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# Functional role of the TGF-β signaling in the *Drosophila* immune response

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ABSTRACT

TGF-β signaling pathways are present in diverse animal species, which indicates their evolutionary importance in modulating several conserved biological processes and maintaining host homeostasis by adjusting the activity of innate immune mechanisms. *Drosophila melanogaster* utilizes two related but separable cascades of the canonical TGF-β signaling pathway: The Bone Morphogenetic Protein and Activin branches. Recent studies have produced significant information on the immune role of TGF-β signaling in the fruit fly model during response against certain bacterial pathogens. Results from further investigations have generated novel insights into the role of *Drosophila* TGF-β signaling molecules as immune regulators opposing infection against nematode parasites and their mutualistic bacterial partners. This knowledge has revealed a previously unknown layer of the host innate immune system. Here we summarize these recent breakthroughs focusing on the participation of TGF-β signaling factors in various *Drosophila* immune processes in relation to infection with potent bacteria and nematode parasites. The presented information provides important clues indicating directions for future research into the design of novel strategies for the effective control of infectious diseases caused by bacterial pathogens and parasitic nematodes.

## 1. Introduction

TGF-β signaling is a well-conserved pathway that directs multiple host physiological functions. *Drosophila melanogaster* utilizes two related but separable branches of the canonical TGF-β signaling pathway: The Bone Morphogenetic Protein (BMP) and Activin branches (Peterson and O'Connor, 2014) (Fig. 1).

In the BMP branch, the three extracellular ligands – Decapentaplegic (Dpp), Glass bottom boat (Gbb), Screw (Scw) – attract a Type II receptor and one of the two BMP-specific Type I receptors, Thickveins (Tkv) or Saxophone (Sax). Formation of this complex results in the phosphorylation of the Response (R)-Smad of the BMP branch, Mothers against decapentaplegic (Mad). In the Activin branch, there are four extracellular ligands: Activin-β, (Actβ), Dawdle (Daw), Myoglianin (Myg), and Maverick (Mav). An Activin ligand binds a Type II receptor and the Type I receptor Baboon (Babo), which phosphorylates the Activin R-Smad, Smad on X (Smox). Babo may also phosphorylate Mad. The two Type II receptors – Punt (Put) and Wishful thinking (Wit) – can function in both the BMP and Activin branches, as can the Co-Smad, Medea (Med) (Li et al., 2006).

Here, we outline recent findings that implicate the TGF-β signaling branches in the *Drosophila* antibacterial and anti-nematode response. This information forms the critical first step for unraveling previously undescribed host innate immune activities, which may in turn provide new therapeutic targets for more efficient protection against infectious pathogens.

## 2. The role of the TGF-β pathway in the *Drosophila* anti-nematode response

The TGF-β pathway and its involved signaling molecules are implicated in the innate immunological response of *Drosophila* to a wide range of pathogen classes, including defense against parasitic nematode infection (Fig. 2). The *Drosophila* innate immune response is characterized by the detection of specific pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors (PRRs) that aid in activating downstream immune signaling events (Lu et al., 2020). Once activated, PRRs induce upregulated expression of Nuclear factor kappa-light-chain-enhancer of activated B cells, or NF-κB (Cammarata-Mouchtouris et al., 2022). NF-κB is a critical player in the *Drosophila* immune system, involving ligands *Dorsal, Dorsal-related immunity factor* (DIF), and *Relish* that are critical in the production of antimicrobial peptides (AMPs) that form an integral part of the *Drosophila* humoral immune response (Ganesan et al., 2011). This

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upregulation of NF-κB in adult flies induces the expression of TGF-β ligands *Dpp* and *Daw,* which are instrumental in initiating the signaling pathways of both the Activin and BMP branches (Patrnogic et al., 2019). Subsequent downstream signaling of each branch involves Smad and Mad signaling that induces Activin and BMP branch activation and the expression of effector molecules (Upadhyay et al., 2017).

During a parasitic nematode infection, such as with the entomopathogenic nematodes (EPNs) *Heterorhabditis* sp., the parasites enter the adult fly through piercing the cuticle or through natural openings such as the mouth parts or anus (Brivio and Mastore, 2018). Upon infection with these nematode parasites, the TGF-β pathway ligands *Daw* and *Dpp* are transcriptionally activated in adult flies (Eleftherianos et al., 2016). Although the EPN *Heterorhabditis bacteriophora* naturally carries the symbiotic bacteria *Photorhabdus luminescens*, TGF-β pathway activity can be induced with or without the presence of the associated bacteria (Castillo et al., 2013). Activation of the TGF-β pathway in *Drosophila* induces a wide range of effects opposing nematode infection. Upon infection with axenic *Steinernema carpocapsae,* the survival of *Drosophila* larvae increases as a result of TGF-β pathway activity from nematode infection, attributed to the inactivation of ligand *dpp* and the large production of AMPs (Yadav et al., 2018). The BMP branch TGF-β receptor *Sax* and transcription factor Mad are also upregulated upon sterile injury in adult flies (Patrnogic et al., 2018a). In *Drosophila* larvae, the Mad transcription factor has been shown to unite the two TGF-β branches, becoming upregulated as a result of alterations in gene expression from either branch's intracellular receptors (Patrnogic et al., 2018b). In addition to Smad signaling-induced gene expression, TGF-β pathway activity during nematode infection also leads to increased dual oxidase expression. Dual oxidase is an enzyme responsible for the production of reactive oxygen species, which have been observed to be beneficial to the gut immune response of *Drosophila* (Ozakman and Eleftherianos, 2019). When challenged with EPNs, *Drosophila* larval mutant for the *Daw* ligand show increased levels of dual oxidase compared to their wild type counterparts, thus providing evidence for the involvement of this enzyme in the immune response to nematode infection in *Drosophila* (Ozakman and Eleftherianos, 2019). Taken together, the effects of TGF-β pathway ligands and effectors contribute to the innate immune response of *Drosophila* towards enhancing host defense against parasitic nematode infection, and increasing organismal survival. Many of these mechanisms and molecules are not specific to the anti-nematode defense, but provide protection against bacterial infections as well.

## 3. The role of the TGF-β pathway in the *Drosophila* antibacterial response

Similar to the anti-nematode defense regulated by the TGF-β pathway, the antibacterial defense from this conserved signaling cascade begins with the stimulation of specific ligands (Fig. 3). Though the production of the TGF-β ligand *Dpp* is normally stimulated by injury – in which the infecting bacteria are introduced, or the injury remains sterile – the ligand *Daw* is dependent on the Gram-positive or Gram-negative status of the infecting bacteria. While Gram-positive bacteria (like *Micrococcus luteus*) activate *Daw* ligand production, Gram-negative bacteria (like *Escherichia coli*) repress *Daw* production, indicating the importance of membrane lipopolysaccharide in the determination of the immune response (Clark et al., 2011). Following activation of the corresponding receptor by these ligands and downstream signaling resulting in Activin and BMP branch gene expression, the antibacterial effects of the TGF-β signaling pathway are induced. Activation of the TGF-β

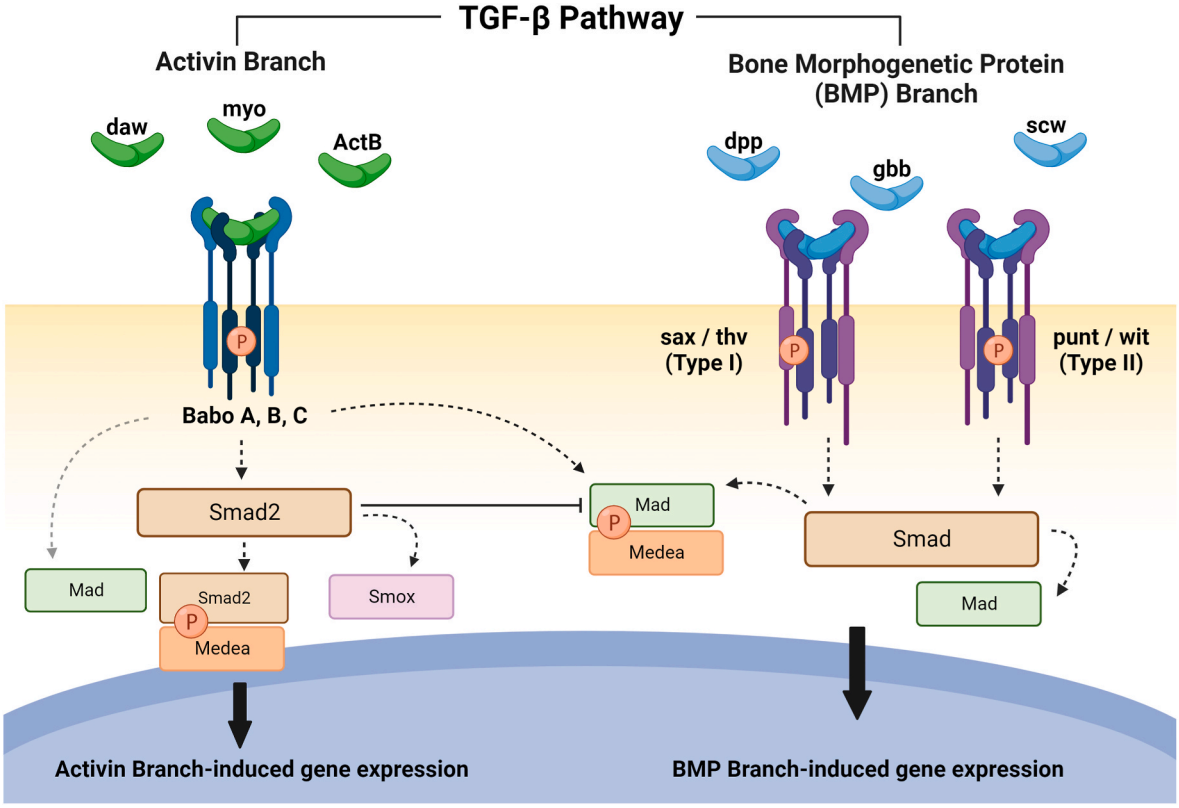


**Fig. 1.** TGF-β signaling pathways (Activin and Bone Morphogenetic Protein). The Activin branch contains the ligands Dawdle *(*daw*)*, Myoglianin *(*myo*), and Activin-B (*ActB*)* which bind to Babo A, B, and C receptors. This initiates Smad2, Mothers against decapentaplegic (Mad), Smad on X (Smox), and Medea downstream signaling that allows for Activin branch-induced gene expression in the nucleus. The Bone Morphogenetic Protein (BMP) branch utilizes two types of receptors, sax/thv (Type I) and punt/wit (Type II) which bind to ligands Decapentaplegic (dpp), Glass Bottom Boat *(*gbb*),* and Screw *(*scw*)*. This signaling pathway also induces Smad and Mad signaling, leading to BMP branch-induced gene expression in the nucleus.

pathway ligand *Dpp* leads to a decrease in the production of AMPs, while flies deficient in *Dpp* show continual AMP production even when the size of the wound is minimal (Clark et al., 2011). The decrease in AMP production by *Dpp* following injury is thought to be a preventative measure to regulate inflammation, particularly during sterile injury in which the need for AMPs would be significantly reduced (Clark et al., 2011). This regulation by *Dpp* is one example of the inflammation modulation by the TGF-β pathway; while inflammation can be a powerful mechanism in combating infection or responding to injury, inflammation in excess can be detrimental for organismal survival. In addition to the regulation of signaling events in *Drosophila*, certain immune functions such as the melanization process are also impacted by the TGF-β ligands. The melanization response is one of the hallmark signs of injury in *Drosophila,* characterized by a darkening of the cuticle in the ruptured or injured area (Ozakman et al., 2021). Together with serine proteases such as *Serine protease 7 (Sp7),* ligands of the TGF-β pathway such as *Daw* are critical regulators of melanization, specifically as it relates to discerning sterile from infectious injury (Clark et al., 2011). While *Daw* expression has been shown to limit melanization activity through the inhibition of *Sp7* in adult flies, overexpression of *Daw* can trigger the melanization response in an infection-induced manner even during sterile injury (Clark et al., 2011). Thus, the delicate balance and co-regulation of TGF-β ligands and serine proteases in *Drosophila* is instrumental for the regulation of innate immune responses to injury and bacterial infection.

## 4. The impact of the TGF-β pathway on *Drosophila* metabolism during nematode or bacterial infection

Although many of the immune functions of the *Drosophila* TGF-β pathway against pathogenic infection are pathogen-specific, metabolism has shown to be impacted by the TGF-β pathway uniquely during nematode and bacterial infection (Figs. 2 and 3). Recent research has indicated that the ligands of the TGF-β pathway, particularly *Dpp* and *Daw*, have crucial roles in the regulation of key metabolic indicators, such as lipid droplet size and glycogen levels, in *Drosophila* larvae responding to parasitic nematode infection (Ozakman et al., 2020). During nematode infection, lipid droplet size and glycogen production levels are both elevated in *Daw*-mutant larvae, suggesting that the entire pathway or certain components singularly participate in altering the metabolic rate of *Drosophila* as a means of defending against parasitic nematode infection (Ozakman and Eleftherianos, 2019). Similar to the

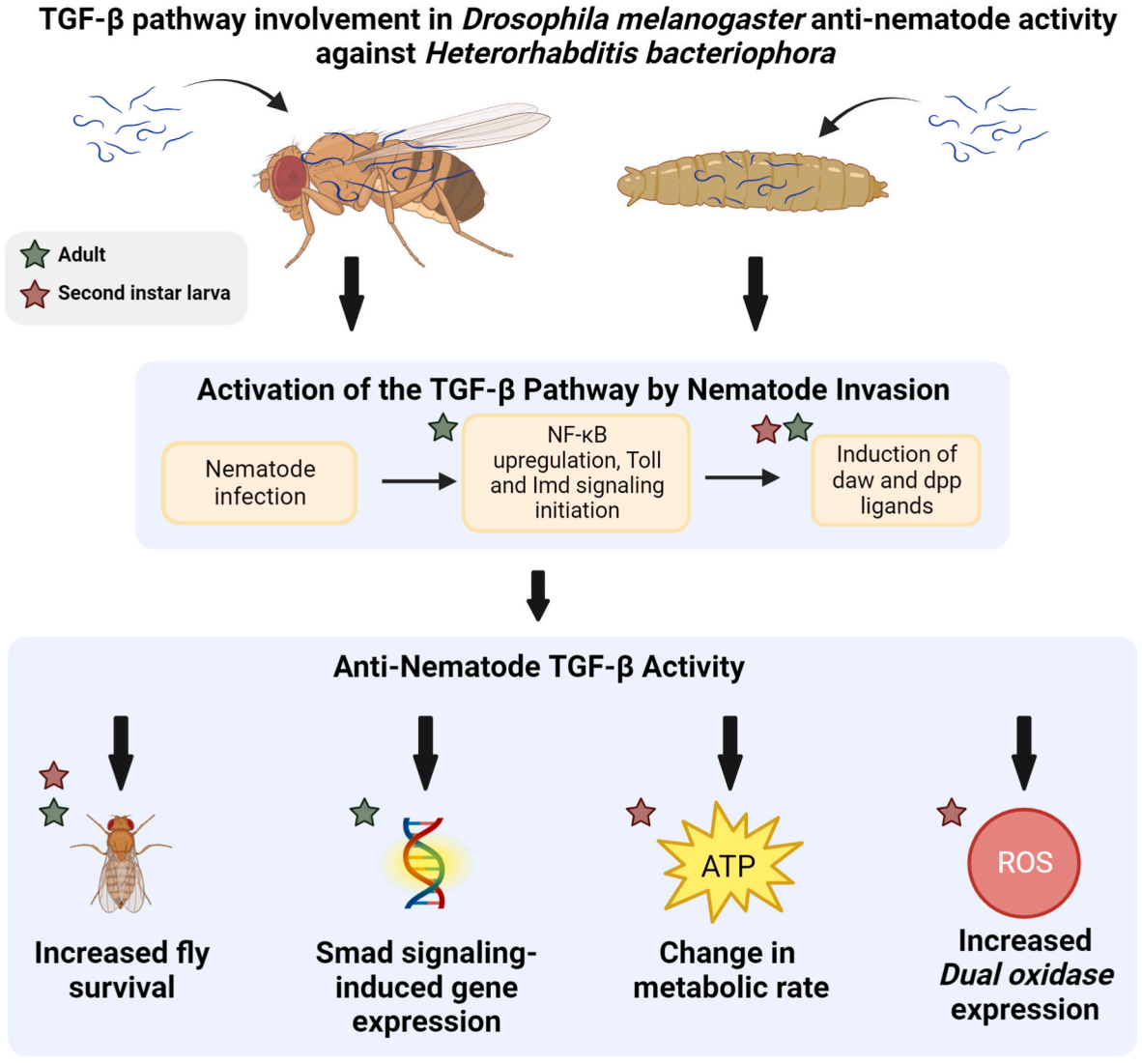


**Fig. 2.** Role of TGF-β signaling in the *Drosophila* anti-nematode response. A series of signaling events are triggered after initial nematode entry into the organism, with NF-κB upregulation spurring the initiation of Toll and Imd signaling, which activates the TGF-β ligands *Daw* and *Dpp*. Once activated, TGF-β signaling is implicated in four primary responses to nematode infection: Increased fly survival, Smad signaling-induced gene expression, changes in metabolic rate, and increased *Dual oxidase* expression. Each of these responses is critical to the defense of the organism against the parasitic nematode infection.

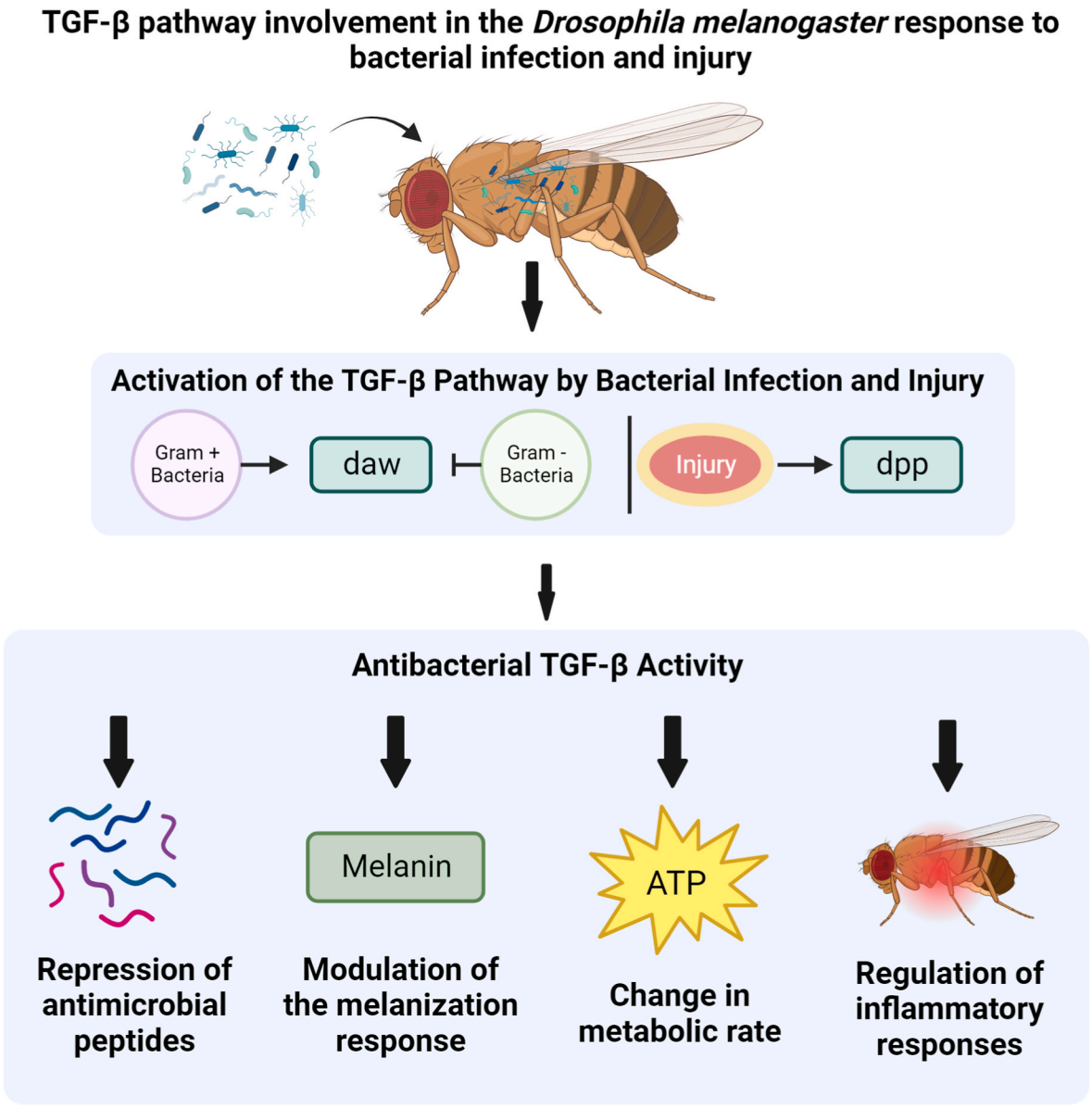


**Fig. 3.** Role of TGF-β signaling in the *Drosophila* antibacterial response. The TGF-β pathway ligands dpp and daw are activated differently during bacterial infection, with dpp activated by injury and the daw ligand activated by Gram-positive bacteria (*Micrococcus luteus*) but repressed by Gram-negative bacteria (*Escherichia coli*). Once activated, the TGF-β pathway is implicated in four primary responses to bacterial infection: Repression of antimicrobial peptides, modulation of the melanization response, changes in metabolic rates, and regulation of inflammatory responses. Each of these responses functions to promote organismal survival during infection.

anti-nematode response, the TGF-β pathway contributes to an altered metabolic rate during bacterial infection. Experiments with bacterial species which are harbored by EPNs, including *P. luminescens* and *Xenorhabdus nematophila*, showed differences in lipid droplet size and glycogen levels indicative of metabolic rate, implying that the *Drosophila* larval immune response regulating metabolic changes may be species-specific (Ozakman and Eleftherianos, 2019). Previous research has demonstrated that the antibacterial response in *Drosophila* larvae may also involve metabolic changes, with cytosolic lipid-bound histones aiding in the elimination of Gram-positive and Gram-negative bacteria (Ozakman and Eleftherianos, 2019). The TGF-β pathway has a tremendous role in the regulation of the metabolic processes that fine-tune the host defense against bacterial and nematode pathogens in *Drosophila*, and the implications of future research into this signaling pathway and its impact on insect immunity provide essential insight into the network of signaling molecules and their immunological properties.

## 5. Conclusions and perspectives

The research findings reported herein are critical for integrating our current understanding of the regulation and functional role of TGF-β signaling components in the host response against nematode parasites and bacterial pathogens. This information has also defined a cooperation between TGF-β signaling and innate immune signaling, including the NF-κB pathways in *Drosophila* during parasitic nematode infection. These are pivotal concepts that apply not only to *Drosophila* or other insects or invertebrates, but also to the mammalian innate immune system, and it would be of paramount importance to explore whether the TGF-β pathways participate in the host defense against other pathogens such as viruses and fungi. Therefore, results from this research will resolve some of the unanswered questions in host anti-pathogen immune signaling regulation and function and may help clarify the molecular connection of these immune processes with host metabolism.

Finally, this knowledge will offer further interpretation on the biological role of conserved TGF-β signaling molecules as regulators of host anti-pathogen innate immunity and therefore is expected to contribute to the development of novel strategies for treating bacterial and nematode infections in humans.

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## CRediT authorship contribution statement

**Ashley Bastin:** Conceptualization, Investigation, Writing – original draft, Visualization. **Ioannis Eleftherianos:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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