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Louise Huot, Audrey Bigourdan, Pierre-Alain Girard, Sylvie Pages, Nicolas Negre, et al.. *Spodoptera frugiperda* immune response to the nematobacterial complex *Steinernema carpocapsae*-*Xenorhabdus nematophila*. Conférences Jacques Monod "Immunologie Intégrative des Insectes: Contrôle des Infections", Jun 2019, Roscoff, France. hal-02166484

**HAL Id: hal-02166484**

**<https://hal.science/hal-02166484>**

Submitted on 5 Jun 2020

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# Spodoptera frugiperda immune response to the nematobacterial complex *Steinernema carpocapsae-Xenorhabdus nematophila*

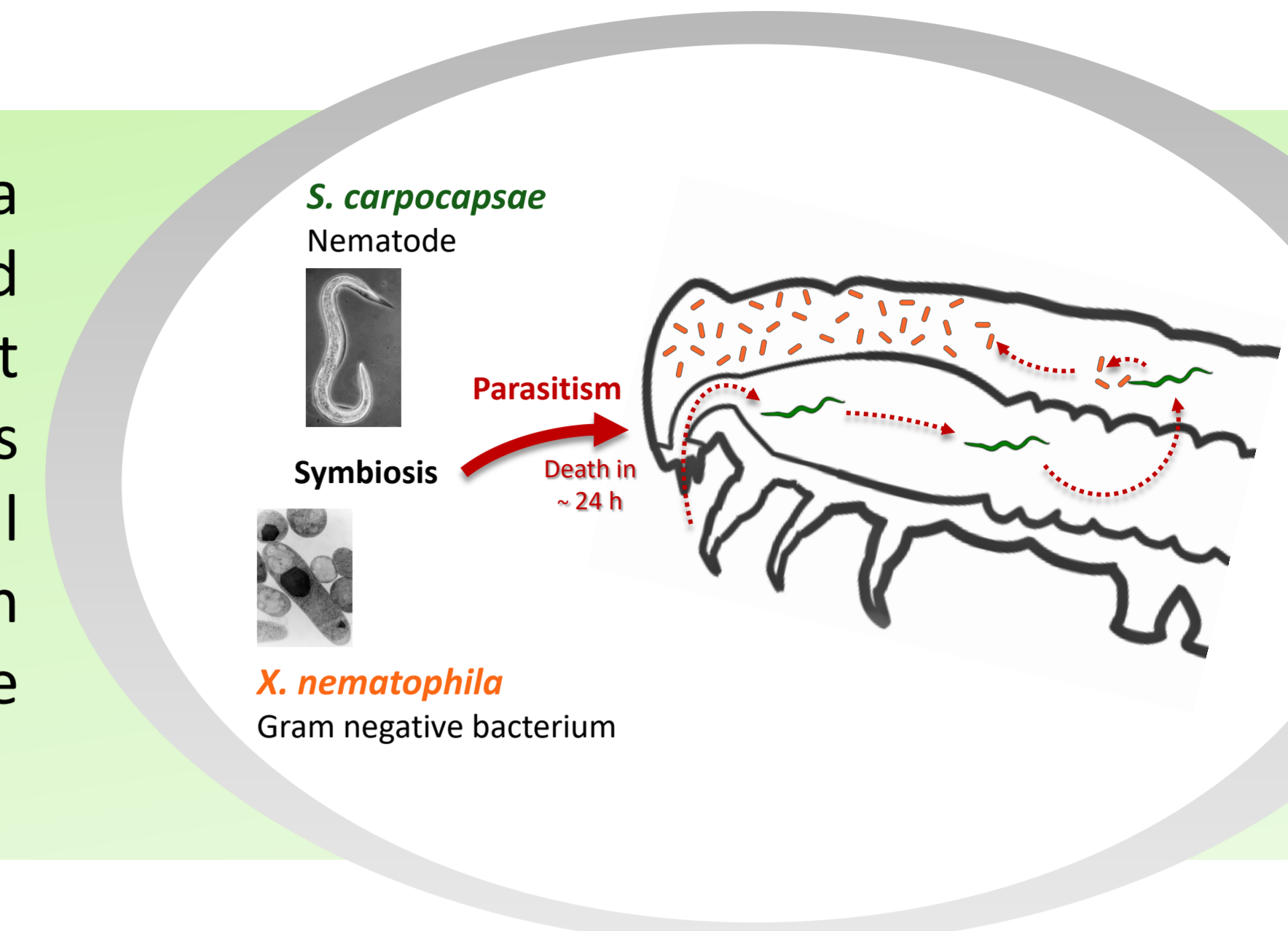
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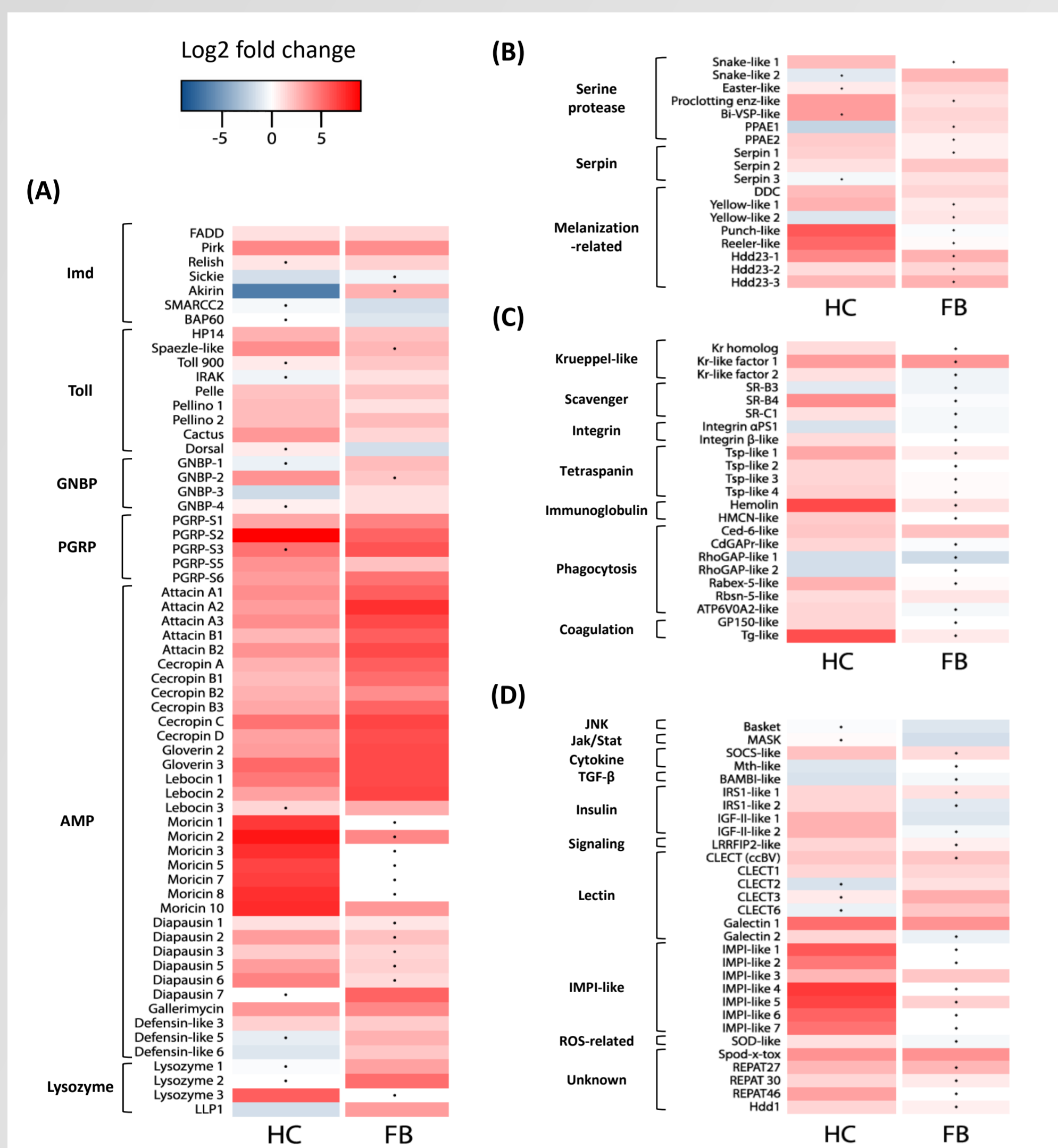
## Introduction

The *S. carpocapsae-X. nematophila* nematobacterial complex (NBC) is a natural symbiotic association between a nematode and a bacterium that is pathogenic for insects. The nematode infests its hosts via the intestinal tract and releases its bacterial symbiont in their hemocoel. The bacterium then grows extracellularly and participates in host killing by secreting virulence factors. Due to its originality and use in biocontrol, this NBC's interactions with insects have been extensively studied. However, still little is known about the insects' immune responses to the dual infection. Here, we provide a topologic and transcriptional analysis of the immune response of a lepidopteran model, *Spodoptera frugiperda*, to the NBC. Our main goals are (i) to describe the structure of the immune response that is induced after infestation and (ii) to identify candidate genes that are likely to interact with each NBC partner.



## Methodology & Results

### 1 RNAseq on immunocompetent tissues

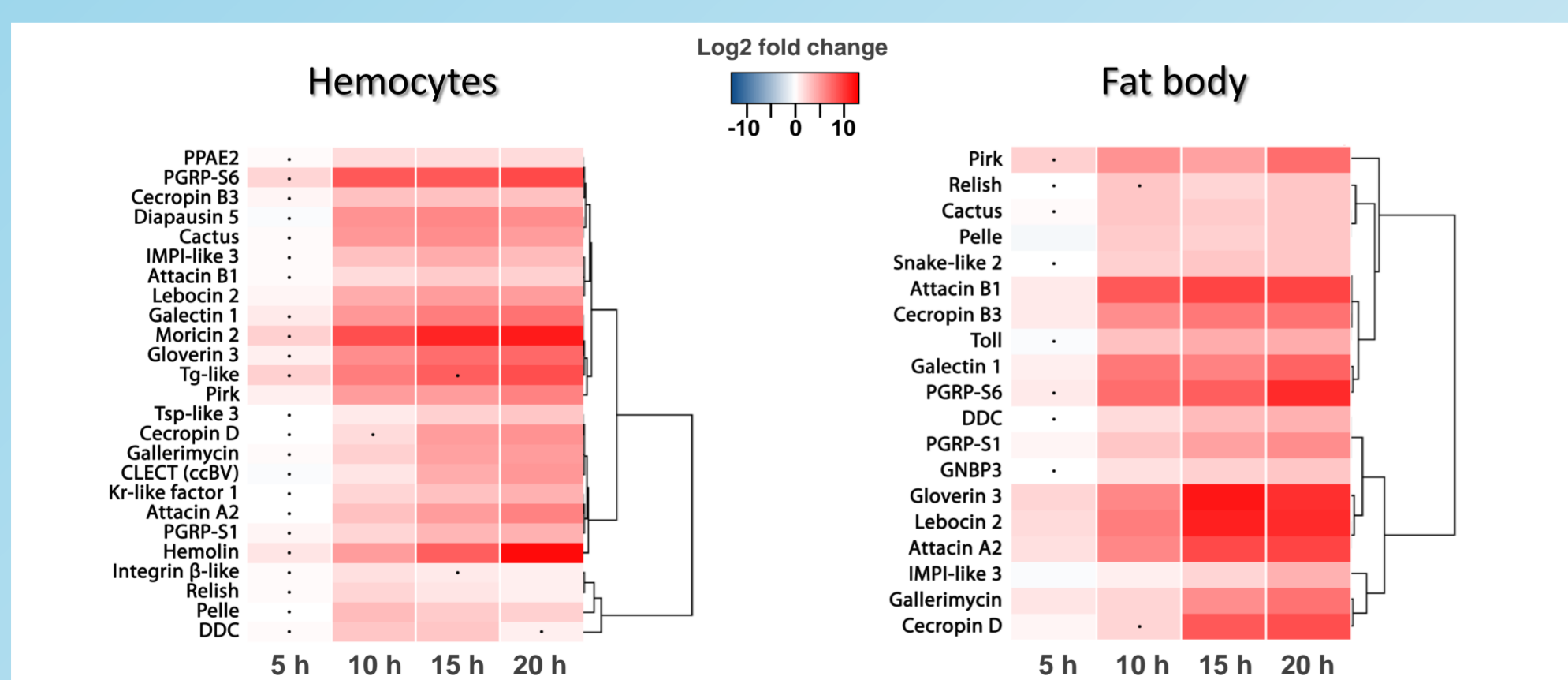


Immune genes expression variations in the hemocytes and in the fat body at a middle time point of 15 h post infestation

Triplicate RNAseq data were analyzed with Kallisto/Sleuth using thresholds of 0.01 for p-values, -1 and +1 for log2 fold changes and 5 for pseudocount means. The immune genes were identified by homology and classified as (A) antimicrobial immunity-related, (B) melanization-related, (C) cellular immunity-related and (D) « undetermined ». Black dots indicate genes with insignificant variation. HC : Hemocytes, FB : Fat body.

Despite only weak and variable immune responses are induced in the midgut (not shown), all the main components of the hemocytes' and fat body's immunities are strongly induced at 15 hpi. The hemocytes' response is particularly diversified at the humoral as well as at the cellular level whereas the fat body's response is more targeted against Gram negative bacteria.

### 2 Temporal RT-qPCR analysis of the immune responses

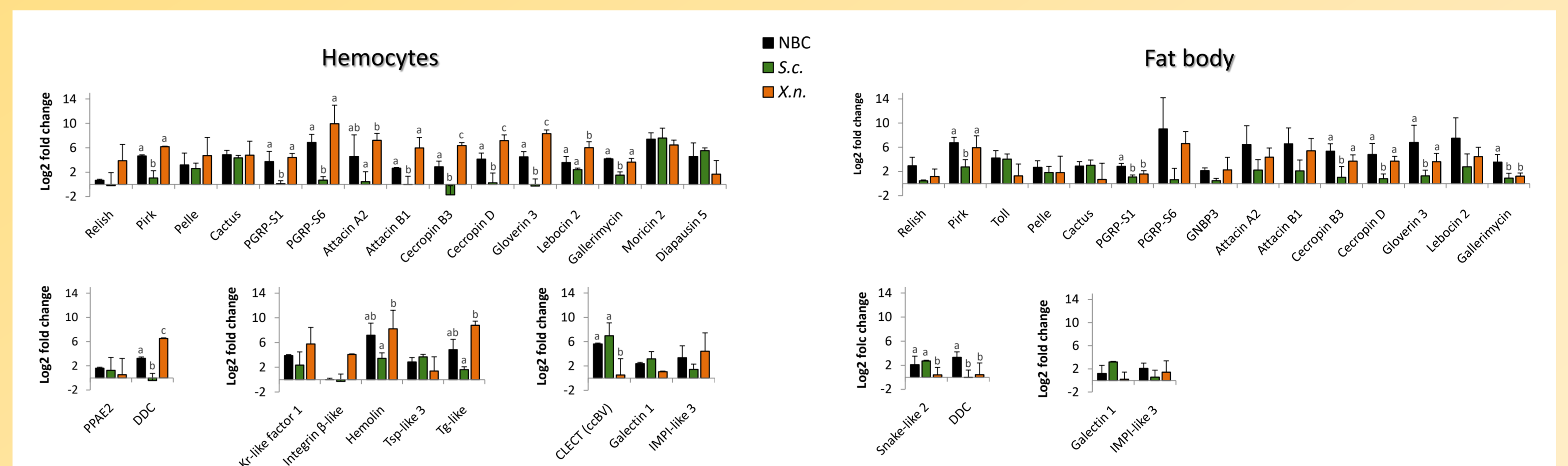


Post-infestation temporal evolution of representative immune genes' induction levels in the hemocytes and in the fat body

Relative RT-qPCR quantifications were performed on triplicate samples with the Rpl32 housekeeping gene as reference. Differential expression was assessed with Student t tests and black dots indicate genes with insignificant variations (p-value > 0.05). Clustering analyses are based on Pearson correlation coefficients.

In both tissues, the immune responses take place between 0 and 10 h post-infestation. Despite little variations, they then remain induced until the end of the infectious process.

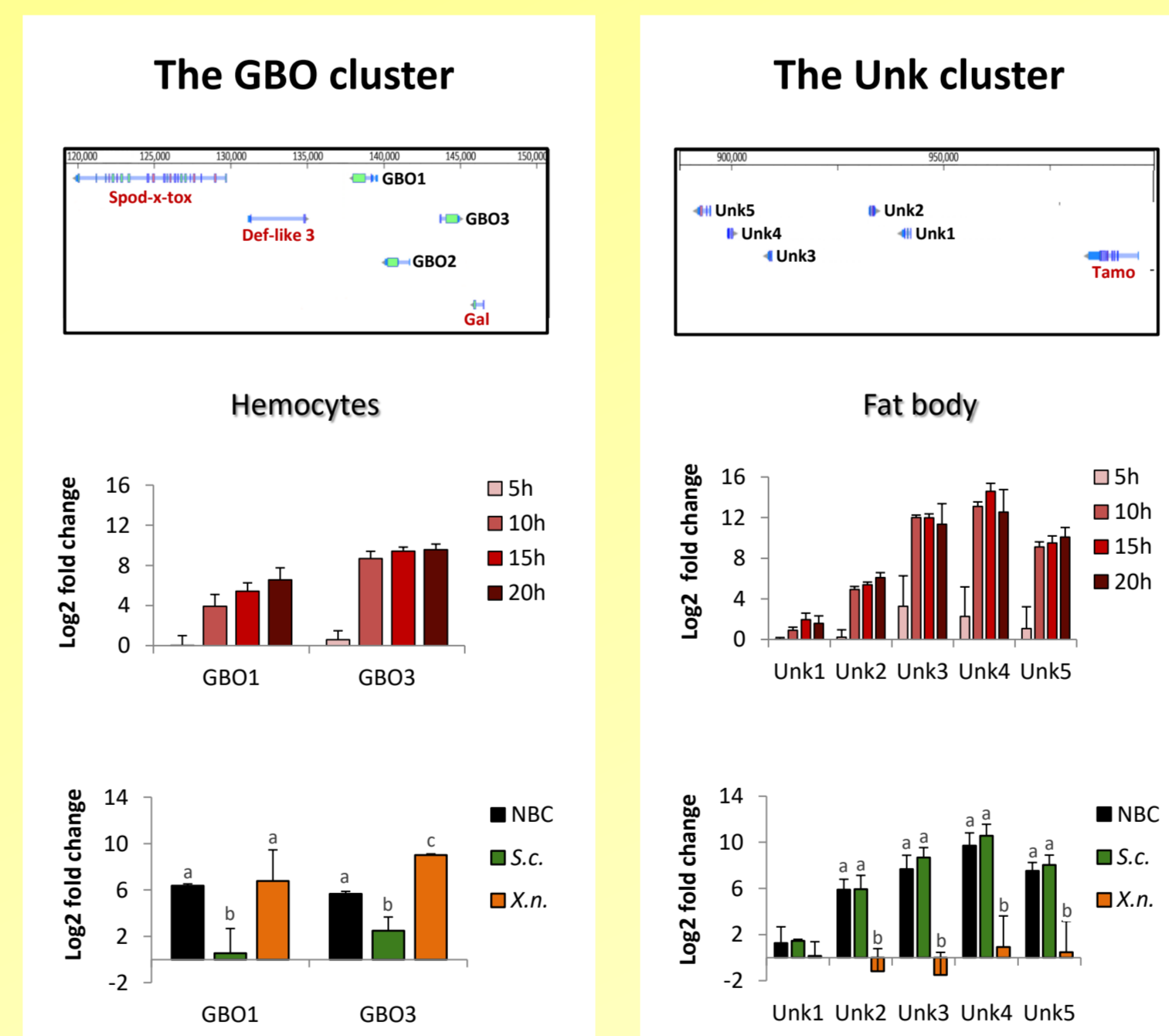
### 3 RT-qPCR dissection of the immune responses



Induction levels of representative immune genes after independent injections of the whole nematobacterial complex and of each partner. The tissues were collected 13 h after injection with either 10 nematobacterial complexes (NBC), 10 axenic *S. carpocapsae* (*S.c.*) or 200 *X. nematophila* symbionts (*X.n.*). RT-qPCR relative quantifications were performed on triplicate samples with the Rpl32 housekeeping gene as reference. Statistical differences between treatments were assessed by one-way ANOVA followed by Tukey tests. Top panels : antimicrobial immunity-related genes. Bottom panels : melanization-related, cellular immunity-related (Hemocytes only) and « undetermined » genes.

In the two tissues, *X. nematophila* is the main inducer of most of the selected genes, and especially of the well known antibacterial ones. However, *S. carpocapsae* is the main inducer of some melanization and encapsulation-related genes as well as of most lectins, which are candidate immune receptors. Overall, these results suggest that the hemocytes and the fat body both responded by adapted ways to each NBC partner.

### 4 Discovery of candidate new immune genes

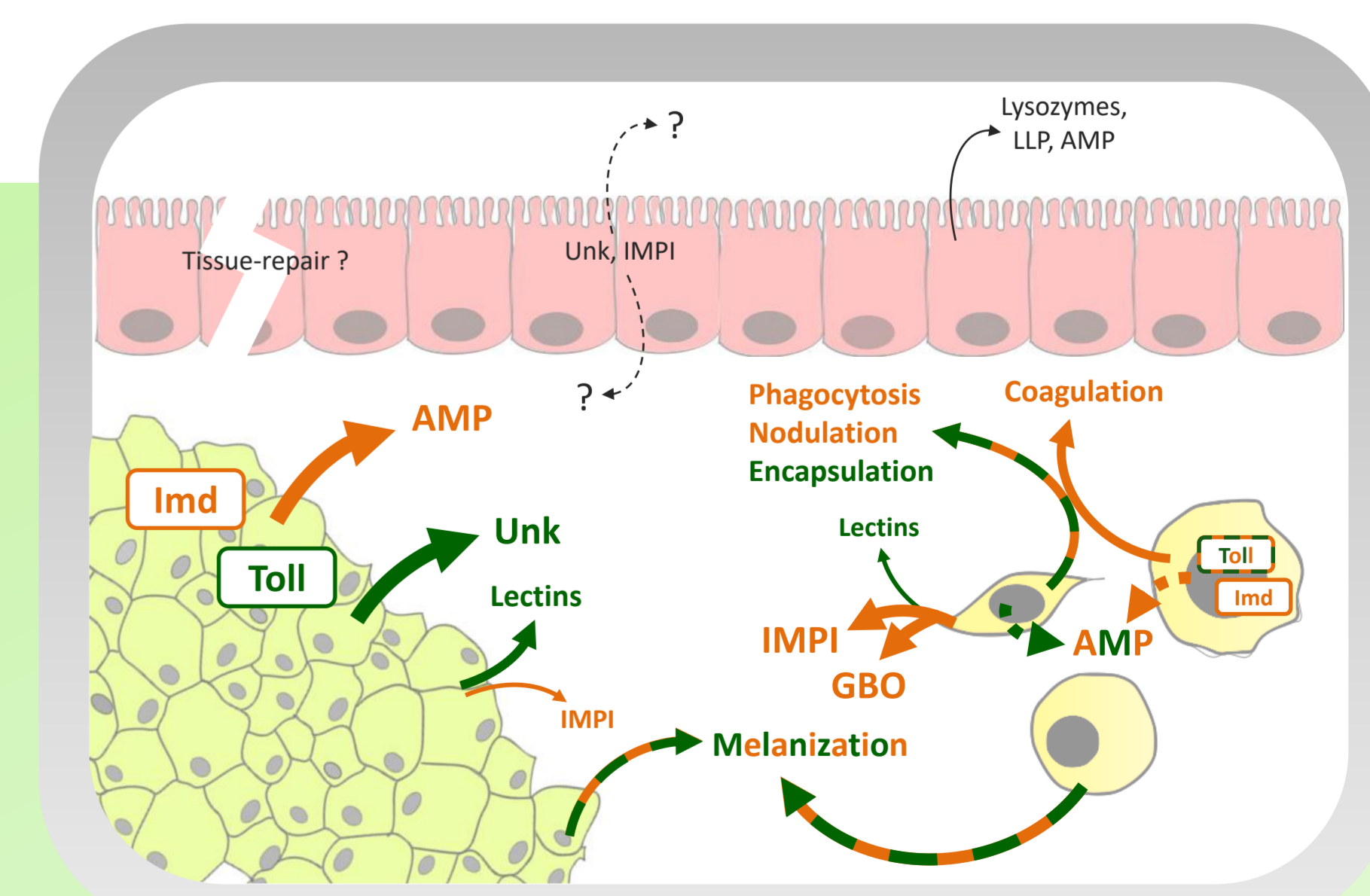


The GBO cluster is predicted to encode secreted proteins with homologs in lepidopterans and bacteria. We hypothesize they were acquired by HGT from bacteria and hijacked to perform antibacterial immune functions.

The Unk cluster is predicted to encode secreted peptides and small proteins that we hypothesize to be anti-nematode immune factors only found in the genus *Spodoptera*.

## Conclusion & Outlook

Here, we show that the hemocytes' and fat body's immunities are strongly induced after infestation by the NBC. The immune responses are diversified and correspond to mixes of bacterium- and nematode-induced ones. They involve many genes that could take part in the response to each partner, including the Unk and GBO ones, which had never been reported in other interaction models. At this stage, our main objectives are (i) to characterize the interactions of these genes with the NBC and (ii) to improve our analysis of the midgut immune response.



In the longer term, our goal is to get an accurate picture of the dialogue that takes place between the three members of the interaction in order to identify the precise causes of the immune system's failure.