL3, TLE1, RALGPS2, SOS1, BCL2L11, CDKN1C, TLR8, CD79B, SPIRE2, CXCL13, MAP3K1, ACE, STMN3
Class R2 Genes (161): GPR85, LDLRAD3, DUSP4, STARD8, C1orf21, LCP2, LONRF1, HCAR3, MAP4K4, SGTB, ARL13B, BCL10, FMNL2, NFKBIZ, KLF6, SRGAP1, BRCA2, CD24, MYO10, GADD45B, RALA, TSHZ3, ADM, FERMT2, QKI, THAP2, EBF1, BCL6, TIFA, S1PR1, VPS9D1, SOCS1, HIVEP2, RAB33A, CDCP1, CPEB4, PAG1, RBMS1, ACTN1, LOC101926963, KLF4, RHOB, LINC01215, SP140, PSD3, RIMKLB, CEMIP, PDGFA, STAP1, PLSCR1, GRB10, IFITM3, IFITM2, USP6NL, CCNYL1, SGMS1, ACSL1, BASP1, LYN, MARCKS, PKIG, CD44, CD274, CD80, PIM1, MYO1G, FAM129A, ADA, IL7, LBH, KREMEN1, LINC01000, GK, PLK3, CCM2L, CEBPD, ANXA3, ENPP2, IL7R, IL4R, STK3, BCL2A1, TNIP3, PTPN2, PTX3, FSD1L, ETS1, EHD1, SLAMF1, SLCO4A1, LILRB1, G0S2, IL10, SOCS3, GAS6, NDST2, LRP12, SBNO2, RNF149, SLC37A3, JAK3, LIMK2, BATF, BLVRA, FPGS, CDYL2, VAMP5, DLL1, NMRK1, ADAM19, STOM, DNAJB4, CXCL1, HECTD2, ZHX2, TP53INP2, HIST1H2BC, LILRB4, SLC41A2, VEGFA, LPCAT1, CDK2, NIT1, ABI1, SERPINB9, GPR137B, NAMPT, DRAM1, PFKFB3, FOSL2, SNX9, FLOT1, AQP9, FAM110B, CD55, LILRB3, ELL3, CCL18, HLX, PPP1R3B, SLC2A3, CHSY1, SLC7A5, UGCG, LINC00884, NEDD4, ACSL4, AGO2, TMEM106A, C2CD4B, PLEKHG2, CREM, ELMO2, LILRB2, ETS2, METRNL, FCGR1B, SLC39A8, NABP1, ADGRE2, LAMB3
Class R3 Genes (36): IL12RB2, MRVI1, SPATA6, ASAP2, FFAR2, PNMA2, SETBP1, C1RL, OSBPL10, SMIM, CBS, PSAT1, PPP1R16B, HMGN5, TLCD2, MAP3K8, RRAS2, JADE3, FAM114A1, MXD1, KANK2, RASGRP2, RASSF4, FAM20A, PAX5, FKBP1B, MUC1, RNF217, AMIGO2, CETP, CASP5, GNG12, GNB4, HEG1, CDKN2D, CHI3L2

Table 6.3: Upregulation profile genes and selected pathways associated with gene expression changes of IL10-induced genes

Profile: **Upregulation** GO Term: **GO:1903037 regulation of leucocyte cell-cell adhesion** P-value: **1.34E-12** Genes (**23**): ADA, BCL10, BCL6, CD24, CD274, CD44, CD55, CD80, ETS1, HLX, IL10, IL4R, IL7, IL7R, JAK3, KLF, LILRB1, LILRB4, LYN, NFKBIZ, PAG1, PTPN2, SOCS1
GO Term: **GO:1903037 immune response** P-value: **5.45E-12** Genes (**53**): ABI1, ADA, ADGRE2, ANXA3, AQP9, BATF, BCL10, BCL6, CCL18, CD24, CD274, CD44, CD55, CD80, CXCL1, DLL1, ELMO2, ENPP2, ETS1, FCGR1B, FLOT1, GAS6, HLX, IFITM2, IFITM3, IL10, IL4R, IL7, IL7R, JAK3, LCP2, LILRB1, LILRB4, LYN, MYO10, MYO1G, NEDD4, NFKBIZ, PAG1, PLSCR1, PTPN2, PTX3, SBNO2, SERPINB9, SLAMF1, SLC2A3, SOCS1, SOCS3, STAP1, STOM, TIFA, TNIP3
KEGG Term: **hsa04630 JAK-STAT signaling pathway** P-value: **0.0044** Genes (**10**): IL10, IL4R, IL7, IL7R, JAK3, PDGFA, PIM1, PTPN2, SOCS1, SOCS3
KEGG Term: **hsa04380 Osteoclast differentiation** P-value: **0.0157** Genes (**8**): FOSL2, LCP2, LILRB1, LILRB2, LILRB3, LILRB4, SOCS1, SOCS3

Table 6.4: Summary of IL10-suppressed genes with gene expression changes

Class R1 Genes (7): CLEC5A, DHRS9, HLA-DOA, **MMP9,** CYSLTR1, FCN1, PID1
Class R2 Genes (42): LMTK2, CD300LB, PC, RNASE6, CYP27A1, MPPE1, NUDT6, LFNG, ABCC3, PCYOX1, CD1D, **SPARC**, ZBED3, HDDC3, FAM120C, C12orf66, OSGEPL1, ASB13, ABHD10, FAM78A, ATP8A1, MYLK, C2CD2, CFAP44, FAXDC2, AGPAT5, IL27RA, LRRK2, MYCL, PRAM1, AKAP1, IL17RA, RASAL1, PDGFC, TRIM16, DEPTOR, MARVELD1, TRERF1, TSC22D1-AS1, OLIG1, DPEP2, FAM117B
Class R3 Genes (34): KCNJ2, A2M, PTGFRN, COLEC12, ADGRE3, OLFM1, TRIT1, PTPN22, TNFSF14, TIAM1, TNFRSF21, MPHOSPH9, SRSF8, APEX1, LOC100507642, FXN

Table 6.5: Inhibition profile genes associated with gene expression changes of IL10-suppressed genes

Profile: **Inhibition** **GO Term:** GO:0050707 regulation of cytokine secretion **P-value:** 0.041 Genes (**7**): CRTAM, IL17RA, LPL, LRRK2, PTPN22, TNFRSF21, TRIM16

VIII References

1. Wolke, S., N. Ackermann, and J. Heesemann, *The Yersinia enterocolitica type 3 secretion system (T3SS) as toolbox for studying the cell biological effects of bacterial Rho GTPase modulating T3SS effector proteins.* Cell Microbiol, 2011. **13**(9): p. 1339-57.
2. Heesemann, J. and M. Aepfelbacher, *The fight between cells and bacteria: modulation of eukaryotic signalling by bacterial pathogens.* Int J Med Microbiol, 2001. **291**(4): p. 241-2.
3. Drancourt, M. and D. Raoult, *Molecular insights into the history of plague.* Microbes Infect, 2002. **4**(1): p. 105-9.
4. Bibel, D.J. and T.H. Chen, *Diagnosis of plaque: an analysis of the Yersin-Kitasato controversy.* Bacteriol Rev, 1976. **40**(3): p. 633-51.
5. Perry, R.D. and J.D. Fetherston, *Yersinia pestis--etiologic agent of plague.* Clin Microbiol Rev, 1997. **10**(1): p. 35-66.
6. Bottone, E.J., *Yersinia enterocolitica: the charisma continues.* Clin Microbiol Rev, 1997. **10**(2): p. 257-76.
7. Grahek-Ogden, D., B. Schimmer, K.S. Cudjoe, K. Nygard, and G. Kapperud, *Outbreak of Yersinia enterocolitica serogroup O:9 infection and processed pork, Norway.* Emerg Infect Dis, 2007. **13**(5): p. 754-6.
8. Jalava, K., M. Hakkinen, M. Valkonen, U.M. Nakari, T. Palo, S. Hallanvuo, J. Ollgren, A. Siitonen, and J.P. Nuorti, *An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with Yersinia pseudotuberculosis.* J Infect Dis, 2006. **194**(9): p. 1209-16.
9. Butt, H.L., D.L. Gordon, T. Lee-Archer, A. Moritz, and W.H. Merrell, *Relationship between clinical and milk isolates of Yersinia enterocolitica.* Pathology, 1991. **23**(2): p. 153-7.
10. Nesbakken, T., T. Iversen, and B. Lium, *Pig herds free from human pathogenic Yersinia enterocolitica.* Emerg Infect Dis, 2007. **13**(12): p. 1860-4.
11. Koch-Institut“, R., *Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2019*. 2020, Berlin.
12. Lian, C.J., W.S. Hwang, J.K. Kelly, and C.H. Pai, *Invasiveness of Yersinia enterocolitica lacking the virulence plasmid: an in-vivo study.* J Med Microbiol, 1987. **24**(3): p. 219-26.
13. Lian, C.J., W.S. Hwang, J.K. Kelly, and C.H. Pai, *Penetration of the intestinal mucosa by Yersinia enterocolitica lacking the virulence plasmid.* Contrib Microbiol Immunol, 1987. **9**: p. 239-42.
14. Trulzsch, K., M.F. Oellerich, and J. Heesemann, *Invasion and dissemination of Yersinia enterocolitica in the mouse infection model.* Adv Exp Med Biol, 2007. **603**: p. 279-85.
15. Wauters, G., K. Kandolo, and M. Janssens, *Revised biogrouping scheme of Yersinia enterocolitica.* Contrib Microbiol Immunol, 1987. **9**: p. 14-21.
16. Heesemann, J. and R. Laufs, *Construction of a mobilizable Yersinia enterocolitica virulence plasmid.* J Bacteriol, 1983. **155**(2): p. 761-7.
17. Lee, L.A., A.R. Gerber, D.R. Lonsway, J.D. Smith, G.P. Carter, N.D. Puhr, C.M. Parrish, R.K. Sikes, R.J. Finton, and R.V. Tauxe, *Yersinia enterocolitica O:3 infections in infants and children, associated with the household preparation of chitterlings.* N Engl J Med, 1990. **322**(14): p. 984-7.
18. Lee, L.A., J. Taylor, G.P. Carter, B. Quinn, J.J. Farmer, 3rd, and R.V. Tauxe, *Yersinia enterocolitica O:3: an emerging cause of pediatric gastroenteritis in the United States. The Yersinia enterocolitica Collaborative Study Group.* J Infect Dis, 1991. **163**(3): p. 660-3.
19. Chlebicz, A. and K. Slizewska, *Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A Review.* Int J Environ Res Public Health, 2018. **15**(5).
20. Fredriksson-Ahomaa, M., A. Stolle, and H. Korkeala, *Molecular epidemiology of Yersinia enterocolitica infections.* FEMS Immunol Med Microbiol, 2006. **47**(3): p. 315-29.
21. Vantrappen, G., K. Geboes, and E. Ponette, *Yersinia enteritis.* Med Clin North Am, 1982. **66**(3): p. 639-53.
22. Laitenen, O., J. Tuuhea, and P. Ahvonen, *Polyarthritis associated with Yersinia enterocolitica infection. Clinical features and laboratory findings in nine cases with severe joint symptoms.* Ann Rheum Dis, 1972. **31**(1): p. 34-9.
23. Blei, F. and D.R. Puder, *Yersinia enterocolitica bacteremia in a chronically transfused patient with sickle cell anemia. Case report and review of the literature.* Am J Pediatr Hematol Oncol, 1993. **15**(4): p. 430-4.

24. Viboud, G.I. and J.B. Bliska, *Yersinia outer proteins: role in modulation of host cell signaling responses and pathogenesis.* Annu Rev Microbiol, 2005. **59**: p. 69-89.
25. Clark, M.A., B.H. Hirst, and M.A. Jepson, *M-cell surface beta1 integrin expression and invasin-mediated targeting of Yersinia pseudotuberculosis to mouse Peyer's patch M cells.* Infect Immun, 1998. **66**(3): p. 1237-43.
26. Forman, S., C.R. Wulff, T. Myers-Morales, C. Cowan, R.D. Perry, and S.C. Straley, *yadBC of Yersinia pestis, a new virulence determinant for bubonic plague.* Infect Immun, 2008. **76**(2): p. 578-87.
27. Isberg, R.R., D.L. Voorhis, and S. Falkow, *Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells.* Cell, 1987. **50**(5): p. 769-78.
28. Mikula, K.M., R. Kolodziejczyk, and A. Goldman, *Yersinia infection tools-characterization of structure and function of adhesins.* Front Cell Infect Microbiol, 2012. **2**: p. 169.
29. Miller, V.L., K.B. Beer, G. Heusipp, B.M. Young, and M.R. Wachtel, *Identification of regions of Ail required for the invasion and serum resistance phenotypes.* Mol Microbiol, 2001. **41**(5): p. 1053-62.
30. Miller, V.L., J.B. Bliska, and S. Falkow, *Nucleotide sequence of the Yersinia enterocolitica ail gene and characterization of the Ail protein product.* J Bacteriol, 1990. **172**(2): p. 1062-9.
31. Bliska, J.B., J.E. Galan, and S. Falkow, *Signal transduction in the mammalian cell during bacterial attachment and entry.* Cell, 1993. **73**(5): p. 903-20.
32. Felek, S. and E.S. Krukonis, *The Yersinia pestis Ail protein mediates binding and Yop delivery to host cells required for plague virulence.* Infect Immun, 2009. **77**(2): p. 825-36.
33. Michiels, T. and G.R. Cornelis, *Secretion of hybrid proteins by the Yersinia Yop export system.* J Bacteriol, 1991. **173**(5): p. 1677-85.
34. Mota, L.J. and G.R. Cornelis, *The bacterial injection kit: type III secretion systems.* Ann Med, 2005. **37**(4): p. 234-49.
35. Paczosa, M.K., M.L. Fisher, F.J. Maldonado-Arocho, and J. Mecsas, *Yersinia pseudotuberculosis uses Ail and YadA to circumvent neutrophils by directing Yop translocation during lung infection.* Cell Microbiol, 2014. **16**(2): p. 247-68.
36. Straley, S.C., *Adhesins in Yersinia pestis.* Trends Microbiol, 1993. **1**(8): p. 285-6.
37. Tsang, T.M., S. Felek, and E.S. Krukonis, *Ail binding to fibronectin facilitates Yersinia pestis binding to host cells and Yop delivery.* Infect Immun, 2010. **78**(8): p. 3358-68.
38. Tsang, T.M., J.S. Wiese, S. Felek, M. Kronshage, and E.S. Krukonis, *Ail proteins of Yersinia pestis and Y. pseudotuberculosis have different cell binding and invasion activities.* PLoS One, 2013. **8**(12): p. e83621.
39. Eitel, J. and P. Dersch, *The YadA protein of Yersinia pseudotuberculosis mediates high-efficiency uptake into human cells under environmental conditions in which invasin is repressed.* Infect Immun, 2002. **70**(9): p. 4880-91.
40. Bliska, J.B. and S. Falkow, *Bacterial resistance to complement killing mediated by the Ail protein of Yersinia enterocolitica.* Proc Natl Acad Sci U S A, 1992. **89**(8): p. 3561-5.
41. Cover, T.L. and R.C. Aber, *Yersinia enterocolitica.* N Engl J Med, 1989. **321**(1): p. 16-24.
42. Amirmozafari, N. and D.C. Robertson, *Nutritional requirements for synthesis of heat-stable enterotoxin by Yersinia enterocolitica.* Appl Environ Microbiol, 1993. **59**(10): p. 3314-20.
43. Straley, S.C. and R.D. Perry, *Environmental modulation of gene expression and pathogenesis in Yersinia.* Trends Microbiol, 1995. **3**(8): p. 310-7.
44. Carniel, E., I. Guilvout, and M. Prentice, *Characterization of a large chromosomal "high-pathogenicity island" in biotype 1B Yersinia enterocolitica.* J Bacteriol, 1996. **178**(23): p. 6743-51.
45. Heesemann, J., K. Hantke, T. Vocke, E. Saken, A. Rakin, I. Stojiljkovic, and R. Berner, *Virulence of Yersinia enterocolitica is closely associated with siderophore production, expression of an iron-repressible outer membrane polypeptide of 65,000 Da and pesticin sensitivity.* Mol Microbiol, 1993. **8**(2): p. 397-408.
46. Simonet, M. and S. Falkow, *Invasin expression in Yersinia pseudotuberculosis.* Infect Immun, 1992. **60**(10): p. 4414-7.
47. Revell, P.A. and V.L. Miller, *Yersinia virulence: more than a plasmid.* FEMS Microbiol Lett, 2001. **205**(2): p. 159-64.
48. Pierson, D.E. and S. Falkow, *The ail gene of Yersinia enterocolitica has a role in the ability of the organism to survive serum killing.* Infect Immun, 1993. **61**(5): p. 1846-52.
49. Dewoody, R.S., P.M. Merritt, and M.M. Marketon, *Regulation of the Yersinia type III secretion system: traffic control.* Front Cell Infect Microbiol, 2013. **3**: p. 4.

50. Nauth, T., F. Huschka, M. Schweizer, J.B. Bosse, A. Diepold, A.V. Failla, A. Steffen, T.E.B. Stradal, M. Wolters, and M. Aepfelbacher, *Visualization of translocons in Yersinia type III protein secretion machines during host cell infection.* PLoS Pathog, 2018. **14**(12): p. e1007527.
51. Cornelis, G.R., A. Boland, A.P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M.P. Sory, and I. Stainier, *The virulence plasmid of Yersinia, an antihost genome.* Microbiol Mol Biol Rev, 1998. **62**(4): p. 1315-52.
52. Wagner, S., I. Grin, S. Malmsheimer, N. Singh, C.E. Torres-Vargas, and S. Westerhausen, *Bacterial type III secretion systems: a complex device for the delivery of bacterial effector proteins into eukaryotic host cells.* FEMS Microbiol Lett, 2018. **365**(19).
53. Cornelis, G.R., *Yersinia type III secretion: send in the effectors.* J Cell Biol, 2002. **158**(3): p. 401-8.
54. Brubaker, R.R., *Interleukin-10 and inhibition of innate immunity to Yersiniae: roles of Yops and LcrV (V antigen).* Infect Immun, 2003. **71**(7): p. 3673-81.
55. Aepfelbacher, M., C. Trasak, and K. Ruckdeschel, *Effector functions of pathogenic Yersinia species.* Thromb Haemost, 2007. **98**(3): p. 521-9.
56. Aepfelbacher, M. and J. Heesemann, *Modulation of Rho GTPases and the actin cytoskeleton by Yersinia outer proteins (Yops).* Int J Med Microbiol, 2001. **291**(4): p. 269-76.
57. Viboud, G.I., E. Mejia, and J.B. Bliska, *Comparison of YopE and YopT activities in counteracting host signalling responses to Yersinia pseudotuberculosis infection.* Cell Microbiol, 2006. **8**(9): p. 1504-15.
58. Aepfelbacher, M. and M. Wolters, *Acting on Actin: Rac and Rho Played by Yersinia.* Curr Top Microbiol Immunol, 2017. **399**: p. 201-220.
59. Black, D.S. and J.B. Bliska, *The RhoGAP activity of the Yersinia pseudotuberculosis cytotoxin YopE is required for antiphagocytic function and virulence.* Mol Microbiol, 2000. **37**(3): p. 515-27.
60. Rosqvist, R., A. Forsberg, M. Rimpilainen, T. Bergman, and H. Wolf-Watz, *The cytotoxic protein YopE of Yersinia obstructs the primary host defence.* Mol Microbiol, 1990. **4**(4): p. 657-67.
61. Trulzsch, K., T. Sporleder, E.I. Igwe, H. Russmann, and J. Heesemann, *Contribution of the major secreted yops of Yersinia enterocolitica O:8 to pathogenicity in the mouse infection model.* Infect Immun, 2004. **72**(9): p. 5227-34.
62. Galyov, E.E., S. Hakansson, A. Forsberg, and H. Wolf-Watz, *A secreted protein kinase of Yersinia pseudotuberculosis is an indispensable virulence determinant.* Nature, 1993. **361**(6414): p. 730-2.
63. Logsdon, L.K. and J. Mecsas, *Requirement of the Yersinia pseudotuberculosis effectors YopH and YopE in colonization and persistence in intestinal and lymph tissues.* Infect Immun, 2003. **71**(8): p. 4595-607.
64. Dave, M.N., J.E. Silva, R.J. Elicabe, M.B. Jerez, V.P. Filippa, C.V. Gorlino, S. Autenrieth, I.B. Autenrieth, and M.S. Di Genaro, *Yersinia enterocolitica YopH-Deficient Strain Activates Neutrophil Recruitment to Peyer's Patches and Promotes Clearance of the Virulent Strain.* Infect Immun, 2016. **84**(11): p. 3172-3181.
65. Chung, L.K., N.H. Philip, V.A. Schmidt, A. Koller, T. Strowig, R.A. Flavell, I.E. Brodsky, and J.B. Bliska, *IQGAP1 is important for activation of caspase-1 in macrophages and is targeted by Yersinia pestis type III effector YopM.* MBio, 2014. **5**(4): p. e01402-14.
66. Ratner, D., M.P. Orning, K.K. Starheim, R. Marty-Roix, M.K. Proulx, J.D. Goguen, and E. Lien, *Manipulation of interleukin-1beta and interleukin-18 production by Yersinia pestis effectors YopJ and YopM and redundant impact on virulence.* J Biol Chem, 2016. **291**(31): p. 16417.
67. Schotte, P., G. Denecker, A. Van Den Broeke, P. Vandenabeele, G.R. Cornelis, and R. Beyaert, *Targeting Rac1 by the Yersinia effector protein YopE inhibits caspase-1-mediated maturation and release of interleukin-1beta.* J Biol Chem, 2004. **279**(24): p. 25134-42.
68. Iriarte, M. and G.R. Cornelis, *YopT, a new Yersinia Yop effector protein, affects the cytoskeleton of host cells.* Mol Microbiol, 1998. **29**(3): p. 915-29.
69. Shao, F., P.M. Merritt, Z. Bao, R.W. Innes, and J.E. Dixon, *A Yersinia effector and a Pseudomonas avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis.* Cell, 2002. **109**(5): p. 575-88.
70. Hakansson, S., E.E. Galyov, R. Rosqvist, and H. Wolf-Watz, *The Yersinia YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane.* Mol Microbiol, 1996. **20**(3): p. 593-603.

71. Juris, S.J., A.E. Rudolph, D. Huddler, K. Orth, and J.E. Dixon, *A distinctive role for the Yersinia protein kinase: actin binding, kinase activation, and cytoskeleton disruption.* Proc Natl Acad Sci U S A, 2000. **97**(17): p. 9431-6.
72. Trasak, C., G. Zenner, A. Vogel, G. Yuksekdag, R. Rost, I. Haase, M. Fischer, L. Israel, A. Imhof, S. Linder, M. Schleicher, and M. Aepfelbacher, *Yersinia protein kinase YopO is activated by a novel G-actin binding process.* J Biol Chem, 2007. **282**(4): p. 2268-77.
73. Dukuzumuremyi, J.M., R. Rosqvist, B. Hallberg, B. Akerstrom, H. Wolf-Watz, and K. Schesser, *The Yersinia protein kinase A is a host factor inducible RhoA/Rac-binding virulence factor.* J Biol Chem, 2000. **275**(45): p. 35281-90.
74. Groves, E., K. Rittinger, M. Amstutz, S. Berry, D.W. Holden, G.R. Cornelis, and E. Caron, *Sequestering of Rac by the Yersinia effector YopO blocks Fcgamma receptor-mediated phagocytosis.* J Biol Chem, 2010. **285**(6): p. 4087-4098.
75. Prehna, G., M.I. Ivanov, J.B. Bliska, and C.E. Stebbins, *Yersinia virulence depends on mimicry of host Rho-family nucleotide dissociation inhibitors.* Cell, 2006. **126**(5): p. 869-80.
76. Guan, K.L. and J.E. Dixon, *Protein tyrosine phosphatase activity of an essential virulence determinant in Yersinia.* Science, 1990. **249**(4968): p. 553-6.
77. Fallman, M., K. Andersson, S. Hakansson, K.E. Magnusson, O. Stendahl, and H. Wolf-Watz, *Yersinia pseudotuberculosis inhibits Fc receptor-mediated phagocytosis in J774 cells.* Infect Immun, 1995. **63**(8): p. 3117-24.
78. Grabowski, B., M.A. Schmidt, and C. Ruter, *Immunomodulatory Yersinia outer proteins (Yops)-useful tools for bacteria and humans alike.* Virulence, 2017. **8**(7): p. 1124-1147.
79. Ruckdeschel, K., A. Roggenkamp, S. Schubert, and J. Heesemann, *Differential contribution of Yersinia enterocolitica virulence factors to evasion of microbicidal action of neutrophils.* Infect Immun, 1996. **64**(3): p. 724-33.
80. Yao, T., J. Mecsas, J.I. Healy, S. Falkow, and Y. Chien, *Suppression of T and B lymphocyte activation by a Yersinia pseudotuberculosis virulence factor, yopH.* J Exp Med, 1999. **190**(9): p. 1343-50.
81. Bliska, J.B. and D.S. Black, *Inhibition of the Fc receptor-mediated oxidative burst in macrophages by the Yersinia pseudotuberculosis tyrosine phosphatase.* Infect Immun, 1995. **63**(2): p. 681-5.
82. Rolan, H.G., E.A. Durand, and J. Mecsas, *Identifying Yersinia YopH-targeted signal transduction pathways that impair neutrophil responses during in vivo murine infection.* Cell Host Microbe, 2013. **14**(3): p. 306-17.
83. Dewoody, R., P.M. Merritt, and M.M. Marketon, *YopK controls both rate and fidelity of Yop translocation.* Mol Microbiol, 2013. **87**(2): p. 301-17.
84. Zwack, E.E., A.G. Snyder, M.A. Wynosky-Dolfi, G. Ruthel, N.H. Philip, M.M. Marketon, M.S. Francis, J.B. Bliska, and I.E. Brodsky, *Inflammasome activation in response to the Yersinia type III secretion system requires hyperinjection of translocon proteins YopB and YopD.* mBio, 2015. **6**(1): p. e02095-14.
85. Zwack, E.E., E.M. Feeley, A.R. Burton, B. Hu, M. Yamamoto, T.D. Kanneganti, J.B. Bliska, J. Coers, and I.E. Brodsky, *Guanylate Binding Proteins Regulate Inflammasome Activation in Response to Hyperinjected Yersinia Translocon Components.* Infect Immun, 2017. **85**(10).
86. Brodsky, I.E., N.W. Palm, S. Sadanand, M.B. Ryndak, F.S. Sutterwala, R.A. Flavell, J.B. Bliska, and R. Medzhitov, *A Yersinia effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system.* Cell Host Microbe, 2010. **7**(5): p. 376-87.
87. Meinzer, U., F. Barreau, S. Esmiol-Welterlin, C. Jung, C. Villard, T. Leger, S. Ben-Mkaddem, D. Berrebi, M. Dussaillant, Z. Alnabhani, M. Roy, S. Bonacorsi, H. Wolf-Watz, J. Perroy, V. Ollendorff, and J.P. Hugot, *Yersinia pseudotuberculosis effector YopJ subverts the Nod2/RICK/TAK1 pathway and activates caspase-1 to induce intestinal barrier dysfunction.* Cell Host Microbe, 2012. **11**(4): p. 337-51.
88. Mittal, R., S.Y. Peak-Chew, and H.T. McMahon, *Acetylation of MEK2 and I kappa B kinase (IKK) activation loop residues by YopJ inhibits signaling.* Proc Natl Acad Sci U S A, 2006. **103**(49): p. 18574-9.
89. Mittal, R., S.Y. Peak-Chew, R.S. Sade, Y. Vallis, and H.T. McMahon, *The acetyltransferase activity of the bacterial toxin YopJ of Yersinia is activated by eukaryotic host cell inositol hexakisphosphate.* J Biol Chem, 2010. **285**(26): p. 19927-34.
90. Mukherjee, S., G. Keitany, Y. Li, Y. Wang, H.L. Ball, E.J. Goldsmith, and K. Orth, *Yersinia YopJ acetylates and inhibits kinase activation by blocking phosphorylation.* Science, 2006. **312**(5777): p. 1211-4.

91. Paquette, N., J. Conlon, C. Sweet, F. Rus, L. Wilson, A. Pereira, C.V. Rosadini, N. Goutagny, A.N. Weber, W.S. Lane, S.A. Shaffer, S. Maniatis, K.A. Fitzgerald, L. Stuart, and N. Silverman, *Serine/threonine acetylation of TGFbeta-activated kinase (TAK1) by Yersinia pestis YopJ inhibits innate immune signaling.* Proc Natl Acad Sci U S A, 2012. **109**(31): p. 12710-5.
92. Orth, K., L.E. Palmer, Z.Q. Bao, S. Stewart, A.E. Rudolph, J.B. Bliska, and J.E. Dixon, *Inhibition of the mitogen-activated protein kinase kinase superfamily by a Yersinia effector.* Science, 1999. **285**(5435): p. 1920-3.
93. Denecker, G., S. Totemeyer, L.J. Mota, P. Troisfontaines, I. Lambermont, C. Youta, I. Stainier, M. Ackermann, and G.R. Cornelis, *Effect of low- and high-virulence Yersinia enterocolitica strains on the inflammatory response of human umbilical vein endothelial cells.* Infect Immun, 2002. **70**(7): p. 3510-20.
94. Schesser, K., A.K. Spiik, J.M. Dukuzumuremyi, M.F. Neurath, S. Pettersson, and H. Wolf-Watz, *The yopJ locus is required for Yersinia-mediated inhibition of NF-kappaB activation and cytokine expression: YopJ contains a eukaryotic SH2-like domain that is essential for its repressive activity.* Mol Microbiol, 1998. **28**(6): p. 1067-79.
95. Ruckdeschel, K., J. Machold, A. Roggenkamp, S. Schubert, J. Pierre, R. Zumbihl, J.P. Liautard, J. Heesemann, and B. Rouot, *Yersinia enterocolitica promotes deactivation of macrophage mitogen-activated protein kinases extracellular signal-regulated kinase-1/2, p38, and c-Jun NH2-terminal kinase. Correlation with its inhibitory effect on tumor necrosis factor-alpha production.* J Biol Chem, 1997. **272**(25): p. 15920-7.
96. Monack, D.M., J. Mecsas, N. Ghori, and S. Falkow, *Yersinia signals macrophages to undergo apoptosis and YopJ is necessary for this cell death.* Proc Natl Acad Sci U S A, 1997. **94**(19): p. 10385-90.
97. Ruckdeschel, K., S. Harb, A. Roggenkamp, M. Hornef, R. Zumbihl, S. Kohler, J. Heesemann, and B. Rouot, *Yersinia enterocolitica impairs activation of transcription factor NF-kappaB: involvement in the induction of programmed cell death and in the suppression of the macrophage tumor necrosis factor alpha production.* J Exp Med, 1998. **187**(7): p. 1069-79.
98. Boland, A. and G.R. Cornelis, *Role of YopP in suppression of tumor necrosis factor alpha release by macrophages during Yersinia infection.* Infect Immun, 1998. **66**(5): p. 1878-84.
99. Schubert, K.A., Y. Xu, F. Shao, and V. Auerbuch, *The Yersinia Type III Secretion System as a Tool for Studying Cytosolic Innate Immune Surveillance.* Annu Rev Microbiol, 2020. **74**: p. 221-245.
100. Weng, D., R. Marty-Roix, S. Ganesan, M.K. Proulx, G.I. Vladimer, W.J. Kaiser, E.S. Mocarski, K. Pouliot, F.K. Chan, M.A. Kelliher, P.A. Harris, J. Bertin, P.J. Gough, D.M. Shayakhmetov, J.D. Goguen, K.A. Fitzgerald, N. Silverman, and E. Lien, *Caspase-8 and RIP kinases regulate bacteria-induced innate immune responses and cell death.* Proc Natl Acad Sci U S A, 2014. **111**(20): p. 7391-6.
101. Philip, N.H., C.P. Dillon, A.G. Snyder, P. Fitzgerald, M.A. Wynosky-Dolfi, E.E. Zwack, B. Hu, L. Fitzgerald, E.A. Mauldin, A.M. Copenhaver, S. Shin, L. Wei, M. Parker, J. Zhang, A. Oberst, D.R. Green, and I.E. Brodsky, *Caspase-8 mediates caspase-1 processing and innate immune defense in response to bacterial blockade of NF-kappaB and MAPK signaling.* Proc Natl Acad Sci U S A, 2014. **111**(20): p. 7385-90.
102. Orning, P., D. Weng, K. Starheim, D. Ratner, Z. Best, B. Lee, A. Brooks, S. Xia, H. Wu, M.A. Kelliher, S.B. Berger, P.J. Gough, J. Bertin, M.M. Proulx, J.D. Goguen, N. Kayagaki, K.A. Fitzgerald, and E. Lien, *Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death.* Science, 2018. **362**(6418): p. 1064-1069.
103. Lilo, S., Y. Zheng, and J.B. Bliska, *Caspase-1 activation in macrophages infected with Yersinia pestis KIM requires the type III secretion system effector YopJ.* Infect Immun, 2008. **76**(9): p. 3911-23.
104. Schoberle, T.J., L.K. Chung, J.B. McPhee, B. Bogin, and J.B. Bliska, *Uncovering an Important Role for YopJ in the Inhibition of Caspase-1 in Activated Macrophages and Promoting Yersinia pseudotuberculosis Virulence.* Infect Immun, 2016. **84**(4): p. 1062-1072.
105. Brodsky, I.E. and R. Medzhitov, *Reduced secretion of YopJ by Yersinia limits in vivo cell death but enhances bacterial virulence.* PLoS Pathog, 2008. **4**(5): p. e1000067.
106. Philip, N.H. and I.E. Brodsky, *Cell death programs in Yersinia immunity and pathogenesis.* Front Cell Infect Microbiol, 2012. **2**: p. 149.
107. Peterson, L.W., N.H. Philip, A. DeLaney, M.A. Wynosky-Dolfi, K. Asklof, F. Gray, R. Choa, E. Bjanes, E.L. Buza, B. Hu, C.P. Dillon, D.R. Green, S.B. Berger, P.J. Gough, J. Bertin, and I.E.

Brodsky, *RIPK1-dependent apoptosis bypasses pathogen blockade of innate signaling to promote immune defense.* J Exp Med, 2017. **214**(11): p. 3171-3182.
108. Evdokimov, A.G., D.E. Anderson, K.M. Routzahn, and D.S. Waugh, *Overproduction, purification, crystallization and preliminary X-ray diffraction analysis of YopM, an essential virulence factor extruded by the plague bacterium Yersinia pestis.* Acta Crystallogr D Biol Crystallogr, 2000. **56**(Pt 12): p. 1676-9.
109. Evdokimov, A.G., D.E. Anderson, K.M. Routzahn, and D.S. Waugh, *Unusual molecular architecture of the Yersinia pestis cytotoxin YopM: a leucine-rich repeat protein with the shortest repeating unit.* J Mol Biol, 2001. **312**(4): p. 807-21.
110. Benabdillah, R., L.J. Mota, S. Lutzelschwab, E. Demoinet, and G.R. Cornelis, *Identification of a nuclear targeting signal in YopM from Yersinia spp.* Microb Pathog, 2004. **36**(5): p. 247-61.
111. McCoy, M.W., M.L. Marre, C.F. Lesser, and J. Mecsas, *The C-terminal tail of Yersinia pseudotuberculosis YopM is critical for interacting with RSK1 and for virulence.* Infect Immun, 2010. **78**(6): p. 2584-98.
112. Kerschen, E.J., D.A. Cohen, A.M. Kaplan, and S.C. Straley, *The plague virulence protein YopM targets the innate immune response by causing a global depletion of NK cells.* Infect Immun, 2004. **72**(8): p. 4589-602.
113. Oberhettinger, P., M. Schutz, G. Raddatz, H. Keller, I.B. Autenrieth, and D. Linke, *The sequence of the pYV virulence plasmid from Yersinia enterocolitica strain WA-314 biogroup 1B serotype O:8.* Plasmid, 2011. **65**(1): p. 20-4.
114. Ng, A. and R.J. Xavier, *Leucine-rich repeat (LRR) proteins: integrators of pattern recognition and signaling in immunity.* Autophagy, 2011. **7**(9): p. 1082-4.
115. Berneking, L., M. Schnapp, A. Rumm, C. Trasak, K. Ruckdeschel, M. Alawi, A. Grundhoff, A.G. Kikhney, F. Koch-Nolte, F. Buck, M. Perbandt, C. Betzel, D.I. Svergun, M. Hentschke, and M. Aepfelbacher, *Immunosuppressive Yersinia Effector YopM Binds DEAD Box Helicase DDX3 to Control Ribosomal S6 Kinase in the Nucleus of Host Cells.* PLoS Pathog, 2016. **12**(6): p. e1005660.
116. McDonald, C., P.O. Vacratsis, J.B. Bliska, and J.E. Dixon, *The yersinia virulence factor YopM forms a novel protein complex with two cellular kinases.* J Biol Chem, 2003. **278**(20): p. 18514-23.
117. Hentschke, M., L. Berneking, C. Belmar Campos, F. Buck, K. Ruckdeschel, and M. Aepfelbacher, *Yersinia virulence factor YopM induces sustained RSK activation by interfering with dephosphorylation.* PLoS One, 2010. **5**(10).
118. LaRock, C.N. and B.T. Cookson, *The Yersinia virulence effector YopM binds caspase-1 to arrest inflammasome assembly and processing.* Cell Host Microbe, 2012. **12**(6): p. 799-805.
119. Skrzypek, E. and S.C. Straley, *Interaction between Yersinia pestis YopM protein and human alpha-thrombin.* Thromb Res, 1996. **84**(1): p. 33-43.
120. Boland, A., M.P. Sory, M. Iriarte, C. Kerbourch, P. Wattiau, and G.R. Cornelis, *Status of YopM and YopN in the Yersinia Yop virulon: YopM of Y.enterocolitica is internalized inside the cytosol of PU5-1.8 macrophages by the YopB, D, N delivery apparatus.* EMBO J, 1996. **15**(19): p. 5191-201.
121. Skrzypek, E., C. Cowan, and S.C. Straley, *Targeting of the Yersinia pestis YopM protein into HeLa cells and intracellular trafficking to the nucleus.* Mol Microbiol, 1998. **30**(5): p. 1051-65.
122. Scharnert, J., L. Greune, D. Zeuschner, M.L. Lubos, M. Alexander Schmidt, and C. Ruter, *Autonomous translocation and intracellular trafficking of the cell-penetrating and immune-suppressive effector protein YopM.* Cell Mol Life Sci, 2013. **70**(24): p. 4809-23.
123. Skrzypek, E., T. Myers-Morales, S.W. Whiteheart, and S.C. Straley, *Application of a Saccharomyces cerevisiae model to study requirements for trafficking of Yersinia pestis YopM in eucaryotic cells.* Infect Immun, 2003. **71**(2): p. 937-47.
124. Schnappauf, O., J.J. Chae, D.L. Kastner, and I. Aksentijevich, *The Pyrin Inflammasome in Health and Disease.* Front Immunol, 2019. **10**: p. 1745.
125. Chung, L.K., Y.H. Park, Y. Zheng, I.E. Brodsky, P. Hearing, D.L. Kastner, J.J. Chae, and J.B. Bliska, *The Yersinia Virulence Factor YopM Hijacks Host Kinases to Inhibit Type III Effector-Triggered Activation of the Pyrin Inflammasome.* Cell Host Microbe, 2016. **20**(3): p. 296-306.
126. Medici, N.P., M. Rashid, and J.B. Bliska, *Characterization of Pyrin Dephosphorylation and Inflammasome Activation in Macrophages as Triggered by the Yersinia Effectors YopE and YopT.* Infect Immun, 2019. **87**(3).
127. Loeven, N.A., N.P. Medici, and J.B. Bliska, *The pyrin inflammasome in host-microbe interactions.* Curr Opin Microbiol, 2020. **54**: p. 77-86.

128. Malik, H.S. and J.B. Bliska, *The pyrin inflammasome and the Yersinia effector interaction.* Immunol Rev, 2020. **297**(1): p. 96-107.
129. Leung, K.Y., B.S. Reisner, and S.C. Straley, *YopM inhibits platelet aggregation and is necessary for virulence of Yersinia pestis in mice.* Infect Immun, 1990. **58**(10): p. 3262-71.
130. Trulzsch, K., A. Roggenkamp, M. Aepfelbacher, G. Wilharm, K. Ruckdeschel, and J. Heesemann, *Analysis of chaperone-dependent Yop secretion/translocation and effector function using a mini-virulence plasmid of Yersinia enterocolitica.* Int J Med Microbiol, 2003. **293**(2-3): p. 167-77.
131. McPhee, J.B., P. Mena, and J.B. Bliska, *Delineation of regions of the Yersinia YopM protein required for interaction with the RSK1 and PRK2 host kinases and their requirement for interleukin-10 production and virulence.* Infect Immun, 2010. **78**(8): p. 3529-39.
132. Ye, Z., E.J. Kerschen, D.A. Cohen, A.M. Kaplan, N. van Rooijen, and S.C. Straley, *Gr1+ cells control growth of YopM-negative yersinia pestis during systemic plague.* Infect Immun, 2009. **77**(9): p. 3791-806.
133. Ye, Z., A.M. Uittenbogaard, D.A. Cohen, A.M. Kaplan, J. Ambati, and S.C. Straley, *Distinct CCR2(+) Gr1(+) cells control growth of the Yersinia pestis DeltayopM mutant in liver and spleen during systemic plague.* Infect Immun, 2011. **79**(2): p. 674-87.
134. Zeniou, M., T. Ding, E. Trivier, and A. Hanauer, *Expression analysis of RSK gene family members: the RSK2 gene, mutated in Coffin-Lowry syndrome, is prominently expressed in brain structures essential for cognitive function and learning.* Hum Mol Genet, 2002. **11**(23): p. 2929-40.
135. Dummler, B.A., C. Hauge, J. Silber, H.G. Yntema, L.S. Kruse, B. Kofoed, B.A. Hemmings, D.R. Alessi, and M. Frodin, *Functional characterization of human RSK4, a new 90-kDa ribosomal S6 kinase, reveals constitutive activation in most cell types.* J Biol Chem, 2005. **280**(14): p. 13304-14.
136. Romeo, Y., X. Zhang, and P.P. Roux, *Regulation and function of the RSK family of protein kinases.* Biochem J, 2012. **441**(2): p. 553-69.
137. Bjorbaek, C., Y. Zhao, and D.E. Moller, *Divergent functional roles for p90rsk kinase domains.* J Biol Chem, 1995. **270**(32): p. 18848-52.
138. Dalby, K.N., N. Morrice, F.B. Caudwell, J. Avruch, and P. Cohen, *Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90rsk that are inducible by MAPK.* J Biol Chem, 1998. **273**(3): p. 1496-505.
139. Frodin, M. and S. Gammeltoft, *Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction.* Mol Cell Endocrinol, 1999. **151**(1-2): p. 65-77.
140. Roux, P.P., S.A. Richards, and J. Blenis, *Phosphorylation of p90 ribosomal S6 kinase (RSK) regulates extracellular signal-regulated kinase docking and RSK activity.* Mol Cell Biol, 2003. **23**(14): p. 4796-804.
141. Anjum, R. and J. Blenis, *The RSK family of kinases: emerging roles in cellular signalling.* Nat Rev Mol Cell Biol, 2008. **9**(10): p. 747-58.
142. Joel, P.B., J. Smith, T.W. Sturgill, T.L. Fisher, J. Blenis, and D.A. Lannigan, *pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167.* Mol Cell Biol, 1998. **18**(4): p. 1978-84.
143. Ginty, D.D., A. Bonni, and M.E. Greenberg, *Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-fos transcription via phosphorylation of CREB.* Cell, 1994. **77**(5): p. 713-25.
144. Xing, J., D.D. Ginty, and M.E. Greenberg, *Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase.* Science, 1996. **273**(5277): p. 959-63.
145. Nakajima, T., A. Fukamizu, J. Takahashi, F.H. Gage, T. Fisher, J. Blenis, and M.R. Montminy, *The signal-dependent coactivator CBP is a nuclear target for pp90RSK.* Cell, 1996. **86**(3): p. 465-74.
146. Blenis, J., *Signal transduction via the MAP kinases: proceed at your own RSK.* Proc Natl Acad Sci U S A, 1993. **90**(13): p. 5889-92.
147. DiDonato, J.A., F. Mercurio, and M. Karin, *Phosphorylation of I kappa B alpha precedes but is not sufficient for its dissociation from NF-kappa B.* Mol Cell Biol, 1995. **15**(3): p. 1302-11.
148. Hoesel, B. and J.A. Schmid, *The complexity of NF-kappaB signaling in inflammation and cancer.* Mol Cancer, 2013. **12**: p. 86.
149. Oeckinghaus, A. and S. Ghosh, *The NF-kappaB family of transcription factors and its regulation.* Cold Spring Harb Perspect Biol, 2009. **1**(4): p. a000034.

150. Kawai, T. and S. Akira, *Toll-like receptor and RIG-I-like receptor signaling.* Ann N Y Acad Sci, 2008. **1143**: p. 1-20.
151. Kawasaki, T. and T. Kawai, *Toll-like receptor signaling pathways.* Front Immunol, 2014. **5**: p. 461.
152. Park, B.S. and J.O. Lee, *Recognition of lipopolysaccharide pattern by TLR4 complexes.* Exp Mol Med, 2013. **45**: p. e66.
153. Pandey, S., T. Kawai, and S. Akira, *Microbial sensing by Toll-like receptors and intracellular nucleic acid sensors.* Cold Spring Harb Perspect Biol, 2014. **7**(1): p. a016246.
154. Cargnello, M. and P.P. Roux, *Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases.* Microbiol Mol Biol Rev, 2011. **75**(1): p. 50-83.
155. Grutz, G., *New insights into the molecular mechanism of interleukin-10-mediated immunosuppression.* J Leukoc Biol, 2005. **77**(1): p. 3-15.
156. Riley, J.K., K. Takeda, S. Akira, and R.D. Schreiber, *Interleukin-10 receptor signaling through the JAK-STAT pathway. Requirement for two distinct receptor-derived signals for anti-inflammatory action.* J Biol Chem, 1999. **274**(23): p. 16513-21.
157. McPhee, J.B., P. Mena, Y. Zhang, and J.B. Bliska, *Interleukin-10 induction is an important virulence function of the Yersinia pseudotuberculosis type III effector YopM.* Infect Immun, 2012. **80**(7): p. 2519-27.
158. Imada, K. and W.J. Leonard, *The Jak-STAT pathway.* Mol Immunol, 2000. **37**(1-2): p. 1-11.
159. Stark, G.R. and J.E. Darnell, Jr., *The JAK-STAT pathway at twenty.* Immunity, 2012. **36**(4): p. 503-14.
160. Carey, A.J., C.K. Tan, and G.C. Ulett, *Infection-induced IL-10 and JAK-STAT: A review of the molecular circuitry controlling immune hyperactivity in response to pathogenic microbes.* JAKSTAT, 2012. **1**(3): p. 159-67.
161. !!! INVALID CITATION !!! {Carey, 2012 #1196}.
162. Seif, F., M. Khoshmirsafa, H. Aazami, M. Mohsenzadegan, G. Sedighi, and M. Bahar, *The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells.* Cell Commun Signal, 2017. **15**(1): p. 23.
163. Murray, P.J., *The JAK-STAT signaling pathway: input and output integration.* J Immunol, 2007. **178**(5): p. 2623-9.
164. Darnell, J.E., Jr., I.M. Kerr, and G.R. Stark, *Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins.* Science, 1994. **264**(5164): p. 1415-21.
165. Boulton, T.G., Z. Zhong, Z. Wen, J.E. Darnell, Jr., N. Stahl, and G.D. Yancopoulos, *STAT3 activation by cytokines utilizing gp130 and related transducers involves a secondary modification requiring an H7-sensitive kinase.* Proc Natl Acad Sci U S A, 1995. **92**(15): p. 6915-9.
166. Shen, Y., K. Schlessinger, X. Zhu, E. Meffre, F. Quimby, D.E. Levy, and J.E. Darnell, Jr., *Essential role of STAT3 in postnatal survival and growth revealed by mice lacking STAT3 serine 727 phosphorylation.* Mol Cell Biol, 2004. **24**(1): p. 407-19.
167. Wen, Z. and J.E. Darnell, Jr., *Mapping of Stat3 serine phosphorylation to a single residue (727) and evidence that serine phosphorylation has no influence on DNA binding of Stat1 and Stat3.* Nucleic Acids Res, 1997. **25**(11): p. 2062-7.
168. Wen, Z., Z. Zhong, and J.E. Darnell, Jr., *Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation.* Cell, 1995. **82**(2): p. 241-50.
169. Williams, L., L. Bradley, A. Smith, and B. Foxwell, *Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages.* J Immunol, 2004. **172**(1): p. 567-76.
170. Takeda, K., B.E. Clausen, T. Kaisho, T. Tsujimura, N. Terada, I. Forster, and S. Akira, *Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils.* Immunity, 1999. **10**(1): p. 39-49.
171. El Kasmi, K.C., A.M. Smith, L. Williams, G. Neale, A.D. Panopoulos, S.S. Watowich, H. Hacker, B.M. Foxwell, and P.J. Murray, *Cutting edge: A transcriptional repressor and corepressor induced by the STAT3-regulated anti-inflammatory signaling pathway.* J Immunol, 2007. **179**(11): p. 7215-9.
172. Donnelly, R.P., H. Dickensheets, and D.S. Finbloom, *The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes.* J Interferon Cytokine Res, 1999. **19**(6): p. 563-73.
173. Yoshimura, A., T. Naka, and M. Kubo, *SOCS proteins, cytokine signalling and immune regulation.* Nat Rev Immunol, 2007. **7**(6): p. 454-65.

174. Berlato, C., M.A. Cassatella, I. Kinjyo, L. Gatto, A. Yoshimura, and F. Bazzoni, *Involvement of suppressor of cytokine signaling-3 as a mediator of the inhibitory effects of IL-10 on lipopolysaccharide-induced macrophage activation.* J Immunol, 2002. **168**(12): p. 6404-11.
175. Jenkins, B.J., *Transcriptional regulation of pattern recognition receptors by Jak/STAT signaling, and the implications for disease pathogenesis.* J Interferon Cytokine Res, 2014. **34**(10): p. 750-8.
176. Zhang, L., M. Mei, C. Yu, W. Shen, L. Ma, J. He, and L. Yi, *The Functions of Effector Proteins in Yersinia Virulence.* Pol J Microbiol, 2016. **65**(1): p. 5-12.
177. Nakajima, R. and R.R. Brubaker, *Association between virulence of Yersinia pestis and suppression of gamma interferon and tumor necrosis factor alpha.* Infect Immun, 1993. **61**(1): p. 23-31.
178. Ruter, J., S.J. Antonia, H.A. Burris, R.D. Huhn, and R.H. Vonderheide, *Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors.* Cancer Biol Ther, 2010. **10**(10): p. 983-93.
179. Uittenbogaard, A.M., R.L. Chelvarajan, T. Myers-Morales, A.A. Gorman, W.J. Brickey, Z. Ye, A.M. Kaplan, D.A. Cohen, J.P. Ting, and S.C. Straley, *Toward a molecular pathogenic pathway for Yersinia pestis YopM.* Front Cell Infect Microbiol, 2012. **2**: p. 155.
180. Hofling, S., J. Scharnert, C. Cromme, J. Bertrand, T. Pap, M.A. Schmidt, and C. Ruter, *Manipulation of pro-inflammatory cytokine production by the bacterial cell-penetrating effector protein YopM is independent of its interaction with host cell kinases RSK1 and PRK2.* Virulence, 2014. **5**(7): p. 761-71.
181. Ruter, C., C. Buss, J. Scharnert, G. Heusipp, and M.A. Schmidt, *A newly identified bacterial cell-penetrating peptide that reduces the transcription of pro-inflammatory cytokines.* J Cell Sci, 2010. **123**(Pt 13): p. 2190-8.
182. Auerbuch, V. and R.R. Isberg, *Growth of Yersinia pseudotuberculosis in mice occurs independently of Toll-like receptor 2 expression and induction of interleukin-10.* Infect Immun, 2007. **75**(7): p. 3561-70.
183. Zhang, Y., A.T. Ting, K.B. Marcu, and J.B. Bliska, *Inhibition of MAPK and NF-kappa B pathways is necessary for rapid apoptosis in macrophages infected with Yersinia.* J Immunol, 2005. **174**(12): p. 7939-49.
184. Nuss, A.M., M. Beckstette, M. Pimenova, C. Schmuhl, W. Opitz, F. Pisano, A.K. Heroven, and P. Dersch, *Tissue dual RNA-seq allows fast discovery of infection-specific functions and riboregulators shaping host-pathogen transcriptomes.* Proc Natl Acad Sci U S A, 2017. **114**(5): p. E791-E800.
185. Bohn, E., S. Muller, J. Lauber, R. Geffers, N. Speer, C. Spieth, J. Krejci, B. Manncke, J. Buer, A. Zell, and I.B. Autenrieth, *Gene expression patterns of epithelial cells modulated by pathogenicity factors of Yersinia enterocolitica.* Cell Microbiol, 2004. **6**(2): p. 129-41.
186. Koch, I., K. Dach, J. Heesemann, and R. Hoffmann, *Yersinia enterocolitica inactivates NK cells.* Int J Med Microbiol, 2013. **303**(8): p. 433-42.
187. Subrahmanyam, Y.V., S. Yamaga, Y. Prashar, H.H. Lee, N.P. Hoe, Y. Kluger, M. Gerstein, J.D. Goguen, P.E. Newburger, and S.M. Weissman, *RNA expression patterns change dramatically in human neutrophils exposed to bacteria.* Blood, 2001. **97**(8): p. 2457-68.
188. Auerbuch, V., D.T. Golenbock, and R.R. Isberg, *Innate immune recognition of Yersinia pseudotuberculosis type III secretion.* PLoS Pathog, 2009. **5**(12): p. e1000686.
189. Sauvonnet, N., B. Pradet-Balade, J.A. Garcia-Sanz, and G.R. Cornelis, *Regulation of mRNA expression in macrophages after Yersinia enterocolitica infection. Role of different Yop effectors.* J Biol Chem, 2002. **277**(28): p. 25133-42.
190. Hoffmann, R., K. van Erp, K. Trulzsch, and J. Heesemann, *Transcriptional responses of murine macrophages to infection with Yersinia enterocolitica.* Cell Microbiol, 2004. **6**(4): p. 377-90.
191. Lamkanfi, M. and V.M. Dixit, *Modulation of inflammasome pathways by bacterial and viral pathogens.* J Immunol, 2011. **187**(2): p. 597-602.
192. Sahoo, M., I. Ceballos-Olvera, L. del Barrio, and F. Re, *Role of the inflammasome, IL-1beta, and IL-18 in bacterial infections.* ScientificWorldJournal, 2011. **11**: p. 2037-50.
193. Bekere, I., *Modulation of gene expression and epigenetic modifications in human macrophages by Yersinia enterocolitica*. 2021.
194. Schnapp, M., *Characterization of novel interaction partners of the Yersinia enterocolitica effector protein YopM and their role in macrophage cytokine expression*. 2016.

195. Kopp, P., R. Lammers, M. Aepfelbacher, G. Woehlke, T. Rudel, N. Machuy, W. Steffen, and S. Linder, *The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages.* Mol Biol Cell, 2006. **17**(6): p. 2811-23.
196. Dasgupta, M., H. Unal, B. Willard, J. Yang, S.S. Karnik, and G.R. Stark, *Critical role for lysine 685 in gene expression mediated by transcription factor unphosphorylated STAT3.* J Biol Chem, 2014. **289**(44): p. 30763-71.
197. Davies, A.H., K. Reipas, K. Hu, R. Berns, N. Firmino, A.L. Stratford, and S.E. Dunn, *Inhibition of RSK with the novel small-molecule inhibitor LJI308 overcomes chemoresistance by eliminating cancer stem cells.* Oncotarget, 2015. **6**(24): p. 20570-7.
198. Aronchik, I., B.A. Appleton, S.E. Basham, K. Crawford, M. Del Rosario, L.V. Doyle, W.F. Estacio, J. Lan, M.K. Lindvall, C.A. Luu, E. Ornelas, E. Venetsanakos, C.M. Shafer, and A.B. Jefferson, *Novel potent and selective inhibitors of p90 ribosomal S6 kinase reveal the heterogeneity of RSK function in MAPK-driven cancers.* Mol Cancer Res, 2014. **12**(5): p. 803-12.
199. Mooney, R.A., *Use of digitonin-permeabilized adipocytes for cAMP studies.* Methods Enzymol, 1988. **159**: p. 193-202.
200. Dobin, A., C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T.R. Gingeras, *STAR: ultrafast universal RNA-seq aligner.* Bioinformatics, 2013. **29**(1): p. 15-21.
201. Huang, J. and B. Voss, *Analysing RNA-kinetics based on folding space abstraction.* BMC Bioinformatics, 2014. **15**: p. 60.
202. Love, M.I., W. Huber, and S. Anders, *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2.* Genome Biol, 2014. **15**(12): p. 550.
203. Liao, Y., J. Wang, E.J. Jaehnig, Z. Shi, and B. Zhang, *WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs.* Nucleic Acids Res, 2019. **47**(W1): p. W199-W205.
204. Chen, R.H., C. Sarnecki, and J. Blenis, *Nuclear localization and regulation of erk- and rsk-encoded protein kinases.* Mol Cell Biol, 1992. **12**(3): p. 915-27.
205. Strelkov, I.S. and J.R. Davie, *Ser-10 phosphorylation of histone H3 and immediate early gene expression in oncogene-transformed mouse fibroblasts.* Cancer Res, 2002. **62**(1): p. 75-8.
206. Teles, R.M., T.G. Graeber, S.R. Krutzik, D. Montoya, M. Schenk, D.J. Lee, E. Komisopoulou, K. Kelly-Scumpia, R. Chun, S.S. Iyer, E.N. Sarno, T.H. Rea, M. Hewison, J.S. Adams, S.J. Popper, D.A. Relman, S. Stenger, B.R. Bloom, G. Cheng, and R.L. Modlin, *Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses.* Science, 2013. **339**(6126): p. 1448-53.
207. He, K., X. Wang, J. Jiang, R. Guan, K.E. Bernstein, P.P. Sayeski, and S.J. Frank, *Janus kinase 2 determinants for growth hormone receptor association, surface assembly, and signaling.* Mol Endocrinol, 2003. **17**(11): p. 2211-27.
208. Veenstra, C., E. Karlsson, S.M. Mirwani, B. Nordenskjold, T. Fornander, G. Perez-Tenorio, and O. Stal, *The effects of PTPN2 loss on cell signalling and clinical outcome in relation to breast cancer subtype.* J Cancer Res Clin Oncol, 2019. **145**(7): p. 1845-1856.
209. Tursynbay, Y., J. Zhang, Z. Li, T. Tokay, Z. Zhumadilov, D. Wu, and Y. Xie, *Pim-1 kinase as cancer drug target: An update.* Biomed Rep, 2016. **4**(2): p. 140-146.
210. Renoux, F., M. Stellato, C. Haftmann, A. Vogetseder, R. Huang, A. Subramaniam, M.O. Becker, P. Blyszczuk, B. Becher, J.H.W. Distler, G. Kania, O. Boyman, and O. Distler, *The AP1 Transcription Factor Fosl2 Promotes Systemic Autoimmunity and Inflammation by Repressing Treg Development.* Cell Rep, 2020. **31**(13): p. 107826.
211. Guler, R., T. Mpotje, M. Ozturk, J.K. Nono, S.P. Parihar, J.E. Chia, N. Abdel Aziz, L. Hlaka, S. Kumar, S. Roy, A. Penn-Nicholson, W.A. Hanekom, D.E. Zak, T.J. Scriba, H. Suzuki, and F. Brombacher, *Batf2 differentially regulates tissue immunopathology in Type 1 and Type 2 diseases.* Mucosal Immunol, 2019. **12**(2): p. 390-402.
212. Leopold Wager, C.M., E. Arnett, and L.S. Schlesinger, *Macrophage nuclear receptors: Emerging key players in infectious diseases.* PLoS Pathog, 2019. **15**(3): p. e1007585.
213. Verger, A. and M. Duterque-Coquillaud, *When Ets transcription factors meet their partners.* Bioessays, 2002. **24**(4): p. 362-70.
214. Cai, D.H., D. Wang, J. Keefer, C. Yeamans, K. Hensley, and A.D. Friedman, *C/EBP alpha:AP-1 leucine zipper heterodimers bind novel DNA elements, activate the PU.1 promoter and direct monocyte lineage commitment more potently than C/EBP alpha homodimers or AP-1.* Oncogene, 2008. **27**(19): p. 2772-9.

215. Sanin, D.E., C.T. Prendergast, and A.P. Mountford, *IL-10 Production in Macrophages Is Regulated by a TLR-Driven CREB-Mediated Mechanism That Is Linked to Genes Involved in Cell Metabolism.* J Immunol, 2015. **195**(3): p. 1218-32.
216. Herbst, S. and M.G. Gutierrez, *LRRK2 in Infection: Friend or Foe?* ACS Infect Dis, 2019. **5**(6): p. 809-815.
217. Singh, B., C. Fleury, F. Jalalvand, and K. Riesbeck, *Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host.* FEMS Microbiol Rev, 2012. **36**(6): p. 1122-80.
218. Li, W.X., *Canonical and non-canonical JAK-STAT signaling.* Trends Cell Biol, 2008. **18**(11): p. 545-51.
219. Ohmori, Y. and T.A. Hamilton, *Requirement for STAT1 in LPS-induced gene expression in macrophages.* J Leukoc Biol, 2001. **69**(4): p. 598-604.
220. Patel, A.A. and D.M. Anderson, *Innate immune responses during infection with Yersinia pestis.* Adv Exp Med Biol, 2012. **954**: p. 151-7.
221. Yokogami, K., S. Wakisaka, J. Avruch, and S.A. Reeves, *Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR.* Curr Biol, 2000. **10**(1): p. 47-50.
222. Ihle, J.N., *Cytokine receptor signalling.* Nature, 1995. **377**(6550): p. 591-4.
223. Mohr, A., D. Fahrenkamp, N. Rinis, and G. Muller-Newen, *Dominant-negative activity of the STAT3-Y705F mutant depends on the N-terminal domain.* Cell Commun Signal, 2013. **11**: p. 83.
224. Boonstra, A., R. Rajsbaum, M. Holman, R. Marques, C. Asselin-Paturel, J.P. Pereira, E.E. Bates, S. Akira, P. Vieira, Y.J. Liu, G. Trinchieri, and A. O'Garra, *Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals.* J Immunol, 2006. **177**(11): p. 7551-8.
225. Wen, A.Y., K.M. Sakamoto, and L.S. Miller, *The role of the transcription factor CREB in immune function.* J Immunol, 2010. **185**(11): p. 6413-9.
226. Ghoda, L., X. Lin, and W.C. Greene, *The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of IkappaBalpha and stimulates its degradation in vitro.* J Biol Chem, 1997. **272**(34): p. 21281-8.
227. Chen, R.H., C. Abate, and J. Blenis, *Phosphorylation of the c-Fos transrepression domain by mitogen-activated protein kinase and 90-kDa ribosomal S6 kinase.* Proc Natl Acad Sci U S A, 1993. **90**(23): p. 10952-6.
228. Chen, R.H., P.C. Juo, T. Curran, and J. Blenis, *Phosphorylation of c-Fos at the C-terminus enhances its transforming activity.* Oncogene, 1996. **12**(7): p. 1493-502.
229. Bonni, A., A. Brunet, A.E. West, S.R. Datta, M.A. Takasu, and M.E. Greenberg, *Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms.* Science, 1999. **286**(5443): p. 1358-62.
230. Schroder, K., K.M. Irvine, M.S. Taylor, N.J. Bokil, K.A. Le Cao, K.A. Masterman, L.I. Labzin, C.A. Semple, R. Kapetanovic, L. Fairbairn, A. Akalin, G.J. Faulkner, J.K. Baillie, M. Gongora, C.O. Daub, H. Kawaji, G.J. McLachlan, N. Goldman, S.M. Grimmond, P. Carninci, H. Suzuki, Y. Hayashizaki, B. Lenhard, D.A. Hume, and M.J. Sweet, *Conservation and divergence in Toll-like receptor 4-regulated gene expression in primary human versus mouse macrophages.* Proc Natl Acad Sci U S A, 2012. **109**(16): p. E944-53.
231. Rao, M.S., T.R. Van Vleet, R. Ciurlionis, W.R. Buck, S.W. Mittelstadt, E.A.G. Blomme, and M.J. Liguori, *Comparison of RNA-Seq and Microarray Gene Expression Platforms for the Toxicogenomic Evaluation of Liver From Short-Term Rat Toxicity Studies.* Front Genet, 2018. **9**: p. 636.
232. Ruckdeschel, K., A. Roggenkamp, V. Lafont, P. Mangeat, J. Heesemann, and B. Rouot, *Interaction of Yersinia enterocolitica with macrophages leads to macrophage cell death through apoptosis.* Infect Immun, 1997. **65**(11): p. 4813-21.
233. Sarhan, J., B.C. Liu, H.I. Muendlein, P. Li, R. Nilson, A.Y. Tang, A. Rongvaux, S.C. Bunnell, F. Shao, D.R. Green, and A. Poltorak, *Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during Yersinia infection.* Proc Natl Acad Sci U S A, 2018. **115**(46): p. E10888-E10897.
234. O'Neill, L.A., R.J. Kishton, and J. Rathmell, *A guide to immunometabolism for immunologists.* Nat Rev Immunol, 2016. **16**(9): p. 553-65.
235. Trinchieri, G., *Type I interferon: friend or foe?* J Exp Med, 2010. **207**(10): p. 2053-63.

236. Platanitis, E. and T. Decker, *Regulatory Networks Involving STATs, IRFs, and NFkappaB in Inflammation.* Front Immunol, 2018. **9**: p. 2542.
237. Hoey, T., S. Zhang, N. Schmidt, Q. Yu, S. Ramchandani, X. Xu, L.K. Naeger, Y.L. Sun, and M.H. Kaplan, *Distinct requirements for the naturally occurring splice forms Stat4alpha and Stat4beta in IL-12 responses.* EMBO J, 2003. **22**(16): p. 4237-48.
238. Carow, B. and M.E. Rottenberg, *SOCS3, a Major Regulator of Infection and Inflammation.* Front Immunol, 2014. **5**: p. 58.
239. Liu, X., R. Lu, Y. Xia, S. Wu, and J. Sun, *Eukaryotic signaling pathways targeted by Salmonella effector protein AvrA in intestinal infection in vivo.* BMC Microbiol, 2010. **10**: p. 326.
240. Uchiya, K. and T. Nikai, *Salmonella pathogenicity island 2-dependent expression of suppressor of cytokine signaling 3 in macrophages.* Infect Immun, 2005. **73**(9): p. 5587-94.
241. Svensson, M.N., K.M. Doody, B.J. Schmiedel, S. Bhattacharyya, B. Panwar, F. Wiede, S. Yang, E. Santelli, D.J. Wu, C. Sacchetti, R. Gujar, G. Seumois, W.B. Kiosses, I. Aubry, G. Kim, P. Mydel, S. Sakaguchi, M. Kronenberg, T. Tiganis, M.L. Tremblay, F. Ay, P. Vijayanand, and N. Bottini, *Reduced expression of phosphatase PTPN2 promotes pathogenic conversion of Tregs in autoimmunity.* J Clin Invest, 2019. **129**(3): p. 1193-1210.
242. Zhang, Y., H. Ding, X. Wang, and S.D. Ye, *Modulation of STAT3 phosphorylation by PTPN2 inhibits naive pluripotency of embryonic stem cells.* FEBS Lett, 2018. **592**(13): p. 2227-2237.
243. Doni, A., M. Stravalaci, A. Inforzato, E. Magrini, A. Mantovani, C. Garlanda, and B. Bottazzi, *The Long Pentraxin PTX3 as a Link Between Innate Immunity, Tissue Remodeling, and Cancer.* Front Immunol, 2019. **10**: p. 712.
244. Gupta, G., Z. Mou, P. Jia, R. Sharma, R. Zayats, S.M. Viana, L. Shan, A. Barral, V.S. Boaventura, T.T. Murooka, A. Soussi-Gounni, C.I. de Oliveira, and J.E. Uzonna, *The Long Pentraxin 3 (PTX3) Suppresses Immunity to Cutaneous Leishmaniasis by Regulating CD4(+) T Helper Cell Response.* Cell Rep, 2020. **33**(11): p. 108513.
245. Chetoui, N., M. Boisvert, S. Gendron, and F. Aoudjit, *Interleukin-7 promotes the survival of human CD4+ effector/memory T cells by up-regulating Bcl-2 proteins and activating the JAK/STAT signalling pathway.* Immunology, 2010. **130**(3): p. 418-26.
246. Waris, G., K.W. Huh, and A. Siddiqui, *Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress.* Mol Cell Biol, 2001. **21**(22): p. 7721-30.
247. Tacke, R.S., A. Tosello-Trampont, V. Nguyen, D.W. Mullins, and Y.S. Hahn, *Extracellular hepatitis C virus core protein activates STAT3 in human monocytes/macrophages/dendritic cells via an IL-6 autocrine pathway.* J Biol Chem, 2011. **286**(12): p. 10847-55.
248. Punjabi, A.S., P.A. Carroll, L. Chen, and M. Lagunoff, *Persistent activation of STAT3 by latent Kaposi's sarcoma-associated herpesvirus infection of endothelial cells.* J Virol, 2007. **81**(5): p. 2449-58.
249. Percario, Z., E. Olivetta, G. Fiorucci, G. Mangino, S. Peretti, G. Romeo, E. Affabris, and M. Federico, *Human immunodeficiency virus type 1 (HIV-1) Nef activates STAT3 in primary human monocyte/macrophages through the release of soluble factors: involvement of Nef domains interacting with the cell endocytotic machinery.* J Leukoc Biol, 2003. **74**(5): p. 821-32.
250. Butcher, B.A., L. Kim, A.D. Panopoulos, S.S. Watowich, P.J. Murray, and E.Y. Denkers, *IL-10-independent STAT3 activation by Toxoplasma gondii mediates suppression of IL-12 and TNF-alpha in host macrophages.* J Immunol, 2005. **174**(6): p. 3148-52.
251. Murray, P.J., *The primary mechanism of the IL-10-regulated antiinflammatory response is to selectively inhibit transcription.* Proc Natl Acad Sci U S A, 2005. **102**(24): p. 8686-91.
252. Lang, R., D. Patel, J.J. Morris, R.L. Rutschman, and P.J. Murray, *Shaping gene expression in activated and resting primary macrophages by IL-10.* J Immunol, 2002. **169**(5): p. 2253-63.
253. Williams, L., G. Jarai, A. Smith, and P. Finan, *IL-10 expression profiling in human monocytes.* J Leukoc Biol, 2002. **72**(4): p. 800-9.
254. Lentsch, A.B., T.P. Shanley, V. Sarma, and P.A. Ward, *In vivo suppression of NF-kappa B and preservation of I kappa B alpha by interleukin-10 and interleukin-13.* J Clin Invest, 1997. **100**(10): p. 2443-8.
255. Schottelius, A.J., M.W. Mayo, R.B. Sartor, and A.S. Baldwin, Jr., *Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding.* J Biol Chem, 1999. **274**(45): p. 31868-74.
256. Wang, P., P. Wu, M.I. Siegel, R.W. Egan, and M.M. Billah, *Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms.* J Biol Chem, 1995. **270**(16): p. 9558-63.

257. Liu, W., X. Liu, Y. Li, J. Zhao, Z. Liu, Z. Hu, Y. Wang, Y. Yao, A.W. Miller, B. Su, M.R. Cookson, X. Li, and Z. Kang, *LRRK2 promotes the activation of NLRC4 inflammasome during Salmonella Typhimurium infection.* J Exp Med, 2017. **214**(10): p. 3051-3066.
258. Wang, Y., I. Shaked, S.M. Stanford, W. Zhou, J.M. Curtsinger, Z. Mikulski, Z.R. Shaheen, G. Cheng, K. Sawatzke, A.M. Campbell, J.L. Auger, H. Bilgic, F.M. Shoyama, D.O. Schmeling, H.H. Balfour, Jr., K. Hasegawa, A.C. Chan, J.A. Corbett, B.A. Binstadt, M.F. Mescher, K. Ley, N. Bottini, and E.J. Peterson, *The autoimmunity-associated gene PTPN22 potentiates toll-like receptor-driven, type 1 interferon-dependent immunity.* Immunity, 2013. **39**(1): p. 111-22.
259. Tomlin, H. and A.M. Piccinini, *A complex interplay between the extracellular matrix and the innate immune response to microbial pathogens.* Immunology, 2018. **155**(2): p. 186-201.
260. Ramu, P., L.A. Lobo, M. Kukkonen, E. Bjur, M. Suomalainen, H. Raukola, M. Miettinen, I. Julkunen, O. Holst, M. Rhen, T.K. Korhonen, and K. Lahteenmaki, *Activation of pro-matrix metalloproteinase-9 and degradation of gelatin by the surface protease PgtE of Salmonella enterica serovar Typhimurium.* Int J Med Microbiol, 2008. **298**(3-4): p. 263-78.
261. Ma, J. and X. Cao, *Regulation of Stat3 nuclear import by importin alpha5 and importin alpha7 via two different functional sequence elements.* Cell Signal, 2006. **18**(8): p. 1117-26.
262. Ushijima, R., N. Sakaguchi, A. Kano, A. Maruyama, Y. Miyamoto, T. Sekimoto, Y. Yoneda, K. Ogino, and T. Tachibana, *Extracellular signal-dependent nuclear import of STAT3 is mediated by various importin alphas.* Biochem Biophys Res Commun, 2005. **330**(3): p. 880-6.
263. Cimica, V., H.C. Chen, J.K. Iyer, and N.C. Reich, *Dynamics of the STAT3 transcription factor: nuclear import dependent on Ran and importin-beta1.* PLoS One, 2011. **6**(5): p. e20188.
264. Liu, L., K.M. McBride, and N.C. Reich, *STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin-alpha3.* Proc Natl Acad Sci U S A, 2005. **102**(23): p. 8150-5.
265. Debidda, M., L. Wang, H. Zang, V. Poli, and Y. Zheng, *A role of STAT3 in Rho GTPase-regulated cell migration and proliferation.* J Biol Chem, 2005. **280**(17): p. 17275-85.
266. Raptis, L., R. Arulanandam, M. Geletu, and J. Turkson, *The R(h)oads to Stat3: Stat3 activation by the Rho GTPases.* Exp Cell Res, 2011. **317**(13): p. 1787-95.
267. Reitsma, J.M., H. Sato, M. Nevels, S.S. Terhune, and C. Paulus, *Human cytomegalovirus IE1 protein disrupts interleukin-6 signaling by sequestering STAT3 in the nucleus.* J Virol, 2013. **87**(19): p. 10763-76.
268. Dutta, P., L. Zhang, H. Zhang, Q. Peng, P.R. Montgrain, Y. Wang, Y. Song, J. Li, and W.X. Li, *Unphosphorylated STAT3 in heterochromatin formation and tumor suppression in lung cancer.* BMC Cancer, 2020. **20**(1): p. 145.
269. Brown, S. and M.P. Zeidler, *Unphosphorylated STATs go nuclear.* Curr Opin Genet Dev, 2008. **18**(5): p. 455-60.
270. Yang, J., X. Liao, M.K. Agarwal, L. Barnes, P.E. Auron, and G.R. Stark, *Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFkappaB.* Genes Dev, 2007. **21**(11): p. 1396-408.
271. Yang, J. and G.R. Stark, *Roles of unphosphorylated STATs in signaling.* Cell Res, 2008. **18**(4): p. 443-51.
272. Yang, J., M. Chatterjee-Kishore, S.M. Staugaitis, H. Nguyen, K. Schlessinger, D.E. Levy, and G.R. Stark, *Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation.* Cancer Res, 2005. **65**(3): p. 939-47.
273. Yu, Z., W. Zhang, and B.C. Kone, *Signal transducers and activators of transcription 3 (STAT3) inhibits transcription of the inducible nitric oxide synthase gene by interacting with nuclear factor kappaB.* Biochem J, 2002. **367**(Pt 1): p. 97-105.
274. Yoshida, Y., A. Kumar, Y. Koyama, H. Peng, A. Arman, J.A. Boch, and P.E. Auron, *Interleukin 1 activates STAT3/nuclear factor-kappaB cross-talk via a unique TRAF6- and p65-dependent mechanism.* J Biol Chem, 2004. **279**(3): p. 1768-76.
275. Kojima, H., K. Nakajima, and T. Hirano, *IL-6-inducible complexes on an IL-6 response element of the junB promoter contain Stat3 and 36 kDa CRE-like site binding protein(s).* Oncogene, 1996. **12**(3): p. 547-54.
276. Schaefer, T.S., L.K. Sanders, and D. Nathans, *Cooperative transcriptional activity of Jun and Stat3 beta, a short form of Stat3.* Proc Natl Acad Sci U S A, 1995. **92**(20): p. 9097-101.
277. Niehof, M., K. Streetz, T. Rakemann, S.C. Bischoff, M.P. Manns, F. Horn, and C. Trautwein, *Interleukin-6-induced tethering of STAT3 to the LAP/C/EBPbeta promoter suggests a new mechanism of transcriptional regulation by STAT3.* J Biol Chem, 2001. **276**(12): p. 9016-27.
278. Ciofani, M., A. Madar, C. Galan, M. Sellars, K. Mace, F. Pauli, A. Agarwal, W. Huang, C.N. Parkhurst, M. Muratet, K.M. Newberry, S. Meadows, A. Greenfield, Y. Yang, P. Jain, F.K.

Kirigin, C. Birchmeier, E.F. Wagner, K.M. Murphy, R.M. Myers, R. Bonneau, and D.R. Littman, *A validated regulatory network for Th17 cell specification.* Cell, 2012. **151**(2): p. 289-303.
279. Mohr, A., N. Chatain, T. Domoszlai, N. Rinis, M. Sommerauer, M. Vogt, and G. Muller-Newen, *Dynamics and non-canonical aspects of JAK/STAT signalling.* Eur J Cell Biol, 2012. **91**(6-7): p. 524-32.
280. Kuchipudi, S.V., *The Complex Role of STAT3 in Viral Infections.* J Immunol Res, 2015. **2015**: p. 272359.

IX List of Tables

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XI List of Abbreviations

°C	degree celsius
µF	microfarrad
µg	microgram
µl	microliter
µm	micrometer
µM	micromolar
aa	amino acid
ad	add
Ail	Attachment invasion locus
AP-1	activator protein 1
APRE	Acute Phase Response Element
B2M	Beta-2 microglobulin
BAD	Bcl-2-associated death promoter *protein*
BATF	Basic Leucine Zipper ATF-Like Transcription Factor
BMDM	bone marrow-derived macrophages
BSA	Bovine Serum Albumin
bZIP	Basic Leucine Zipper Domain
CBP	CREB-binding protein
CD	cluster of differentiation
cDNA	copy DNA
cm	centimeter
CPP	cell penetrating peptide
CREB	cAMP response element binding protein
CRM1	exportin chromosome region maintenance 1
CTKD	C-terminal kinase domain
DAMPs	danger-associated molecular patterns
dd	double distilled
ddH_2O	distilled water
DDX3	DEAD box helicase 3
DEG	differentially expressed genes
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ECM	extracellular matrix
EDTA	Ethylenediamine tetra-acetic acid
ERK	extracellular signal-regulated kinase
FCS	Fetal calf serum
FKHRL1	forkhead homolog (rhabdomyosarcoma) like 1
Flag	peptide sequence DYKDDDDK
Fosl2	Fos-like 2
g	gram
g	relative centrifugal force
GAS	INF-γ -activated sequence
GAP	GTPase activating protein
GAPDH	Glyceraldehyde-3-phosphate-Dehydrogenase
GBPs	guanylate-binding proteins
GDI	GDP (Guanosine diphosphate) dissociation inhibitors
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GILZ	glucocorticoid-induced leucine zipper

GO	Gene Ontology
GPCRs	G protein-coupled receptors
GSDM	gasdermin D pore
GSK3	glycogen synthase kinase 3
GTP	Guanosine 5'-Triphosphate
h	hour
HP1	heterochromatin protein 1
HPI	High Pathogenicity Island
HRP	Horseradish peroxidase
IF	Immunofluorescence
IFN	interferon
IFNAR	IFN-a receptor
IKBα	NF-kappa-B inhibitor alpha
IKK	inhibitor of nuclear factor kappa-B kinase
IL	interleukin
ILRN	interleukin-1 receptor antagonist
iNOS	Inducible nitric oxide synthase
IP	Immunoprecipitation
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IQGAP1	IQ Motif Containing GTPase Activating Protein 1
ISGs	interferon-stimulated genes
ISREs	interferon-sensitive response elements
IRAK 1/4	Interleukin-1 receptor-associated kinase 1/4
IRF	*Interferon regulatory factor*
JAK	Janus kinase
kDa	kilo Dalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
KLF	Kruppel like factor
L	liter
LAMP1	lysosomal-associated membrane protein 1
LB	Lysogeny broth (Luria-Bertani)
LPS	Lipopolysaccharides
LRRs	leucine-rich-repeats
LRRK2	leucin-rich repeat kinase 2
M	molar
MACS	Magnetic Cell Sorting System
MAPK	mitogen activated protein kinase
MAPKK	mitogen activated protein kinase (MAPK) kinase
MCP-1	macrophage chemotactic factor 1
MD-2	myeloid differentiation factor 2
mg	milligram
min	minute
ml	millilitre
mM	millimolar
MMP9	Matrix metallopeptidase 9
mRNA	messenger-RNA
mRNPs	messenger ribonucleoprotein particles
MRTF	myocardin related transcription factor
mTOR	mechanistic Target of Rapamycin
Myd88	Myeloid differentiation primary response gene 88
NF-κB	nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
ng	nanogram
NK	Natural killer
NLRP3	nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3

NLRs	NOD-like receptors
nM	Nanomolar
nt	non-targeting
NTKD	N- terminal kinase domain
OD	Optical density
p	phosphorylated
PAGE	Polyacrylamide gel electrophoresis
PAMPs	pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PBST	PBS with Tween-20
PCA	principal component analysis
PCR	Polymerase chain reaction
PDK	3′-phosphoinositide-dependent kinase
PEI	polyethylenimine
PFA	Paraformaldehyde
Phe	Phenylalanine
PI	phosphoinositide
PKN/PRK	Protein Kinase C-Related Kinase
pM	Pikomolar
pmol	Pikomol
PRGs	primary response genes
PRRs	Pattern recognition receptors
pYV	Yersinia virulence plasmid
RHD	rel homology domain
Rho	Ras homolog gene family, member
RIPK1	receptor-interacting serine/threonine protein kinase 1
rlog	log transformation
RLRs	RIG-I- like receptors
RNA	Ribonucleic acid
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
RSK	ribosomal S6 Kinase
RT	room temperature
RT-PCR	Real time PCR
s	second
SBE	STAT-binding elements
SDS	Sodium dodecylsulfate
SEM	Standard error of the mean
Ser	serine
siRNA	small interfering RNA
SOCS3	Suppressor of Cytokine Signaling-3
SPARC	Secreted Protein Acidic And Cysteine Rich
SRE	serum response element
SRF	serum response factor
SRGs	secondary response genes
STAT	signal transducer and activator of transcription
TAE	Tris-acetate-EDTA
Taq	Thermus aquaticus
TBK1	Tank binding kinase 1
TBP	TATA-binding protein
TBS	Tris buffered saline
T	Tween-20
TF	Transcription factor
TGF-β	tumor growth factor- β

Thr	threonine
TIR	Toll-Interleukin 1 Receptor
TIRAP	Toll-Interleukin 1 Receptor (TIR) Domain Containing Adaptor Protei
TLR	toll like receptor
TNF	tumor necrosis factor
Tris	Tris-(hydroxymethyl)-aminomethane
TTSS	type three secretion system
Tyk2	tyrosine kinase 2
u	unphosphorylated
UV	Ultraviolet
V	Volt
Yops	Yersinia outer proteins
YpkA	Yersinia protein kinase A
Ω	Ohm

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XIII Curriculum vitae

Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

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XIV Publications

Brehm TT*, **Berneking L***, Martins MS, Dupke S, Jacob D, Drechsel O, Becker K, Axel Kramer A, Christner M, Aepfelbacher M, Schmiedel S, Rohde H for the German Vibrio Study Group. Heatwave associated Vibrio infections in Germany 2018 and 2019. Eurosurveillance (2021)

Berinson B, Both A, **Berneking L**, Christner M, Lütgehetmann M, Aepfelbacher M, Rohde H. Usefulness of Biofire FilmArray BCID2 for blood culture processing in clinical practice. JCM (2021)

Berneking L, Both A, Asar L, Berinson B, Lütgehetmann M, Rohde H. Performance of a Loop-Mediated Isothermal Amplification-Based Methodology To Detect Carbapenemase Production in gram-negative bacteria. JMM (2021)

Fritzsche A*, **Berneking L***, Nörz D, Reucher S, Fischer N, Roggenkamp H, Aepfelbacher M, Rohde H, Pfefferle S, Lütgehetmann M. Clinical evaluation of a laboratory-developed quantitative BK virus-PCR assay using the cobas® omni Utility Channel. J Virol Methods. (2021)

Berneking L, Both A, Berinson B, Hoffmann A, Lütgehetmann M, Aepfelbacher M, Rohde H. Performance of the BD Phoenix CPO detect assay for detection and classification of carbapenemase-producing organisms. Eur J Clin Microbiol Infect Dis. (2020)

Mannina D, **Berneking L**, Both A, Timm W, Urbanowicz T, Wolschke C, Ayuketang Ayuk F, Fischer N, Fiehler J, Grzyska U, Rösner S, Choe CU, Kröger N, Christopeit M. Major central nervous system complications after allogeneic stem cell transplantation: A large retrospective study on 888 consecutive adult patients. Eur J Haematol. (2020)

Casagrande M, Fitzek A, Püschel K, Aleshcheva G, Schultheiss HP, **Berneking L**, Spitzer MS, Schultheiss M. Detection of SARS-CoV-2 in Human Retinal Biopsies of Deceased COVID-19 Patients. Ocul Immunol Inflamm. (2020)

Samek M, Iversen K, Belmar Campos C, **Berneking L**, Langebrake C, Wolschke C, Ayuk F, Kröger N, Christopeit M. Monocenter study on epidemiology, outcomes, and risk factors of infections in recipients of 166 allogeneic stem cell transplantations during 1 year. Eur J Haematol. (2020)

Both A, **Berneking L**, Berinson B, Lütgehetmann M, Christner M, Aepfelbacher M, Rohde H. Rapid identification of the vanA/vanB resistance determinant in Enterococcus sp. from blood cultures using the Cepheid Xpert vanA/vanB cartridge system. Diagn Microbiol Infect Dis. (2020)

Berneking L, Schnapp M, Nauth T, Hentschke M. Tandem Affinity Purification of SBP-CBP-tagged Type Three Secretion System Effectors. Bio Protoc. (2019)

Eibach D, Dekker D, Gyau Boahen K, Wiafe Akenten C, Sarpong N, Belmar Campos C, **Berneking L**, Aepfelbacher M, Krumkamp R, Owusu-Dabo E, May J; Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in local and imported poultry meat in Ghana. Vet Microbiol. (2018)

Manthey CF, Dranova D, Christner M, **Berneking L**, Kluge S, Lohse AW, Fuhrmann V; Stool cultures at the ICU: get rid of it!, Ann Intensive Care (2018)

Berneking L*, Schnapp M*, Rumm A, Trasak C, Ruckdeschel K, Alawi M, Grundhoff A, Kikhney AG, Koch-Nolte F, Buck F, Perbandt M, Betzel C, Svergun DI, Hentschke M, Aepfelbacher M; The immunosuppressive Yersinia effector YopM binds the DEAD box helicase DDX3 to control nuclear Ribosomal S6 Kinase. Plos Pathogens (2016)

Hentschke M, **Berneking L**, Belmar Campos C, Buck F, Ruckdeschel K, Aepfelbacher M; Yersinia virulence factor YopM induces sustained RSK activation by interfering with dephosphorylation. Plos One (2010)

XV Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

_____

Laura Berneking