Role of immunomodulatory *Yersinia* outer proteins during primary human macrophage infection

Dissertation

zur Erlangung des akademischen Grades eines
Doktors der Humanbiologie (Dr. rer. biol. hum.)
an der
Medizinischen Fakultät der Universität Hamburg

vorgelegt von
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aus
Düsseldorf

2024

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Datum der mündlichen Prüfung: 21.01.2025

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1 Abstract

1.1 Abstract

Yersinia effectors introduced through the type three secretion system by pathogenic *Yersinia* spp. into host cells support the suppression of the host immune response. Three of those effectors YopM, YopP and YopQ fulfill a variety of immunosuppressive functions like impacting the host gene expression, inflammasome formation or histone modifications. However, their potential interplay and their individual effects have not been studied in primary human macrophages yet. In this study a comprehensive RNA-seq analysis of primary human macrophages infected with different *Y. enterocolitica* strains was performed, aiming to determine the individual effects of YopM, YopP and YopQ as well as the effect of a potential interplay between YopM and YopP on gene expression. Furthermore, the impact of YopM, YopP and YopQ on inflammasome formation as well as a specific histone modification, histone H3 serine-10 phosphorylation, was investigated.

RNA-seq analysis revealed that PAMP-induced global changes in gene expression were largely suppressed by YopP. Interestingly YopQ and especially YopM counteracted inhibitory as well as stimulatory effects of YopP even if this effect was abrogated in the case of YopM when YopP was missing. This led to the conclusion that YopM and YopQ fine tune the suppression of the PAMP induced gene expression by YopP, by that optimizing and promoting the immunosuppressive effects of *Yersinia*.

Regarding the impact on inflammasome formation, a significant suppressing effect of YopP and YopQ together was found, whereas whether YopM, YopQ nor YopP alone showed any significant suppression of the inflammasome formation. The reason for that is still unknown, but it could be that one Yop alone is not able to stop the several pathways which lead to inflammasome formation and that in this case at least two are needed to exert a significant reduction. Having a look on the effect of different Yops on the histone modification histone H3 serine-10 phosphorylation, YopP clearly suppressed the phosphorylation by its well-known inhibiting effect on members of the MAPK- and NF-κB pathway and neither YopM, nor YopQ showed any effect.

Taken together this study shows that the three Yops YopM, YopP and YopQ have several different effects on gene expression, inflammasome formation and histone modification not only acting alone but sometimes acting together to promote the survival of pathogenic *Yersinia* spp. during infection.

1.2 Zusammenfassung

Yersinia Effektoren, welche durch das Typ 3 Sekretionssystem von pathogenen *Yersinia* Spezies in die Wirtszelle transloziert werden, tragen zur Unterdrückung der Immunantwort des Wirts bei. Drei dieser Effektoren, YopM, YopP und YopQ üben eine Vielzahl an immunsuppressiven Funktionen wie der Beeinflussung der Genexpression, der Inflammasombildung oder der Histonmodifikation aus. Ihre individuellen Effekte oder ein mögliches Zusammenspiel in Bezug auf die oben genannten Funktionen wurde in primären humanen Makrophagen bisher jedoch nicht untersucht. In dieser Arbeit wurde eine umfassende RNA-seq Analyse von primären humanen Makrophagen, welche mit verschiedenen *Y. enterocolitica* Stämmen infiziert wurden, durchgeführt. Ziel der Arbeit war es, die individuellen Effekte von YopM, YopP und YopQ als auch den Effekt eines möglichen Zusammenspiels von YopM und YopP auf die Genexpression zu untersuchen. Darüber hinaus wurde der Einfluss von YopM, YopP und YopQ auf die Inflammasombildung, sowie die Histon H3 Serin-10 Phosphorylierung, untersucht.

Die RNA-seq Analyse zeigte, dass PAMP induzierte globale Veränderungen in der Genexpression größtenteils durch YopP unterdrückt wurden. YopQ und besonders YopM wirkten diesen inhibitorischen wie stimulatorischen Effekten von YopP entgegen, auch wenn dieser Effekt im Fall von YopM aufgehoben wurde wenn YopP fehlte. YopM und YopQ scheinen die Unterdrückung der PAMP induzierten Genexpression durch YopP zu regulieren, um die immunsuppressiven Effekte von *Yersinia* zu optimieren und zu fördern. Die Inflammasombildung wurde durch YopP und YopQ zusammen signifikant reduziert, wohingegen YopM, YopP und YopQ alleine keinen Einfluss hatten. Möglicherweise reicht ein Yop alleine nicht aus, um die vielen Signalwege, welche zur Inflammasombildung führen, zu unterdrücken und in diesem Fall mindestens 2 Yops vonnöten sind. Die Histon H3 Serin-10 Phosphorylierung wurde deutlich durch die von YopP bekannte Inhibierung von Proteinen des MAPK- und NF-κB Signalwegs, unterdrückt, wohingegen YopM und YopQ keinen Effekt zeigten.

Zusammenfassend zeigt diese Arbeit, dass die drei Yops YopM, YopP, und YopQ viele verschiedene Effekte auf die Genexpression, die Inflammasombildung und eine Histonmodifikation haben und nicht nur alleine wirken, sondern teilweise auch zusammen, um das Überleben von pathogenen *Yersinia* Spezies während der Infektion zu fördern.

2 Introduction

2.1 Pathogenic *Yersinia*

The genus *Yersinia* which belongs to the family of Yersiniaceae contains 28 different gram-negative, rod-shaped, facultative anaerob, oxidase negative and catalase positive species with an optimal growth temperature between 24 and 27 °C, but they are also able to replicate at 4 °C. They include non-pathogenic as well as human and animal pathogenic species. Among them are the well-known enteropathogenic species *Yersinia pseudotuberculosis* & *Yersinia enterocolitica* and *Yersinia pestis,* the causative agent of bubonic plague (Adeolu et al., 2016; Fredriksson-Ahomaa, 2007; McNally et al., 2016; Parte et al., 2020). *Y. pestis* and *Y. pseudotuberculosis* are evolutionary closer related than *Y. enterocolitica*, which separated from their common ancestor around 200 million years ago. *Y. pestis* instead split off around 6000 years ago from *Y. pseudotuberculosis* (Achtman et al., 1999; Andrades Valtuena et al., 2017; Rascovan et al., 2019; Rasmussen et al., 2015; Spyrou et al., 2018). The bacteriologists Alexandre Yersin and Shibasaburō Kitasato were the first ones who isolated *Y. pestis* in 1894, by that discovering the agent of plague (Kitasato, 1894; Treille & Yersin, 1894).

Transmission of *Y. pestis* is commonly known to take place from infected rodents to humans through a bite of the rat flea carrying the bacteria, whereas nowadays infections mostly occur through contact to infected or dead rodents, while having a skin injury, which is an open gate for invasion of bacteria. Once infected, humans transmit *Y. pestis* also via aerosols. Treatment of infection is done by antibiotics leading to a complete recovery, but if untreated, the infection is fatal in most cases (Brubaker, 2003; Kayser, 2010).

Bacterial agents of gastroenteritis in Europe are mainly *Campylobacter* spp., *Salmonella* spp. and thirdly enteropathogenic *Yersinia* spp. of which *Y. enterocolitica* and *Y. pseudotuberculosis* are mostly responsible for (McNally et al., 2016; Rosner et al., 2010; van Pelt et al., 2003). Infection with *Y. enterocolitica* and *Y. pseudotuberculosis* occurs mostly through the intake of raw or undercooked contaminated pork meat as well as carrots and lettuce. They both cause an infection called yersiniosis, which is normally self-limiting, but can also lead to several gut-associated symptoms like enteritis, ileitis and diarrhea (Bottone, 1997; Grahek-Ogden et al., 2007; Jalava et al., 2006).

All three human pathogenic *Yersinia* spp. prefer to colonize in the lympathic tissue of the host even though they differ in their infection routes. Besides, they are also able to replicate extracellularly in micro abscesses (Cornelis & Wolf-Watz, 1997).

In general, the virulence of the three human pathogenic *Yersinia* spp. is mediated via several chromosomal as well as plasmid encoded factors, encoded on a 70 kb virulence plasmid named pYV (plasmid of *Yersinia* virulence). This pYV plasmid encodes for the Yop

(*Yersinia* outer proteins) effectors and also for the Ysc (*Yersinia* secretion) proteins of the type three secretion system (T3SS) through which the Yops are translocated into the host cell cytoplasm where they inhibit phagocytosis on the one side and also affect the immune response of the host on the other side (Pha & Navarro, 2016; Viboud & Bliska, 2005).

2.2 Properties of enteropathogenic *Yersinia*

2.2.1 Classification

The species *Y. enterocolitica* which was used in this study is very heterogeneous and can be biochemically subdivided into six biovars 1A, 1B and 2-5. Further differentiation is possible via antigenic variation in their lipopolysaccharides leading to more than 70 different serovars (Aleksić & Bockemühl, 1984; Sabina et al., 2011; Wauters et al., 1987).
The only non-pathogenic biovar is 1A, as those strains do not have a virulence plasmid, whereas strains of the biovar 1B are considered to have the highest pathogenicity belonging to a mice lethal group (Bottone, 1999; Wren, 2003). However, strains of the biovars 2-5 are less pathogenic and non-lethal to mice as they do not carry a cluster of genes named “high pathogenicity island” (HPI) in contrast to strains of biovar 1B (Carniel, 2002; Wren, 2003). The HPI encodes for the protein Yersiniabactin, a siderophore, which is able to chelate iron with high affinity thus leading to the ability to grow and spread under iron-limited conditions in the host (Carniel, 2002; McNally et al., 2016). Strains are classified depending on their biovar and serovar, which is summarized as bioserovar. In Europe, serovars O:3, O:9 are mostly found in clinical isolates and especially bioserovar 4/O:3 and bioserovar 2/O:9 are quite common as an agent of yersiniosis (EFSA, 2007). In North America the endemic bioserovar 1B/O:8 causes severe diseases and the *Y. enterocolitica* strain WA314, which was used in this study belongs to this bioserovar (Sabina et al., 2011).

2.2.2 Yersiniosis

Yersiniosis, a bacterial zoonosis, is mainly caused by *Y. enterocolitica* taken in by contaminated food, often raw or uncooked pork meat, leading to symptoms of enterocolitis, enteritis, terminal ileitis or mesenteric lymphadenitis combined with often self-regulating symptoms like mild fever, diarrhea, vomiting, nausea and abdominal pain (Bottone, 1997; Drummond et al., 2012; Galindo et al., 2011; Gurry, 1974; Lee et al., 1990; Pai & Mors, 1978; Takao et al., 1984). To cause this, up to 10^9 *Y. enterocolitica* cells are needed for infection (Schaake et al., 2014). In general, symptoms of infection need a few days to develop and normally last for one to three weeks without antibiotic treatment. Depending on the age, the general health condition of the patient and the pathogenic properties of the infecting strain, several clinical phenotypes of the disease are possible (Rosner et al., 2012).

Infected infants normally suffer from a self-limiting gastro-intestinal disease, whereas the infection of older children and teenagers can also lead to a mesenteric lymphadenitis or terminal ileitis with fever and abdominal pain. Adults however can also suffer from pharyngitis with fluish symptoms and in immunosuppressed adults, inflammation of different organs like the pericardium or the pleura was found. Infants under the age of four show the highest rates of infection compared to adults in general. In Europe, around 8000 cases occur per year, most of them in Germany, which could either be due to a higher frequency or method of diagnosis or because of the higher consumption rate of raw pork meat in general (European Centre for Disease Prevention and Control, 2024; Galindo et al., 2011; Rosner et al., 2010).

2.2.3 Pathogenesis

Infection with *Y. enterocolitica* mostly starts with the intake of contaminated food, water or rarely via contaminated blood transfusions. Intake of contaminated food leads to an uptake of the bacteria into the gastrointestinal tract, where the bacteria express urease, an enzyme which protects the cell from the acidic environment in the stomach by hydrolysing urea, thus producing carbonic acid and ammonia, resulting in an increase of the pH (Agrain et al., 2005; Bhagat & Virdi, 2009; de Koning-Ward et al., 1995). Once they reached the intestine, the host-pathogen interaction starts with the *Yersinia* first passing through the gastrointestinal mucus and finally reaching the follicle-associated epithelium (FAE) of the ileum (Autenrieth & Firsching, 1996; Autenrieth et al., 1996). The expression of the proteins “Invasin” (Inv) and “Attachement invasion locus” (Ail) leads to attachment and invasion of the M-cells (microfold cells) which overlay the Peyer’s patches (Felek & Krukonis, 2009; Fredriksson-Ahomaa et al., 2006; Grützkau et al., 1990; Pepe & Miller, 1993). The uptake of *Yersinia* is enabled through Invasin, which activates integrin receptors of the M-cells leading to a restructuring of the actin cytoskeleton of the host (Wong & Isberg, 2005). Release from the M-cells leads to contact between *Yersinia* and immune cells like macrophages or neutrophils (Jepson & Clark, 1998). In order to affect the host immune response, *Yersinia* translocates antiphagocytic bacterial effector proteins through the T3SS into the host cell. By that, extracellular replication in the lymphatic tissue and the formation of microcolonies, which are resistant against phagocytosis of macrophages and neutrophils, is possible (Aepfelbacher et al., 2007; Cornelis et al., 1998; Viboud & Bliska, 2005). Furthermore, *Y. enterocolitica* is able to destroy the FAE and Peyer’s patches within 5 to 7 days after infection as shown in *in vivo* mouse experiments (Autenrieth & Firsching, 1996). Finally, this leads to spreading of *Y. enterocolitica* to the mesenteric lymph nodes allowing a further expansion to inner organs like liver and spleen (Cornelis & Wolf-Watz, 1997; Pepe & Miller, 1993; Pepe et al., 1995).

2.3 *Yersinia* virulence factors

Virulence factors, either chromosomally or plasmid encoded ones, play a crucial role in the pathogenicity of *Y. enterocolitica* enabling the survival and replication inside the host (Atkinson & Williams, 2016). Virulence factors encoded in the chromosome are mainly expressed at temperatures between 25 – 28 °C, while expression of those encoded on the virulence plasmid is only induced at 37 °C, the temperature of the host (Cornelis et al., 1987; de Rouvroit et al., 1992).

2.3.1 Chromosomal and plasmid encoded virulence factors

While chromosomal encoded virulence factors like *inv* and *ail* play a vital role in early stages of the infection for host cell attachment (Atkinson & Williams, 2016; Pepe & Miller, 1993) and intestinal epithelium invasion (Miller & Falkow, 1988), plasmid encoded virulence factors come into action in later stages of infection for maintaining and establishment of the infection.

Invasin is a protein, which covers the bacterial surface (Grosdent et al., 2002) and binds to the host cell's integrin receptors leading to a structural rearrangement of the actin cytoskeleton. This finally ends up in the internalization of *Yersinia* (Atkinson & Williams, 2016; Van Nhieu & Isberg, 1991). The expression of Invasin is stimulated at temperatures between 25 – 28 °C, whereas their expression is lowered at 37 °C (Simonet & Falkow, 1992). However, Ail, another adhesion factor on the bacterial surface, is highly expressed at temperatures between 30 – 37 °C (Miller & Falkow, 1988) and mediates invasion, binding to epithelial cells (Chauhan et al., 2016), serum resistance of *Yersinia* (Miller et al., 1989; Pierson & Falkow, 1993) and also protects the bacteria against complement killing (Kirjavainen et al., 2008). Furthermore, the heat stable enterotoxin Yst (*Yersinia* stable toxin), an activator of the guanylate cyclase inducing diarrhea, is also encoded on the chromosome (Bottone, 1997; Takao et al., 1984). Contact to the host cell is also mediated through flagella on the bacterial surface, encoded by the flagellin genes *fleABC*. The expression of flagella is also induced at around 25 °C but inhibited at 37 °C (Kapatral & Minnich, 1995; Young et al., 2000).

Plasmid encoded virulence factors of *Y. enterocolitica* like the structural Ysc proteins of the T3SS, the Yop effector proteins or the adhesion protein YadA (*Yersinia* adhesion A) are encoded on a 70 kb plasmid called pYV and regulated via temperature and availability of calcium (Horne & Prüss, 2006). Low calcium concentrations and 37 °C lead to a high expression (Cornelis et al., 1998), while cell contact acts as a trigger for secretion and translocation of the effector proteins (Nordfelth & Wolf-Watz, 2001; Rosqvist et al., 1994). The adhesion protein YadA is expressed at 37 °C, forms a fibrillary matrix on the bacterial

surface (Mühlenkamp et al., 2015) and by that mediates the binding to integrins on the host cell surface (Deuschle et al., 2016). Ysc proteins are part of the needle complex and the basal body of the T3SS to which also the tip complex and the translocation pore, formed by LcrV (Low calcium response protein V), YopB and YopD, belongs (Cornelis, 2002b). Yop effector proteins are translocated into the host cell through the T3SS to either suppress phagocytic activities or to alter the immune response of the host cell (Cornelis, 2002a; Pujol & Bliska, 2005; Viboud & Bliska, 2005). Secretion and translocation of the effector proteins can be blocked by a protein complex called “calcium plug”, which is formed by the proteins YopN, TyeA (Translocation of Yops into eukaryotic cells A), YscB and SycN (Specific Yop chaperone N) (Day & Plano, 1998; Ferracci et al., 2005; Joseph & Plano, 2013).

2.3.2 The type three secretion system

Secretion systems are widespread throughout multiple bacterial species to transport small molecules, DNA (deoxyribonucleic acid) or proteins across membranes. The type three secretion system (also termed injectisome) of *Yersinia,* which was first described in 1994, is used to inject Yop effector proteins into the host cell (Rosqvist et al., 1994). Starting from the bacterial cytosol, the inner and outer bacterial membrane is spanned by the basal body, followed by the needle forming an empty channel across the extracellular environment. On top of the needle is the tip complex, which is formed by LcrV. The tip complex connects the needle to the translocation pore (also called translocon), formed by YopB and YopD, which are inserted into the host cell membrane. The translocon completes the channel from the bacterial cytoplasm to the host cytosol, which enables single step transport of effector proteins into the host cell (Dewoody et al., 2013a; Nauth et al., 2018).

As previously mentioned, the expression, secretion and translocation of Yops and components of the T3SS is dependent on the environmental conditions, especially the temperature and calcium concentration (Cornelis, 2002a). The expression of T3SS genes and building of the injectisome is initiated at 37 °C and millimolar calcium concentrations (Dewoody et al., 2013a). *In vitro,* chelation of calcium from the medium leads to a termination of bacterial growth, strong expression of T3SS genes and simultaneous secretion of Yops (Lee et al., 1998), whereas *in vivo* host cell contact stimulates translocation of Yops in the host cell (Cornelis, 2002a; Dewoody et al., 2013a).

2.3.3 Effector proteins

Plasmid encoded *Yersinia* effector proteins which are translocated into the host cell fulfill a variety of different functions to have an impact on phagocytic activities and inflammatory response. In general, they act by either directly interacting with host cell proteins or by mimicking the function of them in a more efficient way (Atkinson & Williams, 2016; Pha &

Navarro, 2016). On the one hand, *Yersinia* effector proteins YopH, YopO, YopE and YopT, manipulate the actin cytoskeleton to inhibit phagocytosis (Aepfelbacher, 2004; Barbieri et al., 2002; Grosdent et al., 2002) and on the other hand, the inflammatory response is modulated by YopM, YopP and YopH (Cornelis, 2002b). Additionally, there are two more *Yersinia* effector proteins YopQ and YopN, which are also translocated into the host cell (Garcia et al., 2006; Lee et al., 1998; Thorslund et al., 2011). Compared to the other six effectors, those two rather regulate secretion and translocation (Forsberg et al., 1991; Holmström et al., 1997) and in the case of YopQ it was also shown that the host cell inflammasome activation was prevented (Bliska et al., 2013; Brodsky et al., 2010). Summarized, all Yops work together in different ways to establish a successful bacterial survival and proliferation by manipulating phagocytic activity and host immune response.

2.3.3.1 YopH, YopO, YopE & YopT inhibit phagocytosis

YopH, a 50 kDa (kilodalton) protein tyrosine phosphatase (Guan & Dixon, 1990), inhibits phagocytosis by targeting components of the focal adhesion or focal adhesion like complexes (Fällman et al., 1995; Grabowski et al., 2017; Ruckdeschel et al., 1996). All interaction partners of YopH like the p130 Crk-associated substrate (p130cas), promyelocytic leukemia (PML) and the retinoic acid receptor-alpha (RARA) regulated adapter molecule 1 (PRAM-1), SH2 domain containing leukocyte protein of 76 kDa (SLP-76), Src kinase-associated phosphoprotein of 55 kDa homologue (SKAP-HOM), Paxilin, Proto-oncogene tyrosine-protein kinase (Fyn) binding protein (Fyb) and focal adhesion kinase (FAK) play a vital role in the contact-dependent signalling, either via integrins or via the T-cell receptor and are dephosphorylated by YopH to prevent downstream signalling (Klinghoffer et al., 1999; Mitra & Schlaepfer, 2006; Ophir et al., 2013). Furthermore, it was shown that YopH is able to prevent T- and B-cell activation (Yao et al., 1999). *In vivo* experiments in mice showed that YopH blocks the neutrophil recruitment to the Peyer's patches (Dave et al., 2016).

YopO, which is also called *Yersinia* protein kinase A (YpkA) in *Y. pseudotuberculosis* and *Y. pestis* is a kinase having anti-phagocytic activities in the host. It comprises three domains, one important for membrane localization, one having a Ser/Thr (serine/ threonine) kinase domain function and one Guanosine diphosphate (GDP) dissociation inhibitor (GDI) domain (Galyov et al., 1993; Håkansson et al., 1996; Juris et al., 2000). Translocation of YopO/YpkA takes place in an inactive state and upon binding of the N-terminal domain to the host cell membrane an interaction with actin leads to autophosphorylation and activation of the kinase (Galyov et al., 1993; Trasak et al., 2007). YopO/YpkA directly targets and phosphorylates many regulators of actin polymerization thus leading to the dissolving of stress fibers and rounding of cells (Prehna et al., 2006). Furthermore, the GDI domain of

YopO binds directly to small Rho (Ras (Rat sarcoma virus) homologue family member)-GTPases, possessing a GDP in their active center, which inhibits the exchange of GDP to GTP (Guanosine triphosphate) and thus to a freeze in an inactive conformation (Dukuzumuremyi et al., 2000).

YopE is a 23 kDa protein having a GTPase activating protein (GAP) activity and is the first translocated effector of *Yersinia* (Mahdavi et al., 2014). It directly targets Rac1 (Ras-related C3 botulinum toxin substrate 1), RhoG and partially RhoA, which are all small Rho-GTPases, thus disturbing the actin cytoskeleton dynamics and finally leading to rounding of the cell and their inability to build phagocytic cups (Andor et al., 2001; Mohammadi & Isberg, 2009; Rosqvist et al., 1990; Viboud & Bliska, 2001). Inhibition of Rac1, which can also activate p38 (p38 mitogen-activated protein kinase) and JNK (c-Jun N-terminal kinase) leads to less IL-8 (Interleukin 8) production and furthermore to an impaired IL-1β (Interleukin 1β) maturation as active Rac1 can also trigger caspase-1 (Coso et al., 1995; Mainiero et al., 2000; Schotte et al., 2004; Viboud et al., 2006). Furthermore, YopE was also shown to reduce the levels of reactive oxygen species (ROS) (Songsungthong et al., 2010) as some of the inhibited Rho-GTPases play a role in the production of ROS (Condliffe et al., 2006; Hordijk, 2006). *In vivo* experiments also showed that polymorphonuclear neutrophils (PMNs) are inactivated by YopE (Westermark et al., 2014). Besides, the inhibition of Rho-GTPases by YopE is sensed from the cell as a danger signal, which leads to the activation of the Pyrin inflammasome in bone-marrow-derived macrophages (BMDMs) (Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016).

YopT, which is a 36 kDa cysteine protease, was shown to cleave the small Rho-GTPases Rac1, RhoA, RhoG and Cdc42 (Cell division control protein 42 homolog) thus leading to their detachment from the membrane and irreversible inhibition, as they do not longer stay in contact with their membrane bound interaction partners. By that, YopT is able to impair the actin cytoskeleton dynamics and inhibits phagocytosis (Iriarte & Cornelis, 1998; Shao et al., 2003; Sorg et al., 2001). The inhibition of the small Rho GTPases further leads to the activation of the Pyrin inflammasome like YopE does (Chung et al., 2016). *In vivo* experiments revealed that RhoA is the preferred target of YopT, whereas Rac1 is preferentially targeted by YopE (Aepfelbacher et al., 2003).

2.3.3.2 YopM

YopM is a leucine rich repeat (LRR) protein, which is found in the cytosol and the nucleus of the host cell as well as in the extracellular space (Benabdillah et al., 2004; Leung et al., 1990; Skrzypek et al., 1998). Dependent of its localization it fulfills different kinds of functions. It is the only Yop without a catalytic activity and its size varies between 41 kDa

(*Y. pestis*) and 57 kDa (*Y. enterocolitica*) due to different numbers and compositions of the LRRs (Boland et al., 1998; Cornelis et al., 1998; Höfling et al., 2015; Leung et al., 1990). In the cytosol, YopM inhibits caspase-1 activation through binding and activation of the two serine/threonine kinases “ribosomal S6 protein kinase” (RSK) and the “protein kinase C-related kinase” (PRK/PKN) thus forming a heterotrimeric protein complex (Hentschke et al., 2010; Höfling et al., 2014; McDonald et al., 2003). Activation of these kinases leads to the phosphorylation of 14-3-3 proteins, which in turn phosphorylate Pyrin leading to the inactivation of Pyrin. By that the formation of the Pyrin inflammasome, consisting of Pyrin, ASC (Apoptosis-associated speck-like protein containing CARD (caspase activation and recruitment domains)) and caspase-1, which is a response to the inhibition of Rho GTPases by YopE and YopT, is prevented (Chung et al., 2016; McDonald et al., 2003). Furthermore, *in vitro* experiments showed that YopM from *Y. pseudotuberculosis* YPIII and *Y. pestis* CO92 were also able to directly bind to caspase-1 through an YLTD motif, thus also inhibiting the inflammasome activation (LaRock & Cookson, 2012). Nuclear import of YopM is either done via the C-terminal domain of YopM without any host factor interaction or via a vesicle associated pathway (Benabdillah et al., 2004; Berneking et al., 2016; Skrzypek et al., 1998). In contrast, nuclear export of YopM is transmitted through the binding to DDX3 (DEAD-box helicase 3) and the exportin CRM1 (Chromosomal maintenance 1) (Berneking et al., 2016). *In vivo* experiments in mice showed that YopM binds to RSK and PRK in the nucleus, leading to an increase in the expression of the immunosuppressive cytokine Interleukin 10 (IL-10). This is an important mechanism of the *Yersinia* virulence as IL-10 downregulates the expression of inflammatory cytokines, which is beneficial for the pathogen (McPhee et al., 2010; McPhee et al., 2012). Furthermore, in primary human macrophages, YopM is an important mediator of the nuclear translocation of STAT3 (signal transducer and activator of transcription 3), which also increases IL-10 gene expression (Berneking et al., 2023). In summary, the activity and function of YopM is highly dependent on its interaction partners and its cellular localization. The two main functions of YopM are the inhibition of the Pyrin inflammasome formation as well as the stimulation of IL-10 gene expression.

2.3.3.3 YopP/J

YopP/J (YopP in *Y. enterocolitica* and YopJ in *Y. pseudotuberculosis* & *Y. pestis*) is a 33 kDa acetyltransferase with specificity for lysines, serines and threonines and a highly potent suppressor of host pro-inflammatory signalling pathways by inhibiting MAPK and NF-κB signalling pathways in the host cell (Meinzer et al., 2012; Mittal et al., 2006; Mittal et al., 2010; Mukherjee et al., 2006; Paquette et al., 2012). Furthermore, it possesses a cysteine protease activity leading to deubiquitination and de-sumoylation of the target proteins

TRAF2, TRAF3 and TRAF6 (TNF (tumor necrosis factor) receptor-associated factor 2/3/6), which was first thought to be the way how YopP inhibits components of the NF-κB and MAPK signalling pathway (Cao et al., 2016; Haase et al., 2005; Orth et al., 2000; Sweet et al., 2007; Zhou et al., 2005). Targeting of MAPK and NF-κB signalling pathways is attractive for the pathogen, because they play a central role in the signal mediation of PAMPs (pattern associated molecular pattern) detected by PRRs (pattern recognition receptors), which finally results in the activation of transcription factors leading to the expression of important genes for the innate immune response (Schubert et al., 2020). In detail, YopP/J acetylates TAK1 (transforming growth factor β-activated kinase 1) (Meinzer et al., 2012; Paquette et al., 2012), IκB (inhibitor of nuclear factor kappaB kinase) (Mittal et al., 2006; Mukherjee et al., 2006) and MEK2, MEK4, MEK6 and MEK7 (Mitogen-activated protein kinase kinase 2/4/6/7) (Meinzer et al., 2012; Mittal et al., 2006; Mukherjee et al., 2006; Paquette et al., 2012) thus blocking immune signalling. The acetyltransferase activity is activated by binding of inositol hexakisphosphate (IP_6) in the host cell (Mittal et al., 2010; Pruneda et al., 2016; Zhang et al., 2016). The inhibition of intracellular signalling by YopP/J also leads to downregulation of several pro-inflammatory cytokines like IL-8 (Interleukin 8) in HEK (human embryonic kidney) cells (Thiefes et al., 2006), IL-6 (Interleukin 6) and IL-8 in human umbilical vein endothelial cells (HUVECs) (Denecker et al., 2002) and TNF-α in murine macrophages (Boland & Cornelis, 1998). Additionally, YopP/J induces cell death in macrophages on the one hand through caspase-8 dependent extrinsic apoptosis pathway, which is activated by the receptor-interacting protein 1 and 3 kinases (RIPK1/K3) leading to the cleavage of caspases-3/7 and 9 (Brentnall et al., 2013; Lamkanfi & Kanneganti, 2010; Nicholson, 1999; Stennicke et al., 1998). On the other hand, activation of caspase-8 further leads to the activation of caspase-1, which is part of the inflammasome thus leading to the cleavage of the pro-inflammatory cytokines pro-IL-1β (pro-Interleukin 1β) and pro-IL-18 (pro-Interleukin 18) as well as the cleavage of Gasdermin D. Cleaved Gasdermin D forms pores in the cell membrane, which leads to cell membrane rupture and efflux of IL-1β and IL-18 as part of a programmed cell death called pyroptosis (Philip et al., 2014). Interestingly, YopP/J inhibits transcription of IL-1β and IL-18 (Ratner, Orning, Proulx, et al., 2016).

2.3.3.4 YopQ/K

YopQ/K (YopQ in *Y. enterocolitica* and YopK in *Y. pseudotuberculosis* & *Y. pestis*) is an important virulence factor and regulatory protein. It is important for the regulation of the Yop effector translocation into the host cell as well as the virulence of *Yersinia* (Aili et al., 2008; Dewoody et al., 2011; Dewoody et al., 2013b; Holmström et al., 1997; Holmström et al., 1995a, 1995b; Thorslund et al., 2013). Loss of YopQ/K leads to hypertranslocation of several Yop proteins, especially the pore proteins YopB and YopD (Dewoody et al., 2013a;

Zwack et al., 2015). Translocation of YopB and YopD is associated with the activation of the NLRP3 (NLR family Pyrin domain containing 3) inflammasome formation mediated by GBPs (Galectin and guanylate binding protein) and Galectin-3 (Zwack et al., 2017). By inhibiting the hypertranslocation of the pore proteins, YopQ/K also inhibits the NLRP3 inflammasome formation (Brodsky et al., 2010; Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016; Zwack et al., 2017; Zwack et al., 2015). *In vivo* experiments in mice showed that infection with *Yersinia* lacking YopQ/K leads to colonization of the Peyer's patches, whereas no systemic infection could be established (Holmström et al., 1995a). Besides, the inflammatory response started much earlier, by that inhibiting dissemination to spleen and liver (Peters & Anderson, 2012; Straley & Bowmer, 1986; Straley & Cibull, 1989). Furthermore, YopQ/K is able to downregulate the activation of caspase-1 and thus also activation of Il-1ß (Brodsky et al., 2010).

2.3.4 Modulation of gene expression

Like already mentioned previously, many Yop effector proteins are able to modulate the gene expression of the host, either through direct interaction with transcription factors or by intervening with signalling pathways of which the inhibition of NF-κB and MAPK signalling pathway by YopP/J is the most important one (Grabowski et al., 2017). For example, YopE and YopH cooperate with YopP/J in suppressing IL-8 expression (Viboud et al., 2003), YopM induces expression of IL-10 (Berneking et al., 2016) and YopT is responsible for the upregulation of the expression of the proteins krüppel-like factor 2 (KLF2) and the glucocorticoid-induced leucine zipper (GILZ) (Dach et al., 2009; Köberle et al., 2012). As *Yersinia* spp. possess many PAMPs like LPS (lipopolysaccharide), YadA, Invasin and the T3SS pore, their Yops effectively try to impair PAMP generated gene expression of pro-inflammatory genes (Auerbuch et al., 2009; Grassl et al., 2003; Palmer et al., 1998; Ruckdeschel et al., 1998; Schmid et al., 2004; Schulte et al., 2000).

The effect of Yops on host gene expression has already been investigated in two studies using microarray analysis of mouse macrophage cell lines infected with *Y. enterocolitica* (Hoffmann et al., 2004; Sauvonnet et al., 2002). While in one study, in J774 cells, 50 differentially expressed genes (DEGs) were found after 2 h of infection (Hoffmann et al., 2004), in another study with PU5-1.8 cells, they found 857 DEGs after 2.5 h of infection (Sauvonnet et al., 2002). Even if there is huge difference in the number of DEGs, both studies have in common that YopP was the main player of the suppression of PAMP induced gene expression. However, in wild type infected cells, also other Yops accounted for changes in gene expression. YopM played a role in PU5-1.8 cells in gene expression changes of genes having a role in cell cycle regulation, cell growth and phagocytosis

(Sauvonnet et al., 2002), but in J774 cells neither YopM nor YopH played a role in gene expression changes (Hoffmann et al., 2004).
Beyond that, wild type *Y. enterocolitica*, avirulent *Y. enterocolitica* and *Y. enterocolitica* lacking YopP were used to infect HeLa cells (Bohn et al., 2004) and primary human macrophages were infected with wild type *Y. enterocolitica*, the avirulent strain, a strain lacking YopM and one lacking YopP (Berneking et al., 2016).

2.4 Pathogen recognition and polarization of primary human macrophages

Macrophages belong to the group of white blood cells of the innate immune system and are found in all tissues. They are important for the detection and digestion of pathogens but are also important for the recruitment of other immune cells and the presentation of antigens to T cells. Macrophages benefit tissue homeostasis under non stimulating conditions, whereas infection or other stimuli lead to an activation resulting in chemo- and cytokine production as well as microbial killing (Ivashkiv, 2013). The polarization and activation of macrophages depends on the external environmental stimuli and leads to either classical activated (M1) macrophages or alternative activated (M2) macrophages. Stimulation with LPS, interferons (IFNs), granulocyte macrophage colony-stimulating factor (GM-CSF) or other microbial stimuli leads to the polarization to M1 macrophages. M1 macrophages kill microbes, produce pro-inflammatory cytokines like TNF, IL-1 & IL-12 and other antimicrobial molecules. Furthermore, they produce reactive oxygen and nitrogen species as well as the chemokines CXCL9 (Chemokine (C-X-C motif) ligand 9) and CXCL10 (Chemokine (C-X-C motif) ligand 10), which are important for T_h1 (T helper cell 1) recruitment. A disadvantage of M1 macrophages is their ability to cause cytotoxicity and tissue damage (Ivashkiv, 2013; Mosser & Edwards, 2008).
In contrast, M2 macrophages are induced by IL-4 (Interleukin 4) & IL-13 (Interleukin 13), IL-10, TGF-β (Transforming growth factor β), glucocorticoids and immune complexes. They promote and preserve tissue function, promote repair and wound healing as well as restrain and resolve inflammation after infection or injury. Inflammatory processes are downregulated by M2 macrophages through the release of anti-inflammatory cytokines like IL-10 and IDO (indoleamine 2,3-dioxygenase) as well as the release of the T_h2 (T helper cell 2) chemokines CCL18 (Chemokine (C-C motif) ligand 18) and CCL22 (Chemokine (C-C motif) ligand 22) (Gordon & Martinez, 2010). While in theory M1 and M2 phenotypes are clearly separated from each other, *in vivo* there might be a mixture of phenotypes (M1, M2 and M1/M2) due to counteracting polarizing factors (Ivashkiv, 2013; Sica & Mantovani, 2012).

Pathogen recognition in macrophages takes place through PRRs, which detect conserved structures called PAMPs, throughout all microbial species. Furthermore, PRRs also recognize endogenous danger signals from damaged cells, which are called damage-associated molecular patterns (DAMPs). PRRs can be divided into four different classes. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are transmembrane proteins, whereas retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are cytoplasmic (Akira et al., 2006; Tang et al., 2012). Detection of PAMPs or DAMPs by PRRs leads to the activation of signalling cascades resulting in the transcriptional upregulation of genes encoding for proteins involved in inflammatory responses like the pro-inflammatory cytokines TNF, IL-1 and IL-6 as well as IFNs, chemokines and other antimicrobial proteins (Pandey et al., 2014). One of the main and best-studied PAMPs is the bacterial LPS, the major component of gram-negative bacterial outer cell membrane. LPS is detected by TLR4, which associates with the myeloid differentiation factor 2 (MD2) upon recognition thus leading to the activation of MAP kinases and the IKK (IκB kinase) complex (Kawai & Akira, 2010; Park et al., 2009). IKK complex activation results in the phosphorylation of IκBα, a NF-κB inhibitory protein, which is proteasomal degraded upon phosphorylation by that releasing NF-κB. Free NF-κB shuttles to the nucleus where it induces pro-inflammatory gene expression (Kawai & Akira, 2010). Activated MAP kinases further phosphorylate several other kinases like p38, extracellular signal-related kinase (ERK) 1 & 2 kinases and JNK resulting in the activation of the transcription factor activator protein 1 (AP-1) and inflammatory gene expression. Furthermore, the TLR4 receptor can be endocytosed where it facilitates activation of interferon regulatory factor (IRF) 3 and NF-κB leading to the induction of inflammatory cytokines and type I interferons (Akira et al., 2006; Zanoni et al., 2011).

2.5 Epigenetics

Even though all cells in a eukaryotic organism possess the same DNA sequence, there is a plethora of phenotypic variability, originating from different DNA readouts. The explanation of this phenomenon is called epigenetics, which means that inheritable changes of the properties of the DNA, proteins or molecules bound to it regulate processes involved in DNA transcription, repair, replication or recombination (Greally, 2018; Henikoff & Greally, 2016; Kouzarides, 2007).

In the nucleus the smallest subunit of a chromosome is called nucleosome consisting of 147 bp of DNA wrapped around a histone (H) octamer, which is built of two copies of each H2A, H2B, H3 and H4 (Gardner et al., 2011). The linker histone H1 acts as a stabilizer of the chromatin structure between the nucleosomes and leads to a further organization into chromosomes. Several epigenetic processes like post-translational modifications (PTMs)

of the histones, non-coding RNAs, DNA methylation and ATP (Adenosine triphosphate) dependent nucleosome remodelling have an influence on the accessibility of the DNA (Bierne et al., 2012). Furthermore, they modulate the recruitment of regulatory proteins which in turn, together with different accessibility of the DNA, leads to either an open chromatin state (Euchromatin) or a more closed chromatin state (Heterochromatin) (Kouzarides, 2007). Combinations of several histone modifications at promoters, which are located closely to the transcription start site (TSS) and enhancers, which are located far away from the TSS are associated with either transcriptional activation or repression of genes (Schoenfelder & Fraser, 2019; Wang et al., 2008; Wittkopp & Kalay, 2011). Post-translational modification of histones like methylation, phosphorylation, acetylation or ubiquitylation normally occurs at various amino acid residues at their flexible N-terminal tail thus influencing the chromatin state (Bierne et al., 2012; Kouzarides, 2007). PTMs are either mediated by enzymes like methyltransferases, acetyltransferases or kinases, which are called “writers”. They are detected by so called “readers” and removed by so called “erasers” like demethylases, deacetylases or phosphatases (Bierne et al., 2012). Histone acetylation at lysine residues neutralizes the basic charge of lysine residues, by that disrupting contacts with the negatively charged DNA leading to transcription promotion (Bierne et al., 2012). Histone methylation occurs at lysine residues and is associated with activation as well as repression of transcription. For example, trimethylation of lysine-9 or -27 on H3 (H3K9me3/H3K27me3) are repressive marks whereas H3K4me3 is activating (Barski et al., 2007). Phosphorylation of histone H3 on serine-10 (H3S10ph) is associated with transcriptional activation and the occurrence of acetylation of histone H3 on lysine-9 (H3K9ac) as well as H3K14ac (Bierne et al., 2012). Besides it was shown that H3S10ph is necessary for the acetylation of lysine-14 on H3 by the histone acetyltransferase (HAT) Gcn5 (Margueron & Reinberg, 2011). Furthermore, infection with several different bacteria leads to either phosphorylation or dephosphorylation of serine-10 on H3. *Listeria monocytogenes* infection of endothelial HUVEC cells leads to phosphorylation of H3 on serine-10 (Opitz et al., 2006; Schmeck et al., 2005). Interestingly, infection of gastric epithelial cells with *H. pylori* leads to dephosphorylation of H3S10, thus impacting the cell cycle (Fehri et al., 2009) as well as the transcription of the oncogene c-jun (positively regulated) and the heat shock gene hsp70 (negatively regulated) (Ding et al., 2010). Additionally, dephosphorylation of H3S10 is a result of infection of TNF-α activated lung epithelial cells with *Bacillus anthracis* lethal toxin (LT), which reduces the immune response by limiting the accessibility of the IL-8 promoter to NF-κB (Raymond et al., 2009). *Shigella flexneri* effector protein OspF (Outer shigella protein F) effectively dephosphorylates MAP kinases, by that preventing H3S10ph and the activation of NF-κB regulated immune response genes (Arbibe et al., 2007). Infection of HeLa cells with *Listeria monocytogenes*

leads to dephosphorylation of H3S10 through the toxin listeriolysin O (LLO), which in fact supresses expression of the immune genes CXCL2, MKP2 (mitogen-activated protein kinase phosphatase 2) or IFIT3 (interferon-induced protein with tetratricopeptide repeats 3) (Hamon et al., 2007). Other pore-forming toxins like pneumolysin (PLY) from *Streptococcus pneumonia*, perfringolysin (PFO) from *Clostridium perfringens* and aerolysin from *Aeromonas hydrophila* show the same effect of serine-10 dephosphorylation, which is triggered by K^+ (Potassium cation) efflux through pore formation (Hamon & Cossart, 2011). The “epigenetic landscape” as an interplay of histone modifications, proteins, chromatin bound RNA and DNA methylation state not only impacts the accessibility of genomic regions and their transcription but also influences the immune response upon pathogen detection in several cell types (Connor et al., 2019; Ivashkiv, 2013).

2.6 The Inflammasome

Multimeric protein complexes consisting of a sensor protein, an adaptor protein and the cysteine protease pro-caspase-1, which assemble as a reaction towards the detection of PAMPs or DAMPs, are called inflammasomes. Assembly of the inflammasome leads to self-cleavage of the pro-caspase-1 leading to the active caspase-1, which proteolitically cleaves pro-IL-1ß and pro-IL-18 leading to mature IL-1ß and IL-18. Furthermore, active caspase-1 cleaves Gasdermin D, which forms a pore in the cell membrane resulting in a type of programmed cell death called pyroptosis (Malik & Kanneganti, 2017).

2.6.1 Pathogen-associated and danger-associated molecular pattern detecting sensors

PAMP and DAMP detecting sensors are located in the cytoplasm and encompass proteins containing a tripartite motif (TRIM) like Pyrin, the absent in melanoma-2 (AIM2)-like receptors (ALRs) as well as NLRs. AIM2, Pyrin and NLRs can assemble with the adaptor molecule ASC and the zymogen caspase-1, forming the inflammasome (Malik & Kanneganti, 2017).

Sensors belonging to the family of the NLR proteins consists of a C-terminal LRR domain, a central nucleotide-binding domain (NBD) and a variable N-terminal tail, which either encompass a CARD or a Pyrin domain (PYD) dividing NLR proteins into NLRP or NLRC (NLR family CARD domain-containing) receptors. 22 and 34 genes encoding for NLRs are present in the human or mouse genome, respectively (Harton et al., 2002). Of those only NLRP1, NLRP3 and NLRC4 can form an inflammasome recruiting pro-caspase-1 (Lamkanfi et al., 2007). The molecular basis for inflammasome assembly is the homotypic interaction between CARD-CARD and PYD-PYD leading to oligomerization (Cai et al., 2014; Lu et al., 2014; Sborgi et al., 2015). Oligomerization of the sensor with ASC is mediated via

interactions of their PYD domains and ASC binding with the pro-caspase-1 relies on CARD-CARD interaction (Lu et al., 2014). The adaptor protein ASC is necessary for inflammasome formation with the sensors NLRP3, AIM2 and Pyrin as they all do not possess a CARD domain. In contrast, NLRP1 and NLRC4 can directly bind pro-caspase-1 through their CARD domains leading to an ASC independent inflammasome formation (Jin et al., 2013; Nour et al., 2009; Ponomareva et al., 2013).

The NLRP3 sensor reacts to several endogenous and infectious DAMPs like microbial cell wall components, pore forming toxins, nucleic acids and environmental crystalline agents like silica as well as ATP and uric acid crystals (Cassel et al., 2008; Cruz et al., 2007; Dostert et al., 2008; Hornung et al., 2008; Kanneganti, Body-Malapel, et al., 2006; Kanneganti, Ozören, et al., 2006). However, NLRP3 probably senses a cellular signal of distress induced by those molecules like K^+ efflux, Ca^{2+} (Calcium cation) signalling, lysosome rupture, cell volume changes or ROS production (Compan et al., 2012; Halle et al., 2008; Kanneganti et al., 2007; Muñoz-Planillo et al., 2013; Schorn et al., 2011; Zhou et al., 2011).

NLRC4 proteins directly bind and activate pro-caspase-1 with their CARD domain (Poyet et al., 2001) as a response towards bacterial flagellin (Franchi et al., 2006; Miao et al., 2006; Molofsky et al., 2006; Ren et al., 2006) and components originating from the flagellin-associated secretion system (Miao et al., 2010; Yang et al., 2013; Zhao et al., 2011). However, the direct sensor of the mentioned PAMPs are NLR family apoptosis inhibitory proteins (NAIPs) of which the human genome encodes one, while the mouse genome encodes for 7 NAIPs (Endrizzi et al., 2000). Human NAIP has the ability to detect flagellin as well as components of bacterial T3SS (Kortmann et al., 2015; Yang et al., 2013).

Modification of cytoskeletal proteins is detected by Pyrin, a PYD containing sensor, leading to the assembly of an inflammasome (Gavrilin et al., 2012; Xu et al., 2014). Many bacteria like *Clostridium difficile* (TcdB), *Vibrio parahemolyticus* (VopS), *Clostridium botulinum* (C3), *Burkholderia cenocepacia* and *Bordetella pertussis* (PT), produce toxins which modify proteins of the Rho protein family (Xu et al., 2014). Even though there is no direct interaction between Rho and Pyrin, Pyrin is essential for inflammasome activation responding to modification of Rho proteins (Dumas et al., 2014; Xu et al., 2014).

3 Aims of the study

T3SS-expressing bacteria like *Yersinia* spp. possess an arsenal of virulence mechanisms mediated by T3SS effector proteins to manipulate the host cell defense aiming for survival and growth (Coburn et al., 2007). Up to this point it is known that seven effector proteins called Yops are translocated into the host cell, where four of them impair the phagocytic activity of the host to enable extracellular survival (Viboud & Bliska, 2005). The other three Yops, YopM, YopP/J and YopQ/K inhibit several inflammatory pathways thus counteracting the immune recognition of the T3SS (Schubert et al., 2020). PAMP induced modulation of gene expression is globally impaired by YopP, whereas for YopM and YopQ no global effect has been shown yet (Bekere et al., 2021; Hoffmann et al., 2004; Sauvonnet et al., 2002). However, YopM has been shown to prevent Pyrin inflammasome formation (Chung et al., 2016; McDonald et al., 2003), YopQ/K inhibits NLRP3 inflammasome formation (Brodsky et al., 2010; Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016; Zwack et al., 2017; Zwack et al., 2015) and YopP/J induces activation of caspase-1 through an unknown mechanism (Brodsky et al., 2010; Zheng et al., 2011). For histone modifications it has been shown that especially YopP contributes a lot to re-modulation after infection, but for YopM and YopQ no effect was shown yet (Bekere et al., 2021). However, none of them has been connected to re-modulation of histone H3 serine-10 phosphorylation, which is altered by many bacterial effectors (Bierne & Pourpre, 2020; Connor et al., 2019). Due to the partly lack of information regarding the influence of YopM, YopP and YopQ on gene expression, inflammasome formation or specific histone modifications this study is aims to:

- Figure out the role of YopP, YopM and YopQ on the influence of gene expression
- Elucidate a potential interplay of YopP and YopM in modulation of gene expression
- Clarify the role of YopM, YopP and YopQ in the impairment of inflammasome formation and figure out if there is an interplay between them
- Determine the occurrence of H3S10ph in primary human macrophages and check for any impact of the translocated T3SS effectors on it

4 Material and Methods

4.1 Materials

4.1.1 Devices

Table 1: Devices

Device	Product, Manufacturer
Accu-jet	Accu-jet® pro, Brand, Wertheim, Germany
Agarose gel electrophoresis	Agarose gel chamber: Roth, Karlsruhe, Germany
Bioanalyzer	Agilent 2100, Agilent Technologies, Santa Clara, USA
Blotting chamber	OWL HEP-1, Thermo Fisher Scientific, Waltham, USA
Cell counting chamber	Neubauer-Zellzählkammer, Hartenstein, Würzburg, Germany
Cell incubator	CB Series, Binder, Tuttlingen, Germany
Centrifuges	Sorvall RC-5B, Thermo Fisher Scientific, Waltham, USA 5417R and 5810R, Eppendorf, Hamburg, Germany Biofuge pico, Heraeus Instruments, Hanau, Germany Sigma 3-18K, Sigma-Aldrich, St. Louis, Missouri, USA Sarstedt MC 6 Centrifuge, Nümbrecht, Germany Strip rotor MC 6 – 0.2 ml, 2x 8f, Sarstedt, Nümbrecht, Germany
Clean bench	Hera Safe, Thermo Fisher Scientific, Waltham, USA
Electroporator	Gene Pulser II electroporator with Puls controller Plus, Bio-Rad
Freezer	-20 °C: comfort, Liebherr-International AG, Bulle, Switzerland -80 °C: HERA freezer, Heraeus, Kendro Laboratory, Hanau, Germany
Freezing containers	Cryo freezing containers, Nalgene Scientific, Rockford, USA
Magnetic stirrer	RCT-Basic, IKA-Labortechnik, Staufen, Germany

Microscope	Laser scanning microscope Olympus FV 3000, 20x Air- & 60x Oil-objective, Olympus, Tokyo, Japan
Microwave	Dimension 4, Panasonic, Osaka, Japan
NanoDrop® ND-1000	PeqLab Biotechnologie GmbH, Erlangen, Germany
pH meter	Seven easy, Mettler-Toledo, Giessen, Germany
Photometer	Ultrospec 3000 pro, Amersham/GE Healthcare Europe, Munich, Germany
Pipettes	2, 10, 100, 200, 1000 µl, Research Plus, Eppendorf, Hamburg, Germany
Power supply unit	Power Pac 2000, Bio-Rad, Munich, Germany
Qubit	Qubit Fluorometer, Thermo Fisher Scientific, Waltham, USA
Refrigerator	4-8 °C, Liebherr Premium, Liebherr-International AG, Bulle, Switzerland
RT-qPCR cycler	LightCycler® 480, Roche, Switzerland
Scanner	CanoScan 4400F, Canon, Amsterdam, Netherlands
SDS-PAGE electrophoresis cell	SDS-PAGE: Mini-Protean II Bio-Rad, Munich, Germany
Sequencer	Nextseq 500, Illumina, San Diego, USA
Shaking incubator	Certomat BS-1, Sartorius, Göttingen, Germany
Thermoblock	Accu Block™ Digital Dry Bath Dual Block, Labnet, Cary, USA
Thermocycler	Thermocycler peqStar, PeqLab Biotechnologie GmbH, Erlangen, Germany
Thermomixer	Thermomixer Comfort, Eppendorf, Hamburg, Germany
Transfection System	Neon® Transfection system, Invitrogen/Life Technologies, Carlsbad, USA
UV-Transilluminator	Vilber Lourmat TFX-20M, Eberhardzell, Germany
Vortexer	REAX Topo, Heidolph Instruments, Schwabach, Germany
Water bath	GFL Type 1013, GFL, Burgwedel, Germany
Weighing scales	440-47N, Kern, Balingen, Germany
Western Blot imaging system	Amersham ImageQuant™ 800, Cytiva, Marlborough, USA

4.1.2 Disposables

Table 2: Disposables

Item	Product, Company
µMacs Columns	MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
µMacs Columns separation column 25LE	MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
Bioanalyzer chips	DNA High Sensitivity Chip, RNA 6000 Nano Chip, Agilent Technologies, Santa Clara, USA
Bottle-top sterile filter units	Steritop Filter Units 0.22 µm, Merck Millipore, Darmstadt, Germany
CD14 Microbeads, human	MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
Cell culture dishes	100 mm, Sarstedt, Nümbrecht, Germany
Glass Coverslips	12mm, No. 1.5H for high resolution, Marienfeld GmbH, Lauda-Königshafen, Germany 18 mm, No. 1.5H, Marienfeld GmbH, Lauda-Königshafen, Germany
Cryo tubes	1.6 ml, Sarstedt, Nümbrecht, Germany
Disposable cuvettes	1.5 ml, 12.5 x 12.5 x 45 mm, BRAND GmbH + CO KG, Wertheim, Germany
Disposable needles	0.40 x 20 mm ,0.55 x 25mm, 0.6 x 25 mm STERICAN disposable needles, B.Braun, Melsungen, Germany
Disposable inoculation loop	10 µl, Sarstedt, Nümbrecht, Germany
Electroporation cuvettes with 1 mm electrode gap	PeqLab Biotechnologie GmbH, Erlangen, Germany
Glass pasteur pipettes	230 mm, Heinz Herenz Medizinalbedarf, Hamburg, Germany
Multi-well-plates	6-/24-well, Sarstedt, Nümbrecht, Germany
Object slides	76x26 mm, Karl Hecht, Sondheim, Germany
Parafilm	M Bemis, Pechiney Plastic Packaging, Neenah, USA
PhaseLock tubes	2 ml, heavy, Quantabio, Beverly, USA
Pipette tips	Sterile filter tips, Biosphere 10, 200, 1.000 µl, Sarstedt, Nümbrecht, Germany
Plastic syringe	Sterile, 2 ml, 5 ml, 10 ml, 20 ml, B. Braun, Melsungen, Germany

PVDF-membrane	Immobilon-P, 0.45 µm pore size, Mollipore, Billerica, USA
Reaction tubes	0.2 ml, Biozym Scientific, Hessisch Odendorf, Germany; 0.5 ml, 1.5 ml, 2 ml, Sarstedt, Nümbrecht, Germany; 15 ml, 50 ml Centrifuge Tubes, CELLSTAR, Greiner Bio-One, Kremsmuenster, Austria; qPCR Strip Tubes and Caps, 0.1 ml, Qiagen, Germany; Qubit assay tubes 0.5 ml, Thermo Fisher Scientific, Waltham, USA
Scalpel	Sterile, B. Braun, Melsungen, Germany
Serological Pipettes	Sterile 2, 5, 10, 25 ml, Sarstedt, Nümbrecht, Germany
Syringe filters	SFCA 0.2 µm, Thermo Scientific/Nalgene, Rockford, Illionois, USA
Whatman paper	190 g/m², Bio-Rad, Munich, Germany

4.1.3 Buffers, kits, enzymes, inhibitors and reagents

Chemicals were received from Amersham/GE Healthcare, Munich (Germany), BD Biosciences, Heidelberg (Germany), Biozyme, Oldendorf (Germany), Dianova, Hamburg (Germany), Fermentas, St. Leon-Rot (Germany), Invitrogen/Life Technologies, Carlsbad (USA), Merck, Darmstadt (Germany), PAA, Pasching (Austria), PromoCell, Heidelberg (Germany), Roth, Karlsruhe (Germany) and Sigma-Aldrich, St. Louis (USA): Buffers were sterile filtered or autoclaved for 20 min at 121 °C and 1.4 bar.

Table 3: Buffers

Buffer	Concentration	Components
SDS-PAGE		
Resolving buffer	1.5 M 0.1 % (w/v)	Tris-HCl, pH 8.8 SDS
Stacking buffer	0.5 M 0.1 % (w/v)	Tris-HCl, pH 6.8 SDS
SDS-PAGE sample buffer	62.5 mM 2 % (w/v) 10 % (w/v) 1.5 % (v/v) 0.01 % (w/v)	Tris-HCl, pH 6.8 SDS Glycerol ß-mercaptoethanol Bromophenol blue

SDS-PAGE running buffer	25 mM 190 mM 0.1 (w/v)	Tris Glycine SDS
Coomassie staining solution	0.1 % (w/v) 25 % (v/v) 10 % (v/v)	Coomassie Brilliant Blue R-250 Methanol Glacial acetic acid
Coomassie destain solution	25 % (v/v) 10 % (v/v)	Methanol Glacial acetic acid
Western-Blot		
Transfer buffer	25 mM 96 mM 20 % (v/v)	Tris Glycine Methanol
Tris buffered saline (TBS)	20 mM 150 mM	Tris-HCl, pH 7.6 NaCl
TBS with Tween® 20 (TBS-T)	1 x 0.05 % (v/v)	TBS Tween® 20
Blocking buffer	1 x 3 % (w/v)	TBS-T BSA
Histone extraction		
Hypotonic lysis buffer	10 mM 1mM 1mM 1.5 mM 1 x 1 x	Tris-HCl, pH 8.0 KCl DTT $MgCl_2$ Complete Protease Inhibitor PhosSTOP Phosphatase Inhibitor
Agarose gel electrophoresis		
Tris acetate EDTA (TAE) buffer	40 mM 20 mM 1 mM	Tris, pH 8.3 Acetic acid EDTA
Immunofluorescence staining		
Triton X-100 permeabilization buffer	1x 0.1 % (v/v)	PBS Triton™ X-100
Blocking solution	1x 3 % (w/v) 0.05 % (v/v)	PBS BSA Triton™ X-100

Table 4: Kits, enzymes, inhibitors and reagents

Kits, enzymes, reagents	Manufacturer
16% paraformaldehyde	Electron Microscopy Science, Hatfield, USA
Accutase™ StemPro™ Cell dissociation reagent	Thermo Fisher Scientific, Waltham, USA
Bio-Rad Protein Assay Dye Reagent Concentrate	Bio-Rad, Hercules, USA
Complete Protease inhibitor	Roche Diagnostics, Risch, Switzerland
DNase, RNase-Free DNase Set	Qiagen, Hilden, Germany
GeneRuler 1 kb plus DNA Ladder	Thermo Fisher Scientific, Waltham, USA
GoTaq® DNA Polymerase	Promega, Walldorf, Germany
iScript cDNA Synthesis Kit	Bio-Rad, Hercules, USA
Lji308 (RSK inhibitor)	Sigma-Aldrich, St. Louis, USA
NEBNext Poly(A) mRNA Magnetic isolation module	New England Biolabs, Ipswich, USA
NEBNext Ultra RNA Library Prep Kit for Illumina	New England Biolabs, Ipswich, USA
Neon™ Transfection system 100 µl-kit	Invitrogen/Life Technologies, Carlsbad, USA
NucleoBond® Xtra Maxi EF	Macherey-Nagel, Düren, Germany
NucleoSpin® Gel and PCR Clean-up	Macherey-Nagel, Düren, Germany
PageRuler Prestained Protein Ladder	Thermo Fisher Scientific, Waltham, USA
PD98059 (MEK1 inhibitor)	Cell Signaling Technology, Danvers, USA
Phosphate buffered saline	Sigma-Aldrich, St. Louis, Missouri, USA
PhosSTOP Phosphatase inhibitor	Roche Diagnostics, Risch, Switzerland
Phusion™ High-Fidelity DNA-Polymerase	Thermo Fisher Scientific, Waltham, USA
Plasmid miniprep kit I (C-Line)	PeqLab Biotechnologie GmbH, Erlangen, Germany
ProLong™ Glass Antifade Mountant	Thermo Fisher Scientific, Waltham, USA
Qubit dsDNA HS Assaykit	Thermo Fisher Scientific, Waltham ,USA
RedSafe™ Nucleic Acid Staining Solution	iNtRON Biotechnology, Korea
RNeasy Mini Kit	Qiagen, Hilden, Germany
ROTIPHORESE®Gel 30 (37,5:1)	Roth, Karlsruhe, Germany
SB203580 (p38 inhibitor)	Cayman Chemical, Ann Arbor, USA
SP600125 (JNK inhibitor)	Cell Signaling Technology, Danvers, USA
SuperSignal™ West Femto/Pico chemiluminescent substrate	Thermo Fisher Scientific, Waltham, USA

Takinib (TAK1 inhibitor)	Cell Signaling Technology, Danvers, USA
TaqMan™ Fast Advanced Master Mix	Applied Biosystems, Waltham, USA
TPCA-1 (IKK inhibitor)	Cell Signaling Technology, Danvers, USA

4.1.4 Antibodies

Immunofluorescence stainings were done with antibodies diluted according to Table 5 in 1 x PBS containing 3 % BSA and 0.05 % Triton™ X-100. Antibodies used in Western blots were diluted in 1x TBS-T containing 3 % BSA according to Table 5.
For western blots secondary antibodies were diluted 1:20000 – 1:30000 in 1x TBS-T. Secondary antibodies used for immunofluorescence staining were 1:200 diluted in the same diluent like the primary antibody according to Table 5.

Table 5: Primary antibodies

Antigen	Provider, catalogue number	Species	Dilution for	
			WB	IF
H3	Cell Signaling, 4499	Rabbit	1:3000	---
H3S10ph	Invitrogen, 701258	Rabbit	1:1000	1:500
H3K9me3	Abcam, ab8898	Rabbit	---	---
ASC	Santa Cruz, sc51414	Mouse	---	1:20
Caspase-1	Invitrogen, PA5-17570	Rabbit	---	1:100

Table 6: Secondary antibodies and labelling substrates

Name/Conjugate	Provider, catalogue number	Dilution
Amersham ECL Rabbit IgG, HRP-linked whole Ab (from donkey)	Cytiva, NA934	1:20000 – 1:30000
Chicken anti-Rabbit IgG (H+L), Alexa Fluor™ 488	Invitrogen, A-21441	1:200
Goat anti-Mouse IgG (H+L), Alexa Fluor™ 647	Invitrogen, A-21235	1:200
Phalloidin CF®568	Biotium, 00044	1:200
DAPI	Invitrogen, 62248	1:5000

4.1.5 Growth media, antibiotics and additives

Sterilization of media was done by autoclaving for 20 min at 121 °C and 1.4 bar. Supplements were sterile filtered with 0.22 µm filter, when autoclaving was not recommended.

Table 7: Media for cultivation of bacteria

Medium	Concentration	Component
Lysogeny broth medium (LB-medium), pH 7.5	10 g/l 5 g/l 10 g/l	Trpytone Yeast extract NaCl
Lysogeny broth agar (LB-agar), pH 7.0	10 g/l 5 g/l 10 g/l 15 g/l	Trpytone Yeast extract NaCl Agar
Super Optimal broth with Catabolite repression medium (SOC-medium), pH 7.0	20 g/l 5 g/l 0.5 g/l 0.186 g/l 20 mM 20 mM	Trpytone Yeast extract NaCl KCl $MgCl_2$ Glucose

Table 8: Antibiotics

Additive	Concentration	Solvent	Provider
Nalidixic acid	100 µg/ml	1 M NaOH	Sigma-Aldrich, St. Louis, USA
Kanamycin	50 µg/ml	ddH_2O	Sigma-Aldrich, St. Louis, USA
Chloramphenicol	20 µg/ml	100 % EtOH	Roth, Karlsruhe, Germany
Spectinomycin	50 µg/ml	ddH_2O	Sigma-Aldrich, St. Louis, USA
Ampicillin	100 µg/ml	ddH_2O	Sigma-Aldrich, St. Louis, USA

Table 9: Media for cultivation of primary human macrophages

Medium	Additive	Concentration	Provider
RPMI1640 Roswell Park Memorial Institute (RPMI) 1640 Medium	---	---	Gibco, Carlsbad, USA

RPMI1640 Roswell Park Memorial Institute (RPMI) 1640 Medium for cultivation of primary human macrophages	---	---	Gibco, Carlsbad, USA
	Autologous human serum	20 % (v/v)	Linder lab, UKE
	Penicillin/Streptomycin	1 % (v/v)	Gibco, Carlsbad, USA

4.1.6 Plasmids

Table 10: Plasmids

Plasmid	Vector	Origin
NLRP3-eGFP	pEGFP-C2	pEGFP-C2-NLRP3 was a gift from Christian Stehlik (Addgene plasmid # 73955 ; http://n2t.net/addgene:73955 ; RRID:Addgene_73955) (Khare et al., 2012)
pKD46-Cas12a-Spec	Contains a temperature-sensitive replicon leading to curation at 42 °C incubation temperature; encodes for *gam*, *bet*, and *exo* (recombination genes) and Cas12a and also for spectinomycin resistance gene	(Rudolph, 2020)
pAC-crRNA-Chlor	Encodes for the pre-crRNA cassettes, the sucrose sensitivity gene (SacB) as well as BpmI and BsaI restriction enzyme sites flanking a gfp gene used as a selection marker,	Yi-Cheng Sun, Beijing, China (Zhao & Sun, 2018)
pAC-crRNA-Chlor-YopQ_3	Encodes for the pre-crRNA cassettes, the sucrose sensitivity gene (SacB) as well as BpmI and BsaI restriction enzyme sites flanking a gfp gene used as a selection marker, crRNA sequences for the deletion of YopQ gene were inserted	This study

4.1.7 Primer

Table 11: Primer sequences.

Name	Sequence 5'-3'
deltaQ HomA fwd	TAAAACAGGGCATGGCAC
deltaQ HomA rev	GTAGTAACTAGCTATATTAAAGAGTTT
deltaQ HomB fwd	AATATAGCTAGTTACTACTCCAAAATT
deltaQ HomB rev	CAACTTATGGGGACAGTG
YopQ-crRNA fwd	TAGATTGGGATGAAGCTATATTAAAGAGTT
YopQ-crRNA rev	AGACAACTCTTTAATATAGCTTCATCCCAA

4.1.8 *Yersinia* strains and eukaryotic cells

Table 12: *Yersinia enterocolitica* strains

Strain	Features	Resistance	Reference
WA314	*Y. enterocolitica* serotype O:8; clinical isolate; virulence plasmid pYVO8+	Nal	(Heesemann & Laufs, 1983)
WAC	Plasmidless derivative of WA314	Nal	(Heesemann & Laufs, 1983)
WAC(pTTSS)	WAC harbouring the pTTSS plasmid, which encodes for the TTSS apparatus bur no Yop effector proteins	Spec	(Heesemann & Laufs, 1983)
WA314ΔYopM	WA314 derivative in which the YopM gene was replaced by a kanamycin resistance gene from pUC4k	Kana	(Trülzsch et al., 2004)
WA314ΔYopP	WA314 derivative in which the YopP gene was replaced by a chloramphenicol resistance gene	Chlor	(Schnapp, 2016)
WA314ΔYopQ	WA314 derivative in which the YopQ gene was replaced by a kanamycin resistance gene	Kana	(Trülzsch et al., 2004)

WA314ΔYopMΔYopP	WA314 derivative in which the YopM gene was replaced by a kanamycin resistance from pUC4k and the YopP gene by a chloramphenicol resistance gene	Kana, Chlor	(Schnapp, 2016)
WA314ΔYopMΔYopQ	WA314ΔYopM derivative with a deletion of YopQ generated using CRISPR-Cas	Kana	This study
WA314ΔYopPΔYopQ	WA314 derivative in which the YopQ gene was replaced by a kanamycin resistance gene and the YopP gene by a chloramphenicol resistance gene	Kana, Chlor	Generated by Konrad Trülzsch, courtesy of K. Ruckdeschel, UKE, Hamburg
WA314ΔYopH **WA314ΔYopO** **WA314ΔYopT** **WA314ΔYopE**	WA314 derivative in which the corresponding Yop gene was replaced by a kanamycin resistance from pACYC177	Kana	(Trülzsch et al., 2004)

Table 13: Eukaryotic cells

Cells	Features	Reference
Human peripheral blood monocytes	Monocytes were incubated with autologous human serum for one week and differentiated into macrophages	Isolation in own lab from buffy coats provided by Frank Bentzien, UKE, Hamburg, according to (Kopp et al., 2006)

4.1.9 Software, data processing and tools

Table 14: Software, tools and data processing programs

Software, Tool	Manufacturer / Provider / Reference
CLC Genomics Workbench	CLC bio, Aarhus, Denmark
DAVID 6.8	(Huang et al., 2009a, 2009b)
DESeq2 package (R-package)	(Love et al., 2014)

FastQC 0.11.5	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
FeatureCounts	(Liao et al., 2014)
Fiji Version 1.53h/ImageJ	(Schindelin et al., 2012) https://fiji.sc/
GEO database	(Barrett et al., 2013)
ggplot2 package (R-package)	(Wickham, 2016)
GraphPad Prism 10	GraphPad Software, San Diego, USA
HOMER 4.11	(Heinz et al., 2010)
limma package (R-package)	(Ritchie et al., 2015)
Multi-symbol checker	(Braschi et al., 2019)
pheatmap (R-package)	https://github.com/raivokolde/pheatmap
SRA toolkit	http://ncbi.github.io/sra-tools/
STAR	(Dobin et al., 2013)
TrimGalore 0.5.0	http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/

4.1.10 RNA sequencing data sets

RNA sequencing data sets for comparison with self-generated data sets were downloaded from the NCBI GEO database (Barrett et al., 2013).

Table 15: Publicly available data sets used for comparisons

Name of dataset	GEO ID	Series
Naïve macrophages	GSM2262901	GSE85243
Naïve macrophages	GSM2262902	GSE85243
LPS stimulated macrophages	GSM2262906	GSE85243
LPS stimulated macrophages	GSM2262907	GSE85243
Naïve macrophages	GSM2679941	GSE100382
Naïve macrophages	GSM2679942	GSE100382
LPS stimulated macrophages	GSM2679944	GSE100382
LPS stimulated macrophages	GSM2679945	GSE100382

4.2 Methods

4.2.1 Microbiological methods

4.2.1.1 Cultivation and conservation of *Yersinia enterocolitica*

Gylcerol stocks were prepared using liquid bacterial culture in exponential growth phase, which were mixed 1:1 with LB medium containing 40 % glycerol, shock frozen in liquid nitrogen and long-term stored at -80 °C. Cultivation of *Yersinia enterocolitica* strains was carried out from frozen glycerol stocks at 27 °C for 24 h on LB agar plates containing the desired antibiotics. LB agar plates could be stored for 4 weeks at 4 °C.

4.2.1.2 *Yersinia* infection

Yersinia precultures were prepared by inoculating 3 ml of LB medium containing the desired antibiotics in a test tube with one single colony from LB agar plates. Bacterial growth was carried out overnight at 27 °C and 180 rpm. The next day the preculture was diluted 1:20 in 20 ml LB medium without antibiotics and incubated for 90 min at 37 °C and 180 rpm. Bacteria were pelleted at 6000 x *g* for 10 min at 4 °C, the supernatant was discarded and the pellet resuspended in 1 ml ice cold PBS containing 1 mM $MgCl_2$ and $CaCl_2$. To account for differences in bacterial growth the optical density at 600 nm (OD_{600}) was measured and adjusted to 3.6. 1 h prior infection the medium of the macrophages was changed to RPMI1640 medium and macrophages were infected using a multiplicity of infection (MOI) from 30 to 500 depending on the experimental aim. Cultivation was done at 37 °C with 5 % CO_2 for the chosen infection time. For subsequent analysis the cells were washed once with PBS and either used for histone extraction (4.2.6.4), RNA isolation (4.2.3.1) or immunofluorescence staining (4.2.7.1).

4.2.2 Cell culture methods

4.2.2.1 Isolation and cultivation of primary human macrophages

Isolation of human peripheral blood monocytes was done from buffy coats according to (Kopp et al., 2006). Cells were kept at 37 °C and 5 % CO_2 atmosphere in RPMI1640 containing 20 % autologous human serum and 1 % Pen/Strep (Monocyte medium) and the medium was changed the first two days after isolation. One week after isolation primary human macrophages were fully differentiated and used for infection experiments.

4.2.2.2 Treatment with NF-κB and MAPK inhibitors

Experiments using inhibitors were done with the help of Marie Schnapp. NF-κB pathway inhibition was done using TPCA, targeting IKK. For the inhibition of MAPK pathway a

combination of equimolar amounts of SB203580, which targets p38 and PD98059, targeting MEK1, was used. Furthermore, Lji308 targeting RSK, SP600125 targeting JNK and Takinib which targets the protein TAK1 were used for inhibition of parts of the MAPK pathway. Inhibitors were used at a final concentration of 10 µM and added 30 – 60 min before infection.

4.2.2.3 Transfection of primary human macrophages

Cells were washed once with PBS following treatment with 500 µl Accutase for 20 – 30 min for cell detachment. Detached cells were scraped, mixed with equal volume of RPMI1640 medium and counted using a neubauer counting chamber. For transfection $1*10^6$ cells were pelleted, washed twice with PBS and finally resuspended in 100 µl R-buffer. 5 µg of DNA were added to the cell mixture, loaded into Neon pipette tip, and electroporated at 1000V for 40 ms with 2 pulses in E2 buffer using the Neon transfection system (Invitrogen, Carlsbad, USA) and immediately transferred into 900 µl of RPMI1640 medium. 100 µl of transfected cells were seeded onto glass coverslips, incubated at 37 °C for 20 – 30 min until cells were attached and the medium was changed to Monocyte medium. 1 h before infection medium was changed to RPMI1640 and infection was started 4 h after transfection according to 4.2.1.2.

4.2.3 Molecular biology techniques

4.2.3.1 Isolation of total RNA

For the isolation of total RNA of primary human macrophages, the RNeasy Mini extraction kit (Qiagen, Hilden, Germany), with additional DNAse treatment, was used according to manufacturer's instructions.

4.2.3.2 Isolation of plasmid DNA

Plasmid DNA isolation was done from from 5 ml bacterial cultures using the "Plasmid Miniprep Kit I" (C-Line, PeqLab, Germany) following the protocol supplied with the kit. If higher amounts of plasmid DNA were needed the NucleoBond® Xtra EF kit (Macherey-Nagel, Düren, Germany) was used, following the protocols supplied by the provider. Isolated plasmid DNA was stored at -20 °C.

4.2.3.3 Measurement of DNA concentration

Determination of the concentration of dsDNA in solution was done using the NanoDrop® ND-1000 spectrophotometer with the ND-1000 V 3.1.0 software (PeqLab, Germany) by measuring the absorbance at 260 nm (A_{260}). DNA concentration was measured against a blank (solvent of the DNA was used as blank medium). The purity of the DNA was checked

by using the absorbance ratio of A_{260}/A_{280} which should be between 1.8 and 2.0 for pure DNA with no protein or phenol contaminations.

4.2.3.4 Polymerase chain reaction (PCR)

The PCR was used to amplify plasmids, DNA fragments or any other type of dsDNA, either for generation of fragments or for checking the correct size of a region of interest. In general, a PCR consists of three steps: Denaturation of the dsDNA strand, primer annealing and elongation of the primer via a thermostable DNA-Polymerase. Annealing temperatures of the primers were calculated using the online melting temperature (Tm) calculator from New England Biolabs. Dependent of the aim of the PCR, either GoTaq® DNA Polymerase (Promega, Walldorf, Germany) or Phusion™ High-Fidelity DNA-Polymerase (Thermo Fisher Scientific, Waltham, USA) were used according to Table 16 - Table 19.
PCR products were analyzed by Agarose gel electrophoresis (4.2.3.6), visualized using a UV-Transilluminator and purified from the Agarose gel using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) for subsequent use.
The following tables show the composition of the reaction mixes for Colony PCR with the GoTaq® DNA Polymerase and the Phusion PCR using the Phusion™ High-Fidelity DNA Polymerase.

Table 16: Composition of the reaction mix for Colony PCR

Composition	Volume	Final concentration
5x Green GoTaq® Reaction buffer	2 µl	1x
dNTP Mix (10 mM)	0.2 µl	0.2 mM
DMSO (100 %)	0.3 µl	3 %
Primer 1 (10 mM)	0.5 µl	0.5 mM
Primer 2 (10 mM)	0.5 µl	0.5 mM
Template DNA (isolated from bacterial colony by heating to 95 °C)	0.5 µl	---
GoTaq® DNA Polymerase (5 U/µl)	0.05 µl	0.25 U
Water	5.95 µl	---
Final volume	10 µl	---

Table 17: Composition of the reaction mix for Phusion PCR

Composition	Volume	Final concentration
5x Phusion™ HF Buffer	10 µl	1x
dNTP Mix (10 mM)	1 µl	0.2 mM
DMSO (100 %)	1.5 µl	3 %
Primer 1 (10 mM)	2.5 µl	0.5 mM
Primer 2 (10 mM)	2.5 µl	0.5 mM
Template DNA	0.5 µl	---
Phusion™ High–Fidelity DNA Polymerase (2 U/µl)	0.5 µl	1 U
Water	31.5 µl	---
Final volume	50 µl	---

Table 18: PCR cycle protocol for Colony PCR

Cycle step	Temperature	Time	Number of cycles
Initial denaturation	95 °C	2 min	1 cycle
Denaturation	95 °C	30 sec	30 – 35 cycles
Annealing	Dependent on primer Tm (Tm minus 3-5 °C)	30 sec	
Extension	72 °C	1 min/kb	
Final extension	72 °C	5 min	1 cycle
Storage	8 °C	∞	1 cycle

Table 19: PCR cycle protocol for Phusion PCR

Cycle step	Temperature	Time	Number of cycles
Initial denaturation	95 °C	30 sec	1 cycle
Denaturation	95 °C	10 sec	30 – 35 cycles
Annealing	Dependent on primer Tm (Tm minus 3-5 °C)	30 sec	
Extension	72 °C	30 sec/kb	
Final extension	72 °C	5 min	1 cycle
Storage	8 °C	∞	1 cycle

4.2.3.5 Quantitative real-time polymerase chain reaction (RT-qPCR)

500 ng to 2 µg of extracted RNA was reverse transcribed with the iScript cDNA Synthesis Kit (Bio-Rad, USA). For the RT-qPCR reaction the TaqMan™ Fast Advanced Master Mix (Applied Biosystems, USA) and gene specific TaqMan probes for the genes of interest IL6 (Hs00985639_m1), TNF (Hs01113624_g1) and IL1B (Hs00174097_m1) as well as for the reference genes Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs02758991_g1), TATA-box binding protein (TBP) (Hs00427620_m1) and beta-2-microglobulin (B2M) (Hs00187842_m1) were used (Thermo Fisher Scientific, USA). RT-qPCR run was done using a the LightCycler® 480 Instrument (Roche, Switzerland) and the analysis was done according to the instruction of the manufacturer (Roche LightCycler® 480 Software; Software release 1.5.1.62). Quantification of the genes of interest was done relatively to the reference genes.

4.2.3.6 Agarose gel electrophoresis

The agarose gel electrophoresis was used to separate DNA fragments of different length from each other to be able to purify, analyze and use them afterwards. 1 % agarose gels were prepared by dissolving the desired amount of agarose in 1 x TAE buffer by boiling in the microwave. iNtron RedSafe™ Nucleic Acid Staining Solution (20.000 x) was added according to manufacturer's instructions as soon as the agarose solution reached a lower temperature. PCR products were mixed in a ratio of 5:1 with TriTrack DNA loading dye (6x) before loading onto the gel. GeneRuler 1 kb plus DNA Ladder was used as a size comparison marker. Agarose gels were run at 120 V constant for at least 30 min or until the relevant loading dye reached the end of the gel. For the visualization of DNA fragments a UV-Transilluminator was used. For purification of DNA fragments the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) was used.

4.2.3.7 CRISPR-Cas12a-assisted recombineering

Generation of YopQ deletion strains in this study was done using a CRISPR-Cas12a-assisted recombineering attempt. Therefor two double stranded "Homology Directed Repair" (HDR) fragments were generated with overlap extension PCR. The first fragment, homology arm A (HomA), which covers an approximately 500 bp fragment directly starting behind the stop codon of YopQ, was amplified from the pYV virulence plasmid in WA314 using the primer deltaQ HomA fwd and deltaQ HomA rev. The second fragment, homology arm B (HomB), covering an around 300 bp fragment ending directly in front of the YopQ sequence, was equally amplified using the primers deltaQ homB fwd and deltaQ homB rev. The final HDR fragment was generated using HomA and HomB as templates in an overlap extension PCR with the outer primers deltaQ HomA fwd and deltaQ HomB rev.

The design of the target sequence, called crRNA, which leads Cas12a to the correct site of cutting, was based on the 20 bp protospacer sequence after the 3'-end of a PAM (5'-TTN-3'). For that the oligonucleotides YopQ-crRNA fwd and YopQ-crRNA rev were designed having Eco31L restriction enzyme overhangs at both ends. Primers were annealed and ligated into the pAC-crRNA vector, digested with Eco31L. The pAC-crRNA vector harbors a sacB sucrose sensitivity gene for later curing.

For the transformation of the desired *Y. enterocolitica* strain (WA314ΔYopM), carrying the plasmid pKD46-Cas12a, which contains the lambda Red recombinase genes controlled by an arabinose inducible promoter, the Enzyme Cas12a from *Francisella novicida* and a temperature sensitive replicon for later curing of the plasmid), bacteria were first made electrocompetent. 100 ml of LB-medium containing the desired antibiotics were inoculated with 10 ml of a preculture of the suitable strain grown over night and incubated at 27 °C at 180 rpm until OD_{600} of 0.2 was reached. Arabinose was added to a final concentration of 1 % for induction of the lambda Red recombinase genes and bacteria were grown until a final OD_{600} of 0.8 at 27 °C at 180 rpm. Bacteria were pelleted at 5000 x *g* for 5 min at 4 °C and washed thrice with 40 ml ice cold sterile filtered ddH_2O. Finally, bacteria were washed once with 10 ml of sterile 10 % glycerol in ddH_2O and resuspended in 400 µl of the last wash medium. 60 µl of electrocompetent bacteria were transformed by electroporation at 1800V, 50µF and 100 Ω with 350 ng of pAC-crRNA and 700 ng of the HDR fragment and directly transferred in 900 µl of SOC medium, incubated at 27 °C and 400 rpm for 2 h and plated onto Agar plates containing the suitable antibiotics for selection. The success of the YopQ deletion was controlled by Colony PCR and sequencing.

4.2.3.8 DNA Sequencing

The success of cloning or generation of PCR products was checked by sequencing of the region of interest. For that suitable primers were mixed with the according dsDNA fragment and send to Seqlab (Göttingen, Germany) for sequencing. Results were confirmed by sequence comparison using CLC Genomics Workbench (CLC bio, Denmark).

4.2.4 RNA-sequencing (RNA-seq)

Isolated RNA was first analyzed for integrity by using the RNA 6000 Nano Chip (Agilent Technologies, USA) on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Extraction of mRNA was done using the NEBNext Poly(A) mRNA Magnetic Isolation module (New England Biolabs, USA). For the generation of the RNA-seq libraries, the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, USA) was used according to the manufacturer's recommendation. Measurement of the concentration of all samples was done with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, USA) and for the analysis of the distribution of the fragment lengths for the final libraries, the DNA High Sensitivity Chip

(Agilent Technologies, USA) was used on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Normalization of all samples was done to 2 nM with equimolar pooling afterwards. For sequencing of the library pool, the NextSeq500 (Illumina, USA) with 1 x 50 bp for samples of batch 1 and 1 x 75 bp for samples of batch 2 was used.

4.2.5 Bioinformatic data analysis

Bioinformatic data analysis was done with the help of Indra Bekere.

4.2.5.1 Trimming and Quality control

For the quality control of FASTQ files containing the clustered sequence data, the FastQC program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) was used. Trimming of sequencing reads, which contained low quality score bases (Phred score cutoff 20) was done with the TrimGalore program (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/).

4.2.5.2 Read alignment

Alignment of the reads to the human reference assembly hg 19 was done using STAR (Dobin et al., 2013). The number of reads which mapped to each gene was gained using FeatureCounts (Liao et al., 2014). Replacement of old gene symbols lacking a match in the RefSeq annotation was done with the Multi-symbol checker tool (Braschi et al., 2019).

4.2.5.3 Differentially expressed genes (DEG) analysis and clustering

Differential expression of genes was statistically analyzed using DESeq2 (Love et al., 2014) with raw counts as input and experimental design as *~batch + condition*. Genes with a log2 fold change ≥ 1 and an adjusted p-value ≤ 0.05 were called significantly enriched genes. Batch effect removal using limma package (Ritchie et al., 2015) generated normalized rlog counts, which were used for visualization and downstream cluster analysis. Principal component analysis (PCA) and sample distance heatmaps were used to confirm the reproducibility of the replicates.

DEGs of comparisons were clustered using rlog counts in R with the pheatmap package. For the generation of the heatmap of 1.5 h DEGs the clustering distance was based on maximum distance and the Ward.D2 clustering method. Heatmaps of DEGs obtained after 6 h were based on Pearson correlation and a complete clustering method. To ensure the identification of all meaningful clusters by the analysis the distance of clustering, the method of clustering and the number of clusters were selected. Row scaling (row Z-score) was used to scale rlog counts from DESeq2 analysis for the generation of heatmaps, in which a blue-white-red color gradient indicate low to high expression levels. For analysis all replicates were used but in the heatmaps only 2 representative replicates are depicted.

4.2.5.4 Pathway analysis

The DAVID (Database for Annotation, Visualization and Integrated Discovery) webtool (Huang et al., 2009a, 2009b) was used to determine Gene Ontology (GO) for the RNA-seq gene list.

4.2.5.5 Enrichment analysis of transcription factor motifs

HOMER (Hypergeometric Optimization of Motif EnRichment) package (Heinz et al., 2010) was used to detect transcription factor motif enrichment. As input, a list of gene symbols was used and the command *findMotifs.pl* was also used. A region 400 bp upstream and 100 bp downstream of the transcription start site was checked for transcription factor motifs using the parameters *–start -400 – end 100*.

4.2.5.6 Boxplots

Ggplot2 in R was used to generate boxplots, in which the boxes contain the twenty-fifth to seventy-fifth percentile, the whiskers reach to the tenth and ninetieth percentiles and the outliers are marked with dots. The median is represented with the central horizontal bar.

4.2.5.7 Publicly available data comparison

RNA-seq data sets from GSE85243 and GSE100382 (Novakovic et al., 2016; Park et al., 2017) were used for comparison with self-generated RNA-seq data sets. The observation of a batch effect when comparing rlog counts from public data sets with data sets from this work, led to the conversion of rlog counts into Z-scores and the calculation of a spearman correlation. Comparison with publicly available data sets was done with DEGs found in comparison between Mock, WA314 and WAC after 1.5 h and 6 h of infection.

4.2.6 Proteinbiochemical methods

4.2.6.1 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to separate proteins according to their molecular weight. For that protein solution of interest was mixed with 4x SDS sample buffer (4.1.3) in a ratio of 3:1, boiled at 95 °C for 5 - 10 min and loaded in equal amounts, measured via Bradford Protein Assay (4.2.6.5), onto the gel. The proteins were electrophoretically separated using 15 % SDS gels in a SDS-PAGE electrophoretic chamber containing 1 x SDS running buffer (4.1.3) at 80 V for 30 min following 125 V for 60 – 75 min until the loading dye reached the end of the SDS gel. SDS gels were casted according to Table 20. First resolving gel was poured until it was 1 cm below the top of the shorter glass plate and after hardening the remaining volume was filled with stacking gel and an adequate gel comb. For comparison

of the molecular weight a prestained page ruler 4.1.3 was used as a molecular weight marker.

Table 20: Composition of one 15 % acrylamide SDS mini gel

Component	4 % stacking gel	15 % resolving gel
ddH_2O	1.55 ml	2.5 ml
Stacking buffer, pH 6.8	625 µl	---
Resolving buffer, pH 8.8	---	2.5 ml
ROTIPHORESE® Gel 30 (37,5:1)	325 µl	5 ml
10 % APS (100 mg/ml)	25 µl	100 µl
TEMED	5 µl	10 µl

4.2.6.2 Coomassie staining of SDS gels

Visualization of proteins electrophoretically separated in a SDS gel was done by incubation in Coomassie staining solution (4.1.3) for 1 h. The gel was destained in an acetic acid based Coomassie destain solution (4.1.3) until the protein bands were distinctly distinguishable from the gel background. Images of the gel were acquired using the Amersham ImageQuant™ 800 Western blot imaging system (4.1.1).

4.2.6.3 Western Blot

Proteins separated in a SDS-PAGE according to their molecular weight were electrophoretically transferred in a semi-dry blotting chamber OWL-HEP1 (4.1.1) onto a polyvinylidene difluoride (PVDF) membrane for subsequent analysis using antibodies against the protein of interest. SDS gels were equilibrated into 1 x Transfer buffer (4.1.3) for 5 – 10 min prior blotting. Starting from the bottom of the chamber (Anode) the stack was composed of 3 Whatman paper, one layer of PVDF membrane activated for 20 – 30 sec in methanol, the SDS gel and finally 3 Whatman paper. All components were equilibrated in 1 x Transfer buffer before building the stack. The transfer was done for 1 h at 1,2 mA/cm^2. Next the membrane was blocked in blocking buffer (4.1.3) for 60 min shaking at RT, followed by incubation overnight at 4 °C with a primary antibody against the protein of interest diluted according to (4.1.4) in blocking buffer. The next day the membrane was washed 3 x in TBS-T (4.1.3) for 5 – 10 min each washing step and finally incubated with a suitable secondary antibody coupled with horseradish peroxidase (HRP) 1:20000 or 1:30000 diluted in TBS-T for 1 h shaking at RT. After washing the membrane 3 x with TBS-T, signal detection was started by incubation with SuperSignal West Femto Maximum sensitivity substrate solution for 5 – 10 min at RT. Images were acquired using the Amersham ImageQuant™ 800 Western blot imaging system (4.1.1).

4.2.6.4 Histone extraction

Histones were isolated from primary human macrophages following the acid extraction protocol from Shechter et al. (Shechter et al., 2007) with little adjustements. 0,5 – 1,5 * 10^6 primary human macrophages (one well of a six well plate) were washed with ice-cold PBS and harvested by scraping off in 1 ml PBS. Cells were pelleted by centrifugation at 700 x *g* for 5 min at 4 °C and the supernatant was discarded. Lysis of the cells was done by incubation of the pellet in 0.5 ml Hypotonic lysis buffer (4.1.3) rotating at 4 °C for 60 – 90 min and controlled by microscopy. Isolated nuclei were pelleted by centrifugation at 10000 x *g* for 10 min at 4 °C, supernatant was discarded and the pellet was resuspended in 0.2 M HCl and lysed by incubating at 4 °C rotating overnight. Nuclear debris was removed by centrifugation at 16000 x *g* for 10 min at 4 °C and the supernatant containing acid soluble proteins was transferred to a new tube. Incubation with trichloric acid (TCA) (final concentration 33 %) for 30 min on ice was used to precipitate histones. Pelleting of precipitated histones was carried out by centrifugation at 16000 x *g* for 10 min at 4 °C and the supernatant was discarded. The pellet was washed twice with 500 µl ice cold acetone, centrifuged in between at 16000 x *g* for 5 min at 4 °C, finally air dried and dissolved in 100 µl millipore water. Concentration of the dissolved histones was determined using Bradford protein assay and the quality and purity of the extracted histones was controlled by SDS-PAGE and subsequent coomassie staining of the gel.

4.2.6.5 Determination of protein concentration (Bradford protein assay)

To determine protein concentration, the Bio-Rad Protein Assay Dye reagent concentrate (4.1.3) was mixed in a ratio of 1:4 with millipore water, 2 µl of the protein solution with unknown concentration was added and incubated for 10 min at RT. The absorbance at 595 nm was measured using a photometer (Ultrospec 3100 pro, GE Healthcare) and the protein concentration was calculated based on a standard curve from varying BSA concentrations (0,1 – 2 mg/ml stock solution).

4.2.7 Microscopy

4.2.7.1 Immunofluorescence staining

For immunofluorescent analysis of primary human macrophages, glass coverslips were used as seeding ground. Cells were washed once with PBS, detached using 500 µl of Accutase for 30 min at 37 °C, mixed with equal volume of RPMI1640 medium and counted in a neubauer counting chamber. 6 * 10^4 cells were seeded onto glass coverslips, incubated at 37 °C for 20 – 30 min until cells were attached and the medium was changed to Monocyte medium afterwards. The next day *Yersinia* infection was done according to 4.2.1.2. Coverslips were washed once with PBS, fixed with 4 % PFA in PBS for 7 min and washed

twice with PBS. Cells were permeabilized with 0.1 % Triton X-100 in PBS for 15 min at RT, washed twice afterwards and blocked with blocking solution for 30 – 60 min at RT in a humid chamber. Next coverslips were transferred into blocking solution containing the desired primary antibodies diluted according to 4.1.4 and incubated overnight at 4 °C. Washing with PBS was done thrice and incubation with secondary antibodies, DAPI and Phalloidin coupled to Alexa Fluor dyes diluted 1:200 in blocking solution was done for 60 min at RT in a humid chamber. Coverslips were washed thrice finally and mounted in Prolong Glass Antifade Mountant on object slide.

4.2.7.2 Confocal microscopy

Microscopy samples prepared according to 4.2.7.1 were observed using the laser scanning microscope Olympus FV 3000 (DFG code: INST 152/933-1) with a 20x air objective (NA 0.8) or the 60x oil objective (NA 1.4) and the Olympus FV3000 Software (Evident Europe GmbH, Germany).

5 Results

5.1 The effect of *Y. enterocolitica* on the transcriptome of primary human macrophages

Yersinia T3SS effectors fulfill a variety of functions within the host cell like impacting the phagocytic activity and impairing the inflammatory response, e.g., by modulating host gene expression (Atkinson & Williams, 2016; Pha & Navarro, 2016). Especially for the T3SS effectors YopM and YopP it has already been shown that they impact inflammatory gene expression, whereas for the other suppressor of inflammation, YopQ, no such activity has been reported yet. We therefore decided to perform a global RNA-seq analysis in primary human macrophages that were either non infected (mock), infected with the *Y. enterocolitica* avirulent strain WAC lacking the virulence plasmid, infected with the fully virulent wild type WA314 or infected with *Y. enterocolitica* strains lacking either YopM (WA314ΔYopM), YopP (WA314ΔYopP), YopQ (WA314ΔYopQ) or YopM & YopP (WA314ΔYopMΔYopP) to gain information about the activity of each single Yop as well as a possible interplay between YopP and YopM as well as the effect of YopQ.

5.1.1 Alteration of gene expression induced by the bacterial PAMPs is modulated through T3SS effectors

To get an overview of the effect of all T3SS effectors together on the modulation of gene expression, primary human macrophages were either mock-infected or infected with WAC or WA314 for 1.5 h and 6 h followed by RNA-seq analysis using next generation sequencing of total RNA (Fig. 1A). As the strain WAC lacks all T3SS effectors as well as the T3SS injectisome, it can be used as a control to check for effects of *Yersinia* PAMPs or other factors which stimulate the immune response, without any interference of the T3SS injectisome and effectors.

First, publicly available data of human macrophages either non treated (naïve) or treated with LPS (Novakovic et al., 2016; Park et al., 2017) were used to evaluate the overlap with our data obtained with macrophages mock-infected or infected for 6 h either with WAC or WA314. Results were depicted in a spearman correlation heatmap (Fig. 1B) showing the emergence of three different groups of infected macrophages: 1) naïve and mock, 2) WA314 and 3) LPS and WAC. The highest distance was found between naïve/mock and LPS/WAC, indicating that WAC infection leads to a large modulation of gene expression in primary human macrophages that is to a large part caused by LPS (Fig. 1B) (Reinés et al., 2012). WA314 infected macrophages show an intermediate pattern ranging between

naïve/mock and WAC/LPS highlighting the strong gene expression modulation by the T3SS effectors (Fig. 1B).

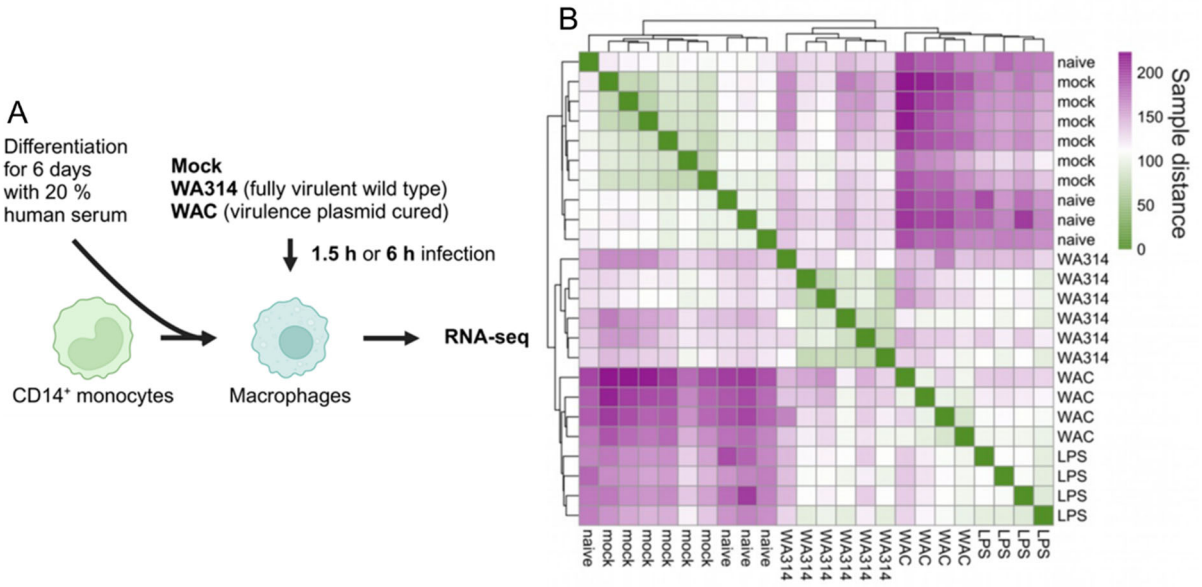

Figure 1: Experimental setup and comparison with published data. (A) Experimental setup. CD14+ monocytes were isolated from human blood samples and differentiated into macrophages by cultivation with 20 % human serum for one week. Macrophages from ≥ two independent donors were either mock infected or infected with avirulent *Y. enterocolitica* strain WAC or wild type strain WA314 for 1.5 h or 6 h with a multiplicity of infection (MOI) of 100. Lysates of infected macrophages were used for RNA-seq analysis. (B) Heatmap representation of sample distance of gene expression between mock, WAC, WA314 infected macrophages (6 h) from this study and naive and LPS-stimulated macrophages from publicly available data (Novakovic et al., 2016; Park et al., 2017).

A Principal component analysis (PCA) verified the clustering of replicates showing the high reproducibility of our experiments (Fig. 2A). Clusters with the replicates of WAC and WA314 infected for 1.5 h were closer to each other than after 6 h of infection. This indicates that the transcriptional response was stronger and more distinctly after 6 h of infection. Like already seen in Fig. 1B, the clusters of WA314 replicates are between mock and WAC clusters independent of the infection time showing the extraordinary modulation of the PAMP induced gene expression by the T3SS effectors (Fig. 2A).

Genes counted as differentially expressed genes (DEGs) showed a log2 fold change of ≥ 1 and a p-adjusted value of ≤ 0.05. After 1.5 h of infection the comparison of WAC vs. mock (1125 DEGs) and WA314 vs. mock (1004 DEGs) showed similar numbers of DEGs, whereas no significant number of DEGs was found in the comparison of WAC vs. WA314 (73 DEGs) (Fig. 2B). This implicates that already after 1.5 h a lot of genes were differentially regulated by the *Yersinia* PAMPs and that the activity of the T3SS effectors is not significantly detectable at this time point. In comparison, 6 h of infection led to a strong

increase in DEGs as we found 7437 DEGs in the comparison WAC vs. mock and 4641 DEGs in WA314 vs. mock. In WAC vs. WA314 we found 2714 DEGs (Fig. 2B). The larger amount of DEGs in the comparison of WAC vs. mock compared with WA314 vs. mock indicates that after 6 h T3SS effectors already strongly suppress the gene expression changes induced the *Yersinia* PAMPs.

As the publicly available data were generated using LPS stimulated human macrophages (Novakovic et al., 2016; Park et al., 2017) we can estimate the amount of DEGs that are presumably regulated by *Yersinia* LPS. Around 25 to 30 % of Up- and downregulated genes were regulated by LPS detection of the macrophages, showing the large impact of LPS on the host immune response (Fig. 2C).

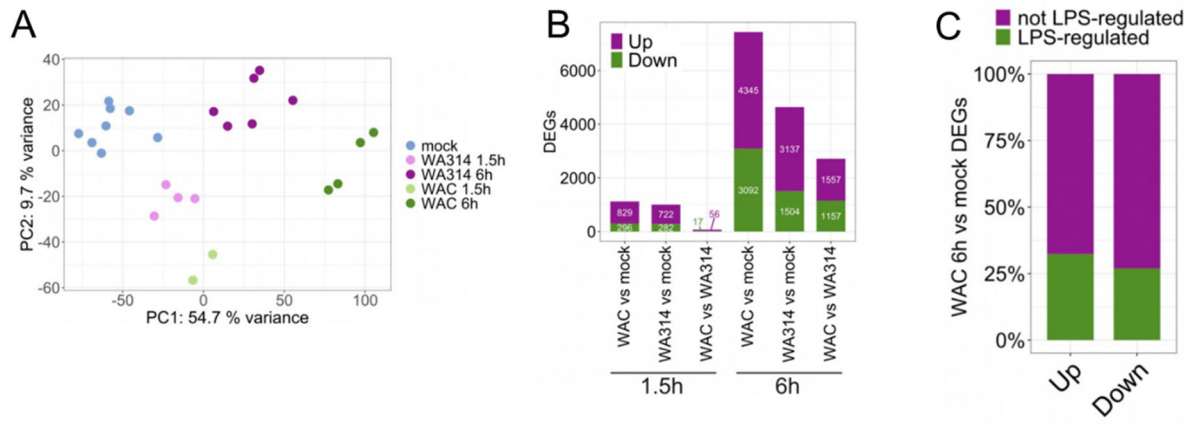

Figure 2: Principal component and general analysis of differentially expressed genes found in all sample replicates. (A) PCA of rlog gene counts of all DEGs from Figure 3A for all sample replicates used in this study. (B) Number of up- and downregulated DEGs in single comparisons between WAC vs. mock, WA314 vs. mock and WAC vs. WA314 after 1.5 h & 6 h. (C) Percentage of LPS- and not LPS-regulated DEGs in a single comparison between WAC 6 h vs. mock.

5.1.2 Gene expression analysis of infection with avirulent and virulent strains reveals 4 separate clusters

Altogether 1512 DEGs were found in mock-, WAC- and WA314-infected macrophages after 1.5 h. A heatmap representation using the Row Z-score showed four clusters E1 - E4 (Early 1/2/3/4) (Fig. 3A). In clusters E1 an E4, upregulated genes in WAC were mostly downregulated in WA314 infected macrophages and downregulated genes by WAC were partly upregulated by WA314, respectively. In cluster E2, WAC as well as WA314 infection upregulated genes compared to mock and in cluster E3 WAC and WA314 infection downregulated genes compared to mock. Figure 3B shows the global expression profiles of clusters E1 - E4 (Fig. 3B). Gene ontology (GO) analysis revealed the pathways enriched

in clusters E1 - E4. DEGs belonging to cluster E1 were highly enriched in inflammatory response pathways as well as the response to LPS and viruses, the regulation of apoptotic processes and in transcriptional regulation. Genes belonging to cluster E3 & E4 were highly enriched in pathways associated with the regulation of transcription (Fig. 3C). The pathway analysis of the cluster E2, in which no difference was found between WAC and WA314, revealed that genes in cluster E2 were enriched in pathways belonging to the positive and negative regulation of transcription (Fig. 3C).

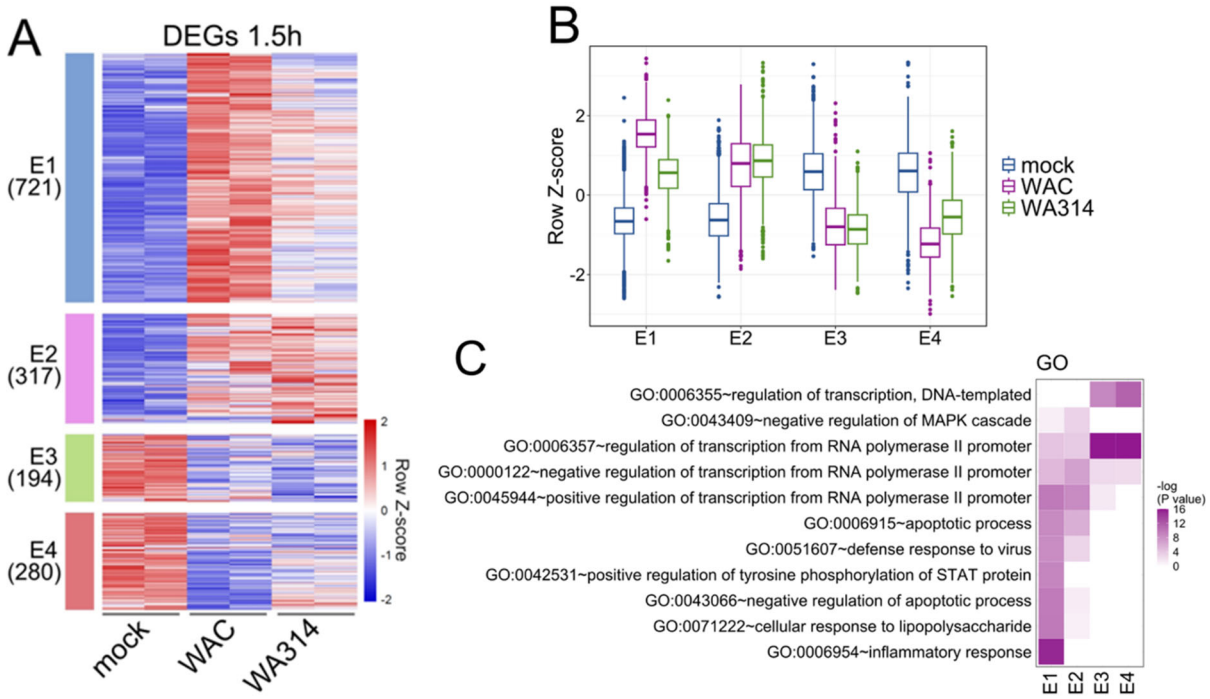

Figure 3: Differentially expressed gene clustering and gene ontology analysis after 1.5 h of infection. Primary human macrophages were either non infected (mock) or infected with *Y. enterocolitica* strain WAC or WA314 for 1.5 h using a MOI of 100. RNA was extracted and used for RNA-seq analysis. (A) Clustered Heatmap of all DEGs for comparisons between mock, WAC and WA314 infected macrophages after 1.5 h of infection. Clustering showed 4 major clusters E1 - E4 (in brackets is the number of genes belonging to each cluster). Gene rlog counts were row-scaled (row Z-score). Two representative replicates are shown for each sample. (B) Boxplots of row Z-scores of rlog counts for genes from clusters E1- E4 in (A) showing global expression profile of each cluster. (C) Heatmap showing –log10 transformed p-values and enriched GO terms for each cluster in (A).

Specific genes belonging to one prominent enriched pathway in each cluster are shown in figure 4A-D. In addition, we checked which transcriptional motifs are enriched in each cluster (Fig. 4E). E1 is highly enriched in inflammatory response pathways as we found several chemokines (CCL1, CCL8, CXCL11) and chemokine receptors (CCR7, CCRL2) as

well as inflammatory cytokines like IL18, IL23 and IL27. Consequently, genes in cluster E1 were enriched for motifs belonging to the transcription factors IRF1, IRF2 & NF-κB (Fig. 4E), which play a central role in the regulation of the inflammatory response. These results show that T3SS effectors already play a role in suppressing the bacterial PAMP induced upregulation of inflammatory response genes already after 1.5 h, even though in our experiments this was not a statistically significant downregulation (Compare Fig. 2B).

Cluster E4 reveals that some genes which are downregulated by WAC are not downregulated by WA314 and many of them function in the regulation of transcription (Fig. 4D). We found many Zinc finger genes in cluster E4 and those genes were associated with the motif of the transcription factor GFY-Staf (Fig. 4E).

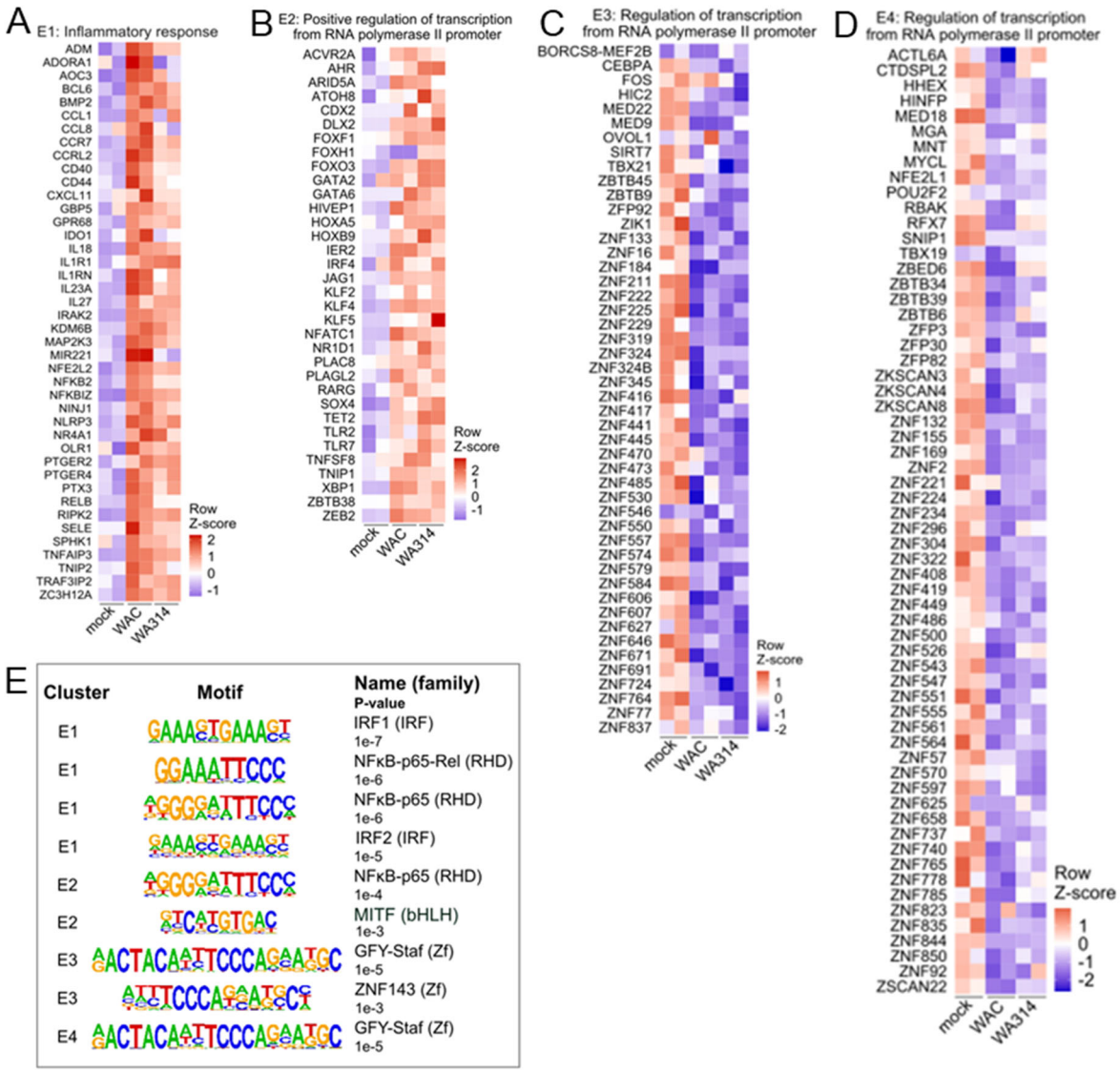

Figure 4: Pathway enrichment and motif analysis in clusters E1 - E4 after 1.5 h of infection. Primary human macrophages were either non infected (mock) or infected with *Y. enterocolitica* strain WAC or WA314 for 1.5 h using a MOI of 100. RNA was extracted and used for RNA-seq analysis. (A) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene

counts for genes from inflammatory response pathway enriched in cluster E1. (B) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes from positive regulation of transcription from RNA polymerase II promoter pathway in cluster E2. (C) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes from regulation of transcription from RNA polymerase II promoter pathway in cluster E3. (D) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes from regulation of transcription from RNA polymerase II promoter pathway in cluster E4. (E) Representative enriched transcription factor motifs in genes from E1 - E4 clusters in Fig. 3A.

5.1.3 T3SS effectors YopM and YopQ vs. YopP show opposing effects on the transcriptome

As we could not detect a significant impact of T3SS effectors on gene expression after 1.5 h but found a large impact on gene expression after 6 h (Fig. 2B) we decided to analyze the influence of the single Yop effectors YopM, YopP, YopQ on gene expression as well as the influence of both YopM and YopP together at the 6 h time point. For that we infected primary human macrophages with either WA314ΔYopM, WA314ΔYopP, WA314ΔYopQ or WA314ΔYopMP for 6 h at a MOI of 100, extracted total RNA and performed RNA-seq analysis using next generation sequencing (Fig. 5A).

PCA analysis revealed that WA314ΔYopP and WA314ΔYopMP clustered close together with large distance to the cluster of WA314 (Fig. 5B). However, the cluster of WA314 is in close proximity to the clusters of WA314ΔYopM and WA314ΔYopQ indicating that the impact of YopM and YopQ on gene expression is smaller than that of YopP (Fig. 5B). Furthermore, it seems that YopM and YopP together do not have any additional effects compared to YopP alone.

In comparison, in WA314ΔYopM vs. WA314 we found a total of 676 DEGs (195 up- and 481 downregulated genes), whereas we found more than double that amount in the comparisons WA314ΔYopP vs. WA314 (1445 DEGs in total) and WA314ΔYopMP vs. WA314 (1539 DEGs in total) (Fig. 5C). Almost no DEGs were found in the comparison WA314ΔYopQ vs. WA314 (84 DEGs in total) (Fig. 5C). Interestingly the comparison of WA314ΔYopP vs. WA314ΔYopMP revealed only 23 DEGs, whereas the comparison WA314ΔYopM vs WA314ΔYopMP identified a total of 2046 DEGs, half of them up- (1011 DEGs) and half of them downregulated (1035 DEGs) (Fig. 5D). Together this shows that YopP has the largest impact on gene expression, YopM a significant but minor impact and YopQ impacts just a few genes. Interestingly, YopM only affects gene expression in the presence of YopP as there is almost no difference between WA314ΔYopP and WA314ΔYopMP (Fig. 5D).

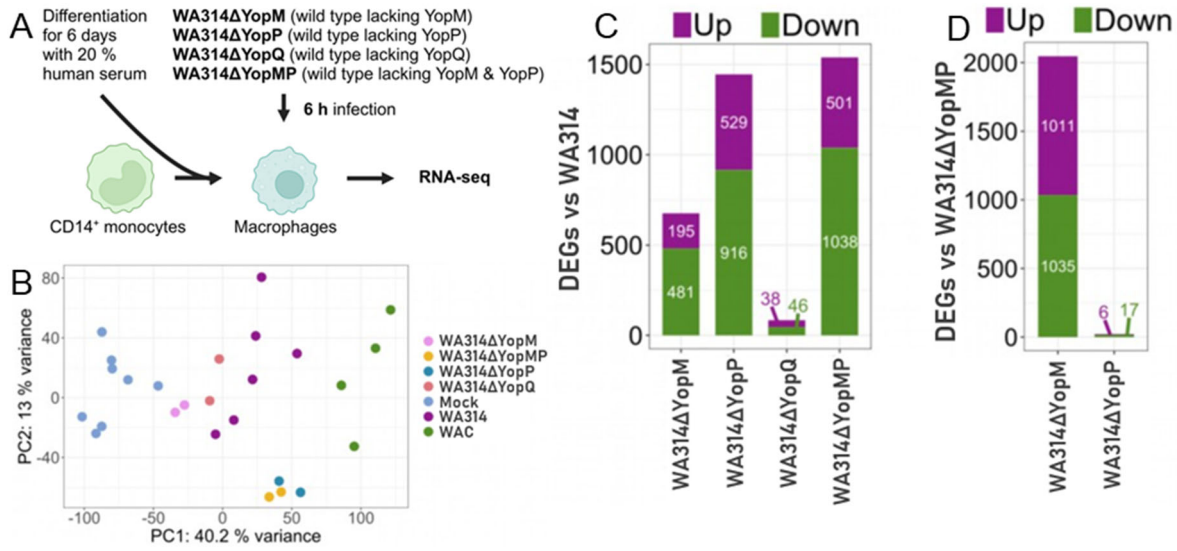

Figure 5: Experimental setup, principal component analysis and general analysis of differentially expressed genes from 6 h infection sample replicates. (A) Experimental setup. CD14+ monocytes were isolated from human blood samples and differentiated into macrophages by cultivation with 20 % human serum for one week. Macrophages from ≥ two independent donors were infected with the Yop-mutant strains WA314ΔYopM, WA314ΔYopP, WA314ΔYopMP & WA314ΔYopQ for 6 h with a MOI of 100. Samples were used for RNA-seq analysis. (B) Principal component analysis (PCA) of rlog gene counts of all DEGs from Fig. 3A & 6A for all sample replicates used in this study. (C) Number of DEGs in multiple comparisons between WA314ΔYopM vs. WA314, WA314ΔYopP vs. WA314, WA314ΔYopQ vs. WA314, WA314ΔYopMP vs. WA314. (D) Number of DEGs in comparison between WA314ΔYopM vs. ΔYopMP, WA314ΔYopP vs. ΔYopMP.

In total 9957 DEGs were found in all infection conditions and 5 clusters were revealed which were termed L1 - L5 (Late 1/2/3/4/5) after 6 h (Fig. 6A & Fig. 6B). Cluster L1 contains 1019 genes which were slightly upregulated in all infected macrophages, even though no distinct pattern was detectable (Fig. 6A & 6B). The 2509 genes in cluster L2 were highly upregulated in WAC infected macrophages, presumably by *Yersinia* PAMPs, and downregulated in all other samples showing a general T3SS effector effect with no impact of YopM, YopP or YopQ (Fig. 6A & 6B). Interestingly 2542 genes in cluster L3 were also upregulated in WAC infected macrophages, but downregulated by T3SS effectors present in WA314 as well as WA314ΔYopQ and WA314ΔYopM. Infection with WA314ΔYopP and WA314ΔYopMP showed a rescue of the WAC infected phenotype indicating that YopP is mostly responsible for the suppression of the PAMP induced upregulation in cluster L3 and that YopQ and YopM slightly counteract the YopP effect (Fig. 6A & 6B). Cluster L3 contains genes of primary and secondary immune response genes and GO analysis showed association with immune response pathways as well as apoptotic processes (Fig. 6C). Furthermore, L3

genes showed enrichment of motifs of IRF and NF-κB transcription factors (Fig. 6D). The 1362 genes in cluster L4 are the opposite of cluster L3, in that WAC induced downregulation of genes and this was prevented by WA314, WA314ΔYopQ and WA314ΔYopM (Fig. 6A & 6B). Like in cluster L3, WA314ΔYopP and WA314ΔYopMP infection rescued the WAC phenotype highlighting that YopP is mostly responsible for the WA314 effect. Similar to cluster L3, YopQ and YopM seem to show opposing effects compared with YopP in cluster L4. GO analysis revealed enrichment of L4 genes in signal transduction pathways including those regulated by small GTPases and a motif enrichment for the transcription factor Oct4 (POU domain containing) (Fig. 6C & 6D). Genes in cluster L5 are similarly regulated by *Yersinia* infection as genes in cluster L4, but infection with WA314ΔYopP and WA314ΔYopMP does not differ from infection with WA314 showing no effect of YopP (Fig. 6A & 6B). Instead YopQ and especially YopM counteracted the effect of the T3SS effectors in WA314. GO analysis of L5 genes depicted enrichment in the regulation of transcription, rRNA processing, cell division and mitotic cell cycle (Fig. 6C) and enrichment of motifs for the ETS family of transcription factors (Fig. 6C & 6D).

In sum, our data suggest that the *Yersinia* effectors in WA314 suppress not only the activation of PAMP induced genes but also suppress the inhibition of genes related to gene transcription in general. YopP is mostly responsible for both effects and YopQ and YopM slightly counteract this effect, maybe as a part of balancing the YopP effects.

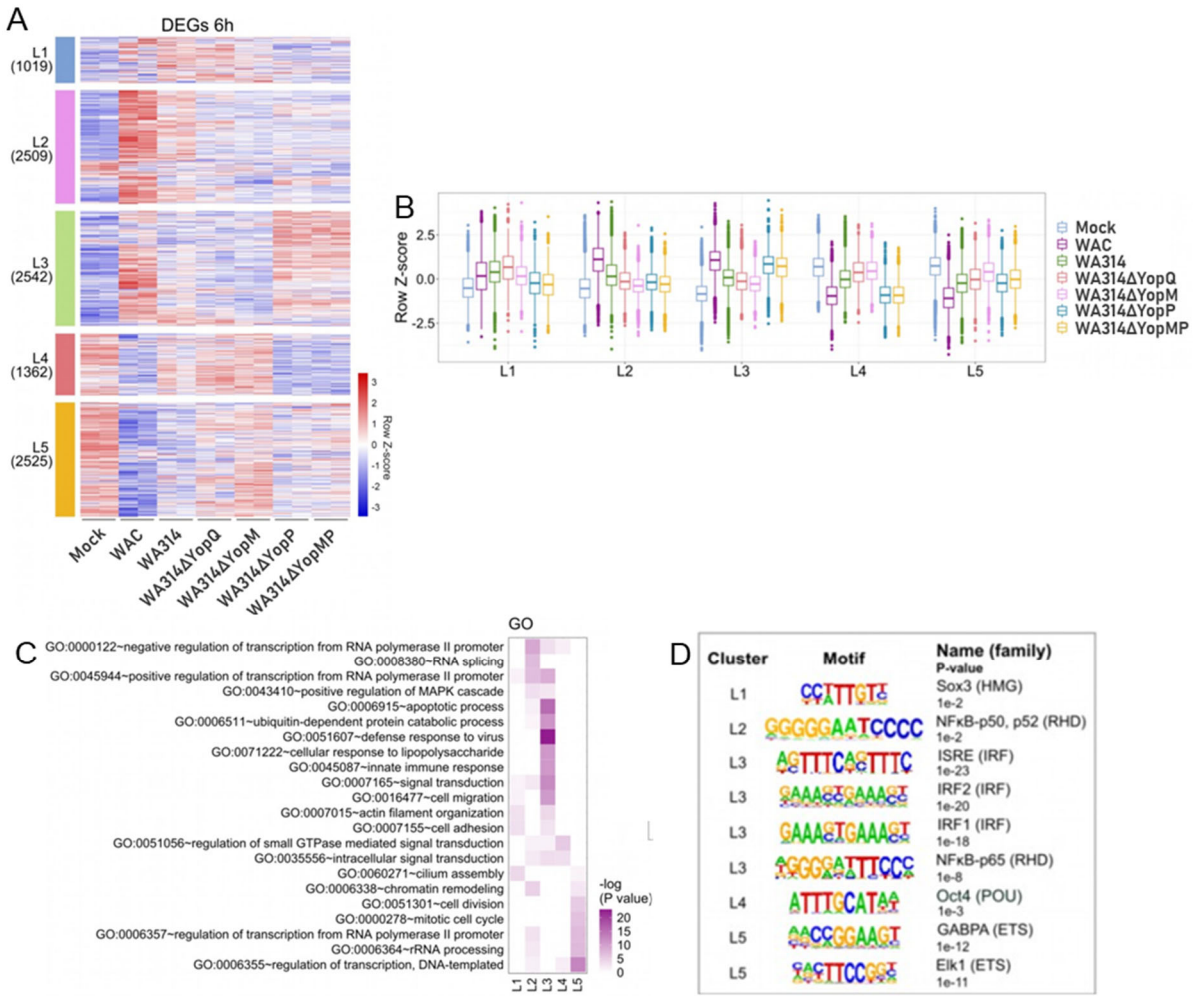

Figure 6: Differentially expressed gene clustering, gene ontology analysis and motif analysis in clusters L1 - L5. (A) Clustered Heatmap of all DEGs for comparisons between mock, WAC, WA314, WA314ΔYopQ, WA314ΔYopM, WA314ΔYopP & WA314ΔYopMP infected macrophages after 6 h of infection. Clustering identified 5 major clusters L1 - L5 (in brackets is the number of genes belonging to each cluster). Gene rlog counts were row-scaled (row Z-score). Two representative replicates are shown for each sample. (B) Boxplots of row Z-scores of rlog counts for genes from clusters L1 - L4 in (A) showing global gene expression profile of each cluster. (C) Heatmap showing -log10 transformed p-values and enriched GO terms for each cluster in (A). (D) Representative enriched transcription factor motifs in genes from L1 - L5 clusters in (A).

As already seen in the clusters L3 - L5, YopM and YopQ induced several genes in opposite directions to YopP, and we therefore had a closer look at these genes. In total 410 DEGs were oppositely regulated by YopQ and YopM compared to YopP and two clusters C1 & C2 emerged with cluster C1 corresponding to cluster L3 and C2 corresponding to cluster L4 (Fig. 7A & 7B).

Cluster C1 contains genes which are stimulated by *Yersinia* PAMPs in WAC and downregulated by T3SS effectors of which mostly YopP is responsible for (Fig. 7A & 7B). Strains lacking the T3SS effectors YopM (WA314ΔYopM) and YopQ (WA314ΔYopQ) even stronger downregulated the PAMP induced upregulation than the wildtype WA314 and thus it seems to be that YopQ and YopM counterregulate the YopP effect. Genes in cluster C1 show enrichment for motifs of IRF transcription factors or NF-κB transcription factor (Fig. 7C).

Genes that are downregulated by the *Yersinia* PAMPs in WAC and mostly upregulated in WA314 by YopP are clustered in C2 (Fig. 7A & 7B). In this case the strains deficient for YopM (WA314ΔYopM) and YopQ (WA314ΔYopQ) upregulate genes even stronger than the wildtype WA314, leading again to the conclusion that YopQ and YopM counterregulate the effect of upregulation by YopP (Fig. 7A & 7B). Genes in cluster C2 show motif enrichment for Forkhead transcription factors (Fig. 7C).

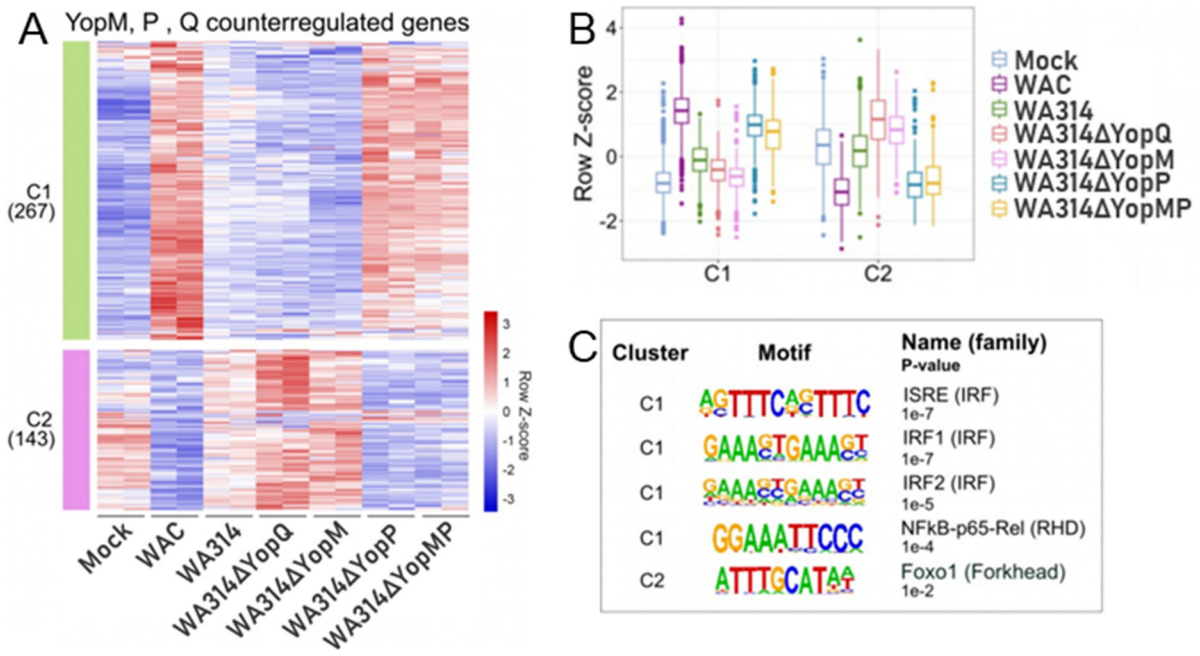

Figure 7: Clustering and motif analysis of counterregulated differentially expressed genes in cluster C1 & C2. (A) Clustered Heatmap of genes counterregulated by YopM, YopP and YopQ for comparisons between mock, WAC, WA314, WA314ΔYopQ, WA314ΔYopM, WA314ΔYopP & WA314ΔYopMP infected macrophages after 6 h of infection. Clustering identified 2 major clusters C1 & C2 (in brackets is the number of genes belonging to each cluster). Gene rlog counts were row-scaled (row Z-score). Two representative replicates are shown for each sample. (B) Boxplots of row Z-scores of rlog counts for genes from clusters C1 & C2 in (A) showing global expression profile of each cluster. (C) Representative enriched transcription factor motifs in genes from C1 & C2 clusters in (A).

To obtain more detailed information on the genes found in each cluster, GO analysis was performed, revealing that genes in cluster C1 play a role in cytokine-cytokine receptor interaction pathway as well as the defense response to virus pathway (Fig. 8A & 8B). Unsurprisingly a lot of inflammatory cytokines were present in those pathways. Genes in cluster C2 belong to the pathways “RNA polymerase II transcription factor activity” and “sequence-specific DNA binding” (Fig. 8C).

To confirm the RNA-seq results we chose the three cytokines TNF, IL6 and IL1B enriched in cluster C1, performed a quantitative RT-qPCR analysis and normalized the expression values to WA314 (Fig. 8D). Consistent with the heatmap, we found that loss of YopM leads to a decrease of gene expression of all three cytokines compared to WA314, meaning that YopM upregulates the expression of these genes. Inversely the deletion of YopP and YopP & YopM together led to the same phenotype like WAC, which shows that YopP is partly responsible for the suppression of WAC induced genes in cluster C1 (Fig. 8D).

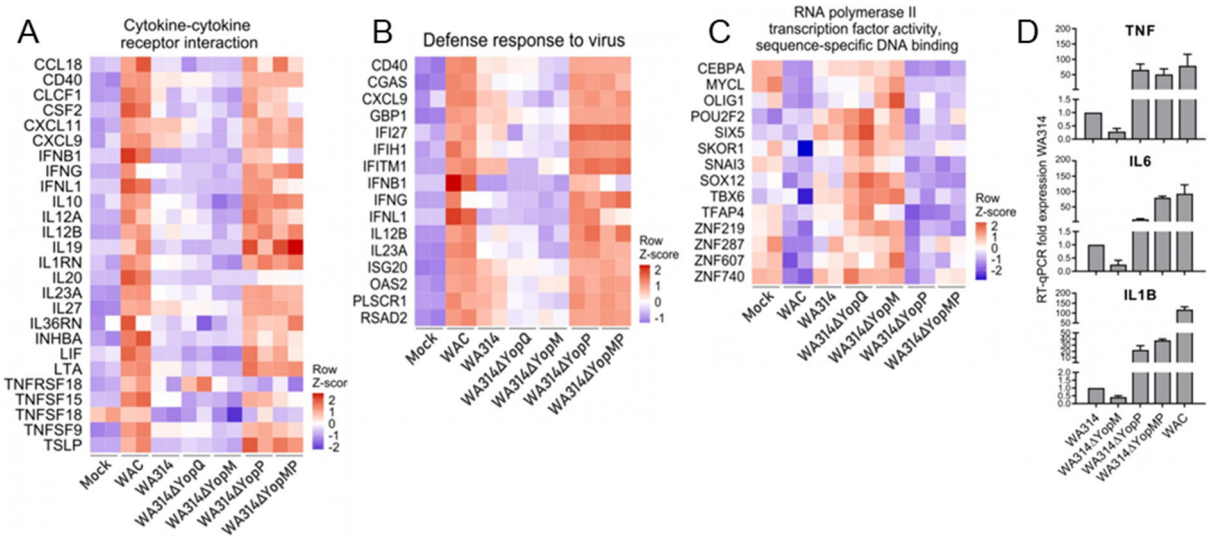

Figure 8: Pathway enrichment in clusters C1 & C2 and cytokine expression levels. (A) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes belonging to cytokine-cytokine receptor interaction pathway enriched in cluster C1. (B) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes belonging to defense response to virus enriched in cluster C1. (C) Heatmap of row scaled (Row Z-score) RNA-seq rlog gene counts for genes belonging to RNA polymerase II transcription factor activity, sequence-specific DNA binding pathway enriched in cluster C2. (D) RT-qPCR expression analysis of TNF, IL6, IL1B genes from primary human macrophages infected with *Y. enterocolitica* strains WA314, WAC, WA314ΔYopM, WA314ΔYopP & WA314ΔYopMP for 6 h. Bars represent mean from at least 2 biological replicates. Error bars indicate standard error of the mean.

Overall our global RNA-seq analysis illustrates that *Yersinia* PAMPs like LPS leads to a massive modulation of gene expression in primary human macrophages. Up- or downregulation of genes takes place in pathways belonging to the inflammatory response, apoptotic processes or the regulation of gene transcription. Inhibition of gene expression changes is due the activities of T3SS effectors in WA314 of which YopP is the main actor. Notably the effectors YopM and YopQ counteract the effects of YopP, potentially leading to a more balanced and regulated suppression of gene expression by YopP.

5.2 *Y. enterocolitica* T3SS effectors suppress inflammasome activation in primary human macrophages

Yersinia T3SS effectors not only impair host gene expression to suppress the immune response and promote the survival of the pathogen, but they also impair cell death pathways which are activated by PRRs like TLRs or NLRs as a response to the detection of a bacterial PAMPs or cellular DAMPs. TLRs detect PAMPs in the extracellular or endosomal surrounding, leading to the activation of pro-inflammatory gene expression through different signalling pathways (Akira et al., 2001). NLRs like NLRP1, NLRP3 or NLRC4 in contrast sense intracellular microbial factors and assemble with ASC and caspase-1 in a multiprotein complex called inflammasome (Chen et al., 2009; Martinon et al., 2002). Three of the T3SS effectors, which are translocated by *Yersinia* into the host cell, YopM, YopP/J and YopQ/K have been reported to alter several steps in different cell death pathways (Chen & Brodsky, 2023). It was shown that YopQ/K prevents formation of the NLRP3 inflammasome, by preventing hypertranslocation of the *Yersinia* pore proteins YopB and YopD. YopB and YopD hypertranslocation induces formation of the non-canonical caspase-11 inflammasome as well as the canonical NLRP3 inflammasome both resulting in the activation of caspase-1 (Brodsky et al., 2010; Zwack et al., 2017; Zwack et al., 2015). However, YopM prevents the activation of the Pyrin inflammasome, which is activated by the inhibition of Rho GTPases by YopE and YopT (Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016). For YopP/J it is known that the inhibition of NF-κB signalling pathway leads to caspase-1 activation likely through caspase-8 activation, but independent of canonical inflammasome components like ASC or different sensors (Brodsky et al., 2010; Orning et al., 2018; Philip et al., 2014; Weng et al., 2014). All of the above mentioned results were obtained using either *Y. pestis or Y. pseudotuberculosis* and only the interaction between YopM and the Pyrin inflammasome was shown in the human monocytic THP-1 cell line. As there is a lack of information regarding the effects of the above mentioned Yops

from *Y. enterocolitica* on the inflammasome formation in primary human macrophages we aimed to uncode their effects.

5.2.1 *Y. enterocolitica* T3SS induces NLRP3 inflammasome formation

To detect the effect of Yops on global inflammasome formation, imaging of ASC speck formation was chosen as a suitable method, as canonical inflammasomes like the NLRP1-, NLRP3- or the Pyrin-inflammasome all possess ASC as a connector between the sensor and (pro-)caspase-1. This makes it possible to gain a general information about the effect of different Yops on inflammasomes while only imaging one component of them. Furthermore, only one speck is formed inside one cell, which makes it easy to detect. First we proved that ASC specks not only contain ASC, but also (pro-)caspase-1 and in our case NLRP3 to show that ASC specks possess all components of a canonical inflammasome and thus can be used as a marker for the impact of Yops on canonical inflammasome formation. For that primary human macrophages were transfected with NLRP3-eGFP and either non infected (mock) or infected with different MOIs (100, 300, 500) with the *Y. enterocolitica* strain pTTSS for different time points (1 h, 2 h, 3 h). *Y. enterocolitica* strain pTTSS only harbours the T3SS, but no T3SS effectors (Heesemann & Laufs, 1983).

Confocal imaging revealed that mock infection does not induce any NLRP3 containing specks (Fig. 9A). In contrast, infection with pTTSS with a MOI of 500 for 2 h leads to a strong induction of one speck per cell in almost all transfected and infected cells. These specks consist of the inflammasome sensor NLRP3, the adaptor molecular ASC as well as the (pro)-caspase-1 (Fig. 9A).

A time course analysis indicated that ASC speck formation was dependent on infection time and MOI (Fig. 9B). After 1 h of infection, from less than 0.5 % to around 1.5 % of infected cells showed ASC specks depending on the MOI. After 2 h, from 5 % to 25 % of cells showed ASC specks and after 3 h of infection from 15 % to 45 % showed ASC specks (Fig. 9B).

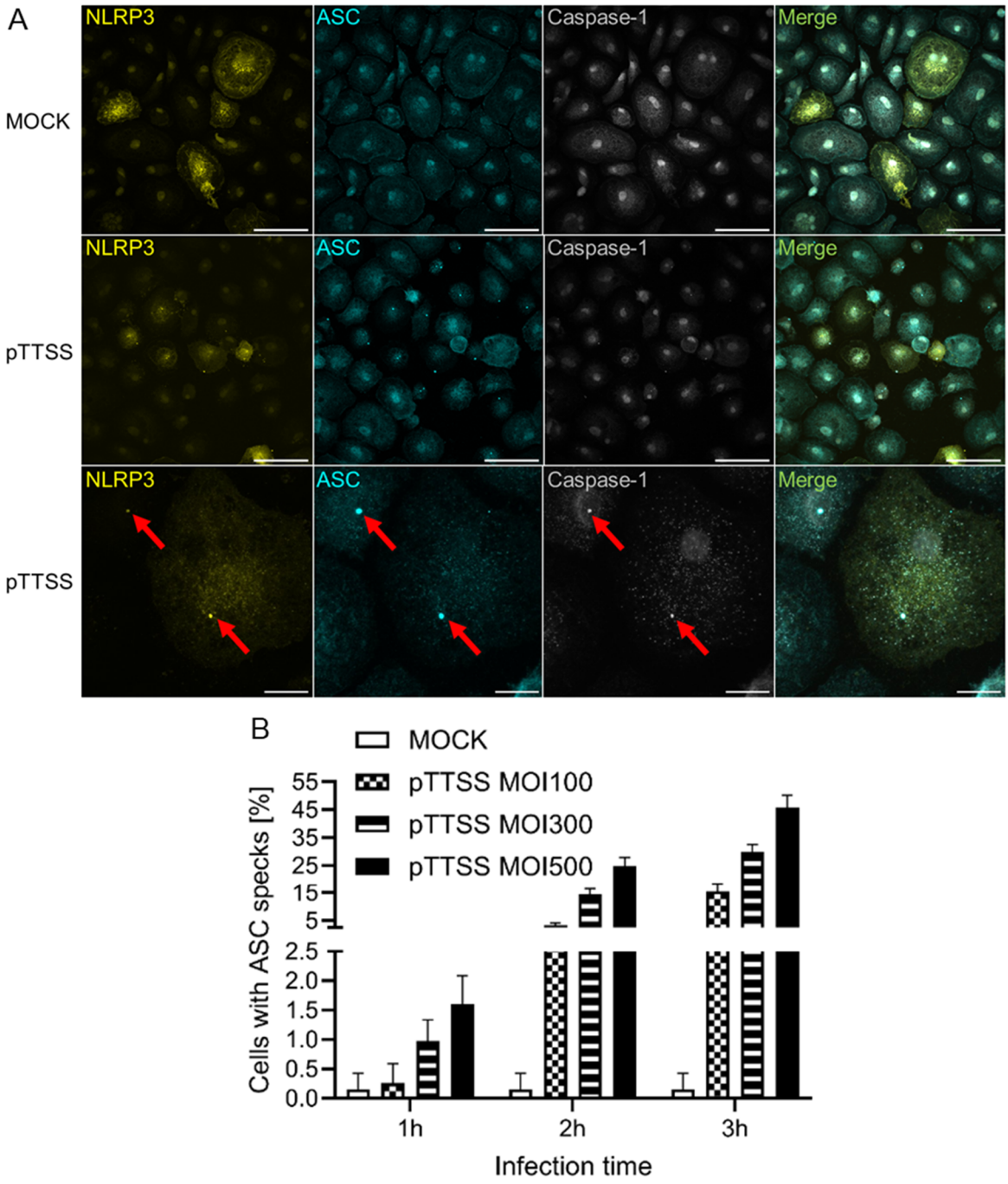

Figure 9: Analysis of ASC inflammasome formation in response to *Y. enterocolitica* infection. (A) NLRP3-eGFP transfected primary human macrophages were either mock- or pTTSS infected with a MOI of 500 for 2 h and immunostained for ASC & caspase-1 using antibodies. Blowup representation of pTTSS infected macrophages is shown in the bottom line. Arrows indicate colocalizing specks. Scale bar: 50 µm for the two upper lines and 10 µm for the bottom line. (B) Analysis of ASC speck formation in primary human macrophages mock-infected or pTTSS-infected with a MOI of 100, 300 and 500 for 1 h, 2 h and 3 h. Bars represent means from at least two biological replicates with two technical replicates respectively. Around 600 cells were counted for each biological replicate. Error bars represent standard deviation.

5.2.2 *Y. enterocolitica* effectors suppress inflammasome formation

To investigate whether the T3SS effectors can block inflammasome formation caused by the T3SS structural components, we infected primary human macrophages for 3 h with different MOIs (30, 100, 300) of the above mentioned strains. Interestingly, mock infection as well as infection with WAC and WA314 induced essentially no ASC speck formation, when compared to the strong dose dependent induction of ASC inflammasome formation by pTTSS (Fig. 10A & 10B). This indicates that none of the bacterial PAMPs from WAC induces ASC dependent inflammasome formation and that the effectors of WA314 completely suppress the ASC speck formation induced by the components of the T3SS (Fig. 10A & 10B).

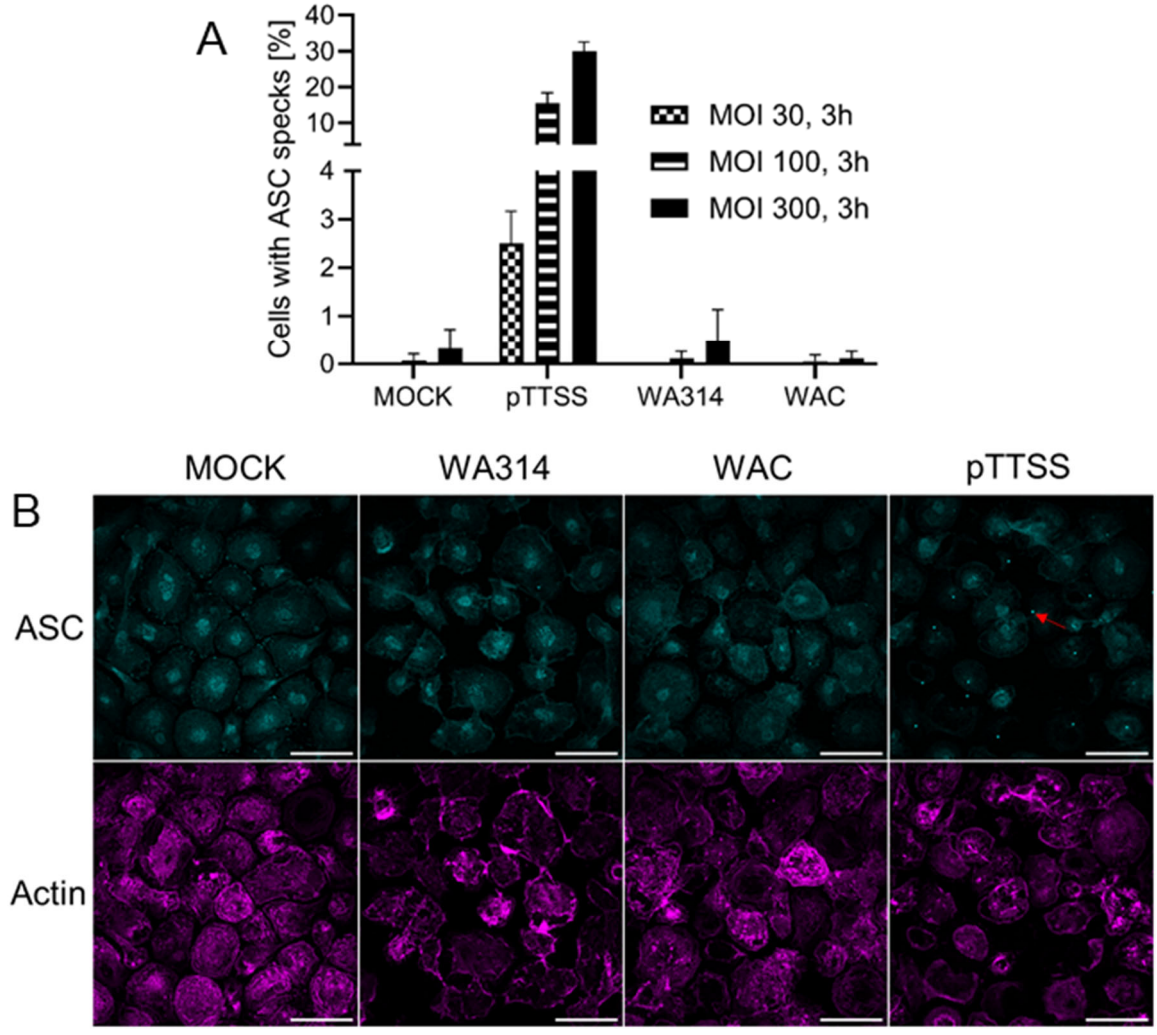

Figure 10: Quantitative analysis & microscopic representation of the effect of T3SS effectors on ASC speck formation. (A) Analysis of ASC speck formation in primary human macrophages infected with mock, pTTSS, WA314 and WAC with MOIs of 30, 100 and 300 for 3 h. Bars represent means from at least two biological replicates with two technical replicates for each. Error bars represent standard deviation. In total around 600 cells were counted for each biological replicate. (B) Representative images of primary human

macrophages infected with mock, WA314, WAC & pTTSS with a MOI of 300 for 3h. Cells were immunostained for ASC & Actin using antibodies. Scale bar: 50 µm. Arrow indicates one representative ASC speck.

5.2.3 T3SS effectors YopP & YopQ cooperate to suppress inflammasome formation

As shown in Fig. 10, T3SS effectors of WA314 almost completely suppressed the formation of ASC inflammasomes. To clarify which of the T3SS effectors is responsible for this effect and if there is a possible cooperation between the effectors, we infected primary human macrophages with single deletion mutants of YopM (WA314ΔYopM), YopP (WA314ΔYopP) and YopQ (WA314ΔYopQ) as well as double deletion mutants of YopM & YopP (WA314ΔYopMΔYopP), YopM & YopQ (WA314ΔYopMΔYopQ) and YopP & YopQ (WA314ΔYopPΔYopQ) with a MOI of 500 for 2 h. Values were normalized to WA314 to facilitate comparison between the different strains. Except for the double mutant lacking YopP and YopQ (WA314ΔYopPQ), none of the employed single or double Yop-mutant strains behaved statistically different from WA314. This indicates that the T3SS effectors act synergistically and cooperatively to block inflammasome formation. It also demonstrates that the combined removal of YopP and YopQ induces a defect in the inflammasome inhibitory activity that cannot anymore be compensated by the remaining Yops (Fig. 11).

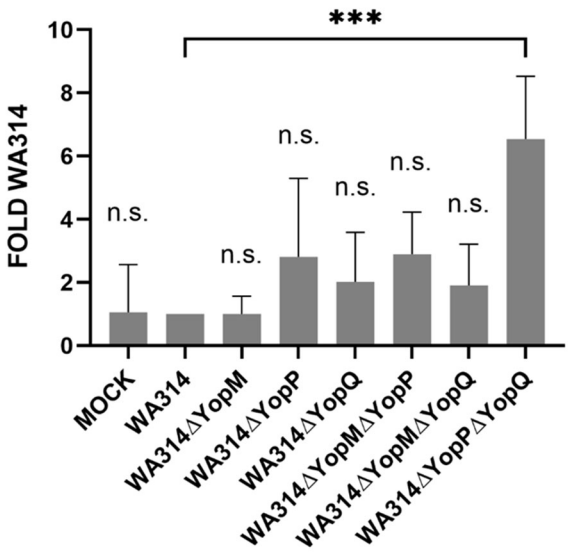

Figure 11: Analysis of the effect of YopM, YopP and YopQ on ASC speck formation. Quantification of ASC speck formation in primary human macrophages infected with mock, WA314, WA314ΔYopM, WA314ΔYopP, WA314ΔYopQ, WA314ΔYopMΔYopP, WA314ΔYopMΔYopQ, WA314ΔYopPΔYopQ with a MOI of 500 for 2 h. Each bar represents mean ± standard deviation of at least two replicates normalized to WA314. Significance was calculated using an ordinary one-way ANOVA. n.s: not significant. ***: p- value ≤ 0.001.

5.3 *Y. enterocolitica* affects histone H3 serine-10 phosphorylation in primary human macrophages

Several bacterial effectors have been shown to influence the epigenetic landscape, often targeting histone H3 serine-10 phosphorylation (H3S10ph), by that interfering with the host immune response (Bierne & Pourpre, 2020; Connor et al., 2019). A previous study in our group with *Y. enterocolitica* has already shown that T3SS effectors largely modulate histone modifications as well as connected gene expression to promote survival inside the host (Bekere et al., 2021). In this study we aimed to find a modulation of H3S10ph by effectors of *Y. enterocolitica*.

5.3.1 *Y. enterocolitica* T3SS effectors suppress histone H3 serine-10 phosphorylation

Primary human macrophages were either non-infected (mock) or infected with WA314 or WAC followed by histone extraction, electrophoretic separation and western blotting to detect differences in H3S10ph pattern. Infection for 10 min just gives a minor but equal H3S10ph signal in WA314 and WAC infected cells, which increases similarly in intensity after 30 min of infection with both strains. The maximum signal intensity in H3S10ph is found after 60 min of infection in WAC infected cells, whereas H3S10ph signal in WA314 infected cells is weaker. After 180 min of infection the H3S10ph signal in WA314 infected cells is not detectable, whereas the WAC infected cells still show a strong H3S10ph signal (Fig. 12A). This effect was not only detectable in Western blotting, but also in immunofluorescently stained macrophages (Fig. 12B). Taken together there is evidence that the T3SS effectors either prevent phosphorylation or exert dephosphorylating functions.

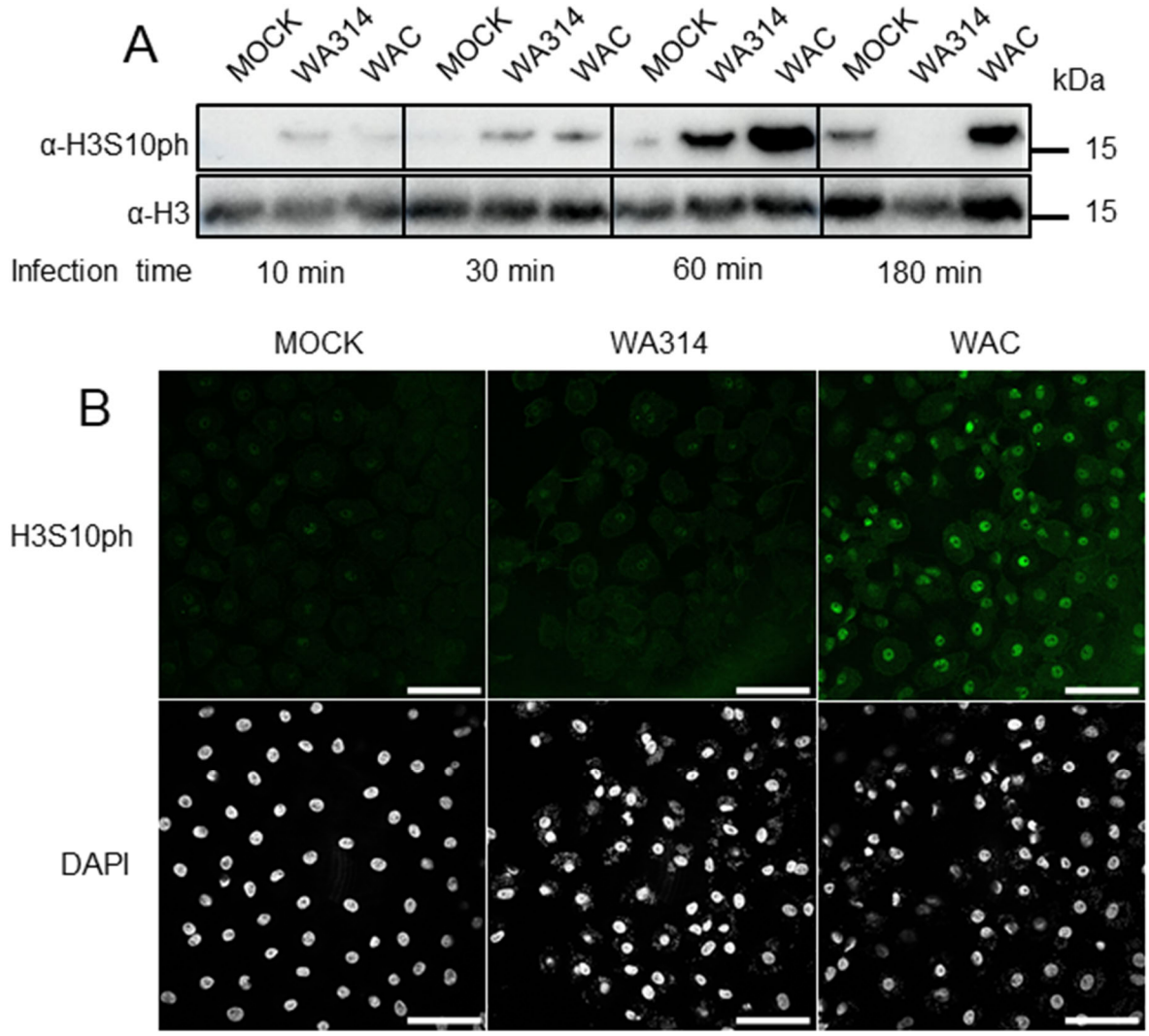

Figure 12: Analysis of the effect of Yops on histone H3 serine-10 phosphorylation. (A) Western blot analysis of H3S10ph in primary human macrophages infected with mock, WA314 and WAC with a MOI of 100 for 10 min, 30 min, 60 min and 180 min. Histone H3 (H3) was used as a loading control. (B) Representative images of primary human macrophages infected for 3 h using the same infection conditions like in (A). Cells were immunostained with antibody for H3S10ph and DAPI for DNA. Scale bar: 50 µm.

5.3.2 T3SS effector YopP partially inhibits H3S10 phosphorylation. Role of MAPK pathway

As seen in Fig. 12 T3SS effector activity led to the disappearance of H3S10ph in infected macrophages. To determine which of the T3SS effectors is or are responsible for this effect we infected primary human macrophages for 3 h with single Yop deletion strains. Mock infection as well as infection with WAC and WA314 showed the same results like already shown in Fig. 12 (Fig. 13A & 13B). Interestingly deletion of YopP rescues approximately

half of the effect as seen with WAC infection, whereas none of the other single deletion mutants showed a large difference to WA314 infection (Fig. 13A & 13B). Because it is already known that YopP is an acetyltransferase, which inhibits MAPK and NF-κB pathways in the host cell (Meinzer et al., 2012; Mittal et al., 2006; Mukherjee et al., 2006; Paquette et al., 2012) we wanted to know which of these pathways needs to be inhibited to prevent H3S10ph. For that we treated macrophages with several inhibitors and infected macrophages with the single YopP deletion strain (WA314ΔYopP) for 3 h. Treatment with the inhibitors for MEKs/ERKs & p38 as well as IKKα/ß leads to a disappearance of the H3S10ph signal showing that those proteins are needed for the phosphorylation of H3S10 and that YopP successfully inhibits those (Fig. 13C).

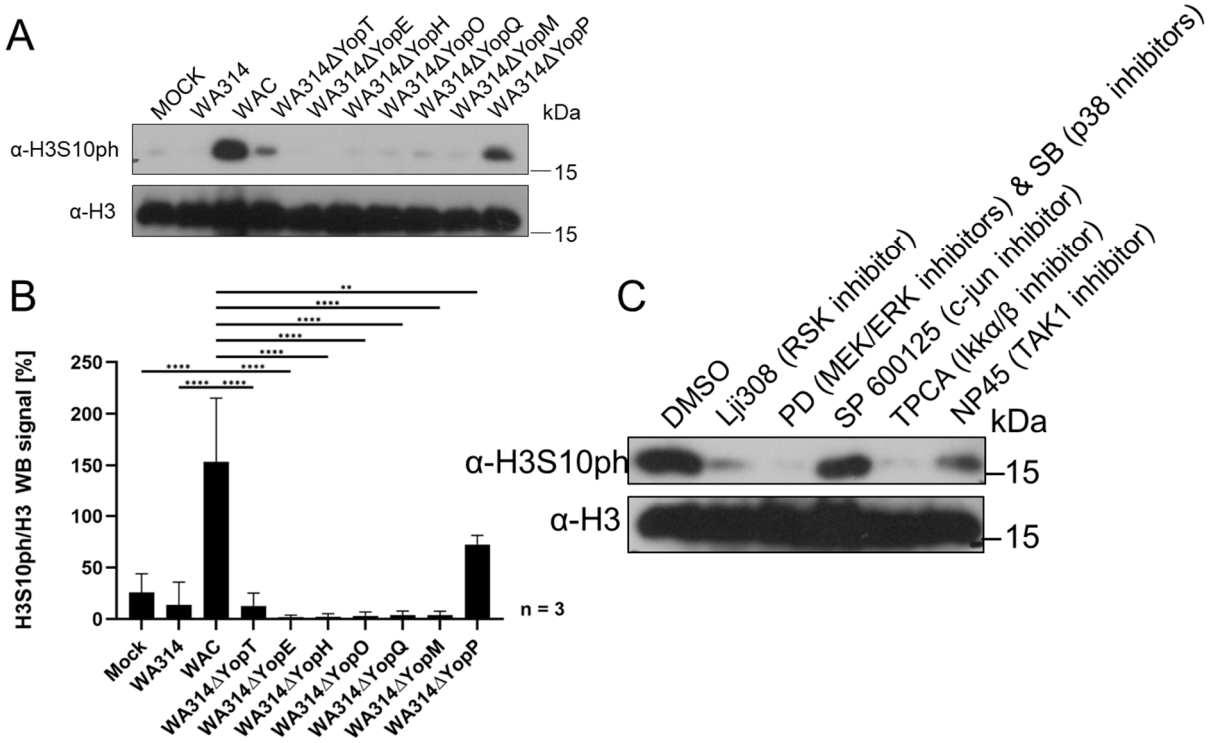

Figure 13: Analysis of the effect of different Yops on histone H3 serine-10 phosphorylation. (A) Representative Western blot analysis of H3S10ph in primary human macrophages infected with mock, WA314, WAC, WA314ΔYopT, WA314ΔYopE, WA314ΔYopH, WA314ΔYopO, WA314ΔYopQ, WA314ΔYopM and WA314ΔYopP with a MOI of 100 for 3h. Histone H3 (H3) was used as a loading control. (B) Statistical analysis of H3S10ph signal from at least three independent blots like shown in (A). Bars represent mean of H3S10ph signal normalized to H3 signal of at least three independent replicates. Error bears represent standard deviation. Significance was calculated using an ordinary one-way ANOVA. **: p-value ≤ 0.01. ****: p-value ≤ 0.0001 (C) Western blot analysis of histone H3S10ph from primary human macrophages infected with WA314ΔYopP with a MOI of 100 for 3 h, which were additionally treated with several inhibitors of MAPK and NF-κB signal pathway. Histone H3 (H3) was used as a loading control.

6 Discussion

6.1 The effect of *Y. enterocolitica* on the transcriptome of primary human macrophages

Pathogenic bacteria like *Y. enterocolitica* modulate gene expression of their host cells as one of their main virulence programs. In this study a global gene expression analysis of primary human macrophages infected with different strains of *Y. enterocolitica* was conducted, aiming to enlighten the role of the T3SS effectors YopM, YopP and YopQ on modulation of gene expression. Furthermore, a deeper focus was on a potential interplay of YopM and YopP in modulating host gene expression.

First of all, comparison with publicly available data and data generated in this study showed an overlap between naïve and mock infected macrophages as well as LPS-stimulated and WAC infected macrophages (Fig. 1B), highlighting the quality of our data and showing that they could be used for further analysis. Further analysis of samples from macrophages infected for 1.5 h with either the wildtype (WA314) or the avirulent strain (WAC) revealed that around 1000 genes, most of them belonging to inflammatory pathways, are differentially regulated vs. mock under these conditions. There were minor differences between WA314 and WAC, indicating that at this time point the T3SS and its effectors are not very active yet and the observed effects on gene transcription are mostly due to the bacterial PAMPs (Fig. 2B). However, after 6 h of infection a strong modulation of gene expression was detected, differing between WAC and WA314 infected macrophages. Whereas 4641 DEGs were found in WA314 infected macrophages and 7437 DEGs were found in WAC infected macrophages compared to mock-infected ones, we found 2714 DEGs in a comparison between WAC and WA314 showing the extensive gene expression modulation caused by T3SS effectors in WA314 (Fig. 2B). It has already been reported that it takes several hours until the effect of many T3SS effectors has a detectable impact (Aepfelbacher et al., 2003; Ruckdeschel et al., 1998; Ruckdeschel et al., 1997; Zumbihl et al., 1999). Furthermore, previous studies already showed that both the avirulent *Y. enterocolitica* strain WAC and the virulent strain WA314 directly induce the modulation of gene expression through the activation of MAPK and NF-κB signaling pathways (Ruckdeschel et al., 1998; Ruckdeschel et al., 1997). This initial activation is followed by a suppression, mainly through YopP, in the virulent strain WA314 (Ruckdeschel et al., 1998; Ruckdeschel et al., 1997). This fits with the results obtained in this study as WA314 already moderately suppresses PAMP-induced genes after 1.5 h and those genes show enrichment of NF-κB motifs and in inflammatory pathways (Fig. 4A). Genes belonging to this group are for example the NF-κB induced genes for TNF, IL23A and IL27 (Carmody et al., 2007; Liu et al., 2007). This states previous

findings showing that the T3SS effector YopP targets inflammatory response genes which are regulated through NF-κB (Ruckdeschel et al., 1998; Ruckdeschel et al., 2001). Interestingly there are not only PAMP-induced genes, but also PAMP-suppressed genes, which are highly enriched for Zinc-finger genes playing a role in transcription regulation (Fig. 4D) and WA314 prevents the suppression of several of them. Unfortunately, it is not really known which influence Zinc finger genes have during the immune response in macrophages (Cassandri et al., 2017). A previous study showed that many Zinc finger proteins promote the repression of genes (Lupo et al., 2013), which led to the conclusion that suppression of Zinc finger genes in turn leads to the activation of many genes. Vice versa it may be beneficial for *Yersinia* to prevent suppression of Zinc finger genes as this could be beneficial for the bacteria by suppressing PAMP induced genes.

Looking more closely to the strong increase in gene expression modulation after 6 h compared to 1.5 h of infection (Fig. 2B), this effect might be due to the stimulation of cyto- and chemokines, which were induced early after infection. Cyto- and chemokines in turn stimulate through paracrine and autocrine effects the expression of secondary response genes (SRG) (Schneider et al., 2014; Shouval et al., 2014).

As we observed a more distinct and clear effect of the T3SS effectors after 6 h compared with 1.5 h of infection, the main goal of this study, enlightening the effects of the single Yops YopM, YopP and YopQ, but also enlightening a potential interplay between YopM and YopP, could be better investigated after the 6 h time point. PCA analysis showed clear separation of WAC from all other samples as well as a tight clustering of WA314ΔYopP and WA314ΔYopMP, which both in turn separated from all other samples (Fig. 5B). Samples from WA314ΔYopM and WA314ΔYopQ infected macrophages however were close to samples from WA314 infected macrophages, which hints to a more attenuated effect of YopM and an even more attenuated one of YopQ compared with YopP (Fig. 5B). A deeper look at DEG comparisons, showed that the deletion of YopQ does not have a large influence on gene expression, whereas the deletion of YopP had the highest impact on gene expression and deletion of YopM was in between the effect of YopP and YopQ (Fig. 5C). Interestingly double deletion of YopM and YopP did not have any additional effect compared to a single deletion of YopP showing that in the absence of YopP, YopM does not have a significant effect on gene expression (Fig. 5D).

We found three clusters of DEGs after 6 h of infection, which showed effects of single Yops (Fig. 6A & 6B), with YopP being mostly responsible for counteracting the PAMP effect on gene expression. Genes suppressed by YopP are enriched in pathways belonging to the innate immune response, response to LPS and virus as well as ubiquitination and transcription regulation and those genes show enrichment of motifs for the IRF and RHD (Rel homology domain) transcription factor family (Fig. 6C & 6D).

Around 400 DEGs in two cluster were found differentially regulated between YopM, YopQ and YopP (Fig. 7A). Surprisingly YopP was the main actor of the suppression of PAMP-induced changes in gene expression, and both YopM and YopQ counteract this effect by promoting the PAMP-induced changes in gene expression (Fig. 7A & 7B). Like already shown in Fig. 6D, PAMP-induced genes which were suppressed by YopP are enriched in motifs belonging to transcription factors from the IRF and RHD family (Fig. 7C) and show enrichment of genes belonging to pathways of cytokine-cytokine receptor interaction and defense response to virus (Fig. 8A & 8B). For example, the gene encoding for INFβ is downregulated by YopP, which inhibits the activation of the STAT1/STAT2/IRF9 complex as well as other interferon stimulated genes (ISGs) (Schneider et al., 2014). However, PAMP suppressed and YopP stimulated genes are enriched for motifs of the transcription factor Foxo1 (Forkhead box protein O1) from the Forkhead family and enriched for genes in the pathway of transcription factor activity (Fig. 7C & 8C). To further support the RNA-seq analysis RT-qPCR was done showing that YopM and YopP have opposing effects on the expression of the cytokines TNF, IL-6 and IL1ß and that a deletion of both YopM and YopP does not lead to an approach to the wildtype effect but more to the WAC induced effect (Fig. 8D), showing that the effect of YopP predominates the effect of YopM.

Previous studies already showed an opposing effect of YopM and YopP/J with regard to inflammasome signaling and cell death (Ratner, Orning, Proulx, et al., 2016; Ratner, Orning, Starheim, et al., 2016; Schoberle et al., 2016). In detail cell death and caspase-1 activation was promoted by YopP/J in naïve (Schoberle et al., 2016) as well as LPS-primed macrophages (Ratner, Orning, Proulx, et al., 2016; Ratner, Orning, Starheim, et al., 2016), whereas YopM prevented inhibition of caspase-1 activation and cell death (Ratner, Orning, Proulx, et al., 2016; Ratner, Orning, Starheim, et al., 2016; Schoberle et al., 2016). In contrast to this opposing effect, a cooperative effect was also shown as inhibition of IL-1β secretion was done in a cooperative manner of YopP/J and YopM together by that promoting *Yersinia* virulence (Ratner, Orning, Proulx, et al., 2016; Ratner, Orning, Starheim, et al., 2016; Schoberle et al., 2016). However, for the regulation of gene expression neither a cooperative nor opposing effect has been shown till now.

YopM stimulates RSK, which is a known regulator of gene expression (Anjum & Blenis, 2008), directly in the nucleus, thus potentially supporting PAMP signaling (Berneking et al., 2016). Through this YopM dependent interaction for example, the expression of IL-10, an anti-inflammatory cytokine, was upregulated in wild type infected primary human macrophages, even though YopP suppresses gene expression of IL-10 (Berneking et al., 2023; Berneking et al., 2016). Our RNA-seq analysis shows that WAC strongly promotes gene expression of IL-10, YopP strongly suppresses it and YopM is slightly counteracting the YopP effect by also promoting the expression of IL-10 (Fig. 8A). This effect was also

shown by RT-qPCR experiments (Berneking et al., 2023), highlighting the complex interplay which is necessary to favor the bacterial infection.

The Yop effector protein YopQ/K is important for the regulation of the Yop effector translocation into the host cell (Aili et al., 2008; Dewoody et al., 2011; Dewoody et al., 2013b; Holmström et al., 1997; Holmström et al., 1995a, 1995b). RNA-seq analysis showed a similar amount of DEGs in macrophages infected with a YopQ deficient strain compared with wildtype infected macrophages even though a small effect was observed (Fig. 5C). This similarity was confirmed by heatmap analysis, but an attenuation of the wild type effect was detectable (Fig. 6A & 7A). Overall YopQ attenuates the wild type effect on gene expression likely due to the prevention of hypertranslocation of several Yop effectors. The deletion of YopK in *Y. pestis* strain KIM5 leads to a hypertranslocation of several Yops, including YopJ and YopM (Dewoody et al., 2013b). As there is a high similarity between YopK from *Y. pestis* strain KIM5 and its homolog YopQ from *Y. enterocolitica* strain WA314 can assume that the effect of YopQ on translocation will be very similar. Having this in mind it might explain, why the deletion of YopQ leads to an even stronger suppression of the PAMP-induced gene expression changes. Hypertranslocation of YopP strongly suppresses PAMP-induced gene expression changes and as the counteracting effect of YopM is weaker than the YopP effect, the PAMP-induced gene expression changes are even stronger suppressed in macrophages infected with a YopQ deficient strain than in wildtype infected macrophages.

Taken together the RNA-seq analysis shows that *Y. enterocolitica* T3SS effectors translocated into the host cell largely suppress the PAMP-induced gene expression changes and that YopP is the main actor of the suppression by inhibiting NF-κB and MAPK pathways like already shown (Ruckdeschel et al., 1998; Ruckdeschel et al., 1997). In contrast YopM, as well as YopQ, both counteract the effect of YopP by slightly promoting the PAMP-induced gene expression changes. Even though its speculating, but the effect of YopM might be due to the stimulation of kinases or transcription factors in the nucleus as previously shown (Berneking et al., 2023; Berneking et al., 2016) and YopQ might promote PAMP-induced gene expression changes by preventing the hypertranslocation of several Yop effector proteins (Dewoody et al., 2013b).

6.2 *Y. enterocolitica* T3SS effectors suppress inflammasome activation in primary human macrophages

The impairment of cell death pathways, which are activated as a response to the detection of bacterial PAMPs, is another mechanism of T3SS effectors of pathogenic *Yersinia* to suppress the host immune response. Previous studies, using mouse cells infected with different *Y. pseudotuberculosis* and *Y. pestis* strains already showed that three Yop effectors, YopM, YopP and YopQ, impair several steps in different cell death pathways (Chen & Brodsky, 2023). Due to the lack of information of the effect of the above mentioned T3SS effectors of *Y. enterocolitica* on cell death pathways in human cells, we performed experiments using primary human macrophages infected with several different strains of *Y. enterocolitica*.

As we wanted to have an easy, fast and reliable method to assess the effects of Yops on global inflammasome formation, we chose to image ASC speck formation, as the adaptor protein ASC is the common component in many canonical inflammasomes (Rathinam et al., 2012). Confocal imaging revealed that ASC specks formed in response to pTTSS infection (a strain only harboring the T3SS, but no Yop effector proteins) also contain the sensor NLRP3, as well as (pro-)caspase-1 showing that all components including the sensor, the caspase and the connector ASC, are present in one ASC speck (Fig. 9A). Furthermore, we also showed that ASC speck formation is highly time and MOI dependent, as longer infection time as well as a higher MOI led to larger amounts of cells containing ASC specks (Fig. 9B).

Next we assessed on the one hand the role of translocated Yop effectors using the wild type strain WA314 and on the other hand the role of the T3SS alone, as present in pTTSS, by using the avirulent strain WAC lacking the complete virulence plasmid.

WA314-infected macrophages show almost no ASC speck formation similar to mock- and WAC-infected ones, in contrast to pTTSS infected macrophages, that showed a MOI- and time-dependent ASC speck formation (Fig. 10A). We conclude that the T3SS and not PAMPs is the main trigger for ASC speck formation, and the translocated Yop effectors in WA314 completely inhibit this T3SS induced ASC speck formation in the infected macrophages (Fig. 10A).

Further, infection of primary human macrophages with either single deletion mutants of YopM, YopP or YopQ, as well as double deletion mutants of YopM & YopP, YopM & YopQ and YopP & YopQ showed that only the deletion of both YopP and YopQ together led to a significant increase in ASC speck formation compared to the wildtype (Fig. 11). Neither a single deletion of YopM, YopP or YopQ nor a double deletion of YopM & YopP or YopM &

YopQ showed a significant difference to the wildtype effect. This really contradicts previous findings showing that YopM alone is responsible for ASC dependent Pyrin inflammasome inhibition (Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016) and YopK alone inhibits NLRP3 inflammasome formation (Brodsky et al., 2010; Zwack et al., 2017; Zwack et al., 2015). As both of those inflammasomes contain ASC, we expected to detect a significant increase in the ASC speck formation upon deletion of YopM and YopQ alone.

The lacking effect of YopM on ASC speck formation could be explained by the short infection time of 2 h. Mangan et al. showed that LPS stimulates the TRIF (TIR-domain-containing adapter-inducing interferon-β) mediated expression of Pyrin after 5 h and 18 h and that low levels of Pyrin are not sufficient to trigger Pyrin inflammasome formation (Mangan et al., 2022). Having this in mind longer infection times of up to 18 h could lead to ASC dependent Pyrin inflammasome formation and the effect of YopM in primary human macrophages may be elucidated with longer infection times. Furthermore, using longer infection times, the role of YopM in Pyrin inflammasome formation or general impairment of cell death pathways could be uncovered by performing several experiments. First of all, Pyrin inflammasome formation upon treatment with a strain lacking all seven translocated Yop effectors except for YopE and YopT (WA314ΔYopHOMPQ) in primary human macrophages should be investigated microscopically. Using this strain should give the largest stimulus for Pyrin inflammasome formation as YopE and YopT induces Pyrin inflammasome formation by inhibiting Rho-GTPases (Chung et al., 2016; Ratner, Orning, Proulx, et al., 2016). In a next step it would be suitable to assess the influence of YopM on Pyrin inflammasome formation by using a strain lacking all Yop effectors except for YopE, YopT and YopM (WA314ΔYopHOPQ) as this strain is supposed to induce Pyrin inflammasome formation by YopE and YopT and similarly suppresses Pyrin inflammasome formation through YopM. As it was shown that YopM from *Y. pestis* strain KIM5 is able to suppress IL-1β secretion in primary human macrophages (Ratner, Orning, Proulx, et al., 2016), it would be of high interest to investigate if YopM from *Y. enterocolitica* strain WA314 also suppresses IL-1β secretion and if so, if this is Pyrin inflammasome dependent. Furthermore, there might also be a promoting effect of YopM on NLRP3 gene expression. As YopM is an important mediator of the nuclear translocation of STAT3 (Berneking et al., 2023) and STAT3 was shown to be important for the gene expression of NLRP3 (Zhu et al., 2021) it might also be that YopM promotes the gene expression of NLRP3.

As there is no effect of YopQ alone on ASC speck formation we conclude that YopQ from *Y. enterocolitica* is not able to inhibit ASC dependent inflammasome formation, thus contradicting previous findings showing that YopK alone inhibits NLRP3 inflammasome formation (Brodsky et al., 2010; Zwack et al., 2017; Zwack et al., 2015). It is important to note that differences can occur due to the difference between human and mouse

macrophages. About other reasons for the difference, we can only speculate. First of all, it shows that any of the other translocated Yops YopH, YopO, YopE or YopT may play a role in suppressing ASC dependent inflammasome formation or that several of the translocated Yops act cooperatively like YopP and YopQ do in our data (Fig. 11). In a next step we aim to investigate ASC speck formation using the triple mutant WA314ΔYopMPQ to detect a potential interplay of YopM, YopP and YopQ. Furthermore, a recent study showed a cooperation of YopE, YopH and YopK from *Y. pseudotuberculosis* strain IP2666 in inhibiting non-canonical caspase-4 inflammasome formation in human intestinal epithelial cells (IECs) and macrophages (Zhang et al., 2023). As the ASC dependent NLRP-3 inflammasome formation is linked with K^+ efflux mediated through caspase-4 activation (Baker et al., 2015; Rühl & Broz, 2015; Schmid-Burgk et al., 2015), it might be that YopE, YopH and YopQ together also inhibit ASC dependent NLRP3 inflammasome formation in primary human macrophages. To further depict the roles of YopE, YopH and YopQ, single as well as double and triple deletion mutants should be used for assessing ASC speck formation upon infection. Infection times for this experiments can be kept at 2 h as we showed that this is sufficient to induce NLRP3 inflammasome formation (Fig. 9C).

Last but not least we found a significant effect of YopP and YopQ together on ASC speck formation and as there is not much known about the effect of both on inflammasome formation we can just speculate about the reason for this effect. The effect of YopP might be the suppression of NLRP3 gene expression by inhibiting MAPK and NF-κB pathway. It was shown that caffeine inhibits NLRP3 gene expression through the inhibition of the MAPK and NF-κB pathway (Zhao et al., 2019) and as YopP inhibits the same pathway (Ruckdeschel et al., 1998; Ruckdeschel et al., 1997) it likely has the same effect. However, deletion of YopP alone is not enough to induce ASC speck formation as other Yops like YopE, YopH, YopQ might inhibit and YopM might promote ASC dependent NLRP3 inflammasome formation by mediating the nuclear translocation of STAT3, which is important for the gene expression of NLRP3. A further deletion of YopQ might attenuate the inhibiting effect of YopE, YopH and YopQ together leading to a slight increase in ASC speck formation as detected in our experiments (Fig. 11).

Finally, it seems to be that many translocated Yop effectors play a role in inflammasome formation. In this study we just got a little insight into the various effects of translocated Yop effectors. They all together nearly completely inhibit ASC speck formation and here we showed that YopP and YopQ together play a vital role in this effect. However, we could not proof previous findings for the effect of YopM or YopQ alone on inflammasome formation, on the one hand likely due to inconvenient time points in the case of YopM and on the other hand likely due to the difference between mouse and human cells in the case of YopQ. Further experiments are needed to enlighten the role of other Yops like YopE and YopH as

they might also influence inflammasome formation. In general, it is important to not only focus on the effect of single Yops but also the effect of several Yops together as they all seem to influence each other in order to gain the best outcome for *Yersinia*.

6.3 *Y. enterocolitica* affects histone H3 serine-10 phosphorylation in primary human macrophages

Bacterial effectors not only impair host gene expression and cell death pathways, but also affect the epigenetic landscape of the host by often targeting H3S10ph to interfere with the host immune response (Bierne & Pourpre, 2020; Connor et al., 2019). Phosphorylation of H3S10 takes place upon LPS sensing, that leads to the activation of MAPK and NF-κB pathways, by that regulating inflammatory gene expression (Connor et al., 2019). Furthermore, H3S10ph recruits other factors like chromatin remodelers or writers, which in turn induces chromatin opening and deposition of other activating histone marks like acetylation, leading to overall transcription promotion (Khan et al., 2017; Sawicka & Seiser, 2014).

In this study we were interested in the effect of the translocated T3SS effectors on H3S10ph. First of all, we detected a time dependent phosphorylation of H3S10 in primary human macrophages infected with the virulent wildtype strain WA314 and the avirulent strain WAC within 1 h and this phosphorylation was abolished after 3 h in wildtype infected macrophages (Fig. 12). This shows that the translocated T3SS effectors are responsible for the removal of H3S10ph after 3 h. Infection with single deletion mutants for 3 h revealed that YopP is responsible for around 50 % of the removal of H3S10ph and other T3SS effectors do not play a role if they act alone (Fig. 13A & 13B). It might be that YopP act not only alone but also in cooperation with other T3SS effectors. In HeLa cells it was shown that *Pseudomonas aeruginosa* T3SS pore proteins suppressed H3S10ph (Dortet et al., 2018), which might also be the case with the T3SS pore proteins YopB and YopD from *Y. enterocolitica*. Further infection experiments with deletion mutants of the pore proteins YopB and YopD, as well as infection experiments with double deletion mutants are needed to uncover the role of the pore proteins and a potential cooperation of T3SS effector proteins. Interestingly both WA314 and WAC induced H3S10ph at early time points of up to 1 h, showing that PAMP-induced signaling is faster than the activity of the translocated T3SS effectors. This fits with our RNA-seq results where we also observed a fast PAMP-induced effect, whereas T3SS effectors did not have a large effect at 1.5 h post infection.

YopP inhibits components of the MAPK and NF-κB signaling pathway (Ruckdeschel et al., 1998; Ruckdeschel et al., 1997) and because of that we aimed to detect if the suppression of H3S10ph is mediated by those pathways. Using several MAPK and NF-κB pathway inhibitors confirmed our assumption (Fig. 13C), showing that YopP suppresses H3S10ph

by inhibiting components of the MAPK and NF-κB signaling pathway. Till now we cannot say if the deposition of H3S10ph has any effect on gene expression or if it just happens by accident and does not have any functional outcome. To further elucidate the role of H3S10ph and find the chromosomal regions which are connected with H3S10ph, ChIP-seq experiments will be highly interesting. ChIP-seq studies using H3S10ph have only been done in BMDMs but not in primary human macrophages (Josefowicz et al., 2016). They showed that LPS stimulation of BMDMs induced phosphorylation of H3S10 and H3S28, but they only found H3S28ph at TSS and enhancers of inflammatory genes, whereas H3S10ph was not found there (Josefowicz et al., 2016). Unfortunately, they did not further investigate the role of H3S10ph. It might be that H3S10ph also controls inflammatory gene expression in human macrophages, even though it could also be that it just acts as an anchor for other histone modifications. Furthermore, it would be highly interesting to find out if H3S28ph is also induced in primary human macrophages and altered by T3SS effectors of *Y. enterocolitica*.

Overall our data show that YopP is the main actor of the suppression of H3S10ph through its well-known inhibition of MAPK and NF-κB pathway. If this suppression of H3S10ph is biological relevant is unclear so far, but ChIP-seq experiments could uncover the role of H3S10ph.

7 Literature

Achtman, M., Zurth, K., Morelli, G., Torrea, G., Guiyoule, A., & Carniel, E. (1999). Yersinia pestis, the cause of plague, is a recently emerged clone of Yersinia pseudotuberculosis. *Proc Natl Acad Sci U S A*, *96*(24), 14043-14048. https://doi.org/10.1073/pnas.96.24.14043

Adeolu, M., Alnajar, S., Naushad, S., & R, S. G. (2016). Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int J Syst Evol Microbiol*, *66*(12), 5575-5599. https://doi.org/10.1099/ijsem.0.001485

Aepfelbacher, M. (2004). Modulation of Rho GTPases by type III secretion system translocated effectors of Yersinia. *Rev Physiol Biochem Pharmacol*, *152*, 65-77. https://doi.org/10.1007/s10254-004-0035-3

Aepfelbacher, M., Trasak, C., & Ruckdeschel, K. (2007). Effector functions of pathogenic Yersinia species. *Thromb Haemost*, *98*(3), 521-529.

Aepfelbacher, M., Trasak, C., Wilharm, G., Wiedemann, A., Trulzsch, K., Krauss, K., Gierschik, P., & Heesemann, J. (2003). Characterization of YopT effects on Rho GTPases in Yersinia enterocolitica-infected cells. *J Biol Chem*, *278*(35), 33217-33223. https://doi.org/10.1074/jbc.M303349200

Agrain, C., Sorg, I., Paroz, C., & Cornelis, G. R. (2005). Secretion of YscP from Yersinia enterocolitica is essential to control the length of the injectisome needle but not to change the type III secretion substrate specificity. *Mol Microbiol*, *57*(5), 1415-1427. https://doi.org/10.1111/j.1365-2958.2005.04758.x

Aili, M., Isaksson, E. L., Carlsson, S. E., Wolf-Watz, H., Rosqvist, R., & Francis, M. S. (2008). Regulation of Yersinia Yop-effector delivery by translocated YopE. *Int J Med Microbiol*, *298*(3-4), 183-192. https://doi.org/10.1016/j.ijmm.2007.04.007

Akira, S., Takeda, K., & Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol*, *2*(8), 675-680. https://doi.org/10.1038/90609

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783-801. https://doi.org/10.1016/j.cell.2006.02.015

Aleksić, S., & Bockemühl, J. (1984). Proposed revision of the Wauters et al. antigenic scheme for serotyping of Yersinia enterocolitica. *Journal of Clinical Microbiology*, *20*(1), 99-102. https://doi.org/10.1128/jcm.20.1.99-102.1984

Andor, A., Trülzsch, K., Essler, M., Roggenkamp, A., Wiedemann, A., Heesemann, J., & Aepfelbacher, M. (2001). YopE of Yersinia, a GAP for Rho GTPases, selectively modulates Rac-dependent actin structures in endothelial cells. *Cell Microbiol*, *3*(5), 301-310. https://doi.org/10.1046/j.1462-5822.2001.00114.x

Andrades Valtuena, A., Mittnik, A., Key, F. M., Haak, W., Allmae, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Jankovic, I., Massy, K., Novak, M., Pfrengle, S., Reinhold, S., Slaus, M., Spyrou, M. A., Szecsenyi-Nagy, A., Torv, M., Hansen, S., . . . Krause, J. (2017). The Stone Age Plague and Its Persistence in Eurasia. *Curr Biol*, *27*(23), 3683-3691 e3688. https://doi.org/10.1016/j.cub.2017.10.025

Anjum, R., & Blenis, J. (2008). The RSK family of kinases: emerging roles in cellular signalling. *Nat Rev Mol Cell Biol*, *9*(10), 747-758. https://doi.org/10.1038/nrm2509

Arbibe, L., Kim, D. W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C., & Sansonetti, P. J. (2007). An injected bacterial effector targets chromatin access for transcription factor NF-kappaB to alter transcription of host genes involved in immune responses. *Nat Immunol*, *8*(1), 47-56. https://doi.org/10.1038/ni1423

Atkinson, S., & Williams, P. (2016). Yersinia virulence factors - a sophisticated arsenal for combating host defences. *F1000Res*, *5*. https://doi.org/10.12688/f1000research.8466.1

Auerbuch, V., Golenbock, D. T., & Isberg, R. R. (2009). Innate immune recognition of Yersinia pseudotuberculosis type III secretion. *PLoS Pathog*, *5*(12), e1000686. https://doi.org/10.1371/journal.ppat.1000686

Autenrieth, I. B., & Firsching, R. (1996). Penetration of M cells and destruction of Peyer's patches by Yersinia enterocolitica: an ultrastructural and histological study. *J Med Microbiol*, *44*(4), 285-294. https://doi.org/10.1099/00222615-44-4-285

Autenrieth, I. B., Kempf, V., Sprinz, T., Preger, S., & Schnell, A. (1996). Defense mechanisms in Peyer's patches and mesenteric lymph nodes against Yersinia enterocolitica involve integrins and cytokines. *Infect Immun*, *64*(4), 1357-1368. https://doi.org/10.1128/iai.64.4.1357-1368.1996

Baker, P. J., Boucher, D., Bierschenk, D., Tebartz, C., Whitney, P. G., D'Silva, D. B., Tanzer, M. C., Monteleone, M., Robertson, A. A., Cooper, M. A., Alvarez-Diaz, S., Herold, M. J., Bedoui, S., Schroder, K., & Masters, S. L. (2015). NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. *Eur J Immunol*, *45*(10), 2918-2926. https://doi.org/10.1002/eji.201545655

Barbieri, J. T., Riese, M. J., & Aktories, K. (2002). Bacterial toxins that modify the actin cytoskeleton. *Annu Rev Cell Dev Biol*, *18*, 315-344. https://doi.org/10.1146/annurev.cellbio.18.012502.134748

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2013). NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res*, *41*(Database issue), D991-995. https://doi.org/10.1093/nar/gks1193

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., Wei, G., Chepelev, I., & Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. *Cell*, *129*(4), 823-837. https://doi.org/10.1016/j.cell.2007.05.009

Bekere, I., Huang, J., Schnapp, M., Rudolph, M., Berneking, L., Ruckdeschel, K., Grundhoff, A., Günther, T., Fischer, N., & Aepfelbacher, M. (2021). Yersinia remodels epigenetic histone modifications in human macrophages. *PLoS Pathog*, *17*(11), e1010074. https://doi.org/10.1371/journal.ppat.1010074

Benabdillah, R., Mota, L. J., Lützelschwab, S., Demoinet, E., & Cornelis, G. R. (2004). Identification of a nuclear targeting signal in YopM from Yersinia spp. *Microb Pathog*, *36*(5), 247-261. https://doi.org/10.1016/j.micpath.2003.12.006

Berneking, L., Bekere, I., Rob, S., Schnapp, M., Huang, J., Ruckdeschel, K., & Aepfelbacher, M. (2023). A bacterial effector protein promotes nuclear translocation of Stat3 to induce IL-10. *Eur J Cell Biol*, *102*(4), 151364. https://doi.org/10.1016/j.ejcb.2023.151364

Berneking, L., Schnapp, M., Rumm, A., Trasak, C., Ruckdeschel, K., Alawi, M., Grundhoff, A., Kikhney, A. G., Koch-Nolte, F., Buck, F., Perbandt, M., Betzel, C., Svergun, D. I., Hentschke, M., & Aepfelbacher, M. (2016). Immunosuppressive Yersinia Effector YopM Binds DEAD Box Helicase DDX3 to Control Ribosomal S6 Kinase in the Nucleus of Host Cells. *PLoS Pathog*, *12*(6), e1005660. https://doi.org/10.1371/journal.ppat.1005660

Bhagat, N., & Virdi, J. S. (2009). Molecular and biochemical characterization of urease and survival of Yersinia enterocolitica biovar 1A in acidic pH in vitro. *BMC Microbiol*, *9*, 262. https://doi.org/10.1186/1471-2180-9-262

Bierne, H., Hamon, M., & Cossart, P. (2012). Epigenetics and bacterial infections. *Cold Spring Harb Perspect Med*, *2*(12), a010272. https://doi.org/10.1101/cshperspect.a010272

Bierne, H., & Pourpre, R. (2020). Bacterial Factors Targeting the Nucleus: The Growing Family of Nucleomodulins. *Toxins (Basel)*, *12*(4). https://doi.org/10.3390/toxins12040220

Bliska, J. B., Wang, X., Viboud, G. I., & Brodsky, I. E. (2013). Modulation of innate immune responses by Yersinia type III secretion system translocators and effectors. *Cell Microbiol*, *15*(10), 1622-1631. https://doi.org/10.1111/cmi.12164

Bohn, E., Müller, S., Lauber, J., Geffers, R., Speer, N., Spieth, C., Krejci, J., Manncke, B., Buer, J., Zell, A., & Autenrieth, I. B. (2004). Gene expression patterns of epithelial cells modulated by pathogenicity factors of Yersinia enterocolitica. *Cell Microbiol*, *6*(2), 129-141. https://doi.org/10.1046/j.1462-5822.2003.00346.x

Boland, A., & Cornelis, G. R. (1998). Role of YopP in suppression of tumor necrosis factor alpha release by macrophages during Yersinia infection. *Infect Immun*, *66*(5), 1878-1884. https://doi.org/10.1128/iai.66.5.1878-1884.1998

Boland, A., Havaux, S., & Cornelis, G. R. (1998). Heterogeneity of the Yersinia YopM protein. *Microb Pathog*, *25*(6), 343-348. https://doi.org/10.1006/mpat.1998.0247

Bottone, E. J. (1997). Yersinia enterocolitica: the charisma continues. *Clin Microbiol Rev*, *10*(2), 257-276. https://doi.org/10.1128/cmr.10.2.257

Bottone, E. J. (1999). Yersinia enterocolitica: overview and epidemiologic correlates. *Microbes and Infection*, *1*(4), 323-333. https://doi.org/https://doi.org/10.1016/S1286-4579(99)80028-8

Braschi, B., Denny, P., Gray, K., Jones, T., Seal, R., Tweedie, S., Yates, B., & Bruford, E. (2019). Genenames.org: the HGNC and VGNC resources in 2019. *Nucleic Acids Res*, *47*(D1), D786-d792. https://doi.org/10.1093/nar/gky930

Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, E., & Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biol*, *14*, 32. https://doi.org/10.1186/1471-2121-14-32

Brodsky, I. E., Palm, N. W., Sadanand, S., Ryndak, M. B., Sutterwala, F. S., Flavell, R. A., Bliska, J. B., & Medzhitov, R. (2010). A Yersinia effector protein promotes virulence by preventing inflammasome recognition of the type III secretion system. *Cell Host Microbe*, *7*(5), 376-387. https://doi.org/10.1016/j.chom.2010.04.009

Brubaker, R. R. (2003). Interleukin-10 and inhibition of innate immunity to Yersiniae: roles of Yops and LcrV (V antigen). *Infect Immun*, *71*(7), 3673-3681. https://doi.org/10.1128/IAI.71.7.3673-3681.2003

Cai, X., Chen, J., Xu, H., Liu, S., Jiang, Q. X., Halfmann, R., & Chen, Z. J. (2014). Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell*, *156*(6), 1207-1222. https://doi.org/10.1016/j.cell.2014.01.063

Cao, Y., Guan, K., He, X., Wei, C., Zheng, Z., Zhang, Y., Ma, S., Zhong, H., & Shi, W. (2016). Yersinia YopJ negatively regulates IRF3-mediated antibacterial response through disruption of STING-mediated cytosolic DNA signaling. *Biochim Biophys Acta*, *1863*(12), 3148-3159. https://doi.org/10.1016/j.bbamcr.2016.10.004

Carmody, R. J., Ruan, Q., Liou, H. C., & Chen, Y. H. (2007). Essential roles of c-Rel in TLR-induced IL-23 p19 gene expression in dendritic cells. *J Immunol*, *178*(1), 186-191. https://doi.org/10.4049/jimmunol.178.1.186

Carniel, E. (2002). Plasmids and pathogenicity islands of Yersinia. *Curr Top Microbiol Immunol*, *264*(1), 89-108.

Cassandri, M., Smirnov, A., Novelli, F., Pitolli, C., Agostini, M., Malewicz, M., Melino, G., & Raschellà, G. (2017). Zinc-finger proteins in health and disease. *Cell Death Discov*, *3*, 17071. https://doi.org/10.1038/cddiscovery.2017.71

Cassel, S. L., Eisenbarth, S. C., Iyer, S. S., Sadler, J. J., Colegio, O. R., Tephly, L. A., Carter, A. B., Rothman, P. B., Flavell, R. A., & Sutterwala, F. S. (2008). The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci U S A*, *105*(26), 9035-9040. https://doi.org/10.1073/pnas.0803933105

Chauhan, N., Wrobel, A., Skurnik, M., & Leo, J. C. (2016). Yersinia adhesins: An arsenal for infection. *Proteomics Clin Appl*, *10*(9-10), 949-963. https://doi.org/10.1002/prca.201600012

Chen, G., Shaw, M. H., Kim, Y. G., & Nuñez, G. (2009). NOD-like receptors: role in innate immunity and inflammatory disease. *Annu Rev Pathol*, *4*, 365-398. https://doi.org/10.1146/annurev.pathol.4.110807.092239

Chen, K. W., & Brodsky, I. E. (2023). Yersinia interactions with regulated cell death pathways. *Curr Opin Microbiol*, *71*, 102256. https://doi.org/10.1016/j.mib.2022.102256

Chung, L. K., Park, Y. H., Zheng, Y., Brodsky, I. E., Hearing, P., Kastner, D. L., Chae, J. J., & Bliska, J. B. (2016). The Yersinia Virulence Factor YopM Hijacks Host Kinases to Inhibit Type III Effector-Triggered Activation of the Pyrin Inflammasome. *Cell Host Microbe*, *20*(3), 296-306. https://doi.org/10.1016/j.chom.2016.07.018

Coburn, B., Sekirov, I., & Finlay, B. B. (2007). Type III secretion systems and disease. *Clin Microbiol Rev*, *20*(4), 535-549. https://doi.org/10.1128/cmr.00013-07

Compan, V., Baroja-Mazo, A., López-Castejón, G., Gomez, A. I., Martínez, C. M., Angosto, D., Montero, M. T., Herranz, A. S., Bazán, E., Reimers, D., Mulero, V., & Pelegrín, P. (2012). Cell volume regulation modulates NLRP3 inflammasome activation. *Immunity*, *37*(3), 487-500. https://doi.org/10.1016/j.immuni.2012.06.013

Condliffe, A. M., Webb, L. M., Ferguson, G. J., Davidson, K., Turner, M., Vigorito, E., Manifava, M., Chilvers, E. R., Stephens, L. R., & Hawkins, P. T. (2006). RhoG regulates the neutrophil NADPH oxidase. *J Immunol*, *176*(9), 5314-5320. https://doi.org/10.4049/jimmunol.176.9.5314

Connor, M., Arbibe, L., & Hamon, M. (2019). Customizing Host Chromatin: a Bacterial Tale. *Microbiol Spectr*, *7*(2). https://doi.org/10.1128/microbiolspec.BAI-0015-2019

Cornelis, G., Vanootegem, J. C., & Sluiters, C. (1987). Transcription of the yop regulon from Y. enterocolitica requires trans acting pYV and chromosomal genes. *Microb Pathog*, *2*(5), 367-379. https://doi.org/10.1016/0882-4010(87)90078-7

Cornelis, G. R. (2002a). Yersinia type III secretion: send in the effectors. *J Cell Biol*, *158*(3), 401-408. https://doi.org/10.1083/jcb.200205077

Cornelis, G. R. (2002b). The Yersinia Ysc-Yop 'type III' weaponry. *Nat Rev Mol Cell Biol*, *3*(10), 742-752. https://doi.org/10.1038/nrm932

Cornelis, G. R., Boland, A., Boyd, A. P., Geuijen, C., Iriarte, M., Neyt, C., Sory, M. P., & Stainier, I. (1998). The virulence plasmid of Yersinia, an antihost genome. *Microbiol Mol Biol Rev*, *62*(4), 1315-1352. https://doi.org/10.1128/mmbr.62.4.1315-1352.1998

Cornelis, G. R., & Wolf-Watz, H. (1997). The Yersinia Yop virulon: a bacterial system for subverting eukaryotic cells. *Mol Microbiol*, *23*(5), 861-867. https://doi.org/10.1046/j.1365-2958.1997.2731623.x

Coso, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T., & Gutkind, J. S. (1995). The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell*, *81*(7), 1137-1146. https://doi.org/10.1016/s0092-8674(05)80018-2

Cruz, C. M., Rinna, A., Forman, H. J., Ventura, A. L., Persechini, P. M., & Ojcius, D. M. (2007). ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J Biol Chem*, *282*(5), 2871-2879. https://doi.org/10.1074/jbc.M608083200

Dach, K., Zovko, J., Hogardt, M., Koch, I., van Erp, K., Heesemann, J., & Hoffmann, R. (2009). Bacterial toxins induce sustained mRNA expression of the silencing transcription factor klf2 via inactivation of RhoA and Rhophilin 1. *Infect Immun*, *77*(12), 5583-5592. https://doi.org/10.1128/iai.00121-09

Dave, M. N., Silva, J. E., Eliçabe, R. J., Jeréz, M. B., Filippa, V. P., Gorlino, C. V., Autenrieth, S., Autenrieth, I. B., & Di Genaro, M. S. (2016). Yersinia enterocolitica YopH-Deficient Strain Activates Neutrophil Recruitment to Peyer's Patches and Promotes Clearance of the Virulent Strain. *Infect Immun*, *84*(11), 3172-3181. https://doi.org/10.1128/iai.00568-16

Day, J. B., & Plano, G. V. (1998). A complex composed of SycN and YscB functions as a specific chaperone for YopN in Yersinia pestis. *Mol Microbiol*, *30*(4), 777-788. https://doi.org/10.1046/j.1365-2958.1998.01110.x

de Koning-Ward, T. F., Ward, A. C., Hartland, E. L., & Robins-Browne, R. M. (1995). The urease complex gene of Yersinia enterocolitica and its role in virulence. *Contrib Microbiol Immunol*, *13*, 262-263.

de Rouvroit, C. L., Sluiters, C., & Cornelis, G. R. (1992). Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of Yersinia enterocolitica. *Mol Microbiol*, *6*(3), 395-409. https://doi.org/10.1111/j.1365-2958.1992.tb01483.x

Denecker, G., Tötemeyer, S., Mota, L. J., Troisfontaines, P., Lambermont, I., Youta, C., Stainier, I., Ackermann, M., & Cornelis, G. R. (2002). Effect of low- and high-virulence Yersinia enterocolitica strains on the inflammatory response of human umbilical vein endothelial cells. *Infect Immun*, *70*(7), 3510-3520. https://doi.org/10.1128/iai.70.7.3510-3520.2002

Deuschle, E., Keller, B., Siegfried, A., Manncke, B., Spaeth, T., Köberle, M., Drechsler-Hake, D., Reber, J., Böttcher, R. T., Autenrieth, S. E., Autenrieth, I. B., Bohn, E., & Schütz, M. (2016). Role of β1 integrins and bacterial adhesins for Yop injection into leukocytes in Yersinia enterocolitica systemic mouse infection. *Int J Med Microbiol*, *306*(2), 77-88. https://doi.org/10.1016/j.ijmm.2015.12.001

Dewoody, R., Merritt, P. M., Houppert, A. S., & Marketon, M. M. (2011). YopK regulates the Yersinia pestis type III secretion system from within host cells. *Mol Microbiol*, *79*(6), 1445-1461. https://doi.org/10.1111/j.1365-2958.2011.07534.x

Dewoody, R., Merritt, P. M., & Marketon, M. M. (2013a). Regulation of the Yersinia type III secretion system: traffic control. *Front Cell Infect Microbiol*, *3*, 4. https://doi.org/10.3389/fcimb.2013.00004

Dewoody, R., Merritt, P. M., & Marketon, M. M. (2013b). YopK controls both rate and fidelity of Yop translocation. *Mol Microbiol*, *87*(2), 301-317. https://doi.org/10.1111/mmi.12099

Ding, S. Z., Fischer, W., Kaparakis-Liaskos, M., Liechti, G., Merrell, D. S., Grant, P. A., Ferrero, R. L., Crowe, S. E., Haas, R., Hatakeyama, M., & Goldberg, J. B. (2010). Helicobacter pylori-induced histone modification, associated gene expression in gastric epithelial cells, and its implication in pathogenesis. *PLoS One*, *5*(4), e9875. https://doi.org/10.1371/journal.pone.0009875

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15-21. https://doi.org/10.1093/bioinformatics/bts635

Dortet, L., Lombardi, C., Cretin, F., Dessen, A., & Filloux, A. (2018). Pore-forming activity of the Pseudomonas aeruginosa type III secretion system translocon alters the host epigenome. *Nat Microbiol*, *3*(3), 378-386. https://doi.org/10.1038/s41564-018-0109-7

Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., & Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science*, *320*(5876), 674-677. https://doi.org/10.1126/science.1156995

Drummond, N., Murphy, B. P., Ringwood, T., Prentice, M. B., Buckley, J. F., & Fanning, S. (2012). Yersinia Enterocolitica: A Brief Review of the Issues Relating to the Zoonotic Pathogen, Public Health Challenges, and the Pork Production Chain. *Foodborne Pathogens and Disease*, *9*(3), 179-189. https://doi.org/10.1089/fpd.2011.0938

Dukuzumuremyi, J. M., Rosqvist, R., Hallberg, B., Akerström, B., Wolf-Watz, H., & Schesser, K. (2000). The Yersinia protein kinase A is a host factor inducible RhoA/Rac-binding virulence factor. *J Biol Chem*, *275*(45), 35281-35290. https://doi.org/10.1074/jbc.M003009200

Dumas, A., Amiable, N., de Rivero Vaccari, J. P., Chae, J. J., Keane, R. W., Lacroix, S., & Vallières, L. (2014). The inflammasome pyrin contributes to pertussis toxin-induced IL-1β synthesis, neutrophil intravascular crawling and autoimmune encephalomyelitis. *PLoS Pathog*, *10*(5), e1004150. https://doi.org/10.1371/journal.ppat.1004150

EFSA. (2007). Monitoring and identification of human enteropathogenic Yersinia spp.-Scientific Opinion of the Panel on Biological Hazards. *EFSA Journal*, *5*(12), 595.

Endrizzi, M. G., Hadinoto, V., Growney, J. D., Miller, W., & Dietrich, W. F. (2000). Genomic sequence analysis of the mouse Naip gene array. *Genome Res*, *10*(8), 1095-1102. https://doi.org/10.1101/gr.10.8.1095

European Centre for Disease Prevention and Control. (2024). *Yersiniosis - Annual Epidemiological Report for 2022*. Retrieved 23.08.24 from https://www.ecdc.europa.eu/sites/default/files/documents/YERS_AER_2022_Report.pdf

Fällman, M., Andersson, K., Håkansson, S., Magnusson, K. E., Stendahl, O., & Wolf-Watz, H. (1995). Yersinia pseudotuberculosis inhibits Fc receptor-mediated phagocytosis in J774 cells. *Infect Immun*, *63*(8), 3117-3124. https://doi.org/10.1128/iai.63.8.3117-3124.1995

Fehri, L. F., Rechner, C., Janssen, S., Mak, T. N., Holland, C., Bartfeld, S., Brüggemann, H., & Meyer, T. F. (2009). Helicobacter pylori-induced modification of the histone H3 phosphorylation status in gastric epithelial cells reflects its impact on cell cycle regulation. *Epigenetics*, *4*(8), 577-586. https://doi.org/10.4161/epi.4.8.10217

Felek, S., & Krukonis, E. S. (2009). The Yersinia pestis Ail protein mediates binding and Yop delivery to host cells required for plague virulence. *Infect Immun*, *77*(2), 825-836. https://doi.org/10.1128/iai.00913-08

Ferracci, F., Schubot, F. D., Waugh, D. S., & Plano, G. V. (2005). Selection and characterization of Yersinia pestis YopN mutants that constitutively block Yop secretion. *Mol Microbiol*, *57*(4), 970-987. https://doi.org/10.1111/j.1365-2958.2005.04738.x

Forsberg, A., Viitanen, A. M., Skurnik, M., & Wolf-Watz, H. (1991). The surface-located YopN protein is involved in calcium signal transduction in Yersinia pseudotuberculosis. *Mol Microbiol*, *5*(4), 977-986. https://doi.org/10.1111/j.1365-2958.1991.tb00773.x

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T. D., Ozören, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., & Núñez, G. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol*, *7*(6), 576-582. https://doi.org/10.1038/ni1346

Fredriksson-Ahomaa, M. (2007). Yersinia enterocolitica and Yersinia pseudotuberculosis. In S. Simjee (Ed.), *Foodborne Diseases* (pp. 79-113). Humana Press. https://doi.org/10.1007/978-1-59745-501-5_4

Fredriksson-Ahomaa, M., Stolle, A., & Korkeala, H. (2006). Molecular epidemiology of Yersinia enterocolitica infections. *FEMS Immunol Med Microbiol*, *47*(3), 315-329. https://doi.org/10.1111/j.1574-695X.2006.00095.x

Galindo, C. L., Rosenzweig, J. A., Kirtley, M. L., & Chopra, A. K. (2011). Pathogenesis of Y. enterocolitica and Y. pseudotuberculosis in Human Yersiniosis. *J Pathog*, *2011*, 182051. https://doi.org/10.4061/2011/182051

Galyov, E. E., Håkansson, S., Forsberg, A., & Wolf-Watz, H. (1993). A secreted protein kinase of Yersinia pseudotuberculosis is an indispensable virulence determinant. *Nature*, *361*(6414), 730-732. https://doi.org/10.1038/361730a0

Garcia, J. T., Ferracci, F., Jackson, M. W., Joseph, S. S., Pattis, I., Plano, L. R., Fischer, W., & Plano, G. V. (2006). Measurement of effector protein injection by type III and type IV secretion systems by using a 13-residue phosphorylatable glycogen synthase kinase tag. *Infect Immun*, *74*(10), 5645-5657. https://doi.org/10.1128/iai.00690-06

Gardner, K. E., Allis, C. D., & Strahl, B. D. (2011). Operating on chromatin, a colorful language where context matters. *J Mol Biol*, *409*(1), 36-46. https://doi.org/10.1016/j.jmb.2011.01.040

Gavrilin, M. A., Abdelaziz, D. H., Mostafa, M., Abdulrahman, B. A., Grandhi, J., Akhter, A., Abu Khweek, A., Aubert, D. F., Valvano, M. A., Wewers, M. D., & Amer, A. O. (2012). Activation of the pyrin inflammasome by intracellular Burkholderia cenocepacia. *J Immunol*, *188*(7), 3469-3477. https://doi.org/10.4049/jimmunol.1102272

Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: mechanism and functions. *Immunity*, *32*(5), 593-604. https://doi.org/10.1016/j.immuni.2010.05.007

Grabowski, B., Schmidt, M. A., & Rüter, C. (2017). Immunomodulatory Yersinia outer proteins (Yops)-useful tools for bacteria and humans alike. *Virulence*, *8*(7), 1124-1147. https://doi.org/10.1080/21505594.2017.1303588

Grahek-Ogden, D., Schimmer, B., Cudjoe, K. S., Nygård, K., & Kapperud, G. (2007). Outbreak of Yersinia enterocolitica Serogroup O:9 Infection and Processed Pork, Norway. *Emerging Infectious Disease journal*, *13*(5), 754. https://doi.org/10.3201/eid1305.061062

Grassl, G. A., Kracht, M., Wiedemann, A., Hoffmann, E., Aepfelbacher, M., von Eichel-Streiber, C., Bohn, E., & Autenrieth, I. B. (2003). Activation of NF-kappaB and IL-8 by Yersinia enterocolitica invasin protein is conferred by engagement of Rac1 and MAP kinase cascades. *Cell Microbiol*, *5*(12), 957-971. https://doi.org/10.1046/j.1462-5822.2003.00339.x

Greally, J. M. (2018). A user's guide to the ambiguous word 'epigenetics'. *Nat Rev Mol Cell Biol*, *19*(4), 207-208. https://doi.org/10.1038/nrm.2017.135

Grosdent, N., Maridonneau-Parini, I., Sory, M. P., & Cornelis, G. R. (2002). Role of Yops and adhesins in resistance of Yersinia enterocolitica to phagocytosis. *Infect Immun*, *70*(8), 4165-4176. https://doi.org/10.1128/iai.70.8.4165-4176.2002

Grützkau, A., Hanski, C., Hahn, H., & Riecken, E. O. (1990). Involvement of M cells in the bacterial invasion of Peyer's patches: a common mechanism shared by Yersinia enterocolitica and other enteroinvasive bacteria. *Gut*, *31*(9), 1011-1015. https://doi.org/10.1136/gut.31.9.1011

Guan, K. L., & Dixon, J. E. (1990). Protein tyrosine phosphatase activity of an essential virulence determinant in Yersinia. *Science*, *249*(4968), 553-556. https://doi.org/10.1126/science.2166336

Gurry, J. F. (1974). Acute Terminal Ileitis and Yersinia Infection. *British Medical Journal*, *2*(5913), 264. https://doi.org/10.1136/bmj.2.5913.264

Haase, R., Richter, K., Pfaffinger, G., Courtois, G., & Ruckdeschel, K. (2005). Yersinia outer protein P suppresses TGF-beta-activated kinase-1 activity to impair innate immune signaling in Yersinia enterocolitica-infected cells. *J Immunol*, *175*(12), 8209-8217. https://doi.org/10.4049/jimmunol.175.12.8209

Håkansson, S., Galyov, E. E., Rosqvist, R., & Wolf-Watz, H. (1996). The Yersinia YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. *Mol Microbiol*, *20*(3), 593-603. https://doi.org/10.1046/j.1365-2958.1996.5251051.x

Halle, A., Hornung, V., Petzold, G. C., Stewart, C. R., Monks, B. G., Reinheckel, T., Fitzgerald, K. A., Latz, E., Moore, K. J., & Golenbock, D. T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol*, *9*(8), 857-865. https://doi.org/10.1038/ni.1636

Hamon, M. A., Batsché, E., Régnault, B., Tham, T. N., Seveau, S., Muchardt, C., & Cossart, P. (2007). Histone modifications induced by a family of bacterial toxins. *Proc Natl Acad Sci U S A*, *104*(33), 13467-13472. https://doi.org/10.1073/pnas.0702729104

Hamon, M. A., & Cossart, P. (2011). K+ efflux is required for histone H3 dephosphorylation by Listeria monocytogenes listeriolysin O and other pore-forming toxins. *Infect Immun*, *79*(7), 2839-2846. https://doi.org/10.1128/iai.01243-10

Harton, J. A., Linhoff, M. W., Zhang, J., & Ting, J. P. (2002). Cutting edge: CATERPILLER: a large family of mammalian genes containing CARD, pyrin, nucleotide-binding, and leucine-rich repeat domains. *J Immunol*, *169*(8), 4088-4093. https://doi.org/10.4049/jimmunol.169.8.4088

Heesemann, J., & Laufs, R. (1983). Construction of a mobilizable Yersinia enterocolitica virulence plasmid. *J Bacteriol*, *155*(2), 761-767. https://doi.org/10.1128/jb.155.2.761-767.1983

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., Cheng, J. X., Murre, C., Singh, H., & Glass, C. K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell*, *38*(4), 576-589. https://doi.org/10.1016/j.molcel.2010.05.004

Henikoff, S., & Greally, J. M. (2016). Epigenetics, cellular memory and gene regulation. *Curr Biol*, *26*(14), R644-648. https://doi.org/10.1016/j.cub.2016.06.011

Hentschke, M., Berneking, L., Belmar Campos, C., Buck, F., Ruckdeschel, K., & Aepfelbacher, M. (2010). Yersinia virulence factor YopM induces sustained RSK activation by interfering with dephosphorylation. *PLoS One*, *5*(10). https://doi.org/10.1371/journal.pone.0013165

Hoffmann, R., van Erp, K., Trülzsch, K., & Heesemann, J. (2004). Transcriptional responses of murine macrophages to infection with Yersinia enterocolitica. *Cell Microbiol*, *6*(4), 377-390. https://doi.org/10.1111/j.1462-5822.2004.00365.x

Höfling, S., Grabowski, B., Norkowski, S., Schmidt, M. A., & Rüter, C. (2015). Current activities of the Yersinia effector protein YopM. *Int J Med Microbiol*, *305*(3), 424-432. https://doi.org/10.1016/j.ijmm.2015.03.009

Höfling, S., Scharnert, J., Cromme, C., Bertrand, J., Pap, T., Schmidt, M. A., & Rüter, C. (2014). 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*, *5*(7), 761-771. https://doi.org/10.4161/viru.29062

Holmström, A., Petterson, J., Rosqvist, R., Håkansson, S., Tafazoli, F., Fällman, M., Magnusson, K. E., Wolf-Watz, H., & Forsberg, A. (1997). YopK of Yersinia pseudotuberculosis controls translocation of Yop effectors across the eukaryotic cell membrane. *Mol Microbiol*, *24*(1), 73-91. https://doi.org/10.1046/j.1365-2958.1997.3211681.x

Holmström, A., Rosqvist, R., Wolf-Watz, H., & Forsberg, A. (1995a). Virulence plasmid-encoded YopK is essential for Yersinia pseudotuberculosis to cause systemic infection in mice. *Infect Immun*, *63*(6), 2269-2276. https://doi.org/10.1128/iai.63.6.2269-2276.1995

Holmström, A., Rosqvist, R., Wolf-Watz, H., & Forsberg, A. (1995b). YopK, a novel virulence determinant of Yersinia pseudotuberculosis. *Contrib Microbiol Immunol*, *13*, 239-243.

Hordijk, P. L. (2006). Regulation of NADPH oxidases: the role of Rac proteins. *Circ Res*, *98*(4), 453-462. https://doi.org/10.1161/01.RES.0000204727.46710.5e

Horne, S. M., & Prüss, B. M. (2006). Global gene regulation in Yersinia enterocolitica: effect of FliA on the expression levels of flagellar and plasmid-encoded virulence genes. *Arch Microbiol*, *185*(2), 115-126. https://doi.org/10.1007/s00203-005-0077-1

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., Fitzgerald, K. A., & Latz, E. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol*, *9*(8), 847-856. https://doi.org/10.1038/ni.1631

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*, *37*(1), 1-13. https://doi.org/10.1093/nar/gkn923

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, *4*(1), 44-57. https://doi.org/10.1038/nprot.2008.211

Iriarte, M., & Cornelis, G. R. (1998). YopT, a new Yersinia Yop effector protein, affects the cytoskeleton of host cells. *Mol Microbiol*, *29*(3), 915-929. https://doi.org/10.1046/j.1365-2958.1998.00992.x

Ivashkiv, L. B. (2013). Epigenetic regulation of macrophage polarization and function. *Trends Immunol*, *34*(5), 216-223. https://doi.org/10.1016/j.it.2012.11.001

Jalava, K., Hakkinen, M., Valkonen, M., Nakari, U.-M., Palo, T., Hallanvuo, S., Ollgren, J., Siitonen, A., & Nuorti, J. P. (2006). An Outbreak of Gastrointestinal Illness and Erythema Nodosum from Grated Carrots Contaminated with Yersinia pseudotuberculosis. *The Journal of Infectious Diseases*, *194*(9), 1209-1216. https://doi.org/10.1086/508191

Jepson, M. A., & Clark, M. A. (1998). Studying M cells and their role in infection. *Trends Microbiol*, *6*(9), 359-365. https://doi.org/10.1016/s0966-842x(98)01337-7

Jin, T., Curry, J., Smith, P., Jiang, J., & Xiao, T. S. (2013). Structure of the NLRP1 caspase recruitment domain suggests potential mechanisms for its association with procaspase-1. *Proteins*, *81*(7), 1266-1270. https://doi.org/10.1002/prot.24287

Josefowicz, S. Z., Shimada, M., Armache, A., Li, C. H., Miller, R. M., Lin, S., Yang, A., Dill, B. D., Molina, H., Park, H. S., Garcia, B. A., Taunton, J., Roeder, R. G., & Allis, C. D. (2016). Chromatin Kinases Act on Transcription Factors and Histone Tails in Regulation of Inducible Transcription. *Mol Cell*, *64*(2), 347-361. https://doi.org/10.1016/j.molcel.2016.09.026

Joseph, S. S., & Plano, G. V. (2013). The SycN/YscB chaperone-binding domain of YopN is required for the calcium-dependent regulation of Yop secretion by Yersinia pestis. *Front Cell Infect Microbiol*, *3*, 1. https://doi.org/10.3389/fcimb.2013.00001

Juris, S. J., Rudolph, A. E., Huddler, D., Orth, K., & Dixon, J. E. (2000). A distinctive role for the Yersinia protein kinase: actin binding, kinase activation, and cytoskeleton disruption. *Proc Natl Acad Sci U S A*, *97*(17), 9431-9436. https://doi.org/10.1073/pnas.170281997

Kanneganti, T. D., Body-Malapel, M., Amer, A., Park, J. H., Whitfield, J., Franchi, L., Taraporewala, Z. F., Miller, D., Patton, J. T., Inohara, N., & Núñez, G. (2006). Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J Biol Chem*, *281*(48), 36560-36568. https://doi.org/10.1074/jbc.M607594200

Kanneganti, T. D., Lamkanfi, M., & Núñez, G. (2007). Intracellular NOD-like receptors in host defense and disease. *Immunity*, *27*(4), 549-559. https://doi.org/10.1016/j.immuni.2007.10.002

Kanneganti, T. D., Ozören, N., Body-Malapel, M., Amer, A., Park, J. H., Franchi, L., Whitfield, J., Barchet, W., Colonna, M., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E. P., Akira, S., & Núñez, G. (2006). Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature*, *440*(7081), 233-236. https://doi.org/10.1038/nature04517

Kapatral, V., & Minnich, S. A. (1995). Co-ordinate, temperature-sensitive regulation of the three Yersinia enterocolitica flagellin genes. *Mol Microbiol*, *17*(1), 49-56. https://doi.org/10.1111/j.1365-2958.1995.mmi_17010049.x

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*, *11*(5), 373-384. https://doi.org/10.1038/ni.1863

Kayser, F. H. (2010). In F. H. Kayser, E. C. Böttger, R. M. Zinkernagel, O. Haller, J. Eckert, & P. Deplazes (Eds.), *Taschenlehrbuch Medizinische Mikrobiologie* (12. Auflage ed.). Georg Thieme Verlag. https://doi.org/10.1055/b-002-44935

Khan, D. H., Healy, S., He, S., Lichtensztejn, D., Klewes, L., Sharma, K. L., Lau, V., Mai, S., Delcuve, G. P., & Davie, J. R. (2017). Mitogen-induced distinct epialleles are phosphorylated at either H3S10 or H3S28, depending on H3K27 acetylation. *Mol Biol Cell*, *28*(6), 817-824. https://doi.org/10.1091/mbc.E16-08-0618

Khare, S., Dorfleutner, A., Bryan, N. B., Yun, C., Radian, A. D., de Almeida, L., Rojanasakul, Y., & Stehlik, C. (2012). An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. *Immunity*, *36*(3), 464-476. https://doi.org/10.1016/j.immuni.2012.02.001

Kirjavainen, V., Jarva, H., Biedzka-Sarek, M., Blom, A. M., Skurnik, M., & Meri, S. (2008). Yersinia enterocolitica serum resistance proteins YadA and ail bind the complement regulator C4b-binding protein. *PLoS Pathog*, *4*(8), e1000140. https://doi.org/10.1371/journal.ppat.1000140

Kitasato, S. (1894). THE BACILLUS OF BUBONIC PLAGUE. *The Lancet*, *144*(3704), 428-430. https://doi.org/https://doi.org/10.1016/S0140-6736(01)58670-5

Klinghoffer, R. A., Sachsenmaier, C., Cooper, J. A., & Soriano, P. (1999). Src family kinases are required for integrin but not PDGFR signal transduction. *Embo j*, *18*(9), 2459-2471. https://doi.org/10.1093/emboj/18.9.2459

Köberle, M., Göppel, D., Grandl, T., Gaentzsch, P., Manncke, B., Berchtold, S., Müller, S., Lüscher, B., Asselin-Labat, M. L., Pallardy, M., Sorg, I., Langer, S., Barth, H., Zumbihl, R., Autenrieth, I. B., & Bohn, E. (2012). Yersinia enterocolitica YopT and Clostridium difficile toxin B induce expression of GILZ in epithelial cells. *PLoS One*, *7*(7), e40730. https://doi.org/10.1371/journal.pone.0040730

Kopp, P., Lammers, R., Aepfelbacher, M., Woehlke, G., Rudel, T., Machuy, N., Steffen, W., & Linder, S. (2006). The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages. *Mol Biol Cell*, *17*(6), 2811-2823. https://doi.org/10.1091/mbc.e05-11-1010

Kortmann, J., Brubaker, S. W., & Monack, D. M. (2015). Cutting Edge: Inflammasome Activation in Primary Human Macrophages Is Dependent on Flagellin. *J Immunol*, *195*(3), 815-819. https://doi.org/10.4049/jimmunol.1403100

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693-705. https://doi.org/10.1016/j.cell.2007.02.005

Lamkanfi, M., & Kanneganti, T. D. (2010). Caspase-7: a protease involved in apoptosis and inflammation. *Int J Biochem Cell Biol*, *42*(1), 21-24. https://doi.org/10.1016/j.biocel.2009.09.013

Lamkanfi, M., Kanneganti, T. D., Franchi, L., & Núñez, G. (2007). Caspase-1 inflammasomes in infection and inflammation. *J Leukoc Biol*, *82*(2), 220-225. https://doi.org/10.1189/jlb.1206756

LaRock, C. N., & Cookson, B. T. (2012). The Yersinia virulence effector YopM binds caspase-1 to arrest inflammasome assembly and processing. *Cell Host Microbe*, *12*(6), 799-805. https://doi.org/10.1016/j.chom.2012.10.020

Lee, L. A., Gerber, A. R., Lonsway, D. R., Smith, J. D., Carter, G. P., Puhr, N. D., Parrish, C. M., Sikes, R. K., Finton, R. J., & Tauxe, R. V. (1990). Yersinia enterocolitica O:3 infections in infants and children, associated with the household preparation of chitterlings. *N Engl J Med*, *322*(14), 984-987. https://doi.org/10.1056/nejm199004053221407

Lee, V. T., Anderson, D. M., & Schneewind, O. (1998). Targeting of Yersinia Yop proteins into the cytosol of HeLa cells: one-step translocation of YopE across bacterial and eukaryotic membranes is dependent on SycE chaperone. *Mol Microbiol*, *28*(3), 593-601. https://doi.org/10.1046/j.1365-2958.1998.00822.x

Leung, K. Y., Reisner, B. S., & Straley, S. C. (1990). YopM inhibits platelet aggregation and is necessary for virulence of Yersinia pestis in mice. *Infect Immun*, *58*(10), 3262-3271. https://doi.org/10.1128/iai.58.10.3262-3271.1990

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923-930. https://doi.org/10.1093/bioinformatics/btt656

Liu, J., Guan, X., & Ma, X. (2007). Regulation of IL-27 p28 gene expression in macrophages through MyD88- and interferon-gamma-mediated pathways. *J Exp Med*, *204*(1), 141-152. https://doi.org/10.1084/jem.20061440

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Lu, A., Magupalli, V. G., Ruan, J., Yin, Q., Atianand, M. K., Vos, M. R., Schröder, G. F., Fitzgerald, K. A., Wu, H., & Egelman, E. H. (2014). Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell*, *156*(6), 1193-1206. https://doi.org/10.1016/j.cell.2014.02.008

Lupo, A., Cesaro, E., Montano, G., Zurlo, D., Izzo, P., & Costanzo, P. (2013). KRAB-Zinc Finger Proteins: A Repressor Family Displaying Multiple Biological Functions. *Curr Genomics*, *14*(4), 268-278. https://doi.org/10.2174/1389202911314999000 2

Mahdavi, A., Szychowski, J., Ngo, J. T., Sweredoski, M. J., Graham, R. L., Hess, S., Schneewind, O., Mazmanian, S. K., & Tirrell, D. A. (2014). Identification of secreted bacterial proteins by noncanonical amino acid tagging. *Proc Natl Acad Sci U S A*, *111*(1), 433-438. https://doi.org/10.1073/pnas.1301740111

Mainiero, F., Soriani, A., Strippoli, R., Jacobelli, J., Gismondi, A., Piccoli, M., Frati, L., & Santoni, A. (2000). RAC1/P38 MAPK signaling pathway controls beta1 integrin-induced interleukin-8 production in human natural killer cells. *Immunity*, *12*(1), 7-16. https://doi.org/10.1016/s1074-7613(00)80154-5

Malik, A., & Kanneganti, T. D. (2017). Inflammasome activation and assembly at a glance. *J Cell Sci*, *130*(23), 3955-3963. https://doi.org/10.1242/jcs.207365

Mangan, M. S. J., Gorki, F., Krause, K., Heinz, A., Pankow, A., Ebert, T., Jahn, D., Hiller, K., Hornung, V., Maurer, M., Schmidt, F. I., Gerhard, R., & Latz, E. (2022). Transcriptional licensing is required for Pyrin inflammasome activation in human macrophages and bypassed by mutations causing familial Mediterranean fever. *PLoS Biol*, *20*(11), e3001351. https://doi.org/10.1371/journal.pbio.3001351

Margueron, R., & Reinberg, D. (2011). The Polycomb complex PRC2 and its mark in life. *Nature*, *469*(7330), 343-349. https://doi.org/10.1038/nature09784

Martinon, F., Burns, K., & Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell*, *10*(2), 417-426. https://doi.org/10.1016/s1097-2765(02)00599-3

McDonald, C., Vacratsis, P. O., Bliska, J. B., & Dixon, J. E. (2003). The yersinia virulence factor YopM forms a novel protein complex with two cellular kinases. *J Biol Chem*, *278*(20), 18514-18523. https://doi.org/10.1074/jbc.M301226200

McNally, A., Thomson, N. R., Reuter, S., & Wren, B. W. (2016). 'Add, stir and reduce': Yersinia spp. as model bacteria for pathogen evolution. *Nat Rev Microbiol*, *14*(3), 177-190. https://doi.org/10.1038/nrmicro.2015.29

McPhee, J. B., Mena, P., & Bliska, J. B. (2010). 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*, *78*(8), 3529-3539. https://doi.org/10.1128/iai.00269-10

McPhee, J. B., Mena, P., Zhang, Y., & Bliska, J. B. (2012). Interleukin-10 induction is an important virulence function of the Yersinia pseudotuberculosis type III effector YopM. *Infect Immun*, *80*(7), 2519-2527. https://doi.org/10.1128/iai.06364-11

Meinzer, U., Barreau, F., Esmiol-Welterlin, S., Jung, C., Villard, C., Léger, T., Ben-Mkaddem, S., Berrebi, D., Dussaillant, M., Alnabhani, Z., Roy, M., Bonacorsi, S., Wolf-Watz, H., Perroy, J., Ollendorff, V., & Hugot, J. P. (2012). Yersinia pseudotuberculosis effector YopJ subverts the Nod2/RICK/TAK1 pathway and activates caspase-1 to induce intestinal barrier dysfunction. *Cell Host Microbe*, *11*(4), 337-351. https://doi.org/10.1016/j.chom.2012.02.009

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., & Aderem, A. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat Immunol*, *7*(6), 569-575. https://doi.org/10.1038/ni1344

Miao, E. A., Mao, D. P., Yudkovsky, N., Bonneau, R., Lorang, C. G., Warren, S. E., Leaf, I. A., & Aderem, A. (2010). Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci U S A*, *107*(7), 3076-3080. https://doi.org/10.1073/pnas.0913087107

Miller, V. L., & Falkow, S. (1988). Evidence for two genetic loci in Yersinia enterocolitica that can promote invasion of epithelial cells. *Infect Immun*, *56*(5), 1242-1248. https://doi.org/10.1128/iai.56.5.1242-1248.1988

Miller, V. L., Farmer, J. J., 3rd, Hill, W. E., & Falkow, S. (1989). The ail locus is found uniquely in Yersinia enterocolitica serotypes commonly associated with disease. *Infect Immun*, *57*(1), 121-131. https://doi.org/10.1128/iai.57.1.121-131.1989

Mitra, S. K., & Schlaepfer, D. D. (2006). Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol*, *18*(5), 516-523. https://doi.org/10.1016/j.ceb.2006.08.011

Mittal, R., Peak-Chew, S. Y., & McMahon, H. T. (2006). Acetylation of MEK2 and I kappa B kinase (IKK) activation loop residues by YopJ inhibits signaling. *Proc Natl Acad Sci U S A*, *103*(49), 18574-18579. https://doi.org/10.1073/pnas.0608995103

Mittal, R., Peak-Chew, S. Y., Sade, R. S., Vallis, Y., & McMahon, H. T. (2010). The acetyltransferase activity of the bacterial toxin YopJ of Yersinia is activated by eukaryotic host cell inositol hexakisphosphate. *J Biol Chem*, *285*(26), 19927-19934. https://doi.org/10.1074/jbc.M110.126581

Mohammadi, S., & Isberg, R. R. (2009). Yersinia pseudotuberculosis virulence determinants invasin, YopE, and YopT modulate RhoG activity and localization. *Infect Immun*, *77*(11), 4771-4782. https://doi.org/10.1128/iai.00850-09

Molofsky, A. B., Byrne, B. G., Whitfield, N. N., Madigan, C. A., Fuse, E. T., Tateda, K., & Swanson, M. S. (2006). Cytosolic recognition of flagellin by mouse macrophages restricts Legionella pneumophila infection. *J Exp Med*, *203*(4), 1093-1104. https://doi.org/10.1084/jem.20051659

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*, *8*(12), 958-969. https://doi.org/10.1038/nri2448

Mühlenkamp, M., Oberhettinger, P., Leo, J. C., Linke, D., & Schütz, M. S. (2015). Yersinia adhesin A (YadA)--beauty & beast. *Int J Med Microbiol*, *305*(2), 252-258. https://doi.org/10.1016/j.ijmm.2014.12.008

Mukherjee, S., Keitany, G., Li, Y., Wang, Y., Ball, H. L., Goldsmith, E. J., & Orth, K. (2006). Yersinia YopJ acetylates and inhibits kinase activation by blocking phosphorylation. *Science*, *312*(5777), 1211-1214. https://doi.org/10.1126/science.1126867

Muñoz-Planillo, R., Kuffa, P., Martínez-Colón, G., Smith, B. L., Rajendiran, T. M., & Núñez, G. (2013). K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity*, *38*(6), 1142-1153. https://doi.org/10.1016/j.immuni.2013.05.016

Nauth, T., Huschka, F., Schweizer, M., Bosse, J. B., Diepold, A., Failla, A. V., Steffen, A., Stradal, T. E. B., Wolters, M., & Aepfelbacher, M. (2018). Visualization of translocons in Yersinia type III protein secretion machines during host cell infection. *PLoS Pathog*, *14*(12), e1007527. https://doi.org/10.1371/journal.ppat.1007527

Nicholson, D. W. (1999). Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ*, *6*(11), 1028-1042. https://doi.org/10.1038/sj.cdd.4400598

Nordfelth, R., & Wolf-Watz, H. (2001). YopB of Yersinia enterocolitica is essential for YopE translocation. *Infect Immun*, *69*(5), 3516-3518. https://doi.org/10.1128/iai.69.5.3516-3518.2001

Nour, A. M., Yeung, Y. G., Santambrogio, L., Boyden, E. D., Stanley, E. R., & Brojatsch, J. (2009). Anthrax lethal toxin triggers the formation of a membrane-associated inflammasome complex in murine macrophages. *Infect Immun*, *77*(3), 1262-1271. https://doi.org/10.1128/iai.01032-08

Novakovic, B., Habibi, E., Wang, S. Y., Arts, R. J. W., Davar, R., Megchelenbrink, W., Kim, B., Kuznetsova, T., Kox, M., Zwaag, J., Matarese, F., van Heeringen, S. J., Janssen-Megens, E. M., Sharifi, N., Wang, C., Keramati, F., Schoonenberg, V., Flicek, P., Clarke, L., . . . Stunnenberg, H. G. (2016). β-Glucan Reverses the Epigenetic State of LPS-Induced Immunological Tolerance. *Cell*, *167*(5), 1354-1368.e1314. https://doi.org/10.1016/j.cell.2016.09.034

Ophir, M. J., Liu, B. C., & Bunnell, S. C. (2013). The N terminus of SKAP55 enables T cell adhesion to TCR and integrin ligands via distinct mechanisms. *J Cell Biol*, *203*(6), 1021-1041. https://doi.org/10.1083/jcb.201305088

Opitz, B., Püschel, A., Beermann, W., Hocke, A. C., Förster, S., Schmeck, B., van Laak, V., Chakraborty, T., Suttorp, N., & Hippenstiel, S. (2006). Listeria monocytogenes activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. *J Immunol*, *176*(1), 484-490. https://doi.org/10.4049/jimmunol.176.1.484

Orning, P., Weng, D., Starheim, K., Ratner, D., Best, Z., Lee, B., Brooks, A., Xia, S., Wu, H., Kelliher, M. A., Berger, S. B., Gough, P. J., Bertin, J., Proulx, M. M., Goguen, J. D., Kayagaki, N., Fitzgerald, K. A., & Lien, E. (2018). Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science*, *362*(6418), 1064-1069. https://doi.org/10.1126/science.aau2818

Orth, K., Xu, Z., Mudgett, M. B., Bao, Z. Q., Palmer, L. E., Bliska, J. B., Mangel, W. F., Staskawicz, B., & Dixon, J. E. (2000). Disruption of signaling by Yersinia effector YopJ, a ubiquitin-like protein protease. *Science*, *290*(5496), 1594-1597. https://doi.org/10.1126/science.290.5496.1594

Pai, C. H., & Mors, V. (1978). Production of enterotoxin by Yersinia enterocolitica. *Infection and Immunity*, *19*(3), 908-911. https://doi.org/10.1128/iai.19.3.908-911.1978

Palmer, L. E., Hobbie, S., Galán, J. E., & Bliska, J. B. (1998). YopJ of Yersinia pseudotuberculosis is required for the inhibition of macrophage TNF-alpha production and downregulation of the MAP kinases p38 and JNK. *Mol Microbiol*, *27*(5), 953-965. https://doi.org/10.1046/j.1365-2958.1998.00740.x

Pandey, S., Kawai, T., & Akira, S. (2014). Microbial sensing by Toll-like receptors and intracellular nucleic acid sensors. *Cold Spring Harb Perspect Biol*, *7*(1), a016246. https://doi.org/10.1101/cshperspect.a016246

Paquette, N., Conlon, J., Sweet, C., Rus, F., Wilson, L., Pereira, A., Rosadini, C. V., Goutagny, N., Weber, A. N., Lane, W. S., Shaffer, S. A., Maniatis, S., Fitzgerald, K. A., Stuart, L., & Silverman, N. (2012). Serine/threonine acetylation of TGFβ-activated kinase (TAK1) by Yersinia pestis YopJ inhibits innate immune signaling. *Proc Natl Acad Sci U S A*, *109*(31), 12710-12715. https://doi.org/10.1073/pnas.1008203109

Park, B. S., Song, D. H., Kim, H. M., Choi, B. S., Lee, H., & Lee, J. O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature*, *458*(7242), 1191-1195. https://doi.org/10.1038/nature07830

Park, S. H., Kang, K., Giannopoulou, E., Qiao, Y., Kang, K., Kim, G., Park-Min, K. H., & Ivashkiv, L. B. (2017). Type I interferons and the cytokine TNF cooperatively reprogram the macrophage epigenome to promote inflammatory activation. *Nat Immunol*, *18*(10), 1104-1116. https://doi.org/10.1038/ni.3818

Parte, A. C., Sarda Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C., & Goker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol*, *70*(11), 5607-5612. https://doi.org/10.1099/ijsem.0.004332

Pepe, J. C., & Miller, V. L. (1993). Yersinia enterocolitica invasin: a primary role in the initiation of infection. *Proc Natl Acad Sci U S A*, *90*(14), 6473-6477. https://doi.org/10.1073/pnas.90.14.6473

Pepe, J. C., Wachtel, M. R., Wagar, E., & Miller, V. L. (1995). Pathogenesis of defined invasion mutants of Yersinia enterocolitica in a BALB/c mouse model of infection. *Infect Immun*, *63*(12), 4837-4848. https://doi.org/10.1128/iai.63.12.4837-4848.1995

Peters, K. N., & Anderson, D. M. (2012). Modulation of host cell death pathways by Yersinia species and the type III effector YopK. *Adv Exp Med Biol*, *954*, 229-236. https://doi.org/10.1007/978-1-4614-3561-7_29

Pha, K., & Navarro, L. (2016). Yersinia type III effectors perturb host innate immune responses. *World J Biol Chem*, *7*(1), 1-13. https://doi.org/10.4331/wjbc.v7.i1.1

Philip, N. H., Dillon, C. P., Snyder, A. G., Fitzgerald, P., Wynosky-Dolfi, M. A., Zwack, E. E., Hu, B., Fitzgerald, L., Mauldin, E. A., Copenhaver, A. M., Shin, S., Wei, L., Parker, M., Zhang, J., Oberst, A., Green, D. R., & Brodsky, I. E. (2014). Caspase-8 mediates caspase-1 processing and innate immune defense in response to bacterial blockade of NF-κB and MAPK signaling. *Proc Natl Acad Sci U S A*, *111*(20), 7385-7390. https://doi.org/10.1073/pnas.1403252111

Pierson, D. E., & Falkow, S. (1993). The ail gene of Yersinia enterocolitica has a role in the ability of the organism to survive serum killing. *Infect Immun*, *61*(5), 1846-1852. https://doi.org/10.1128/iai.61.5.1846-1852.1993

Ponomareva, L., Liu, H., Duan, X., Dickerson, E., Shen, H., Panchanathan, R., & Choubey, D. (2013). AIM2, an IFN-inducible cytosolic DNA sensor, in the development of benign prostate hyperplasia and prostate cancer. *Mol Cancer Res*, *11*(10), 1193-1202. https://doi.org/10.1158/1541-7786.Mcr-13-0145

Poyet, J. L., Srinivasula, S. M., Tnani, M., Razmara, M., Fernandes-Alnemri, T., & Alnemri, E. S. (2001). Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1. *J Biol Chem*, *276*(30), 28309-28313. https://doi.org/10.1074/jbc.C100250200

Prehna, G., Ivanov, M. I., Bliska, J. B., & Stebbins, C. E. (2006). Yersinia virulence depends on mimicry of host Rho-family nucleotide dissociation inhibitors. *Cell*, *126*(5), 869-880. https://doi.org/10.1016/j.cell.2006.06.056

Pruneda, J. N., Durkin, C. H., Geurink, P. P., Ovaa, H., Santhanam, B., Holden, D. W., & Komander, D. (2016). The Molecular Basis for Ubiquitin and Ubiquitin-like Specificities in Bacterial Effector Proteases. *Mol Cell*, *63*(2), 261-276. https://doi.org/10.1016/j.molcel.2016.06.015

Pujol, C., & Bliska, J. B. (2005). Turning Yersinia pathogenesis outside in: subversion of macrophage function by intracellular yersiniae. *Clin Immunol*, *114*(3), 216-226. https://doi.org/10.1016/j.clim.2004.07.013

Rascovan, N., Sjogren, K. G., Kristiansen, K., Nielsen, R., Willerslev, E., Desnues, C., & Rasmussen, S. (2019). Emergence and Spread of Basal Lineages of Yersinia pestis during the Neolithic Decline. *Cell*, *176*(1-2), 295-305 e210. https://doi.org/10.1016/j.cell.2018.11.005

Rasmussen, S., Allentoft, M. E., Nielsen, K., Orlando, L., Sikora, M., Sjogren, K. G., Pedersen, A. G., Schubert, M., Van Dam, A., Kapel, C. M., Nielsen, H. B., Brunak, S., Avetisyan, P., Epimakhov, A., Khalyapin, M. V., Gnuni, A., Kriiska, A., Lasak, I., Metspalu, M., . . . Willerslev, E. (2015). Early divergent strains of Yersinia pestis in Eurasia 5,000 years ago. *Cell*, *163*(3), 571-582. https://doi.org/10.1016/j.cell.2015.10.009

Rathinam, V. A., Vanaja, S. K., & Fitzgerald, K. A. (2012). Regulation of inflammasome signaling. *Nat Immunol*, *13*(4), 333-342. https://doi.org/10.1038/ni.2237

Ratner, D., Orning, M. P., Proulx, M. K., Wang, D., Gavrilin, M. A., Wewers, M. D., Alnemri, E. S., Johnson, P. F., Lee, B., Mecsas, J., Kayagaki, N., Goguen, J. D., & Lien, E. (2016). The Yersinia pestis Effector YopM Inhibits Pyrin Inflammasome Activation. *PLoS Pathog*, *12*(12), e1006035. https://doi.org/10.1371/journal.ppat.1006035

Ratner, D., Orning, M. P., Starheim, K. K., Marty-Roix, R., Proulx, M. K., Goguen, J. D., & Lien, E. (2016). Manipulation of interleukin-1β and interleukin-18 production by Yersinia pestis effectors YopJ and YopM and redundant impact on virulence. *J Biol Chem*, *291*(31), 16417. https://doi.org/10.1074/jbc.A115.697698

Raymond, B., Batsche, E., Boutillon, F., Wu, Y. Z., Leduc, D., Balloy, V., Raoust, E., Muchardt, C., Goossens, P. L., & Touqui, L. (2009). Anthrax lethal toxin impairs IL-8 expression in epithelial cells through inhibition of histone H3 modification. *PLoS Pathog*, *5*(4), e1000359. https://doi.org/10.1371/journal.ppat.1000359

Reinés, M., Llobet, E., Dahlström, K. M., Pérez-Gutiérrez, C., Llompart, C. M., Torrecabota, N., Salminen, T. A., & Bengoechea, J. A. (2012). Deciphering the acylation pattern of Yersinia enterocolitica lipid A. *PLoS Pathog*, *8*(10), e1002978. https://doi.org/10.1371/journal.ppat.1002978

Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., & Vance, R. E. (2006). Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog*, *2*(3), e18. https://doi.org/10.1371/journal.ppat.0020018

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Rosner, B. M., Stark, K., HÖHle, M., & Werber, D. (2012). Risk factors for sporadic Yersinia enterocolitica infections, Germany 2009–2010. *Epidemiology and Infection*, *140*(10), 1738-1747. https://doi.org/10.1017/S0950268811002664

Rosner, B. M., Stark, K., & Werber, D. (2010). Epidemiology of reported Yersinia enterocolitica infections in Germany, 2001-2008. *BMC Public Health*, *10*, 337. https://doi.org/10.1186/1471-2458-10-337

Rosqvist, R., Forsberg, A., Rimpiläinen, M., Bergman, T., & Wolf-Watz, H. (1990). The cytotoxic protein YopE of Yersinia obstructs the primary host defence. *Mol Microbiol*, *4*(4), 657-667. https://doi.org/10.1111/j.1365-2958.1990.tb00635.x

Rosqvist, R., Magnusson, K. E., & Wolf-Watz, H. (1994). Target cell contact triggers expression and polarized transfer of Yersinia YopE cytotoxin into mammalian cells. *Embo j*, *13*(4), 964-972. https://doi.org/10.1002/j.1460-2075.1994.tb06341.x

Ruckdeschel, K., Harb, S., Roggenkamp, A., Hornef, M., Zumbihl, R., Köhler, S., Heesemann, J., & Rouot, B. (1998). 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*, *187*(7), 1069-1079. https://doi.org/10.1084/jem.187.7.1069

Ruckdeschel, K., Machold, J., Roggenkamp, A., Schubert, S., Pierre, J., Zumbihl, R., Liautard, J. P., Heesemann, J., & Rouot, B. (1997). 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*, *272*(25), 15920-15927. https://doi.org/10.1074/jbc.272.25.15920

Ruckdeschel, K., Mannel, O., Richter, K., Jacobi, C. A., Trülzsch, K., Rouot, B., & Heesemann, J. (2001). Yersinia outer protein P of Yersinia enterocolitica simultaneously blocks the nuclear factor-kappa B pathway and exploits lipopolysaccharide signaling to trigger apoptosis in macrophages. *J Immunol*, *166*(3), 1823-1831. https://doi.org/10.4049/jimmunol.166.3.1823

Ruckdeschel, K., Roggenkamp, A., Schubert, S., & Heesemann, J. (1996). Differential contribution of Yersinia enterocolitica virulence factors to evasion of microbicidal action of neutrophils. *Infect Immun*, *64*(3), 724-733. https://doi.org/10.1128/iai.64.3.724-733.1996

Rudolph, M. (2020). Visualization of effector protein translocation and pore formation during *Yersinia enterocolitica* infection of cells. In.

Rühl, S., & Broz, P. (2015). Caspase-11 activates a canonical NLRP3 inflammasome by promoting K(+) efflux. *Eur J Immunol*, *45*(10), 2927-2936. https://doi.org/10.1002/eji.201545772

Sabina, Y., Rahman, A., Ray, R. C., & Montet, D. (2011). Yersinia enterocolitica: Mode of Transmission, Molecular Insights of Virulence, and Pathogenesis of Infection. *J Pathog*, *2011*, 429069. https://doi.org/10.4061/2011/429069

Sauvonnet, N., Pradet-Balade, B., Garcia-Sanz, J. A., & Cornelis, G. R. (2002). Regulation of mRNA expression in macrophages after Yersinia enterocolitica infection. Role of different Yop effectors. *J Biol Chem*, *277*(28), 25133-25142. https://doi.org/10.1074/jbc.M203239200

Sawicka, A., & Seiser, C. (2014). Sensing core histone phosphorylation - a matter of perfect timing. *Biochim Biophys Acta*, *1839*(8), 711-718. https://doi.org/10.1016/j.bbagrm.2014.04.013

Sborgi, L., Ravotti, F., Dandey, V. P., Dick, M. S., Mazur, A., Reckel, S., Chami, M., Scherer, S., Huber, M., Böckmann, A., Egelman, E. H., Stahlberg, H., Broz, P., Meier, B. H., & Hiller, S. (2015). Structure and assembly of the mouse ASC inflammasome by combined NMR spectroscopy and cryo-electron microscopy. *Proc Natl Acad Sci U S A*, *112*(43), 13237-13242. https://doi.org/10.1073/pnas.1507579112

Schaake, J., Drees, A., Grüning, P., Uliczka, F., Pisano, F., Thiermann, T., von Altrock, A., Seehusen, F., Valentin-Weigand, P., & Dersch, P. (2014). Essential Role of Invasin for Colonization and Persistence of Yersinia enterocolitica in Its Natural

Reservoir Host, the Pig. *Infection and Immunity*, *82*(3), 960-969. https://doi.org/10.1128/iai.01001-13

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nat Methods*, *9*(7), 676-682. https://doi.org/10.1038/nmeth.2019

Schmeck, B., Beermann, W., van Laak, V., Zahlten, J., Opitz, B., Witzenrath, M., Hocke, A. C., Chakraborty, T., Kracht, M., Rosseau, S., Suttorp, N., & Hippenstiel, S. (2005). Intracellular bacteria differentially regulated endothelial cytokine release by MAPK-dependent histone modification. *J Immunol*, *175*(5), 2843-2850. https://doi.org/10.4049/jimmunol.175.5.2843

Schmid-Burgk, J. L., Gaidt, M. M., Schmidt, T., Ebert, T. S., Bartok, E., & Hornung, V. (2015). Caspase-4 mediates non-canonical activation of the NLRP3 inflammasome in human myeloid cells. *Eur J Immunol*, *45*(10), 2911-2917. https://doi.org/10.1002/eji.201545523

Schmid, Y., Grassl, G. A., Bühler, O. T., Skurnik, M., Autenrieth, I. B., & Bohn, E. (2004). Yersinia enterocolitica adhesin A induces production of interleukin-8 in epithelial cells. *Infect Immun*, *72*(12), 6780-6789. https://doi.org/10.1128/iai.72.12.6780-6789.2004

Schnapp, M. (2016). Characterization of novel interaction partners of the Yersinia enterocolitica effector protein YopM and their role in macrophage cytokine expression. In.

Schneider, W. M., Chevillotte, M. D., & Rice, C. M. (2014). Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol*, *32*, 513-545. https://doi.org/10.1146/annurev-immunol-032713-120231

Schoberle, T. J., Chung, L. K., McPhee, J. B., Bogin, B., & Bliska, J. B. (2016). Uncovering an Important Role for YopJ in the Inhibition of Caspase-1 in Activated Macrophages and Promoting Yersinia pseudotuberculosis Virulence. *Infect Immun*, *84*(4), 1062-1072. https://doi.org/10.1128/iai.00843-15

Schoenfelder, S., & Fraser, P. (2019). Long-range enhancer-promoter contacts in gene expression control. *Nat Rev Genet*, *20*(8), 437-455. https://doi.org/10.1038/s41576-019-0128-0

Schorn, C., Frey, B., Lauber, K., Janko, C., Strysio, M., Keppeler, H., Gaipl, U. S., Voll, R. E., Springer, E., Munoz, L. E., Schett, G., & Herrmann, M. (2011). Sodium overload and water influx activate the NALP3 inflammasome. *J Biol Chem*, *286*(1), 35-41. https://doi.org/10.1074/jbc.M110.139048

Schotte, P., Denecker, G., Van Den Broeke, A., Vandenabeele, P., Cornelis, G. R., & Beyaert, R. (2004). Targeting Rac1 by the Yersinia effector protein YopE inhibits caspase-1-mediated maturation and release of interleukin-1beta. *J Biol Chem*, *279*(24), 25134-25142. https://doi.org/10.1074/jbc.M401245200

Schubert, K. A., Xu, Y., Shao, F., & Auerbuch, V. (2020). The Yersinia Type III Secretion System as a Tool for Studying Cytosolic Innate Immune Surveillance. *Annu Rev Microbiol*, *74*, 221-245. https://doi.org/10.1146/annurev-micro-020518-120221

Schulte, R., Grassl, G. A., Preger, S., Fessele, S., Jacobi, C. A., Schaller, M., Nelson, P. J., & Autenrieth, I. B. (2000). Yersinia enterocolitica invasin protein triggers IL-8 production in epithelial cells via activation of Rel p65-p65 homodimers. *Faseb j*, *14*(11), 1471-1484. https://doi.org/10.1096/fj.14.11.1471

Shao, F., Vacratsis, P. O., Bao, Z., Bowers, K. E., Fierke, C. A., & Dixon, J. E. (2003). Biochemical characterization of the Yersinia YopT protease: cleavage site and recognition elements in Rho GTPases. *Proc Natl Acad Sci U S A*, *100*(3), 904-909. https://doi.org/10.1073/pnas.252770599

Shechter, D., Dormann, H. L., Allis, C. D., & Hake, S. B. (2007). Extraction, purification and analysis of histones. *Nat Protoc*, *2*(6), 1445-1457. https://doi.org/10.1038/nprot.2007.202

Shouval, D. S., Ouahed, J., Biswas, A., Goettel, J. A., Horwitz, B. H., Klein, C., Muise, A. M., & Snapper, S. B. (2014). Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans. *Adv Immunol*, *122*, 177-210. https://doi.org/10.1016/b978-0-12-800267-4.00005-5

Sica, A., & Mantovani, A. (2012). Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*, *122*(3), 787-795. https://doi.org/10.1172/jci59643

Simonet, M., & Falkow, S. (1992). Invasin expression in Yersinia pseudotuberculosis. *Infect Immun*, *60*(10), 4414-4417. https://doi.org/10.1128/iai.60.10.4414-4417.1992

Skrzypek, E., Cowan, C., & Straley, S. C. (1998). Targeting of the Yersinia pestis YopM protein into HeLa cells and intracellular trafficking to the nucleus. *Mol Microbiol*, *30*(5), 1051-1065. https://doi.org/10.1046/j.1365-2958.1998.01135.x

Songsungthong, W., Higgins, M. C., Rolán, H. G., Murphy, J. L., & Mecsas, J. (2010). ROS-inhibitory activity of YopE is required for full virulence of Yersinia in mice. *Cell Microbiol*, *12*(7), 988-1001. https://doi.org/10.1111/j.1462-5822.2010.01448.x

Sorg, I., Goehring, U. M., Aktories, K., & Schmidt, G. (2001). Recombinant Yersinia YopT leads to uncoupling of RhoA-effector interaction. *Infect Immun*, *69*(12), 7535-7543. https://doi.org/10.1128/iai.69.12.7535-7543.2001

Spyrou, M. A., Tukhbatova, R. I., Wang, C. C., Valtuena, A. A., Lankapalli, A. K., Kondrashin, V. V., Tsybin, V. A., Khokhlov, A., Kuhnert, D., Herbig, A., Bos, K. I., & Krause, J. (2018). Analysis of 3800-year-old Yersinia pestis genomes suggests Bronze Age origin for bubonic plague. *Nat Commun*, *9*(1), 2234. https://doi.org/10.1038/s41467-018-04550-9

Stennicke, H. R., Jürgensmeier, J. M., Shin, H., Deveraux, Q., Wolf, B. B., Yang, X., Zhou, Q., Ellerby, H. M., Ellerby, L. M., Bredesen, D., Green, D. R., Reed, J. C., Froelich, C. J., & Salvesen, G. S. (1998). Pro-caspase-3 is a major physiologic target of caspase-8. *J Biol Chem*, *273*(42), 27084-27090. https://doi.org/10.1074/jbc.273.42.27084

Straley, S. C., & Bowmer, W. S. (1986). Virulence genes regulated at the transcriptional level by Ca2+ in Yersinia pestis include structural genes for outer membrane proteins. *Infect Immun*, *51*(2), 445-454. https://doi.org/10.1128/iai.51.2.445-454.1986

Straley, S. C., & Cibull, M. L. (1989). Differential clearance and host-pathogen interactions of YopE- and YopK- YopL- Yersinia pestis in BALB/c mice. *Infect Immun*, *57*(4), 1200-1210. https://doi.org/10.1128/iai.57.4.1200-1210.1989

Sweet, C. R., Conlon, J., Golenbock, D. T., Goguen, J., & Silverman, N. (2007). YopJ targets TRAF proteins to inhibit TLR-mediated NF-kappaB, MAPK and IRF3 signal transduction. *Cell Microbiol*, *9*(11), 2700-2715. https://doi.org/10.1111/j.1462-5822.2007.00990.x

Takao, T., Tominaga, N., Shimonishi, Y., Hara, S., Inoue, T., & Miyama, A. (1984). Primary structure of heat-stable enterotoxin produced by Yersinia enterocolitica. *Biochemical and Biophysical Research Communications*, *125*(3), 845-851. https://doi.org/https://doi.org/10.1016/0006-291X(84)91360-3

Tang, D., Kang, R., Coyne, C. B., Zeh, H. J., & Lotze, M. T. (2012). PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol Rev*, *249*(1), 158-175. https://doi.org/10.1111/j.1600-065X.2012.01146.x

Thiefes, A., Wolf, A., Doerrie, A., Grassl, G. A., Matsumoto, K., Autenrieth, I., Bohn, E., Sakurai, H., Niedenthal, R., Resch, K., & Kracht, M. (2006). The Yersinia enterocolitica effector YopP inhibits host cell signalling by inactivating the protein kinase TAK1 in the IL-1 signalling pathway. *EMBO Rep*, *7*(8), 838-844. https://doi.org/10.1038/sj.embor.7400754

Thorslund, S. E., Edgren, T., Pettersson, J., Nordfelth, R., Sellin, M. E., Ivanova, E., Francis, M. S., Isaksson, E. L., Wolf-Watz, H., & Fällman, M. (2011). The RACK1 signaling scaffold protein selectively interacts with Yersinia pseudotuberculosis virulence function. *PLoS One*, *6*(2), e16784. https://doi.org/10.1371/journal.pone.0016784

Thorslund, S. E., Ermert, D., Fahlgren, A., Erttmann, S. F., Nilsson, K., Hosseinzadeh, A., Urban, C. F., & Fällman, M. (2013). Role of YopK in Yersinia pseudotuberculosis resistance against polymorphonuclear leukocyte defense. *Infect Immun*, *81*(1), 11-22. https://doi.org/10.1128/iai.00650-12

Trasak, C., Zenner, G., Vogel, A., Yüksekdag, G., Rost, R., Haase, I., Fischer, M., Israel, L., Imhof, A., Linder, S., Schleicher, M., & Aepfelbacher, M. (2007). Yersinia protein kinase YopO is activated by a novel G-actin binding process. *J Biol Chem*, *282*(4), 2268-2277. https://doi.org/10.1074/jbc.M610071200

Treille, G.-F., & Yersin, A. (1894, 1894-09-01). La peste bubonique à Hong Kong. [VIIIe Congrès international d'hygiène et de démographie de Budapest du 1 au 9 septembre 1894]. VIIIe Congrès international d'hygiène et de démographie, Budapest, Hungary.

Trülzsch, K., Sporleder, T., Igwe, E. I., Rüssmann, H., & Heesemann, J. (2004). Contribution of the major secreted yops of Yersinia enterocolitica O:8 to pathogenicity in the mouse infection model. *Infect Immun*, *72*(9), 5227-5234. https://doi.org/10.1128/iai.72.9.5227-5234.2004

Van Nhieu, G. T., & Isberg, R. R. (1991). The Yersinia pseudotuberculosis invasin protein and human fibronectin bind to mutually exclusive sites on the alpha 5 beta 1 integrin receptor. *J Biol Chem*, *266*(36), 24367-24375.

van Pelt, W., de Wit, M. A. S., Wannet, W. J. B., Ligtvoet, E. J. J., Widdowson, M. A., & van Duynhoven, Y. T. H. P. (2003). Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991–2001. *Epidemiology and Infection*, *130*(3), 431-441. https://doi.org/10.1017/s0950268803008392

Viboud, G. I., & Bliska, J. B. (2001). A bacterial type III secretion system inhibits actin polymerization to prevent pore formation in host cell membranes. *Embo j*, *20*(19), 5373-5382. https://doi.org/10.1093/emboj/20.19.5373

Viboud, G. I., & Bliska, J. B. (2005). Yersinia outer proteins: role in modulation of host cell signaling responses and pathogenesis. *Annu Rev Microbiol*, *59*, 69-89. https://doi.org/10.1146/annurev.micro.59.030804.121320

Viboud, G. I., Mejía, E., & Bliska, J. B. (2006). Comparison of YopE and YopT activities in counteracting host signalling responses to Yersinia pseudotuberculosis infection. *Cell Microbiol*, *8*(9), 1504-1515. https://doi.org/10.1111/j.1462-5822.2006.00729.x

Viboud, G. I., So, S. S., Ryndak, M. B., & Bliska, J. B. (2003). Proinflammatory signalling stimulated by the type III translocation factor YopB is counteracted by multiple effectors in epithelial cells infected with Yersinia pseudotuberculosis. *Mol Microbiol*, *47*(5), 1305-1315. https://doi.org/10.1046/j.1365-2958.2003.03350.x

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Peng, W., Zhang, M. Q., & Zhao, K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet*, *40*(7), 897-903. https://doi.org/10.1038/ng.154

Wauters, G., Kandolo, K., & Janssens, M. l. (1987). Revised biogrouping scheme of Yersinia enterocolitica. *Contributions to microbiology and immunology*, *9*, 14-21.

Weng, D., Marty-Roix, R., Ganesan, S., Proulx, M. K., Vladimer, G. I., Kaiser, W. J., Mocarski, E. S., Pouliot, K., Chan, F. K., Kelliher, M. A., Harris, P. A., Bertin, J., Gough, P. J., Shayakhmetov, D. M., Goguen, J. D., Fitzgerald, K. A., Silverman, N., & Lien, E. (2014). Caspase-8 and RIP kinases regulate bacteria-induced innate immune responses and cell death. *Proc Natl Acad Sci U S A*, *111*(20), 7391-7396. https://doi.org/10.1073/pnas.1403477111

Westermark, L., Fahlgren, A., & Fällman, M. (2014). Yersinia pseudotuberculosis efficiently escapes polymorphonuclear neutrophils during early infection. *Infect Immun*, *82*(3), 1181-1191. https://doi.org/10.1128/iai.01634-13

Wickham, H. (2016). Elegant Graphics for Data Analysis. In *ggplot2* (2 ed.). SpringerCham. https://doi.org/https://doi.org/10.1007/978-3-319-24277-4

Wittkopp, P. J., & Kalay, G. (2011). Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. *Nat Rev Genet*, *13*(1), 59-69. https://doi.org/10.1038/nrg3095

Wong, K. W., & Isberg, R. R. (2005). Yersinia pseudotuberculosis spatially controls activation and misregulation of host cell Rac1. *PLoS Pathog*, *1*(2), e16. https://doi.org/10.1371/journal.ppat.0010016

Wren, B. W. (2003). The yersiniae--a model genus to study the rapid evolution of bacterial pathogens. *Nat Rev Microbiol*, *1*(1), 55-64. https://doi.org/10.1038/nrmicro730

Xu, H., Yang, J., Gao, W., Li, L., Li, P., Zhang, L., Gong, Y. N., Peng, X., Xi, J. J., Chen, S., Wang, F., & Shao, F. (2014). Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature*, *513*(7517), 237-241. https://doi.org/10.1038/nature13449

Yang, J., Zhao, Y., Shi, J., & Shao, F. (2013). Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. *Proc Natl Acad Sci U S A*, *110*(35), 14408-14413. https://doi.org/10.1073/pnas.1306376110

Yao, T., Mecsas, J., Healy, J. I., Falkow, S., & Chien, Y. (1999). Suppression of T and B lymphocyte activation by a Yersinia pseudotuberculosis virulence factor, yopH. *J Exp Med*, *190*(9), 1343-1350. https://doi.org/10.1084/jem.190.9.1343

Young, G. M., Badger, J. L., & Miller, V. L. (2000). Motility is required to initiate host cell invasion by Yersinia enterocolitica. *Infect Immun*, *68*(7), 4323-4326. https://doi.org/10.1128/iai.68.7.4323-4326.2000

Zanoni, I., Ostuni, R., Marek, L. R., Barresi, S., Barbalat, R., Barton, G. M., Granucci, F., & Kagan, J. C. (2011). CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. *Cell*, *147*(4), 868-880. https://doi.org/10.1016/j.cell.2011.09.051

Zhang, J., Brodsky, I. E., & Shin, S. (2023). Yersinia deploys type III-secreted effectors to evade caspase-4 inflammasome activation in human cells. *mBio*, *14*(5), e0131023. https://doi.org/10.1128/mbio.01310-23

Zhang, Z. M., Ma, K. W., Yuan, S., Luo, Y., Jiang, S., Hawara, E., Pan, S., Ma, W., & Song, J. (2016). Structure of a pathogen effector reveals the enzymatic mechanism of a novel acetyltransferase family. *Nat Struct Mol Biol*, *23*(9), 847-852. https://doi.org/10.1038/nsmb.3279

Zhao, J., & Sun, Y. (2018). CRISPR-Cas12a-Assisted Recombineering in Yersinia pestis. In R. Yang (Ed.), *Yersinia Pestis Protocols* (pp. 165-172). Springer Singapore. https://doi.org/10.1007/978-981-10-7947-4_20

Zhao, W., Ma, L., Cai, C., & Gong, X. (2019). Caffeine Inhibits NLRP3 Inflammasome Activation by Suppressing MAPK/NF-κB and A2aR Signaling in LPS-Induced THP-1 Macrophages. *Int J Biol Sci*, *15*(8), 1571-1581. https://doi.org/10.7150/ijbs.34211

Zhao, Y., Yang, J., Shi, J., Gong, Y. N., Lu, Q., Xu, H., Liu, L., & Shao, F. (2011). The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*, *477*(7366), 596-600. https://doi.org/10.1038/nature10510

Zheng, Y., Lilo, S., Brodsky, I. E., Zhang, Y., Medzhitov, R., Marcu, K. B., & Bliska, J. B. (2011). A Yersinia effector with enhanced inhibitory activity on the NF-κB pathway activates the NLRP3/ASC/caspase-1 inflammasome in macrophages. *PLoS Pathog*, *7*(4), e1002026. https://doi.org/10.1371/journal.ppat.1002026

Zhou, H., Monack, D. M., Kayagaki, N., Wertz, I., Yin, J., Wolf, B., & Dixit, V. M. (2005). Yersinia virulence factor YopJ acts as a deubiquitinase to inhibit NF-kappa B activation. *J Exp Med*, *202*(10), 1327-1332. https://doi.org/10.1084/jem.20051194

Zhou, R., Yazdi, A. S., Menu, P., & Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature*, *469*(7329), 221-225. https://doi.org/10.1038/nature09663

Zhu, H., Jian, Z., Zhong, Y., Ye, Y., Zhang, Y., Hu, X., Pu, B., Gu, L., & Xiong, X. (2021). Janus Kinase Inhibition Ameliorates Ischemic Stroke Injury and Neuroinflammation Through Reducing NLRP3 Inflammasome Activation via JAK2/STAT3 Pathway Inhibition. *Front Immunol*, *12*, 714943. https://doi.org/10.3389/fimmu.2021.714943

Zumbihl, R., Aepfelbacher, M., Andor, A., Jacobi, C. A., Ruckdeschel, K., Rouot, B., & Heesemann, J. (1999). The cytotoxin YopT of Yersinia enterocolitica induces modification and cellular redistribution of the small GTP-binding protein RhoA. *J Biol Chem*, *274*(41), 29289-29293. https://doi.org/10.1074/jbc.274.41.29289

Zwack, E. E., Feeley, E. M., Burton, A. R., Hu, B., Yamamoto, M., Kanneganti, T. D., Bliska, J. B., Coers, J., & Brodsky, I. E. (2017). Guanylate Binding Proteins Regulate Inflammasome Activation in Response to Hyperinjected Yersinia Translocon Components. *Infect Immun*, *85*(10). https://doi.org/10.1128/iai.00778-16

Zwack, E. E., Snyder, A. G., Wynosky-Dolfi, M. A., Ruthel, G., Philip, N. H., Marketon, M. M., Francis, M. S., Bliska, J. B., & Brodsky, I. E. (2015). Inflammasome activation in response to the Yersinia type III secretion system requires hyperinjection of translocon proteins YopB and YopD. *mBio*, *6*(1), e02095-02014. https://doi.org/10.1128/mBio.02095-14

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10 Abbreviations

%	percentage
°C	degree celsius
µF	mikrofarad
µg/ml	mikrogram per milliliter
µl	mikroliter
µm	mikrometer
µM	mikromolar
∞	infinite
A	absorbance
Ail	attachement invasion locus
AIM2	absent in melanoma-2
ALR	AIM2-like receptor
ANOVA	analysis of variance
AP-1	activator protein 1
APS	ammonium persulfate
ASC	apoptosis-associated speck-like protein containing CARD
ATP	adenosine triphosphate
B2M	beta-2-microglobulin
BMDM	bone-marrow-derived macrophages
bp	base pair
BSA	bovine serum albumine
Ca^{2+}	calcium cation
$CaCl_2$	calcium chloride
CARD	caspase activation and recruitment domains
Cas	CRISPR-associated
CCL	chemokine (C-C motif) ligand
CCR	C-C chemokine receptor
CCRL	C-C chemokine receptor-like
Cdc42	cell division control protein 42 homolog
cDNA	circular DNA
ChIP	Chromatin immunoprecipitation
ChIP-seq	ChIP-sequencing
Chlor	chloramphenicol
CLR	C-type lectin receptor
cm^2	square centimeter

CO_2	carbon dioxide
CRISPR	clustered regularly interspaced short palindromic repeats
CRM1	chromosomal maintenance 1
crRNA	CRISPR RNA
CXCL	chemokine (C-X-C motif) ligand
DAMP	danger-associated molecular pattern
DAPI	4′,6-diamidino-2-phenylindole
DAVID	database for annotation, visualization and integrated discovery
ddH_2O	double distilled water
DDX3	DEAD-box helicase 3
DEAD	aspartic acid-glumatic acid-alanine-aspartic acid
DEG	differentially expressed genes
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dsDNA	double stranded DNA
DTT	dithiothreiotol
E	early
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
eGFP	enhanced green fluorescent protein
ERK	extracellular signal-related kinase
EtOH	ethanol
ETS	erythroblast transformation specific
FAE	follicle-associated epithelium
FAK	focal adhesion kinase
Fig.	figure
Foxo1	forkhead box protein O1
fwd	forward
Fyb	Fyn binding protein
Fyn	proto-oncogene tyrosine-protein kinase
g	gram
g	relative centrifugal force
g/l	gram per liter
GAP	GTPase activating protein
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GBP	galectin and guanylate binding protein

GDI	GDP dissociation inhibitor
GDP	guanosine diphosphate
GEO	gene expression omnibus
GFY	general factor Y
GILZ	glucocorticoid-induced leucine zipper
GM-CSF	granulocyte macrophage colony-stimulating factor
GO	gene ontology
GTP	Guanosine triphosphate
H	histone
h	hour
HAT	histone acetyltransferase
HCl	hydrochloric acid
HDR	homology directed repair
HEK	human embryonic kidney
HF	high fidelity
HOMER	hypergeometric optimization of motif enrichment
HPI	high pathogenicity island
HRP	horseradish peroxidase
HUVEC	human umbilical vein endothelial cells
ID	identity
IDO	indoleamine 2,3-dioxygenase
IEC	intestinal epithelial cells
IF	immunofluorescence
IFIT	interferon-induced protein with tetratricopeptide repeats
IFN	interferon
IgG	immunoglobuline G
IKK	IκB kinase
IL	interleukin
Inv	Invasin
IP_6	hexakisphosphate
IRF	interferon regulatory factor
ISG	interferon stimulated genes
IκB	inihbitor of nuclear factor kappaB kinase
JNK	c-Jun N-terminal kinases
K	lysine
K^+	poatssium cation
Kana	kanamycin

KCl	potassium chloride
kDa	kilodalton
KLF2	krüppel-like factor 2
L	late
LB	lysogeny broth
LcrV	low calcium response protein V
Lji308	2,6-Difluoro-4-(4-(4-morpholinophenyl)pyridin-3-yl)phenol, 2,6-Difluoro-4-[4-[4-(4-morpholinyl)phenyl]-3-pyridinyl]phenol
Log2	binary logarithm
LPS	lipopolysaccharide
LRR	leucine rich repeat
LT	lethal toxin
M	molar
m^2	square meter
mA	milliampere
MAPK	mitogen-activated protein kinase
M-cells	microfold cells
MD2	myeloid differentiation factor 2
MEK	mitogen-activated protein kinase kinase
mg/ml	milligram per milliliter
$MgCl_2$	magnesium chloride
min	minutes
MKP	mitogen-activated protein kinase phosphatase
ml	milliliter
mm	millimeter
mM	millimolar
MOI	multiplicity of infection
ms	millisecond
NaCl	sodium chloride
NAIP	NLR family apoptosis inhibitory protein
Nal	nalidixic acid
NaOH	sodium hydroxide
NBD	nucleotide binding domain
NCBI	national center for biotechnology information
NF-κB	nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells
ng	nanogram
NLR	NOD-like receptor

NLRC	NLR family CARD domain-containing
NLRP	NLR family Pyrin domain-containing
nm	nanometer
nM	nanomolar
NOD	nucleotide-binding oligomerization domain
OD	optical density
Osp	outer shigella protein
p130cas	p130 Crk-associated substrate
p38	p38 mitogen-activated protein kinase
PAM	protospacer adjacent motif
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffered saline
PCA	principal component anylsis
PCR	polymearse chain reaction
PD98059	2'–Amino–3'–methoxyflavone
Pen	penicilline
PFO	perfringolysin
pH	potential/ power of hydrogen
PLY	pneumolysin
PML	promyelocytic leukemia
PMN	polymorphonuclear neutrophils
PRAM1	PML-RARA-regulated adapter molecule 1
PRK/PKN	protein kinase C-related kinase
PRR	pattern recognition receptors
PT	*Pertussis* toxin
PTM	post-translational modification
PVDF	polyvinylidene difluoride
PYD	pyrin domain
pYV	plasmid of *Yersinia* virulence
Rac	Ras-related C3 botulinum toxin substrate
RARA	retinoic acid receptor-alpha
Ras	rat sarcoma virus
RefSeq	reference sequence
rev	reverse
RHD	rel homology domain
Rho	Ras homologue family member
RIG	Retinoic acid-inducible gene

RIPK	receptor-interacting protein kinase
RLR	RIG-I-like receptor
RNA	ribonucleic acid
RNA-seq	RNA-sequencing
ROS	reactive oxygen species
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
rRNA	ribosomal RNA
RSK	ribosomal S6 protein kinase
RT	room temperature
RT-qPCR	reverse transcriptase-quantitative polymerase chain reaction
S	serine
SB203580	4-{4-(4-Fluorophenyl)-2-[4-(methanesulfinyl)phenyl]-1H-imidazol-5-yl}pyridine
SDS-PAGE	sodium dodecylsulfate-polyacrylamide gel electrophoresis
sec	second
Ser	serine
SKAP-HOM	Src kinase-associated phosphoprotein of 55 kDa homologue
SLP-76	SH2 domain containing leukocyte protein of 76 kDa
SOC	super optimal broth with catabolite repression
SP600125	anthra[1,9-cd]pyrazol-6(2H)-one
Spec	spectinomycin
Src	sarcoma
SRG	secondary response genes
STAR	spliced transcripts alignment to a reference
STAT3	signal transducer and activator of transcription 3
Strep	streptomycin
SycN	specific Yop chaperone N
T3SS	type three secretion system
TAE	Tris acetate EDTA
TAK1	transforming growth factor β-activated kinase 1
TBP	TATA-box binding protein
TBS	Tris buffered saline
TBS-T	TBS with Tween
TCA	trichloric acid
Tcd	*Clostridium difficile* toxin
TEMED	tetramethylethylenediamine

TGF-β	transforming growth factor β
T_h	T helper cell
Thr	threonine
TIR	toll-interleukin-1 receptor
TLR	toll-like receptor
Tm	melting temperature
TNF	tumor necrosis factor
TPCA	2-[(aminocarbonyl)amino]-5 -(4-fluorophenyl)-3- thiophenecarboxamide
TRAF	TNF receptor-associated factor
TRIF	TIR-domain-containing adapter-inducing interferon-β
TRIM	tripartite motif
TSS	transcription start site
TyeA	translocation of Yops into eukaryotic cells A
U	units
UV	ultraviolet
V	voltage
v/v	volume per volume
Vop	*Vibrio* outer protein
w/v	weight per volume
WB	western blot
YadA	*Yersinia* adhesion protein
YLTD	tyrosine-leucine-threonine-aspartic acid
Yop	*Yersinia* outer protein
YpkA	*Yersinia* protein kinase A
Ysc	*Yersinia* secretion
Yst	*Yersinia* stable toxin
Δ	delta
Ω	resistance

11 Danksagung

Ich möchte an dieser Stelle allen danken, die mir in irgendeiner Art und Weise den Abschluss dieser Arbeit ermöglicht haben. Ohne eure Hilfe hätte ich es nicht bis hierhin geschafft.

Als erstes danke ich meinem Doktorvater Prof. Dr. Martin Aepfelbacher, welcher mir die Möglichkeit gegeben hat spannende und vielfältige Themengebiete zu bearbeiten und dabei mit seiner umfassenden Erfahrung, seinem großem Interesse und kritischen Diskussionen immer wieder für neuen Input gesorgt hat. Ich danke dir für dein Vertrauen in meine Arbeit.

Herrn Prof. Dr. Adam Grundhoff danke ich für die Bereitwilligkeit zur Begutachtung meiner Dissertation.

Im Allgemeinem möchte ich mich bei allen Mitgliedern des MiBi-Instituts für etwaige Hilfestellungen, lustige Mittagspausen und die gute Stimmung bedanken.

Ein ganz besonderer Dank geht natürlich an alle Mitglieder meiner Arbeitsgruppe, welche jederzeit für mich da waren und auf die ich immer zählen konnte! Für die fantastische Einarbeitung ins Labor danke ich Maren, Alex und Gunni. Vielen Dank Alex für deine großartige Hilfe bei allen Fragen zu Yersinien und Mikroskopie, dein offenes Ohr für alles andere, deine Ausgeglichenheit und für lustige Momente. Gunni, ich danke dir für deine aufmunternde Art, deine ganzen Eigenheiten, deinen Witz und Humor aber auch für dein breites fachliches Wissen. Für deinen großen Erfahrungsschatz, Antworten auf alle möglichen Fragen, das Auffinden auch scheinbar verlorener Dinge im Labor und den Austausch über unsere Lauferfahrungen danke ich dir sehr Claudia. Danke auch an dich Su, für deine „grantige“ Herzlichkeit, den Austausch über Inflammasome aber auch die teilweise ungewollte musikalische Weiterbildung. Indra danke ich für die kompetente Unterstützung bei allen epigenetischen und transkriptionellen Fragestellungen. Ein großer Dank geht auch an dich Gesche, nicht nur für die wöchentliche Präparation der Makrophagen, sondern auch für das jederzeit geduldige Beantworten meiner Fragen. Jonas, ich danke dir für deine sprachliche Eloquenz und deine erfrischende Art. Beides hat mir immer große Freude bereitet.

Virgilio, Bernd und Shuting danke ich für ihren unermüdlichen Einsatz für die Funktionsfähigkeit der Mikroskope.

Danksagung

Für unzählige schlaflose Nächte und eure Zuverlässigkeit möchte ich mich bei Jacqueline, George, Konrad, Waldemar, Roland und Ida bedanken. Ihr habt uns immer sicher ans Ziel gebracht und bleibende Erinnerungen geschaffen.

Den wichtigsten Personen in meinem Leben, meinen Eltern und Irma, danke ich besonders! Ohne euch wäre ich nicht da, wo ich jetzt bin.

Mama und Papa, ich danke euch für eure Hingabe, für euren Einsatz, für eure bedingungslose Liebe, für euer Verständnis und dafür, dass ihr mich auf meinem Weg jederzeit unterstützt habt und unterstützt. Ich weiß, dass ich mich immer auf euch verlassen kann!

Irma, ich weiß gar nicht wie ich dir danken soll. Danke, dass du immer für mich da bist und ich immer auf dich zählen kann! Danke, dafür, dass du eine unbeschreibliche Bereicherung in meinem Leben bist und immer dafür sorgst, dass es mir gut geht. Danke für fantastisches Essen, für unvergessliche Momente und deine Liebe. Ich freue mich auf eine wunderschöne gemeinsame Zukunft mit dir!

12 Lebenslauf

Lebenslauf entfällt aus datenschutzrechtlichen Gründen

13 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe, insbesondere ohne entgeltliche Hilfe von Vermittlungs- und Beratungsdiensten, 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. Das gilt insbesondere auch für alle Informationen aus Internetquellen.

Soweit beim Verfassen der Dissertation KI-basierte Tools („Chatbots“) verwendet wurden, versichere ich ausdrücklich, den daraus generierten Anteil deutlich kenntlich gemacht zu haben. Die „Stellungnahme des Präsidiums der Deutschen Forschungsgemeinschaft (DFG) zum Einfluss generativer Modelle für die Text- und Bilderstellung auf die Wissenschaften und das Förderhandeln der DFG“ aus September 2023 wurde dabei beachtet.

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 damit einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Datum Unterschrift