



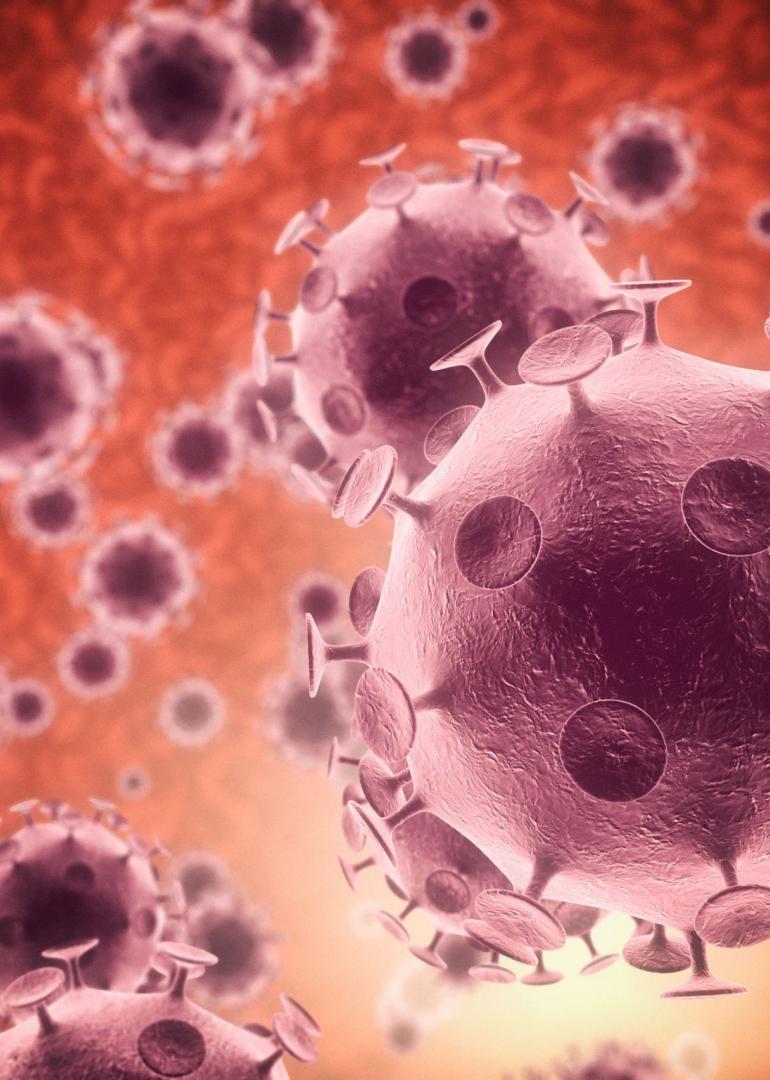
UPLEM
Lemaitre Lab

16th December 2024

**Functional
characterization
of two serine
protease clusters
and one lectin
cluster**



Lab immersion
Semester project



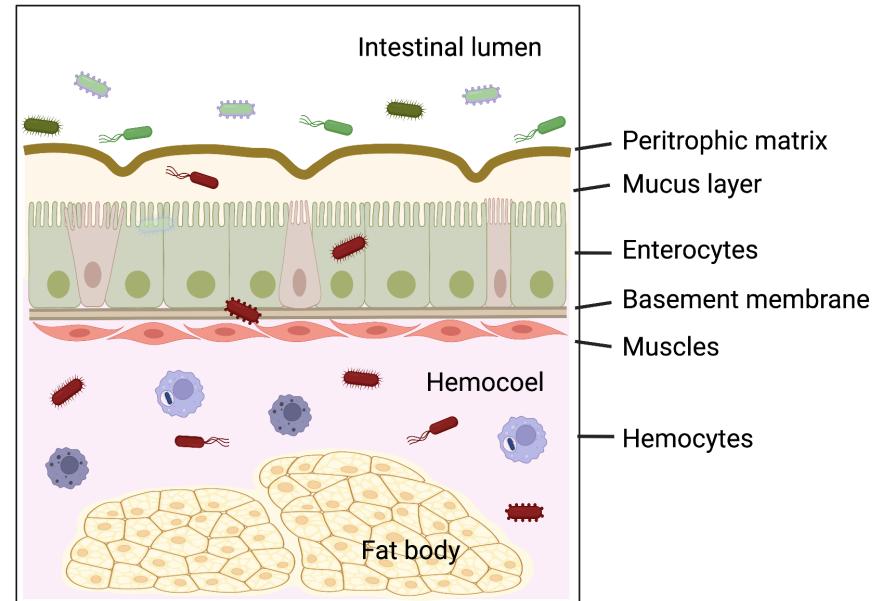
1. INTRODUCTION

Immune system in *Drosophila Melanogaster*

The Drosophila Immune System

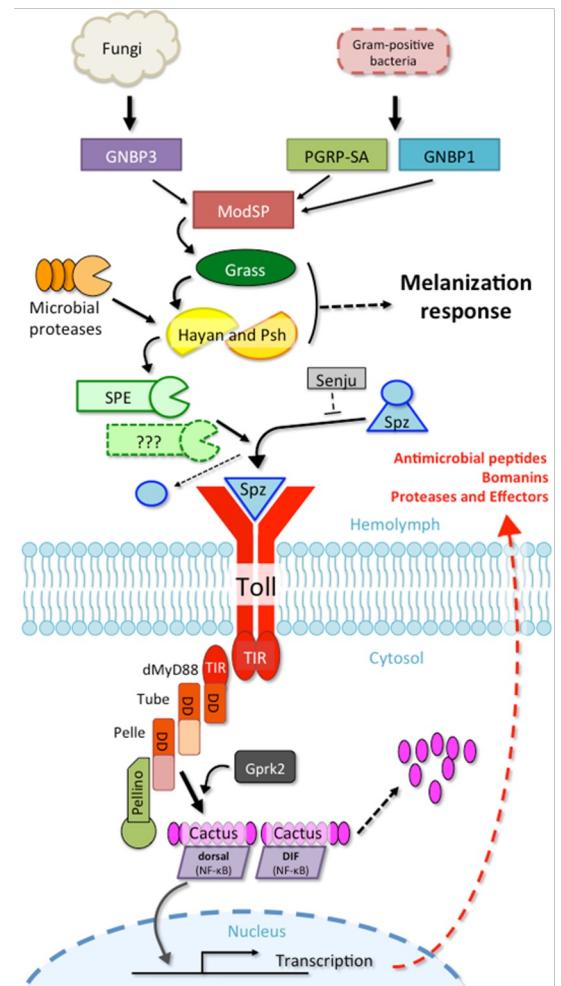


Epithelial
Cellular
Humoral

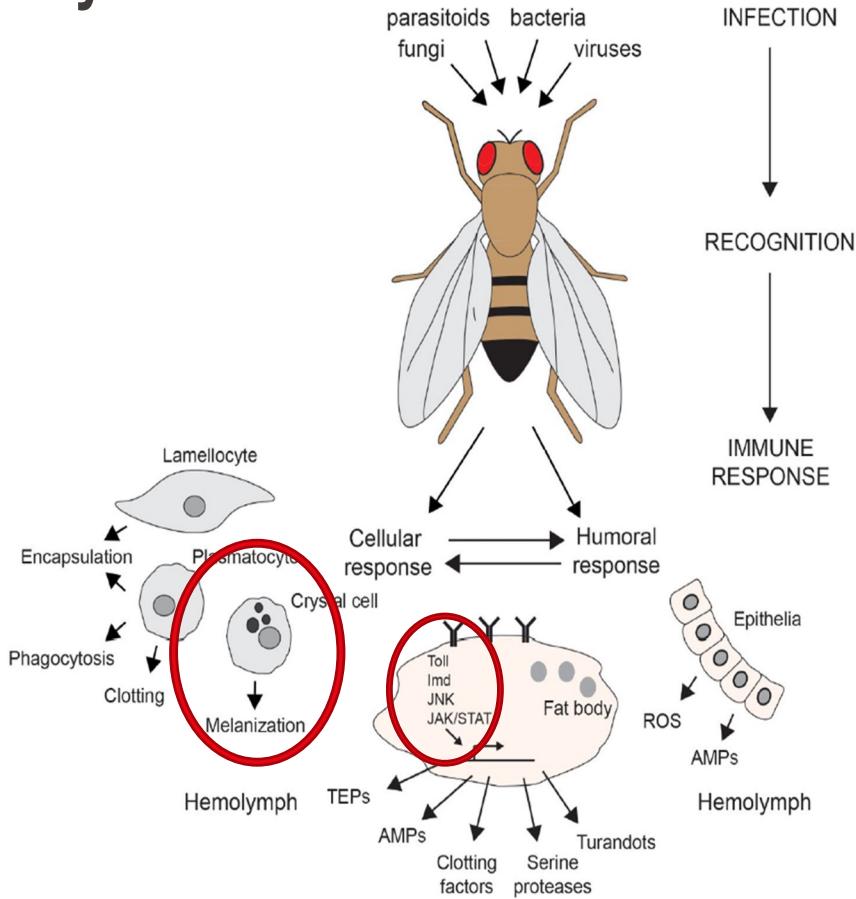


The Toll pathway in Drosophila

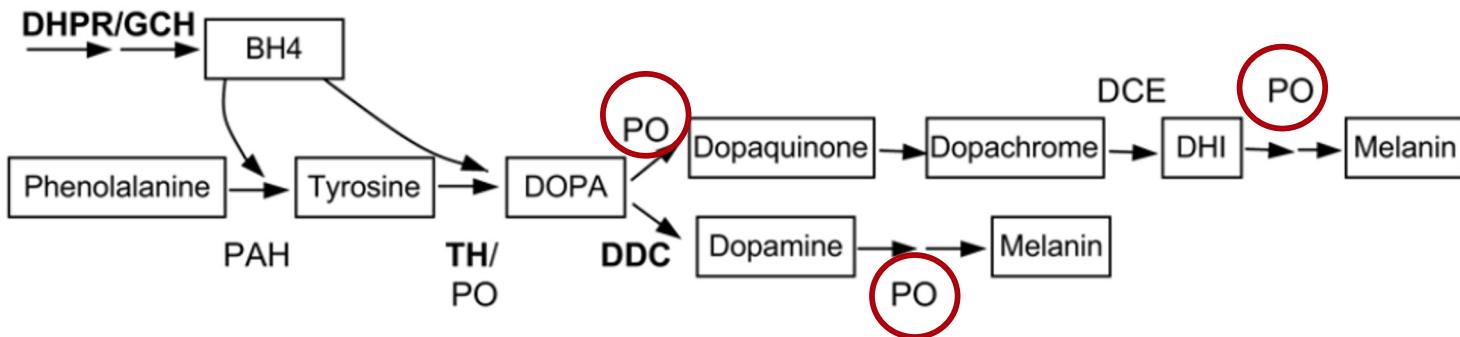
- Used to mount an **immune response** against **gram positive bacteria** and **fungi**
- Pattern Recognition Receptors (PRRs)** upon recognizing **Pathogen-Associated Molecular Patterns (PAMPs)** activate extracellular **serine protease cascades** that lead to production of **microbicidal AMPs**
- Five serine proteases (**ModSP**, **Grass**, **Hayan**, **Persephone**, **SPE**) have been identified to play a role in this **cascade**
- One branch of the pathway involving **Hayan** is activated by **microbial cell wall components**, while another branch involving **Persephone** is activated by **microbial proteases**



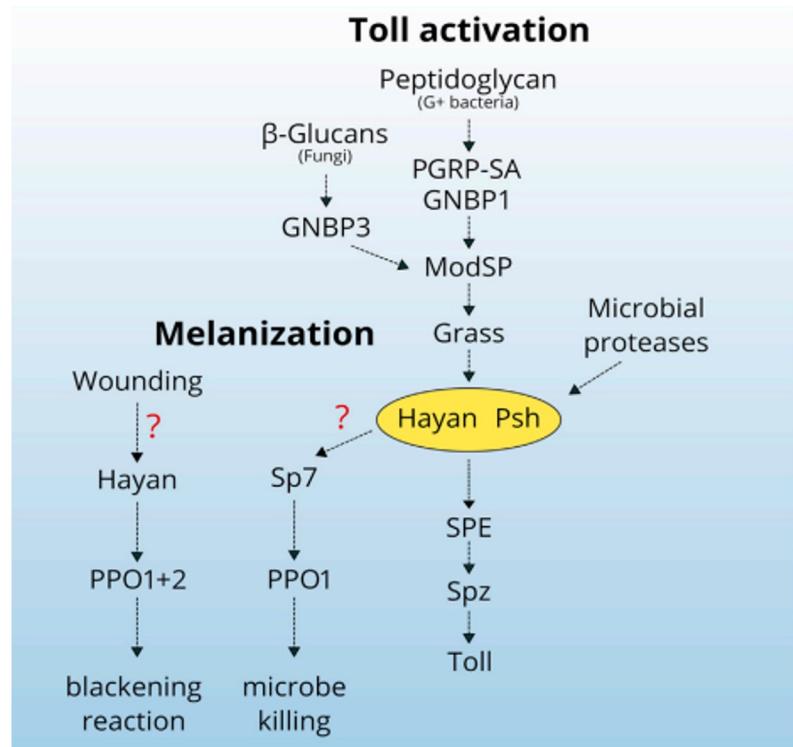
The Drosophila Immune System



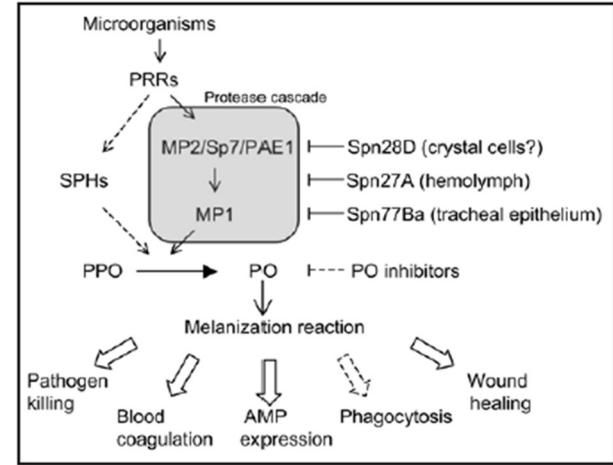
- Arthropod-specific immune response that involves deposition of melanin at wound sites
- Phenoloxidases (POs) catalyze the oxidation of phenols to quinones, which then polymerize to form melanin
- Functions in:
 - Wound healing
 - Sequestering invading microorganisms at the wound site and encapsulating parasites
 - ROS produced during the process could be toxic to microorganisms



Melanization and Serine Proteases in Drosophila



Dudzic et. al., 2019



- Upon injury, crystal cells release Prophenoloxidases (PPOs) into the hemolymph
- Their activation is tightly regulated by SPs and serpins



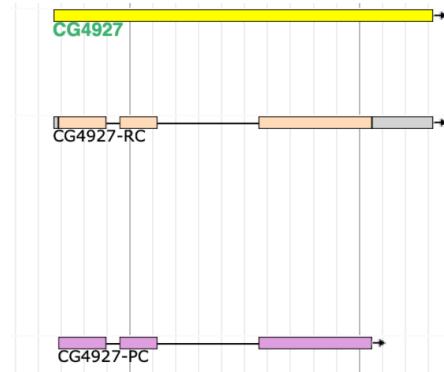
2. What we know: Insights from FlyBase and Literature

Serine protease cluster:
cSP42 (CG4927) and CG9733/37

Lectin cluster:
galC1 (CG9976) and lectin37Da/Db

Sp42 encode a trypsin like serine protease

- **Location:** 2R
- **Family:** S1A SERINE PROTEASES – TRYPSIN LIKE
- **Products:** 1 annotated transcript and 1 polypeptide



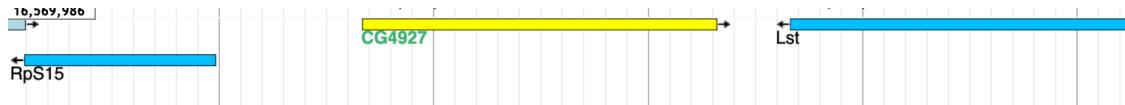
- **Structure**



Trypsin domain (start 104 end at 355)

Predicted by flybase

- **Cluster:** RpS15 (Ribosomal protein), Lst (limostatin; peptide hormone)



Sp42 is a clip-domain containing protein

(Haobo Jiang, personal communication)

- Amino acid sequence

MQVIFGILLILAVICSILSEFCDNGTGECKELSATDC
PSIFFNLHLIRNFVKYCDKSNHIVCCLPNNMQPQSQ
QFSANIGLRRFEKECRRFNEIRTSCRTTPFIVGGAKA
AGREFPFMALLGQRGKNSSQIDWDCGAIIIHPKFVLT
AAHCLETSETKEQLDPNYDGPKYVVRLGELDYNSTT
DDAQPOQDFRVLNYVVHPAYGEDDDTGSRKNDIAVVEL
EMEATFSEYVAPACLPLDGGNEQLQVAAGWGATSES
GHASSHLLKVSLDRYDVAECSQRLEHKIDVRTQLCAG
SRSTSADTCYGDGGPVFVQHPIYSCLKQVIGITSYG
LVCGVQGLPSVYTKVHLYTDWIENIVWGE

- Structure



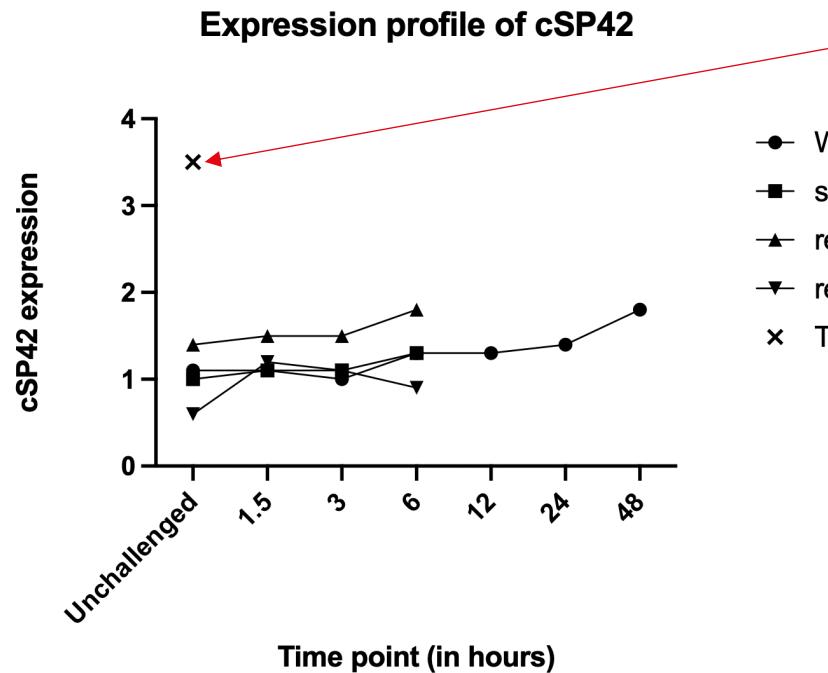
Trypsin domain (start 104 end at 355)

Predicted by flybase

→ cSP42

The underlined region includes multiple cysteines and is likely forming disulfide bonds, which are characteristic of a clip domain.

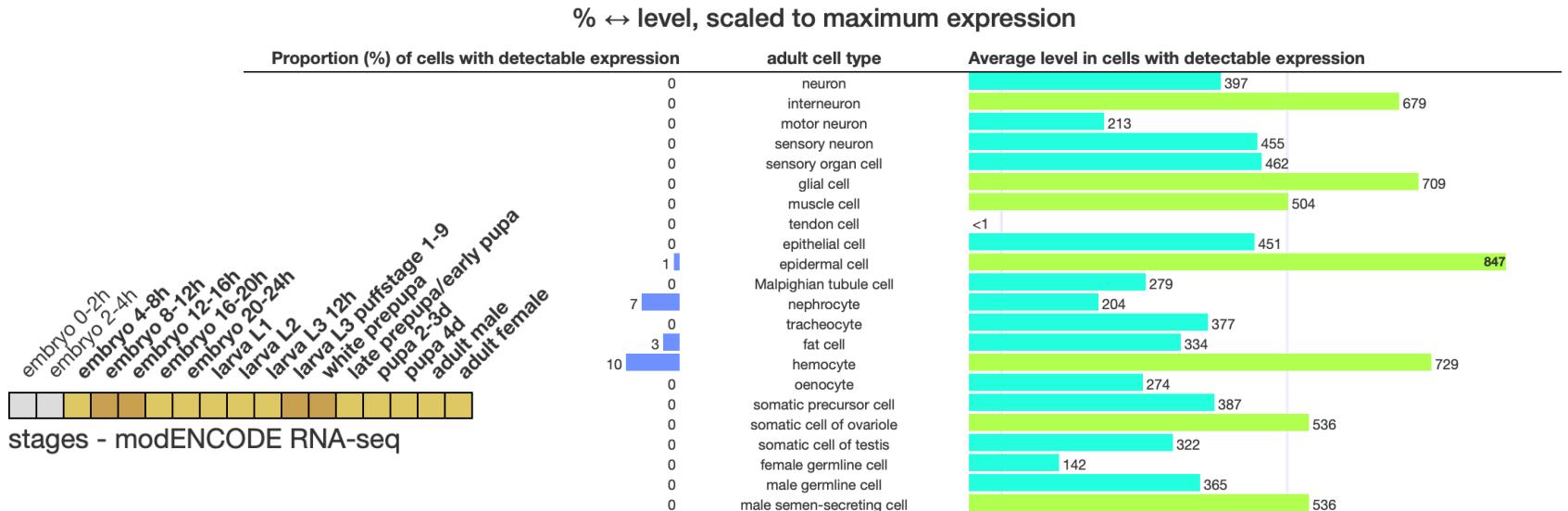
cSP42 is not induced upon septic injury with a mixture of *M.luteus* and *E.coli*



But 3 fold higher expression of cSP42 in Toll 10b mutant.

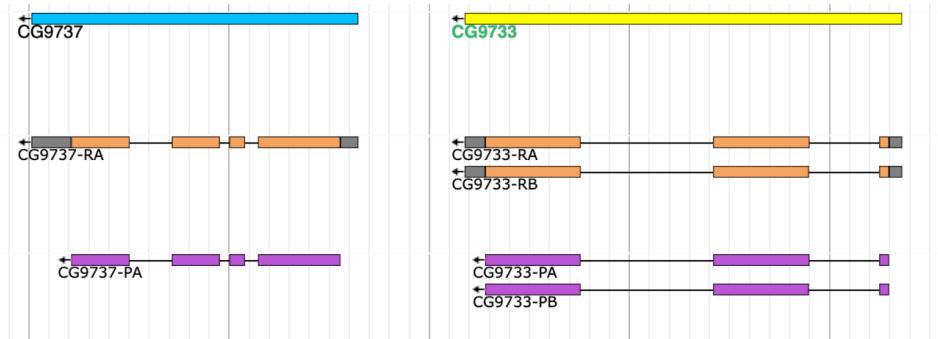
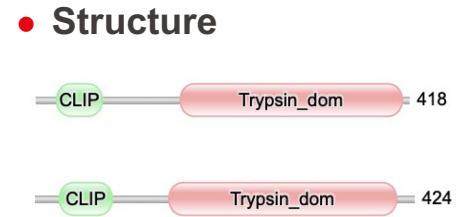
cSP42 is expressed in embryo, larvae and adults in the hemocytes (heart)

- Expression profile: modENCODE RNA-seq and FlyAtlas



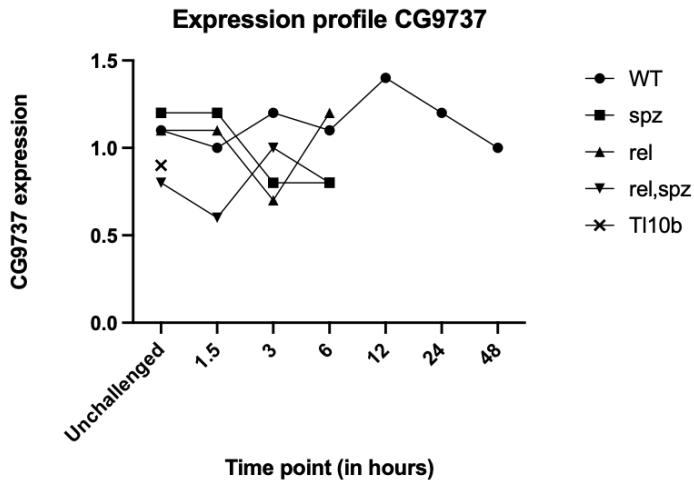
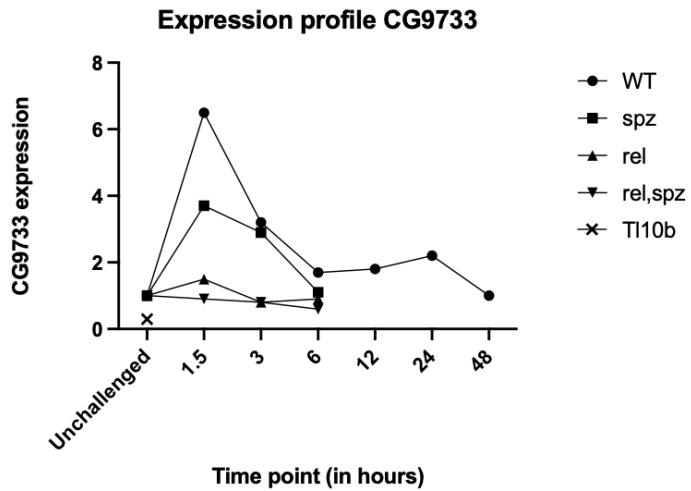
CG9733/37 encodes two clip domain SP

- **Location:** 3R
- **Family:** S1A SERINE PROTEASES – TRYPSIN LIKE
- **Products:** 1 annotated transcript and 1 polypeptide for 37 and 2 annotated transcripts and 2 polypeptides for 33

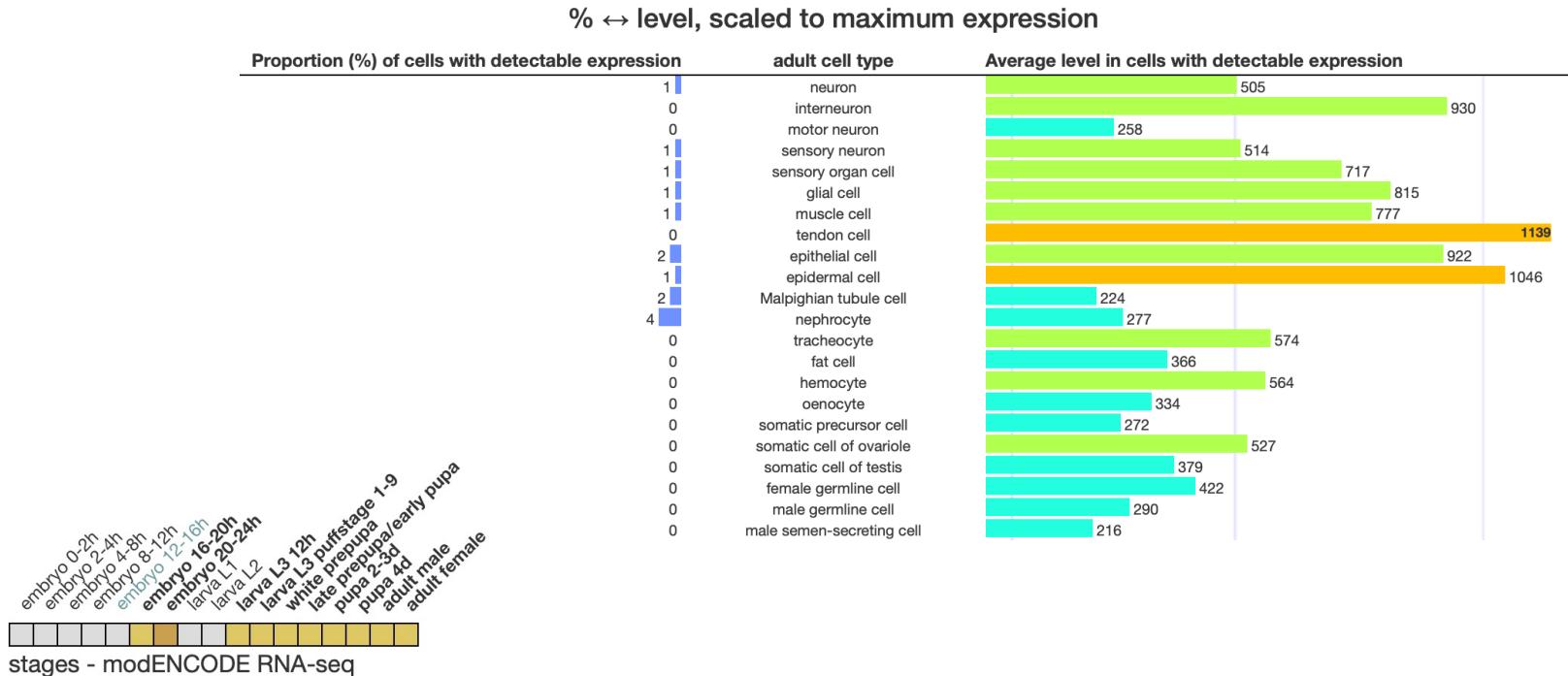


- **Cluster:** tRNA

CG9733 is expressed under an early acute phase profile upon septic injury

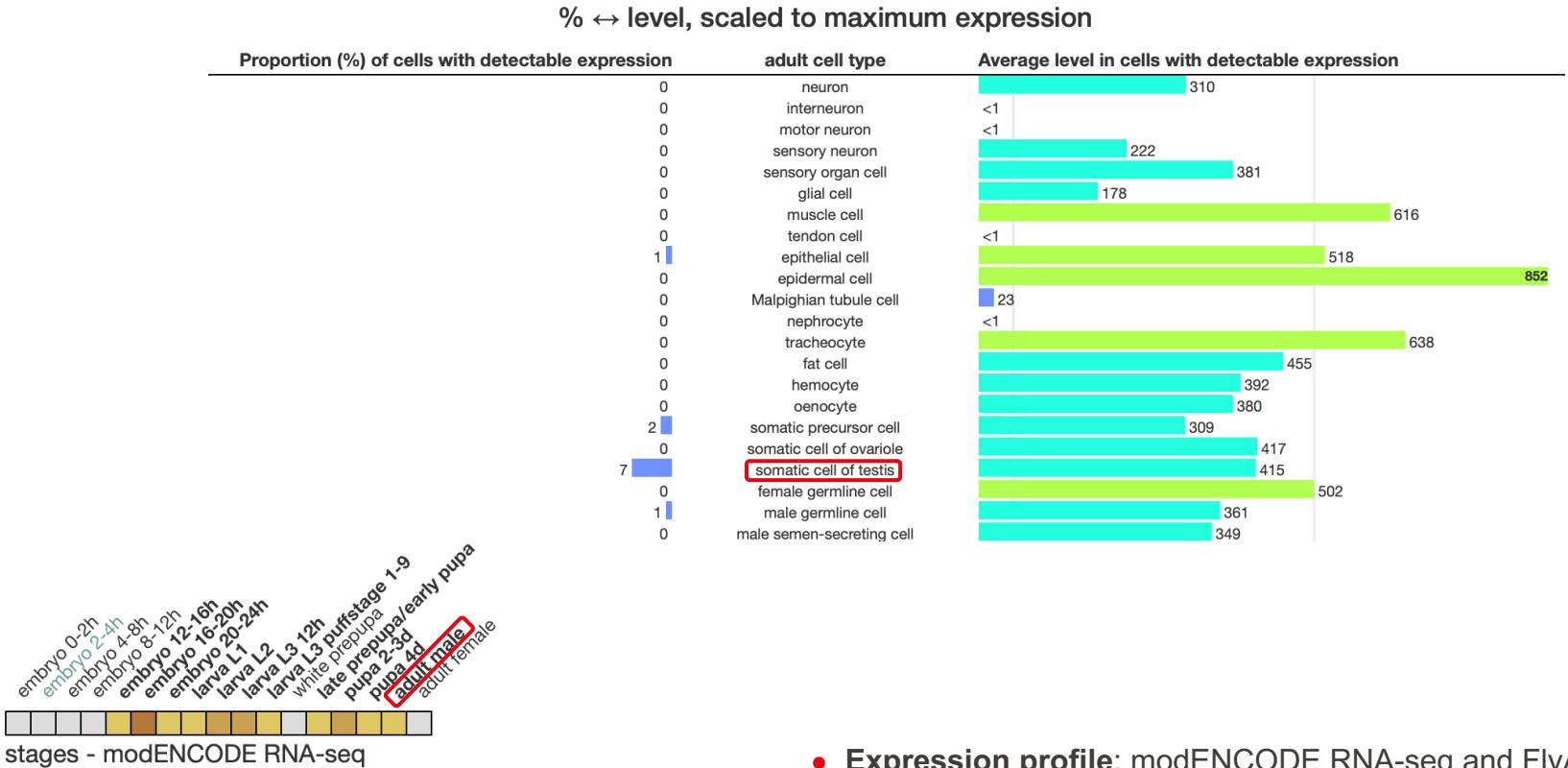


CG9733 is expressed in late stages of embryo and larvae, and in adults without specific locations



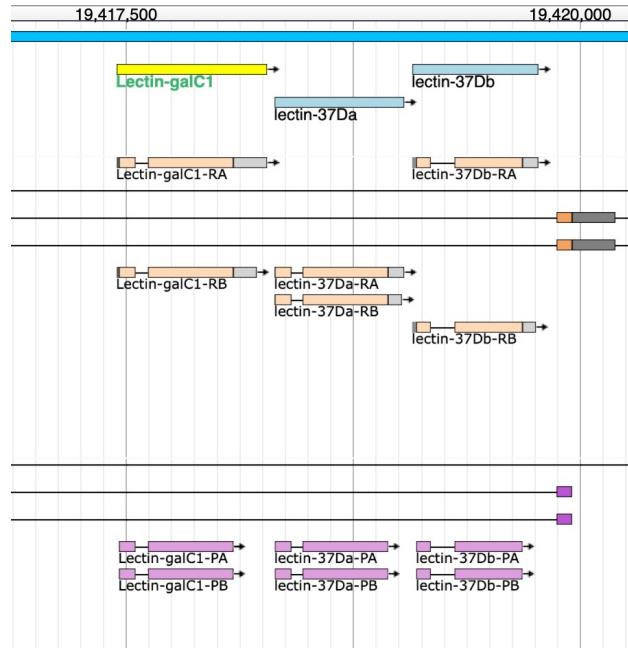
- Expression profile: modENCODE RNA-seq and FlyAtlas

CG9737 is expressed in late stages of embryo, in larvae, and in male adults



galC1 (CG9976) encodes a Galactose-specific C-type lectin

- **Location:** 2L
- **Family:** C-TYPE LECTIN LIKE
- **Products:** 1 annotated transcript and 2 polypeptide



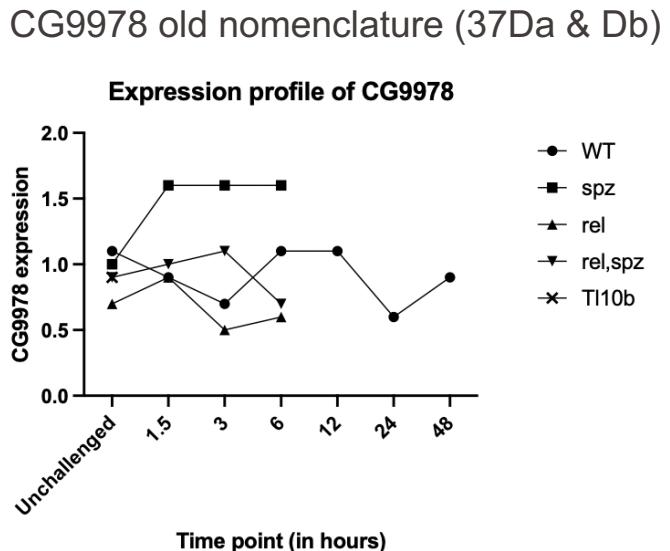
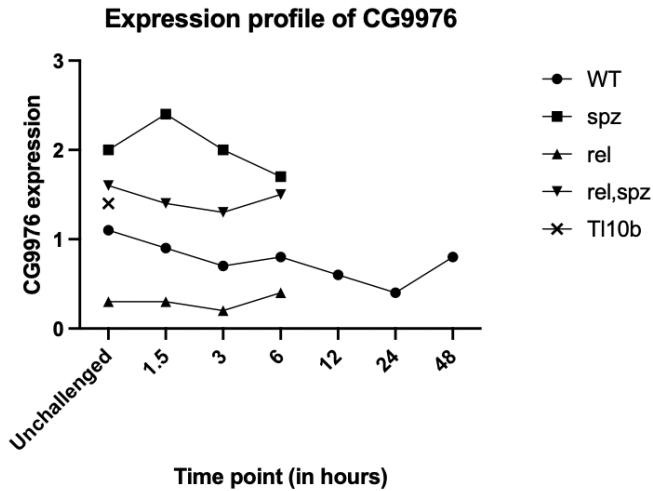
- **Cluster:** 2 other C-TYPE LECTIN LIKE (CG33533 & CG33532)

- **Structure**

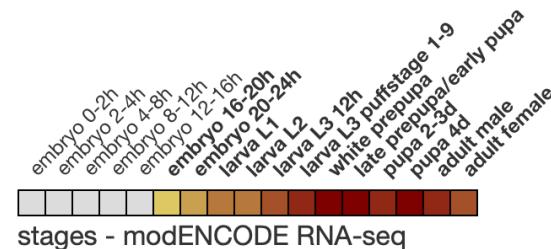
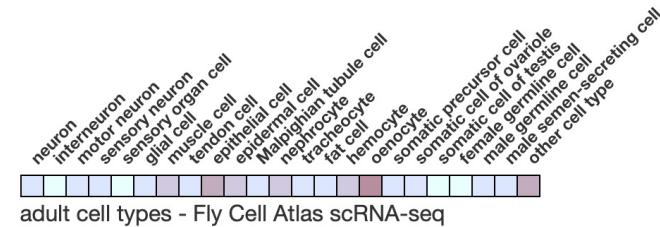


Pfam and SMART protein domains

galC1 is slightly induced under an early acute phase profile upon septic injury in spz mutant

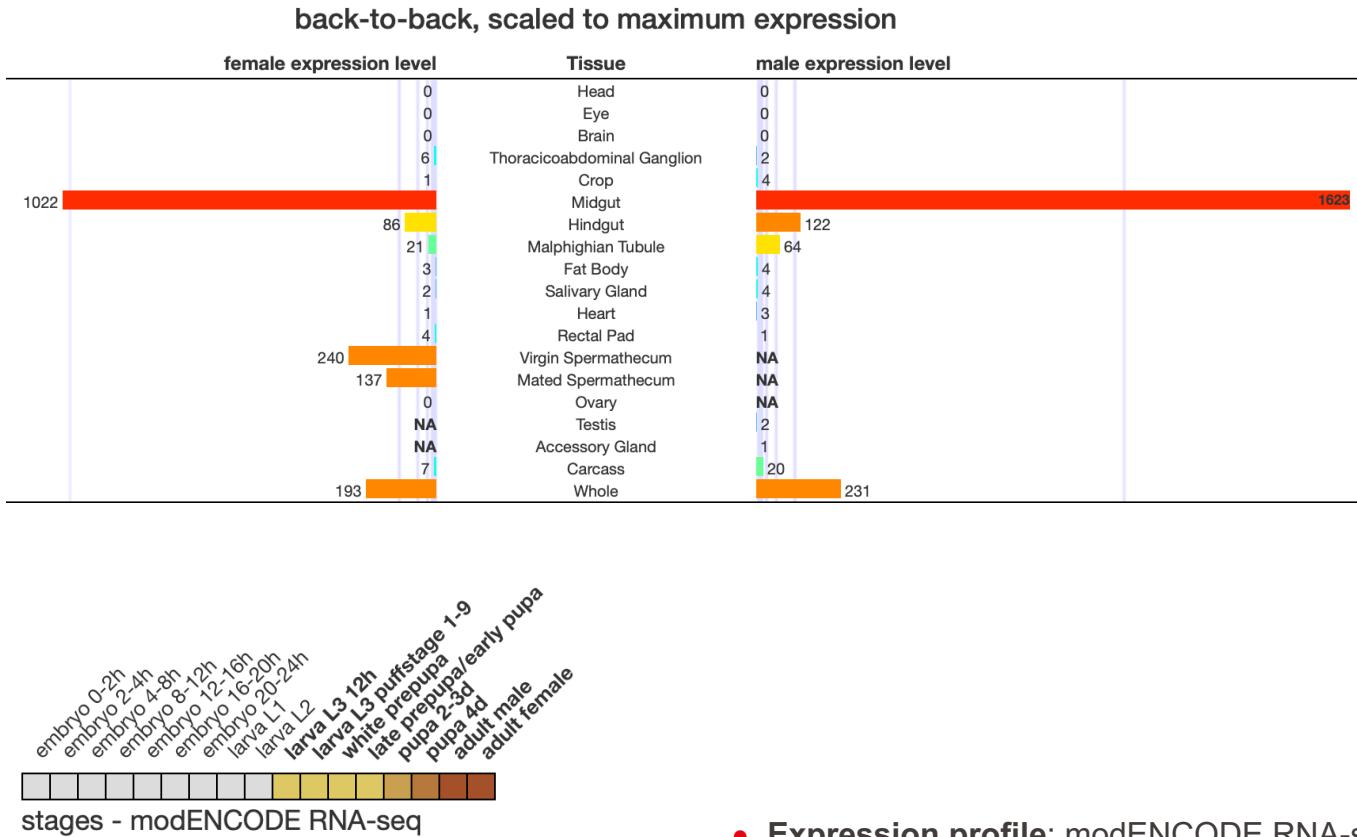


galC1 is highly expressed from late embryo to adult stage in the heart, fat bodies and carcass



- Expression profile: modENCODE RNA-seq and FlyAtlas

Lectin 37Da/Db are highly expressed in adult stage in the midgut



Hypotheses

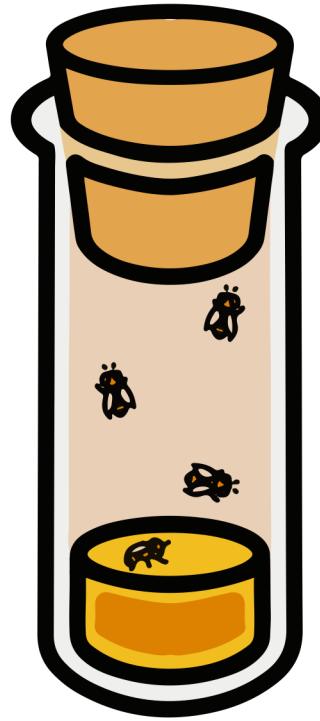
These two SP cluster cSP42
& CG9733/37 could be ...

- Regulator of the Toll pathway
- Regulation of melanization: blackening, pathogene encapsulation
- Redundant activity
- Wound healing/clotting

Hypotheses

The galC1 cluster could be...

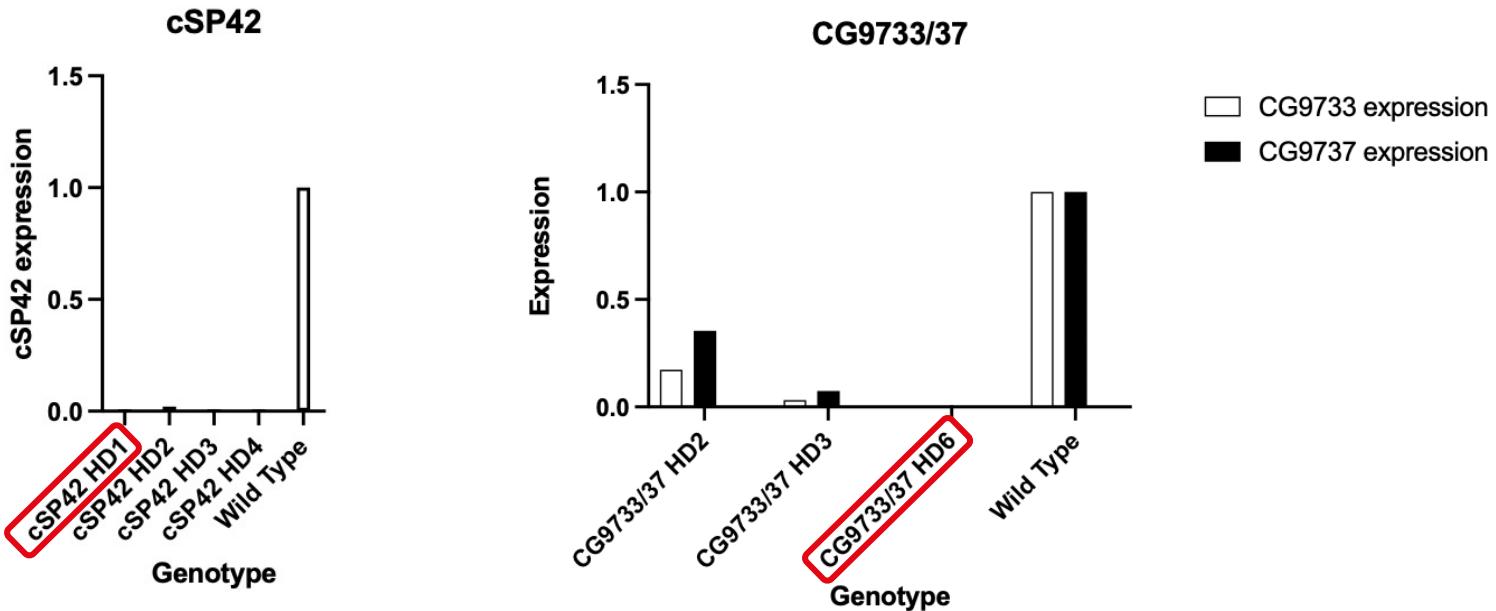
- Regulator of the Toll pathway
- Regulation of melanization: blackening, pathogene encapsulation
- Redundant activity
- Wound healing/clotting
- Binding to carbohydrates on pathogen cell walls
- Recognition of pathogen-associated molecular patterns (PAMPs)



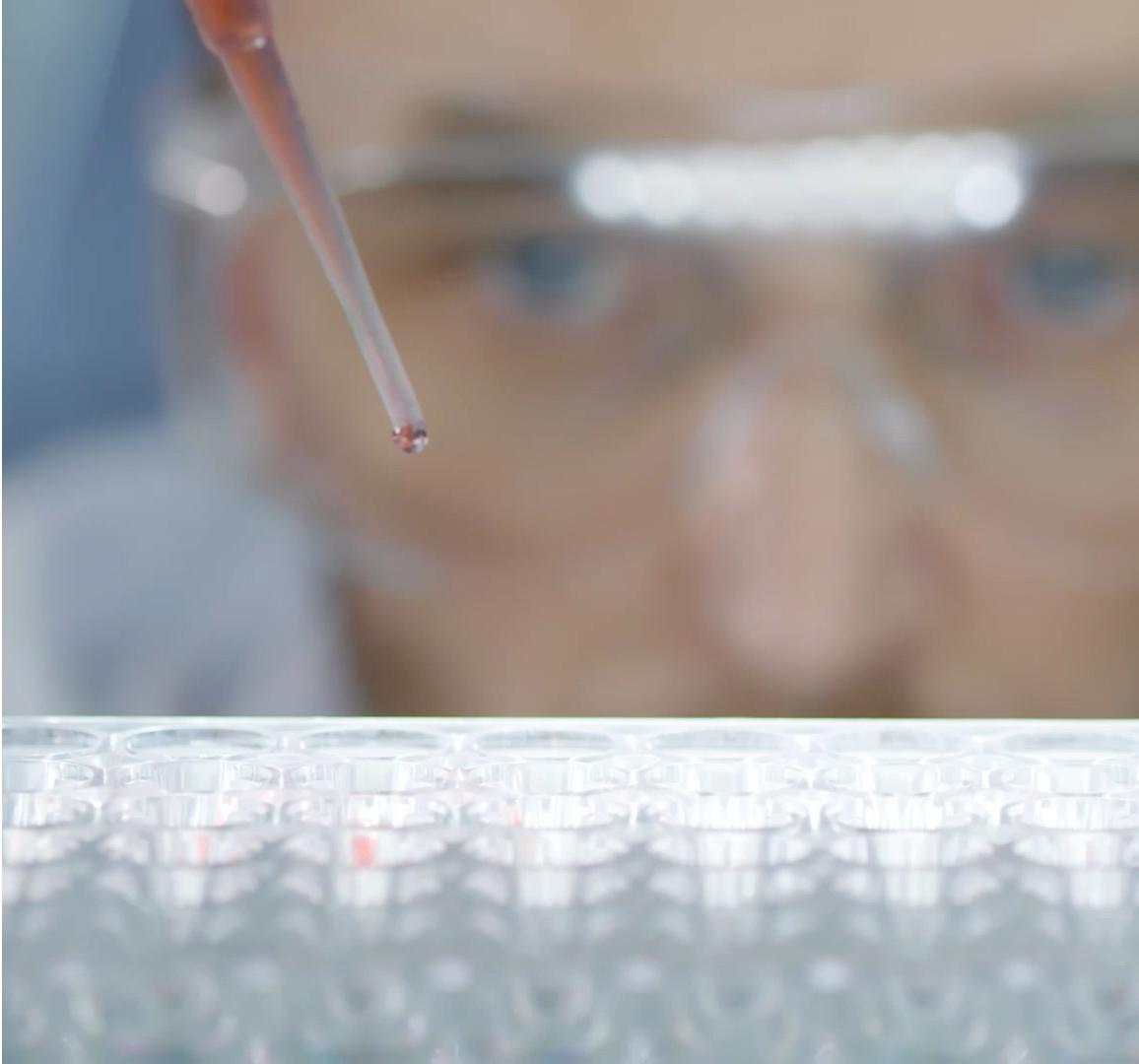
3. Tools

- W^{1118}
- cSP42 (CG4927)
- CG9733/37
- galC1 (CG9976 – CG33533 – CG33532)
- CG11841-43 (do not work yet)
- PPO1 – PPO2
- Spätzle
- Bom^{55C}

Generation of CG9733/37 and cSP42 null mutants

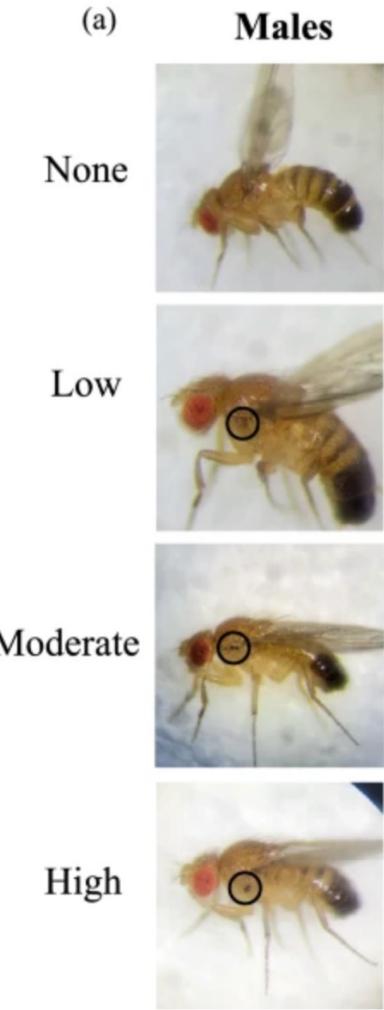


CG9733/37 HD6 and Sp42 HD1 were selected



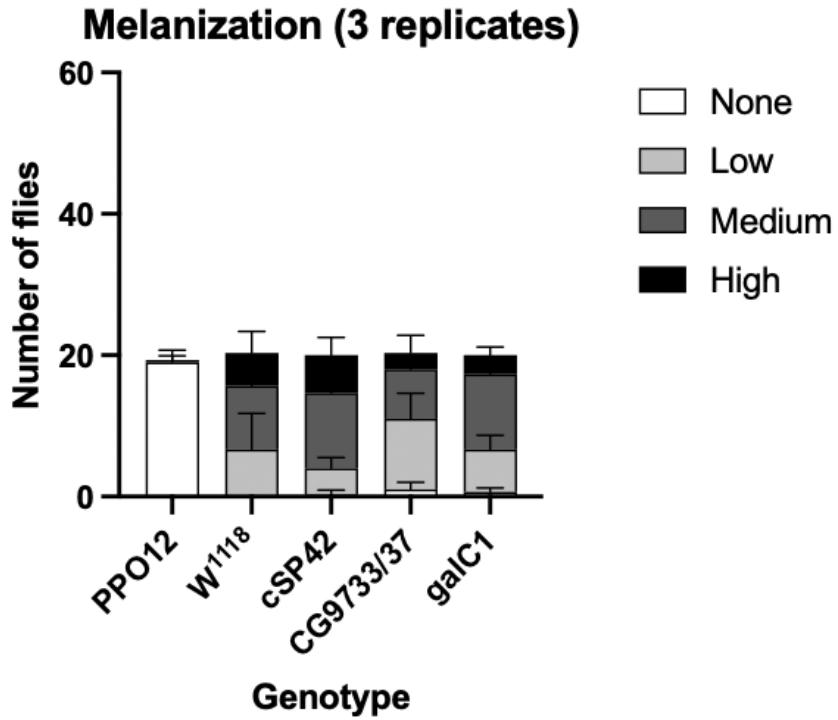
4. Experiments

Melanization assay



*From Kirah Jones et al.
2022, Nature*

cSP42, CG9733/37 and galC1 flies do not show differences in melanization at adult stage

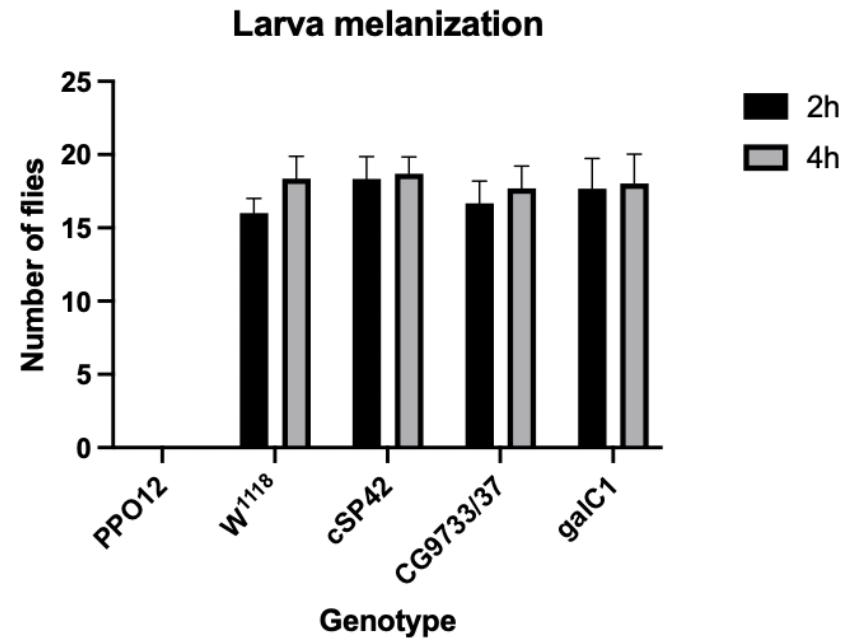


Methods:

Clean injury pricking was performed on flies in the evening. The next morning the score of melanization was observed.

Flies were kept at 25°C

cSP42, CG9733/37 and galC1 flies do not show differences in melanization at L3 stage

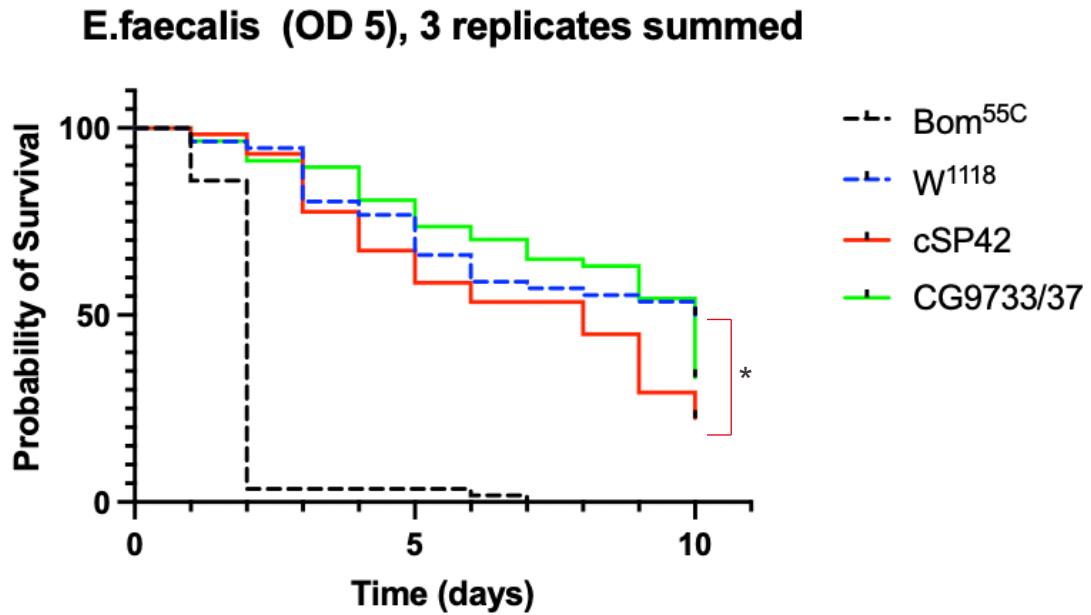


Methods:

Clean injury pricking was performed on larva. The number of melanized larva was observed at different time points

Survival Analysis

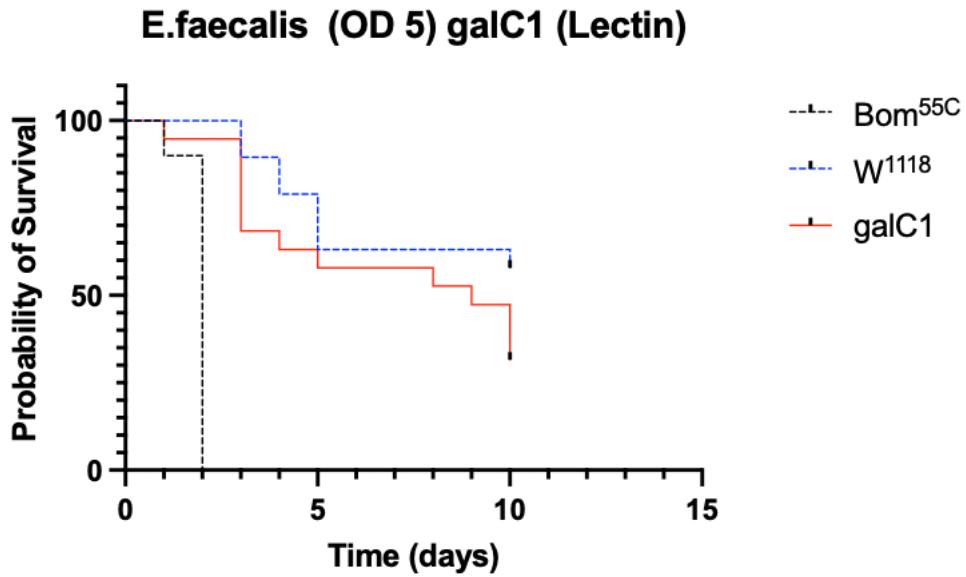
cSP42 & CG9733/37 do not show differences in survival to *E. faecalis*



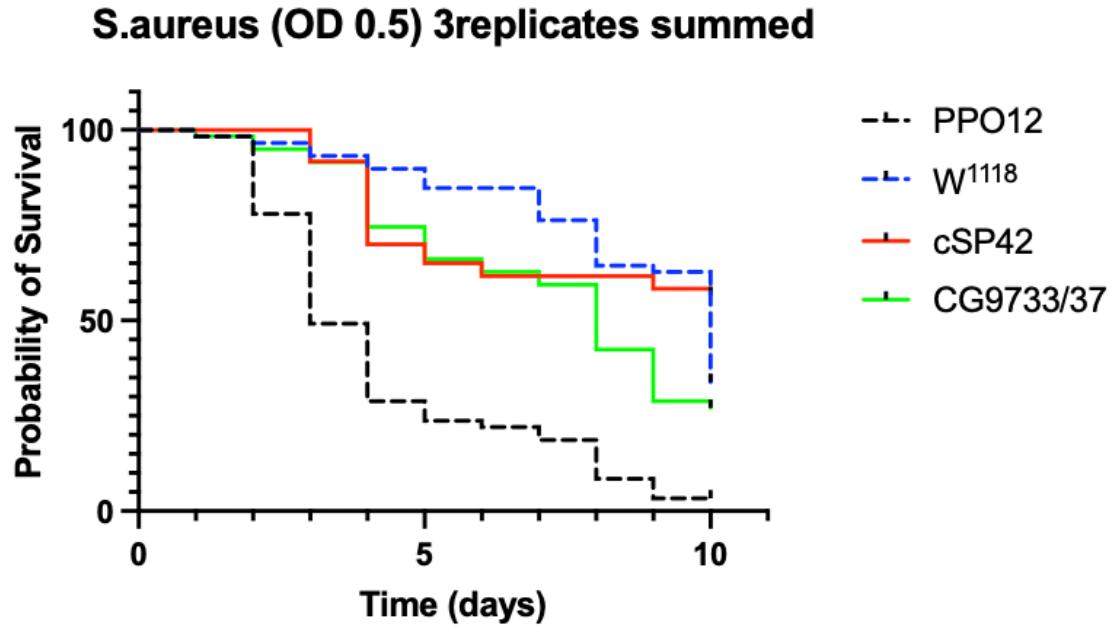
25°C for 10 days

Log-rank (Mantel-Cox) test
P-value = 0.0119

galC1 do not show differences in survival to *E. faecalis*

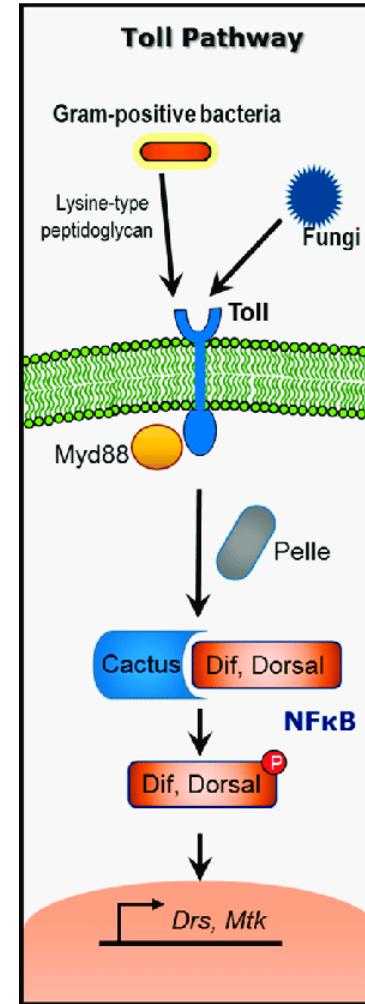


cSP42 & CG9733/37 do not show differences in survival to *S. aureus*

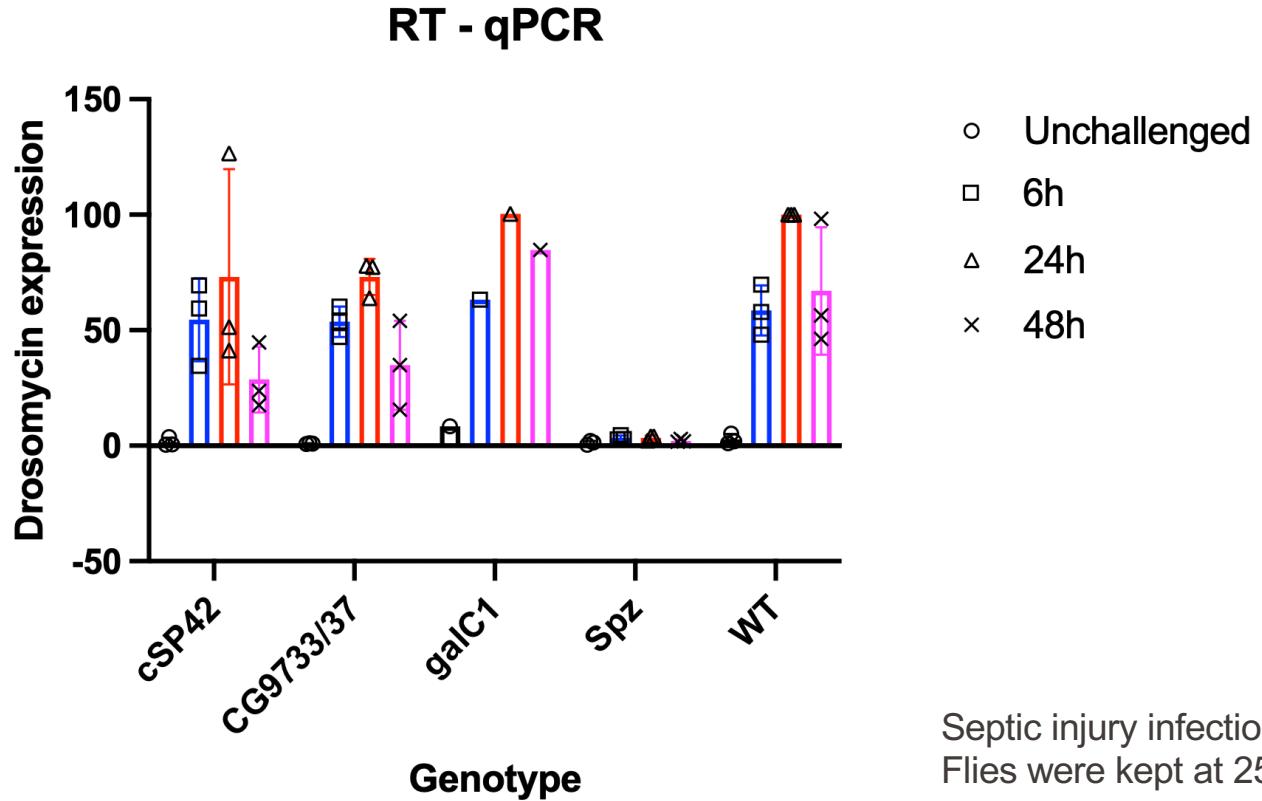


25°C for 10 days

Role of cSP42 , CG9733/37 & galC1 in Toll pathway activation



cSP42, CG9733/37 & galC1 do not show differences in *Drosomycin* expression upon infection



4. Conclusions

cSP42, CG9733/37 and galC1...

- do not appear to regulate melanization in response to sterile injury in adult flies or larvae
- do not appear to regulate the Toll pathway in response to septic injury with
M. luteus
do not exhibit altered survival following septic injury with *S. aureus* (*galC1* need to be tested)

Following septic injury with *E. faecalis*

CG9733/37 and galC1 do not exhibit altered survival following septic injury with *E.faecalis*

cSP42 exhibited a slightly worse but significant survival to *E. faecalis* following a septic injury after 10days.

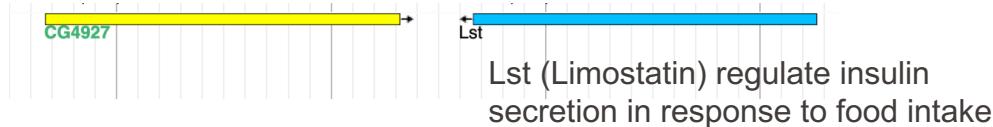
Possible follow-up experiments

Fungal Infection: Survival assay with *Beauveria bassiana* (*on going*)

Toll pathway: *Metchnikowin* (Mtk) expression

Lectin Binding Assay: Test pathogen binding using hemolymph extracts.

Infection under stress condition: Test pathogen on cSP42 with low food availability.



Other fly lines

- Triple mutant (CG11841-43)
- PBac{RB}CG4927^{e03031}
- PBac{WH}CG9733^{f03827}
- UAS – CG9733^{GD6346} (RNAi, Gal4 system)

Thank you !

For your **support, kindness**, and for creating such a **great atmosphere**.

Your guidance and collaboration have been invaluable throughout this project!

Questions ?