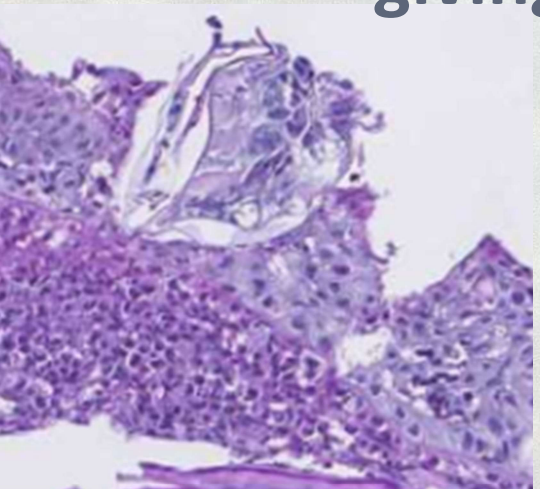
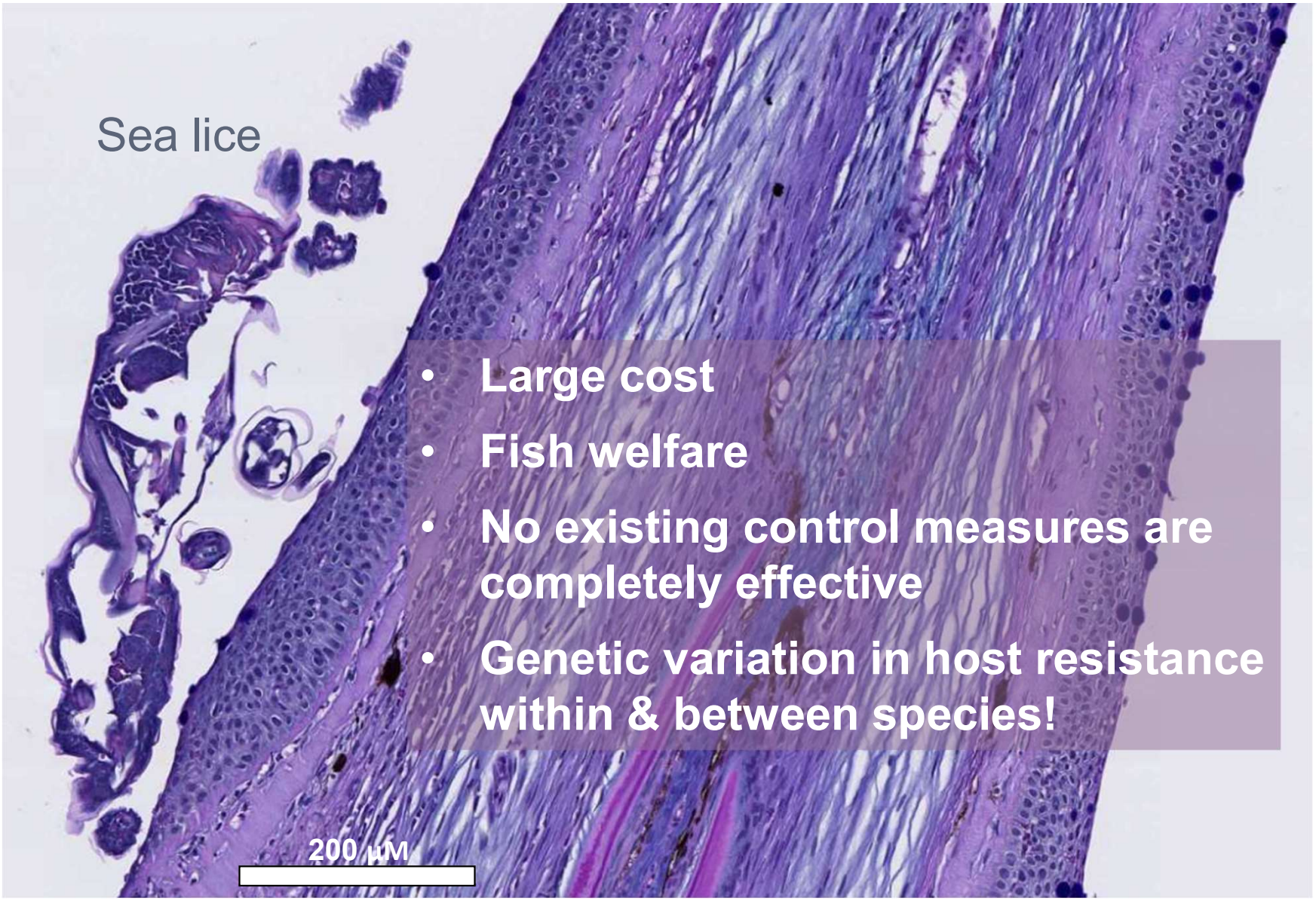
CrispResist

Understanding the mechanisms giving host-resistance against sea lice



Nick Robinson
Project Leader



- Large cost
- Fish welfare
- No existing control measures are completely effective
- Genetic variation in host resistance within & between species!

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Different mechanisms of resistance in coho, pink & Atlantic salmon?

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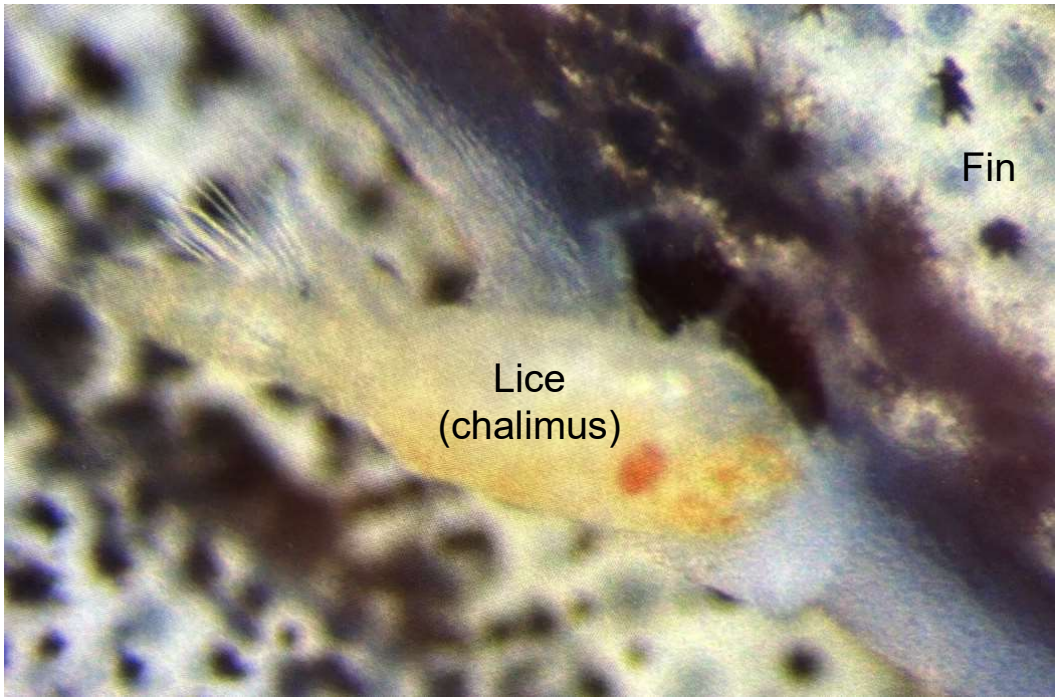


Coho
- 1-7 days PI
  - Immune cell infiltration
  - Epithelial enlargement due to cell division
  - Melanin deposition
- 10 days PI
  - Encapsulation of parasite

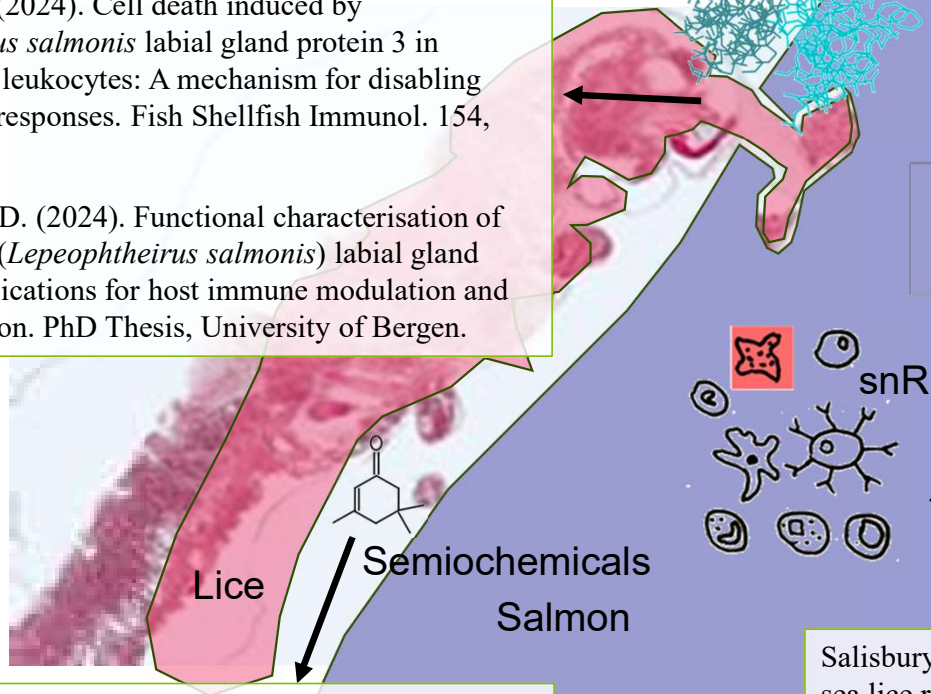
Pink
- Rapid inflammatory response

Atlantic
- Rapid & large-scale immune gene activation
- No effect on lice

CrispResist → Knowledge about genes giving coho & pink salmon ability to resist sea lice

Midtbø *et al*. (2024). Cell death induced by *Lepeophtheirus salmonis* labial gland protein 3 in salmonid fish leukocytes: A mechanism for disabling host immune responses. Fish Shellfish Immunol. 154, 109992.

Midtbø, H.M.D. (2024). Functional characterisation of salmon louse (*Lepeophtheirus salmonis*) labial gland proteins: Implications for host immune modulation and anti-coagulation. PhD Thesis, University of Bergen.



Sveen *et al*. (2023). Transcriptomic landscape of Atlantic salmon (*Salmo salar* L.) skin. *Genes Genomes and Genetics* **13**(11), jkad215

Sveen et al. *(under review)*. Local inflammation at the salmon louse (*Lepeophtheirus salmonis*) attachment site drives copepodid rejection in Coho Salmon (*Oncorhynchus kisutch*). *Cell and Tissue Research*

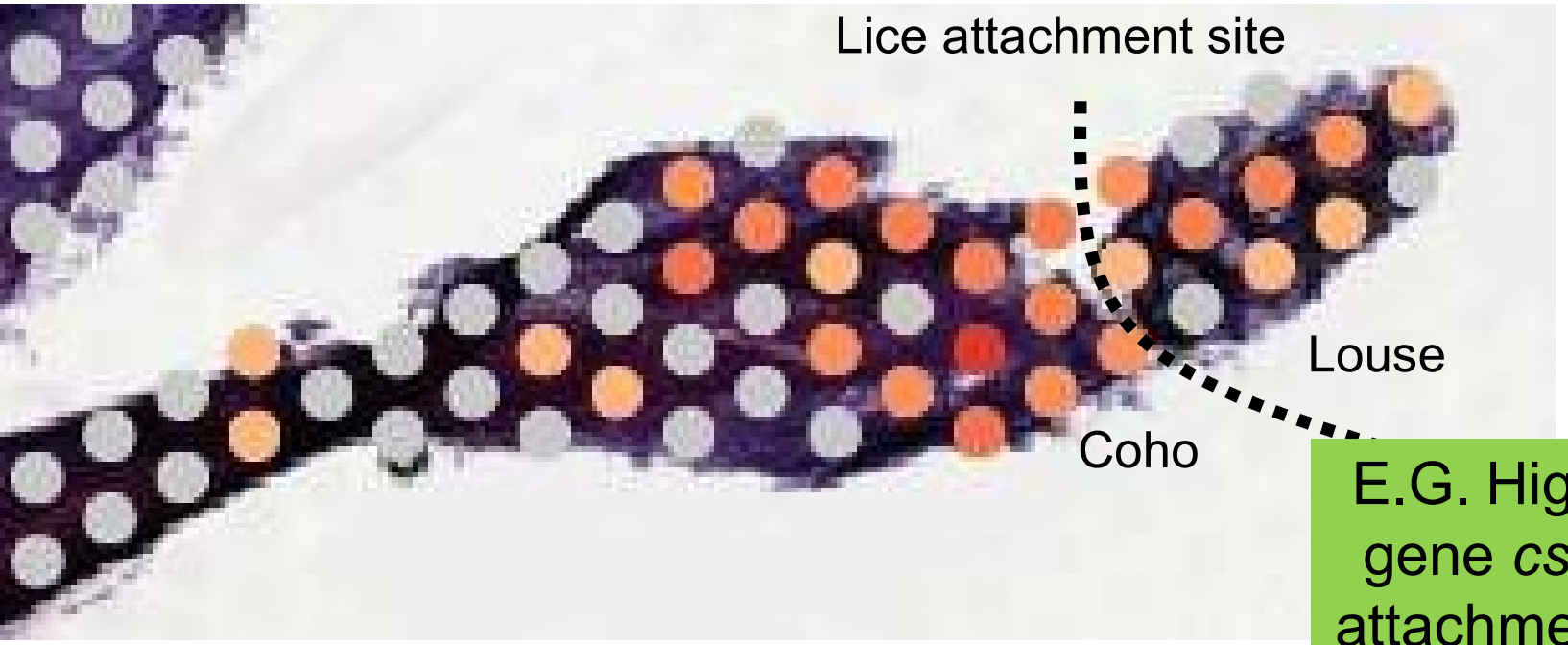
Changes during the first hours/days after infection?

Salisbury S.J *et al*. (2024). Keratinocytes drive the epithelial hyperplasia key to sea lice resistance in coho salmon. *BMC Biology* **22**, 160.

Daniels R.R. *et al*. (*in press*). Transcriptomic characterisation of transitioning cell types in the skin of Atlantic salmon. *BMC Biology*.

Gulliksen, S., 2022. Candidate genes for semiochemical synthesis in salmonid fishes. MSc Thesis, The Arctic University of Norway.

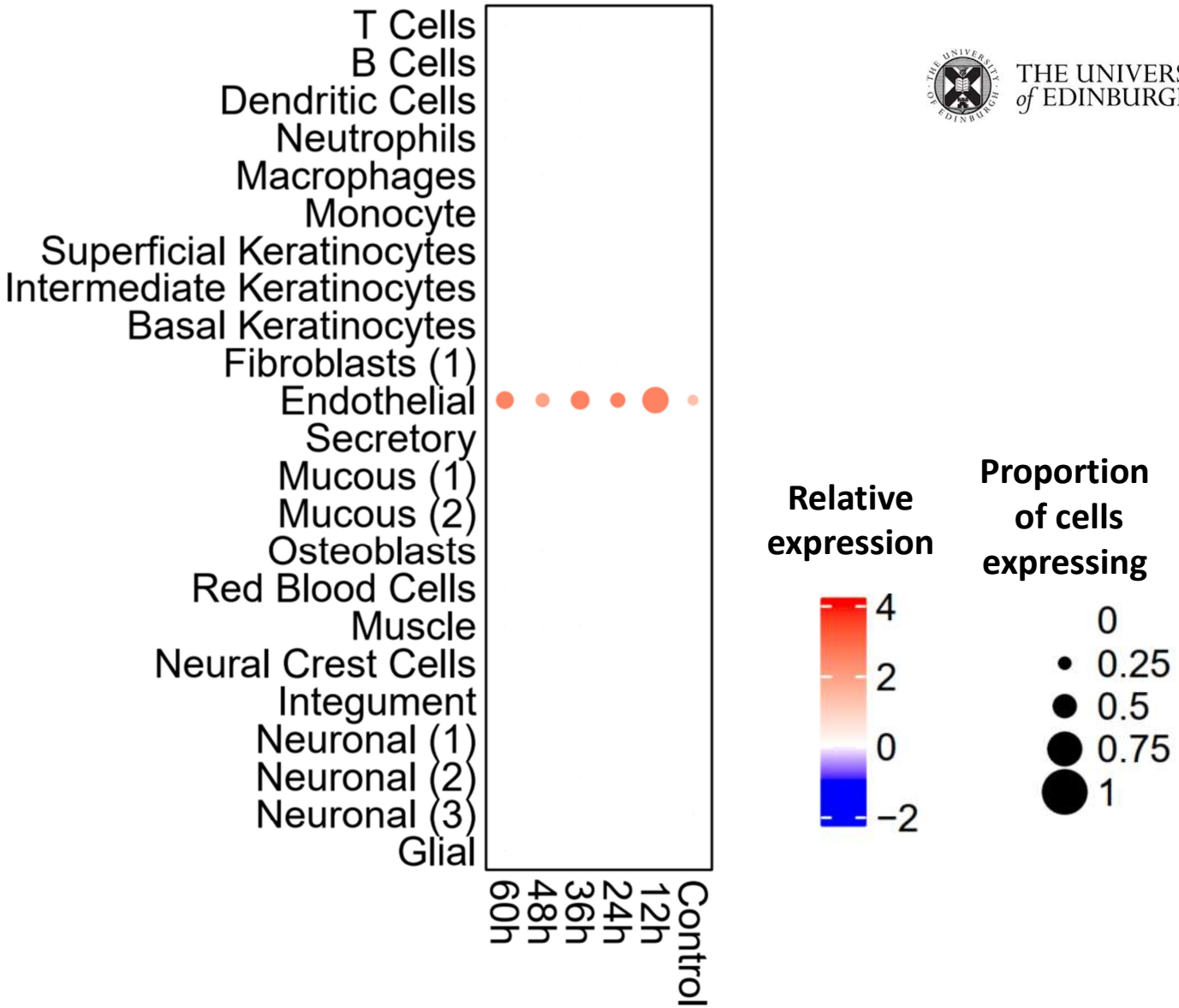
Spatial expression patterns



Redder = higher expression of gene at position on slide

E.G. High expression of gene *csf3r* close to lice attachment site in coho a few hours after infection

mrc1a expression in coho



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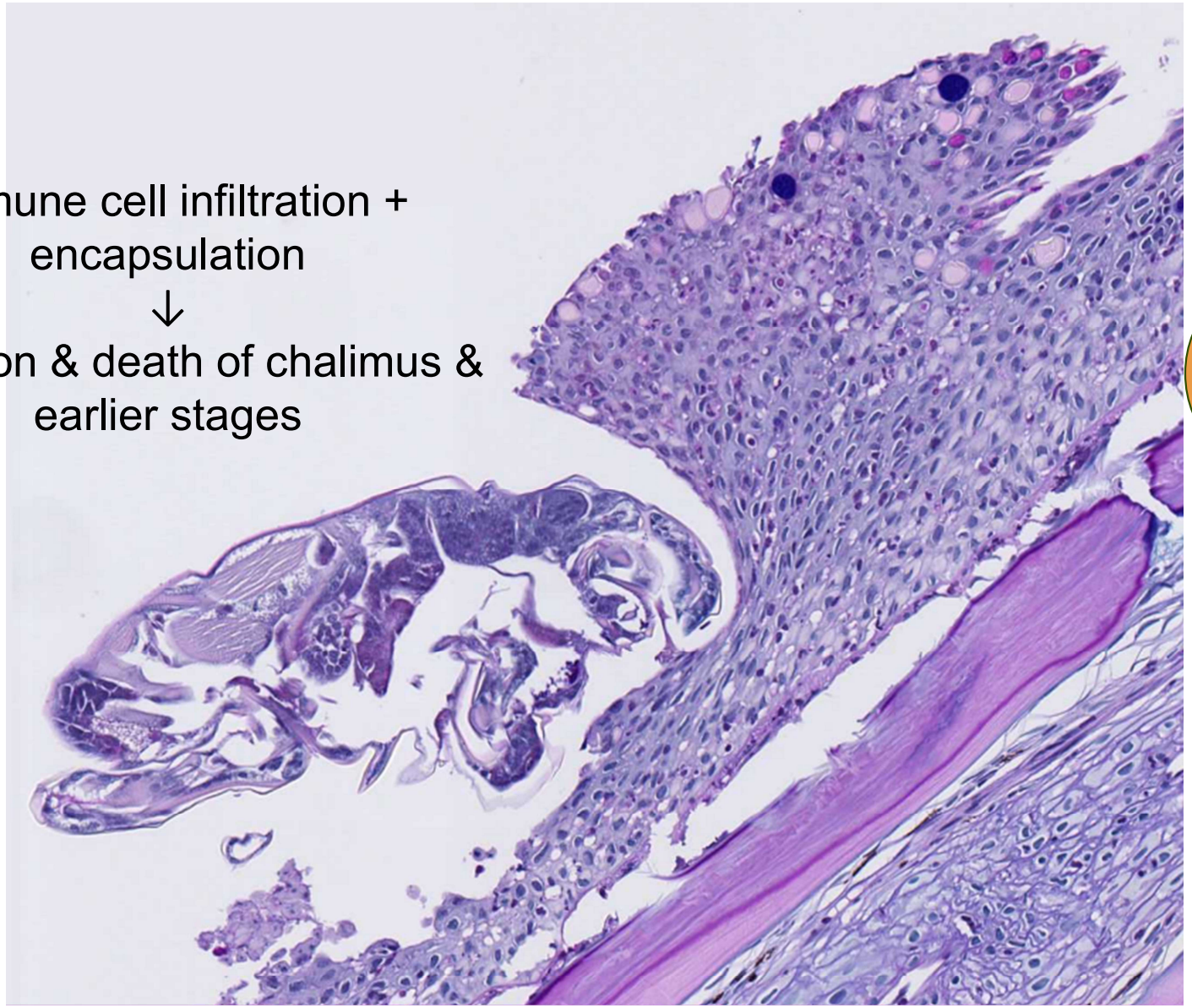
Coho

Immune cell infiltration + encapsulation

↓

Rejection & death of chalimus & earlier stages



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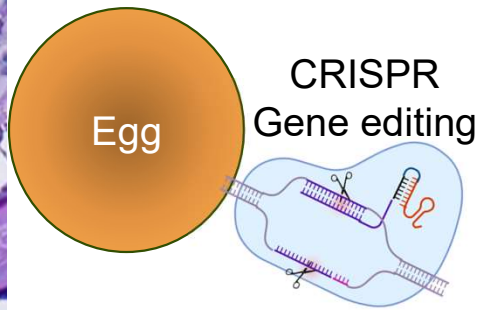
Test gene effects:

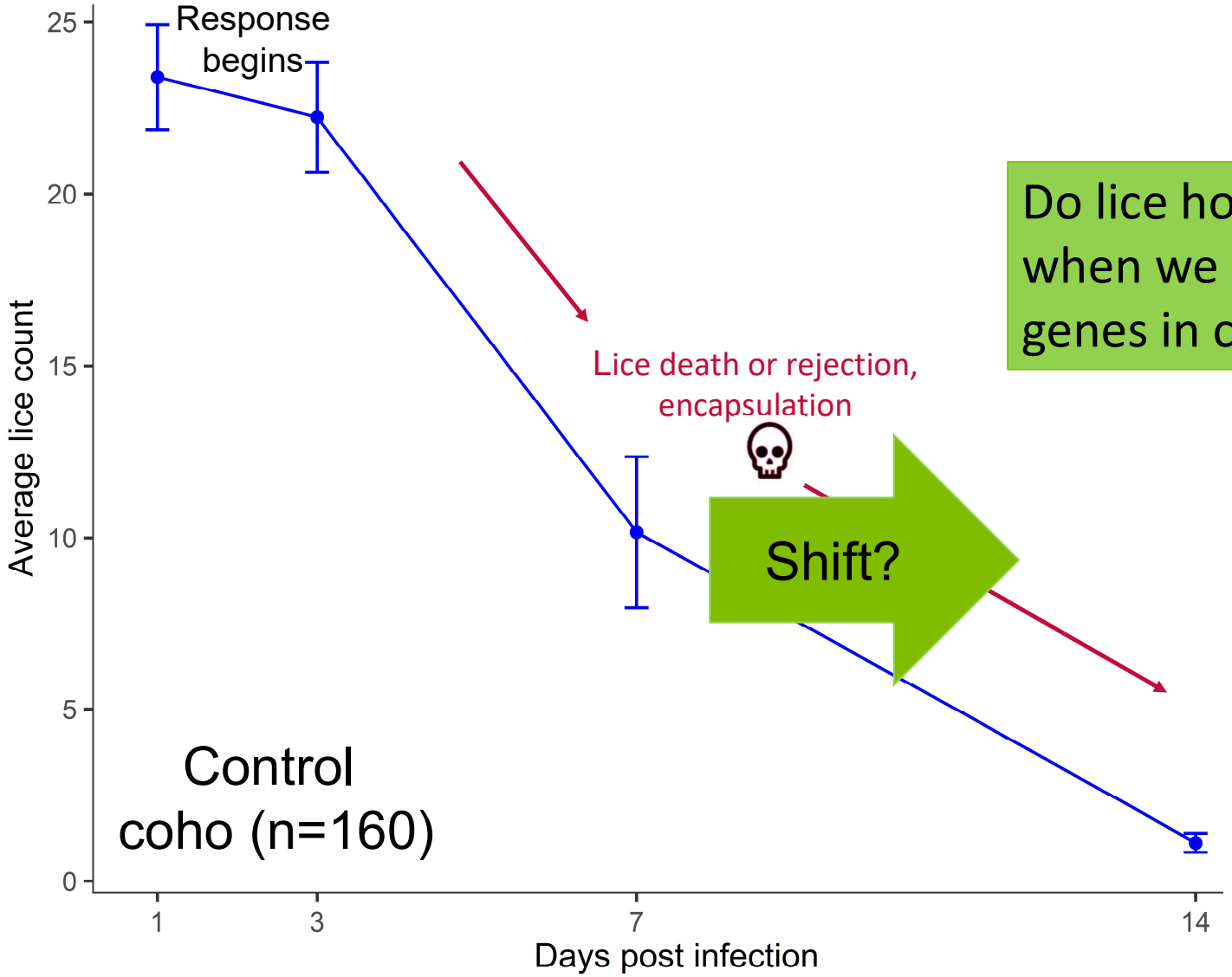


→ elicit "coho-like" response in Atlantic salmon?

alternatively,

→ disrupt this response in coho?

15 different types of gene edited salmon produced for testing



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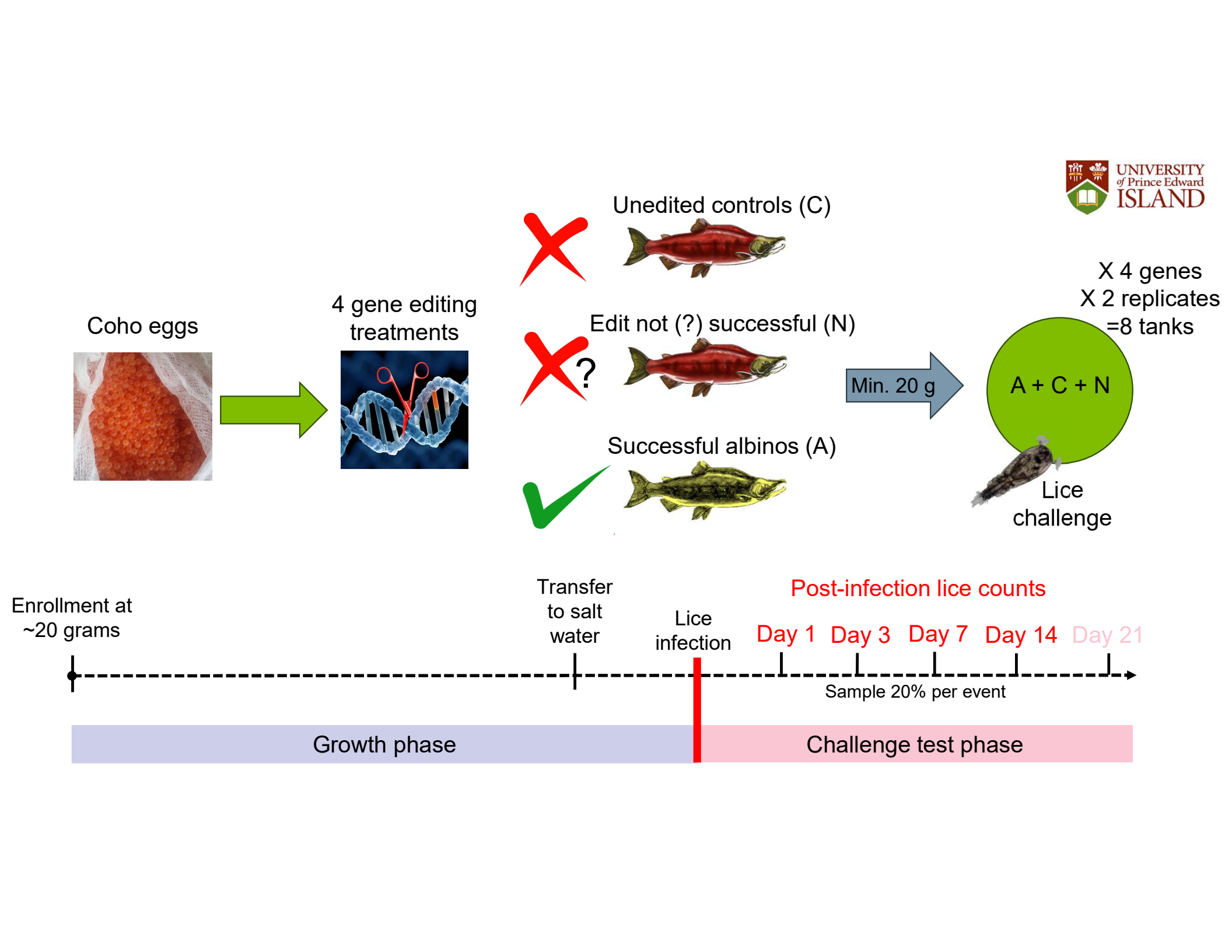
Do lice hold on for longer when we knockout these genes in coho salmon?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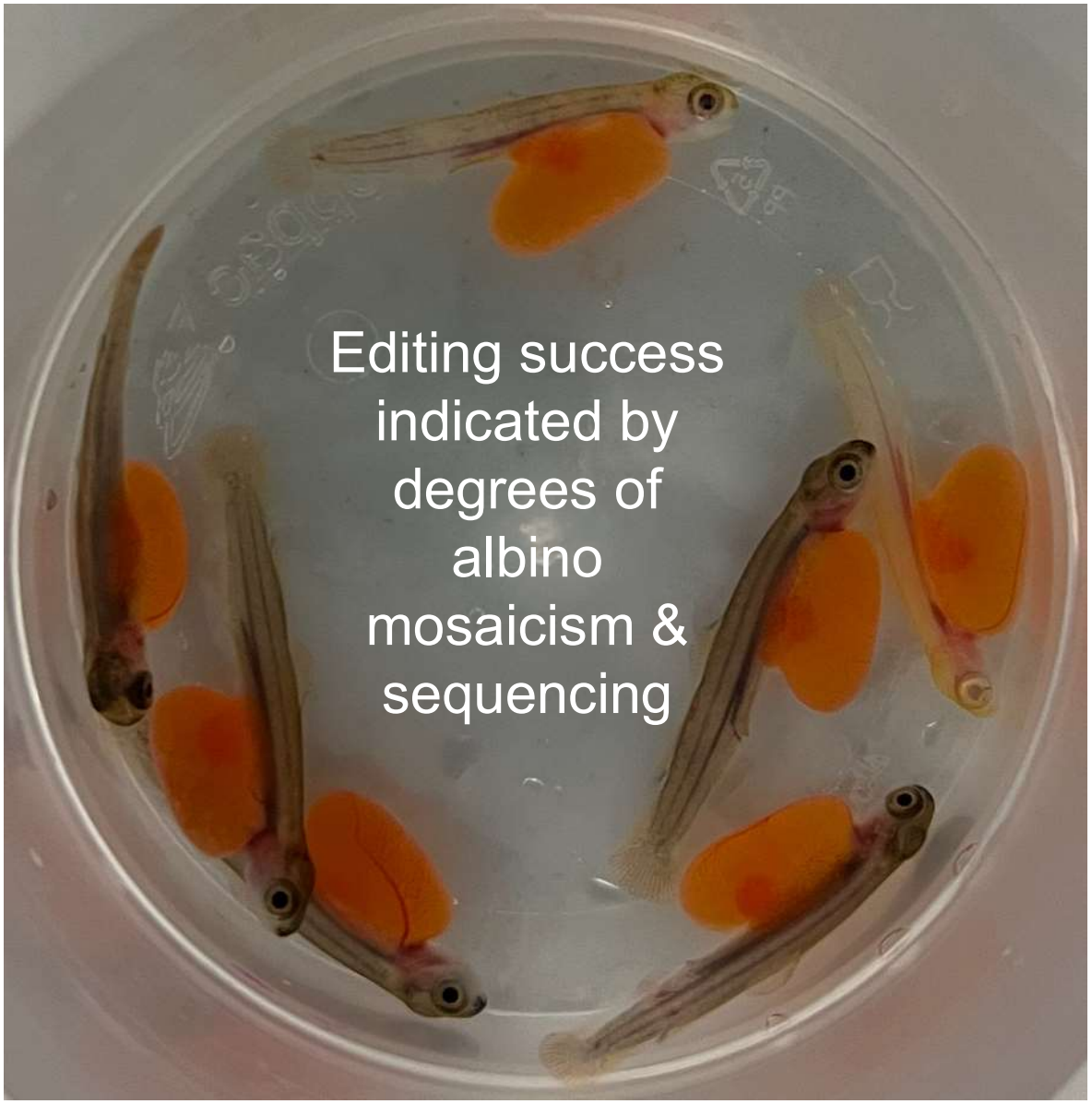
Coho eggs
4 gene editing treatments
Unedited controls (C)
Edit not (?) successful (N)
Successful albinos (A)
Min. 20 g
X 4 genes
X 2 replicates
=8 tanks
A + C + N
Lice challenge
Enrollment at ~20 grams
Transfer to salt water
Lice infection
Post-infection lice counts
Day 1
Day 3
Day 7
Day 14
Day 21
Sample 20% per event
Growth phase
Challenge test phase



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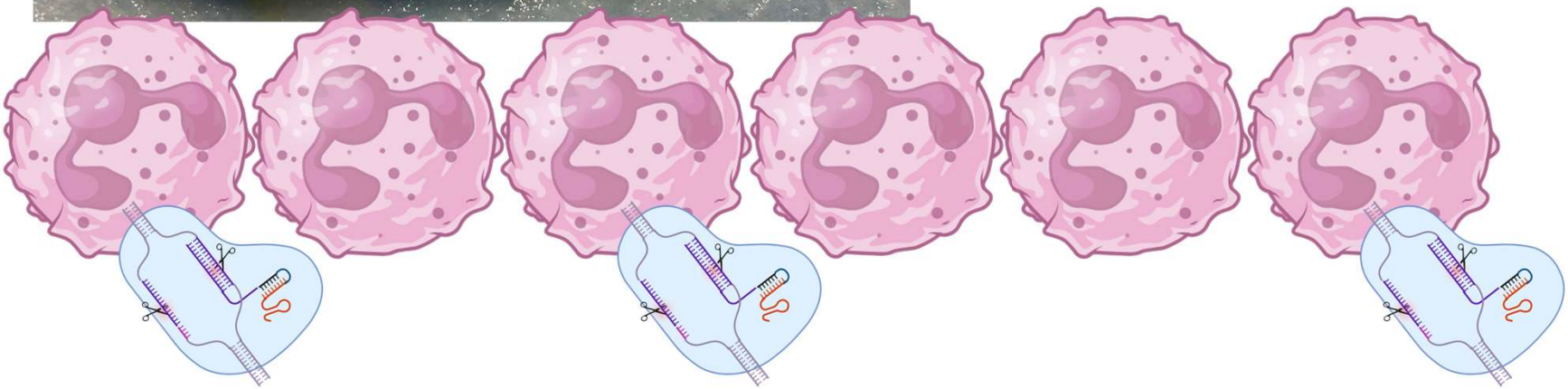
Albinism

Editing Success(?)



26 bases deleted

Stops transcription

23 bases deleted

Stops transcription

3 bases deleted

Deletes one amino acid

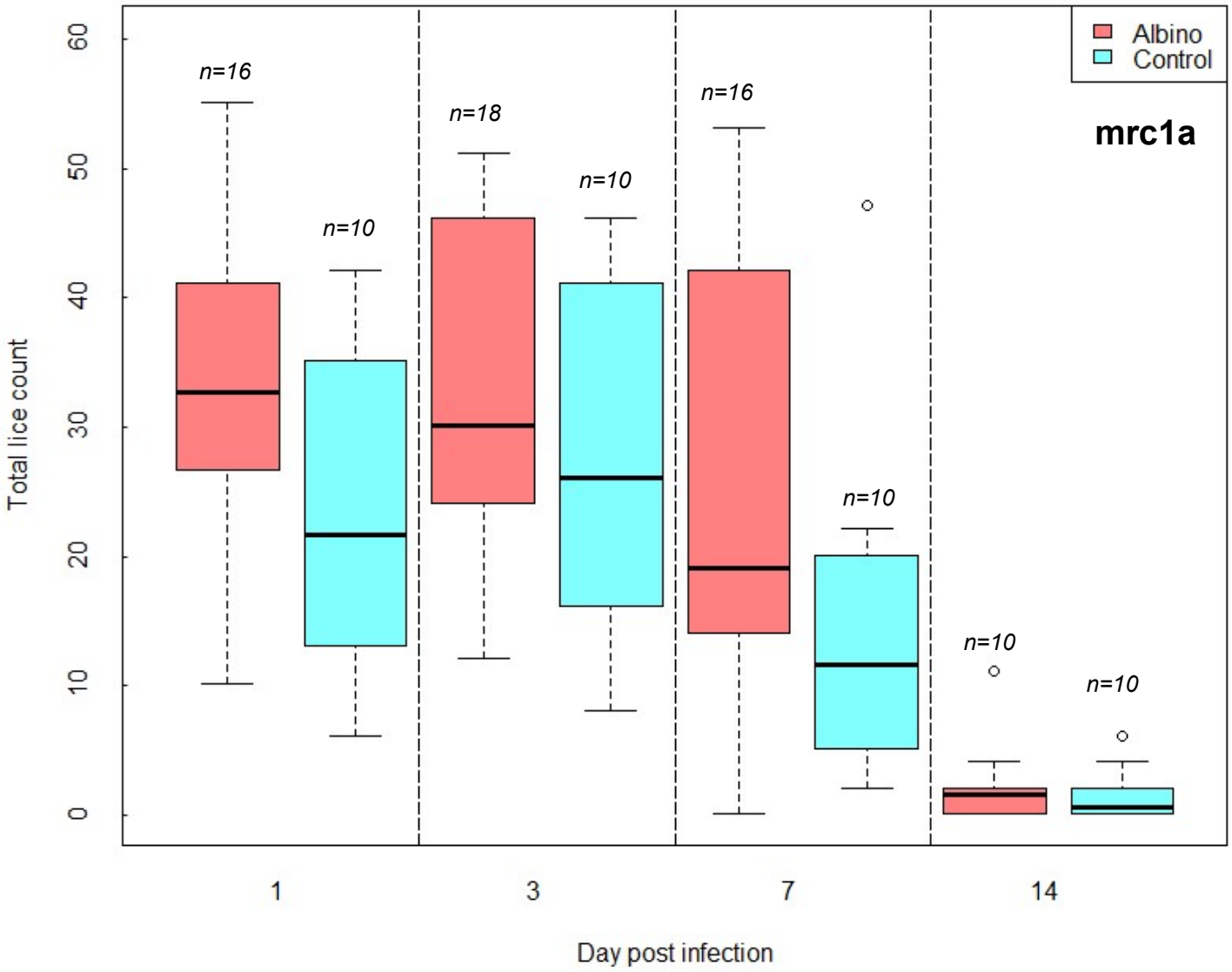
Combined effect of different edit types (and non-edited cells) on each fish?

Therefore:

- Total knockout unlikely
- Expect a range of lice counts if target gene has an effect
- Non-albino fish (N) might still be edited

Analysis:

- How lice count changes over days post-infection for A, N & C groups



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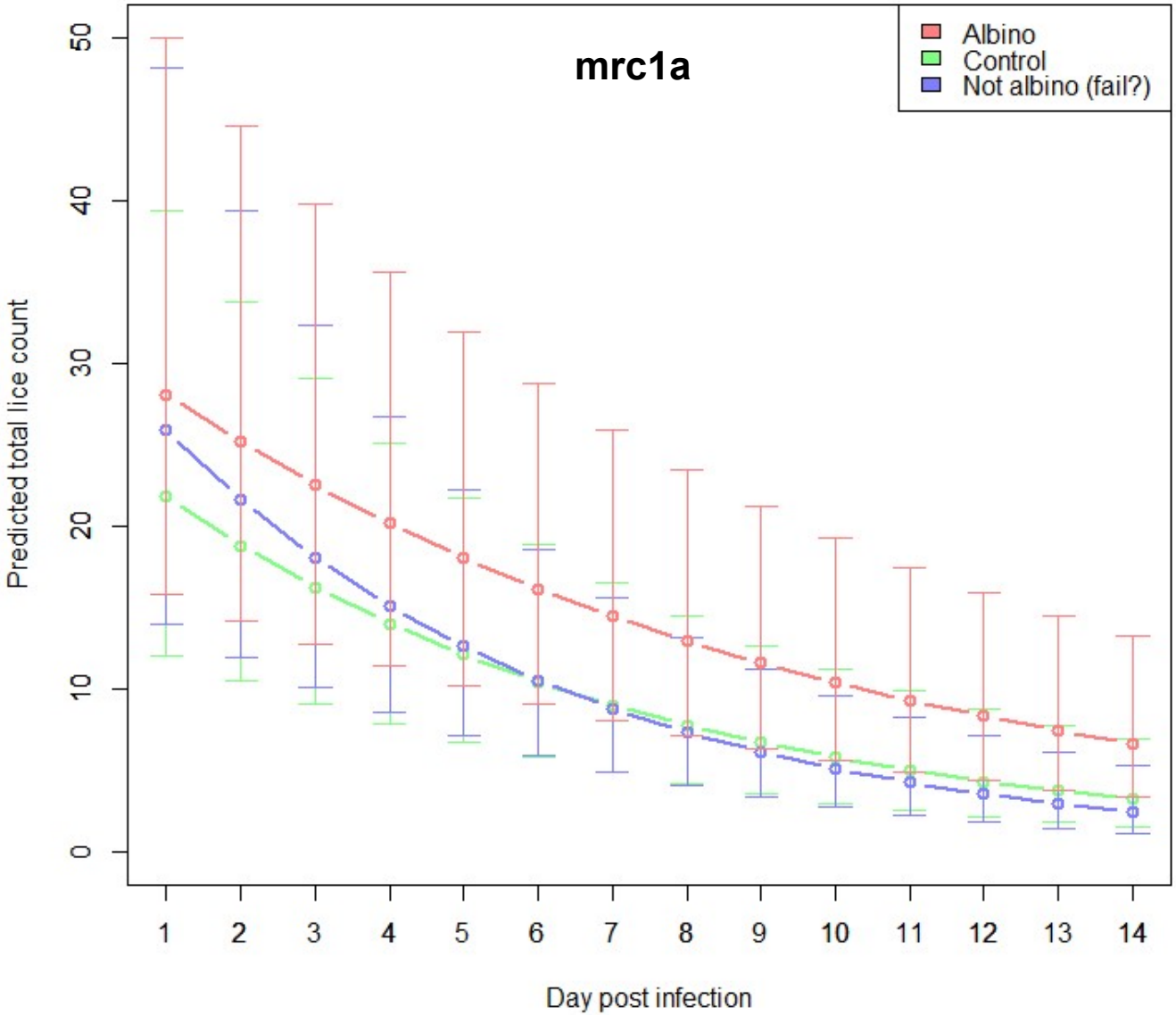
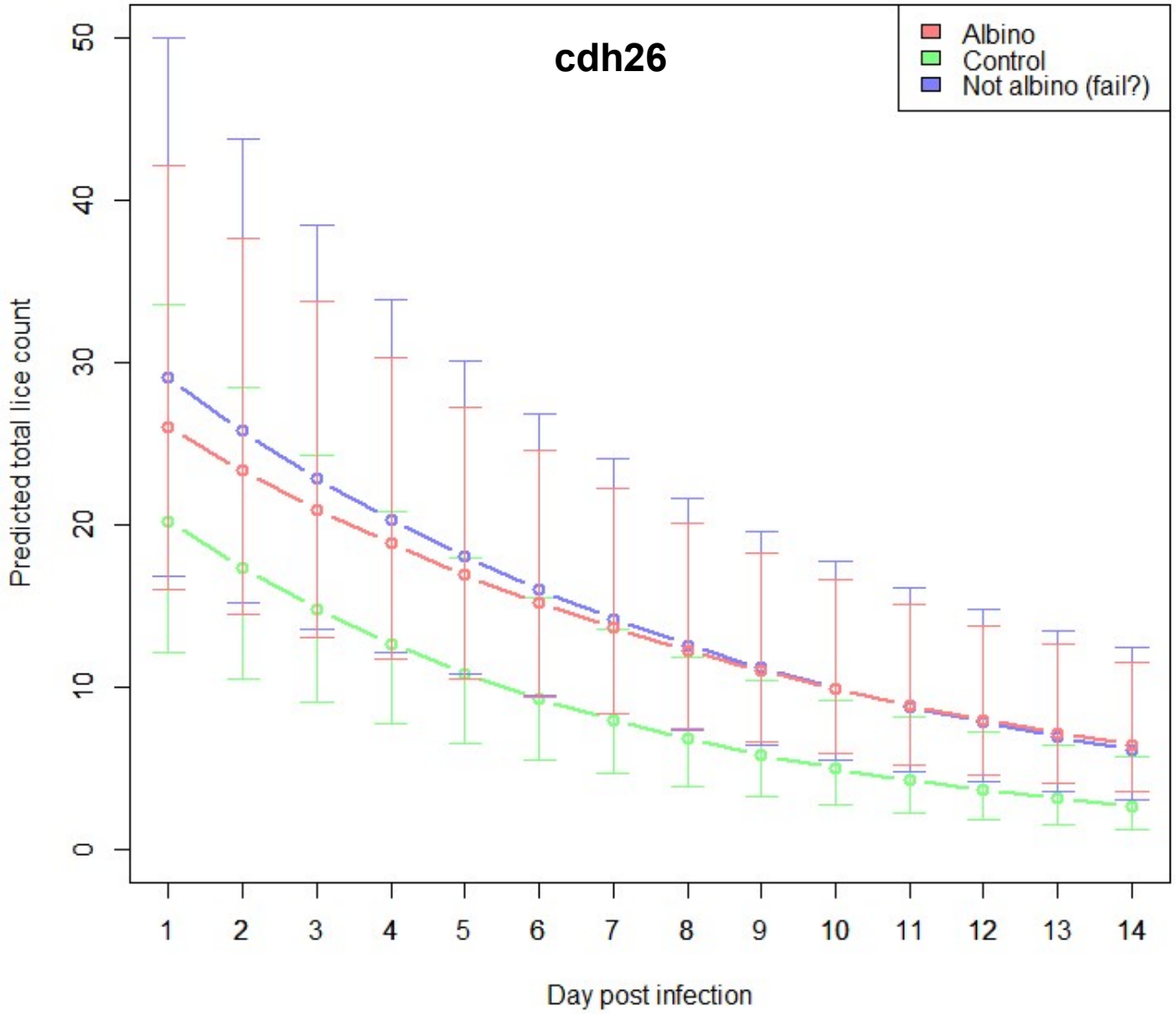
Significantly higher total lice count over days after infection in albino coho than control coho (P_{adj}<0.05)



Predicted response for continuous model (over time) with 95% confidence intervals



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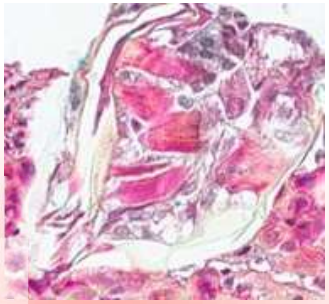
Significantly higher total lice count over days after infection in albino coho than control coho (P_{adj}<0.05)

Significantly higher total lice count over days after infection in non-albino (failed edits?) than control coho (P_{adj}<0.05)



Lice Copepodid

Endothelial cells
- Single layer lining lymph & blood vessels
- Recognition & early innate immune activation

Mrc1a pathogen recognition, activation of responding cells, antigen processing/presentation, cell migration/signalling

Mrc1a involvement in neutrophil trafficking by endothelial junctions

Monocyte
Eosinophil
Basophil
Neutrophil

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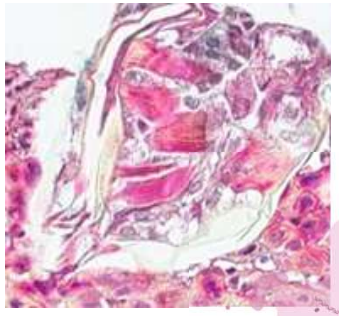
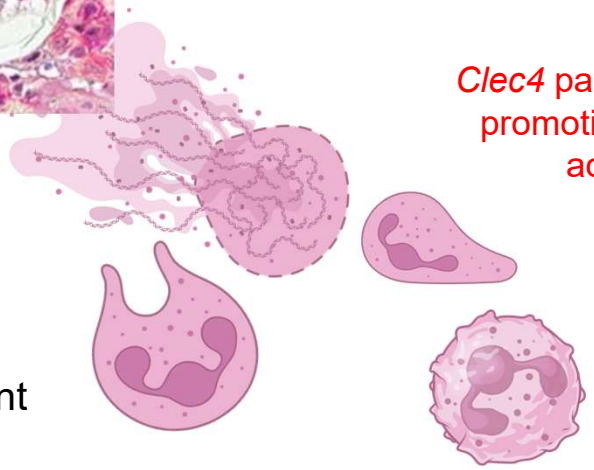
Innate response during first days/hrs after infection in coho



Lice Copepodid



Clec4 pathogen-binding promoting neutrophil activation

Neutrophils

- First responders
- Only function once they cross the endothelial cell barrier
- Swarm/aggregate at attachment site

Function of neutrophils

- NET release (neutrophil extracellular traps)
- release of molecules to kill cells

Impact on lice

- Frontal filament holdfast
- Gut when ingested

Innate response during first days/hrs after infection in coho

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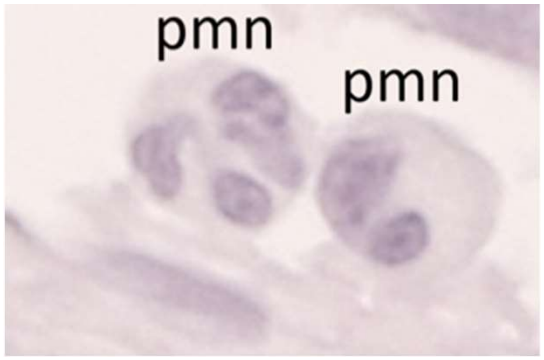
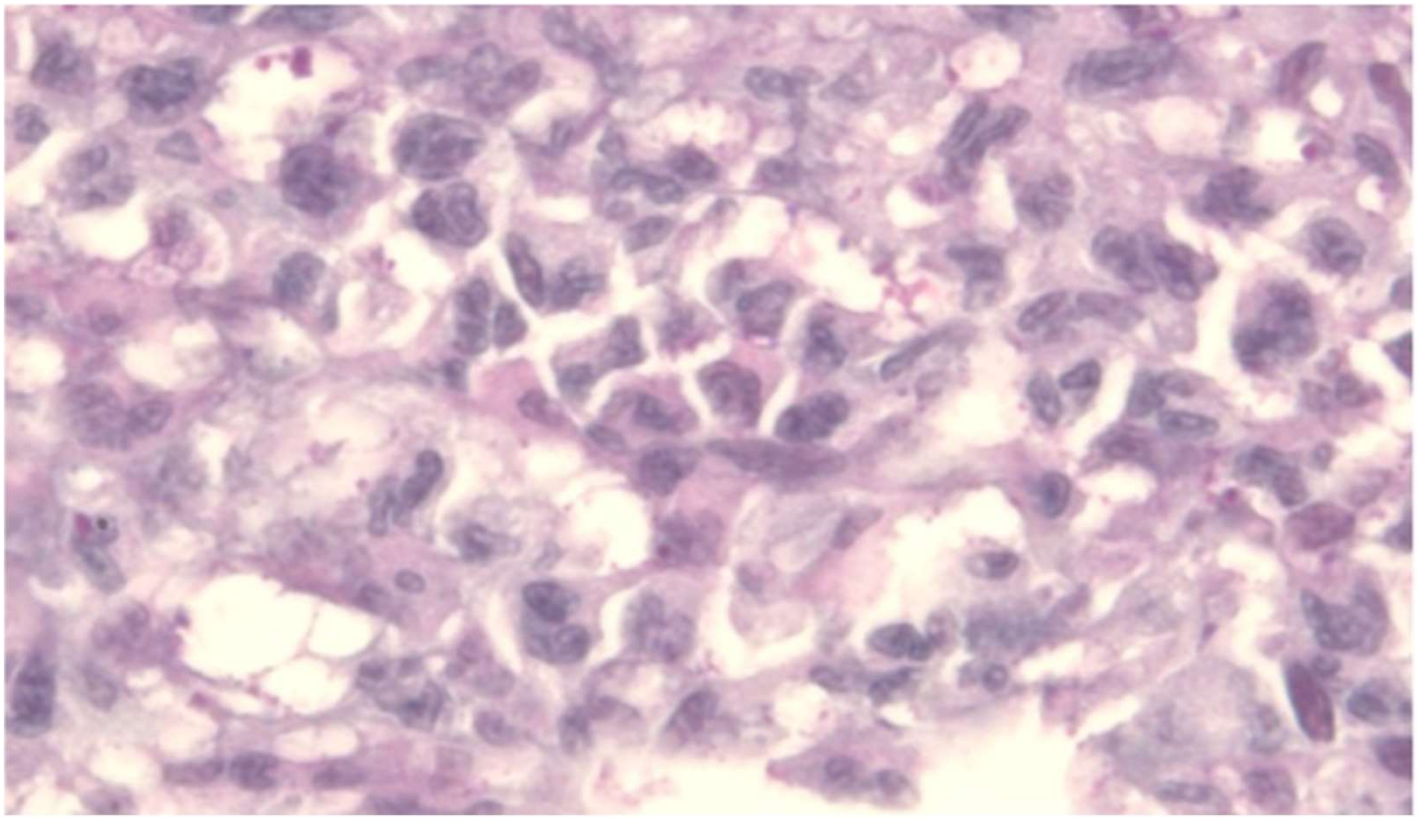


Neutrophils

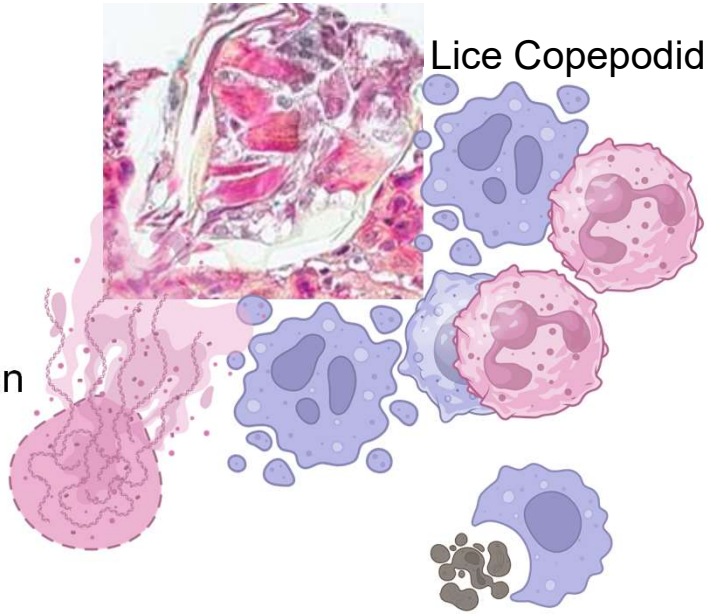
Activated at scene of crime in coho!



Proteomics → enhanced neutrophil mediated immune signalling in coho

Macrophages

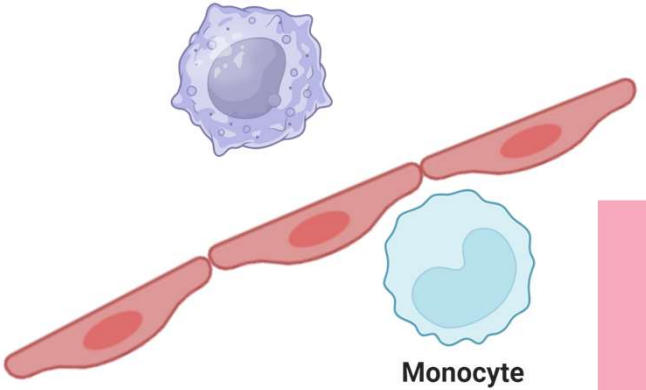
- Inflammatory, derived from blood monocytes
- Release chemokines to direct neutrophils to injury site
- Initiation & resolution of inflammation



Cdh26 - immune modulation, directs/attracts neutrophils

Csf3r - primary regulator of N and M proliferation, survival & differentiation

Socs3 - represses proinflammatory macrophage phenotype, reduces neutrophil recruitment (& *csf* signalling)



Single cell sequencing → genes involved in macrophage activation & infiltration in coho & pink salmon

Innate response during first days/hrs after infection in coho

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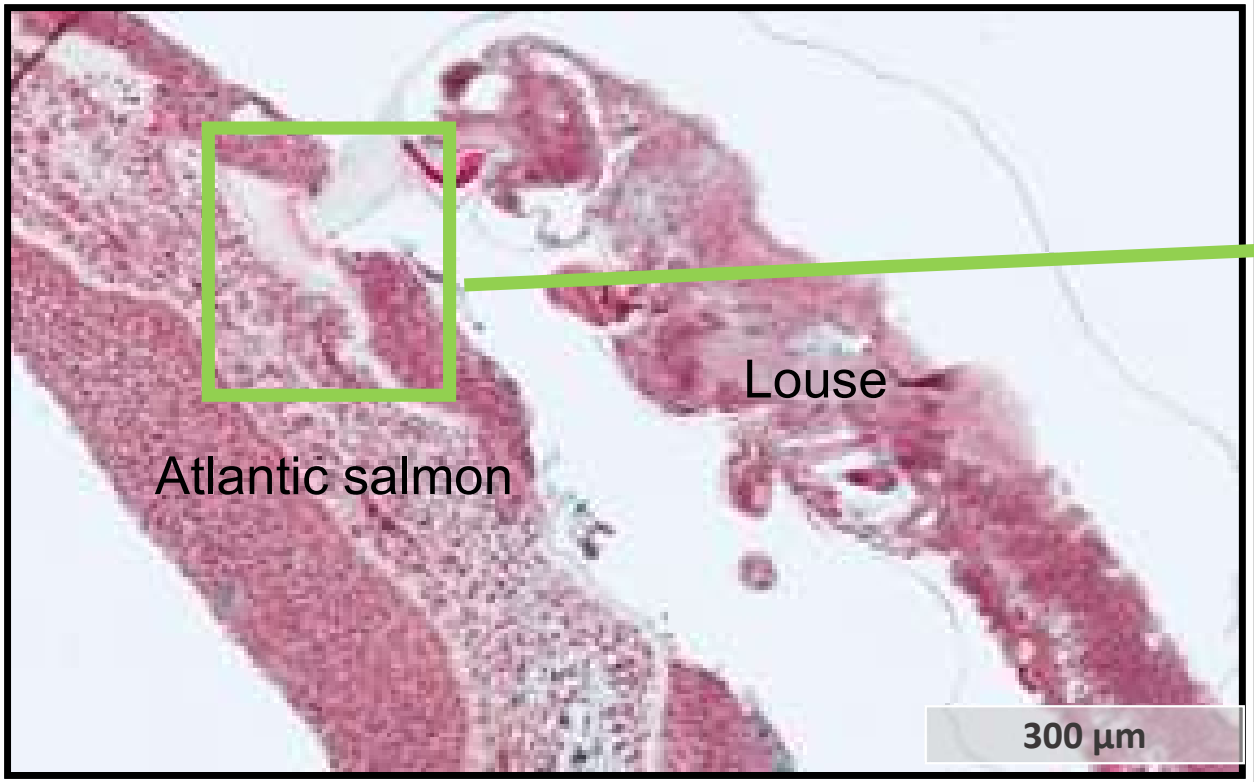
Secure chalimus «anchorage»!

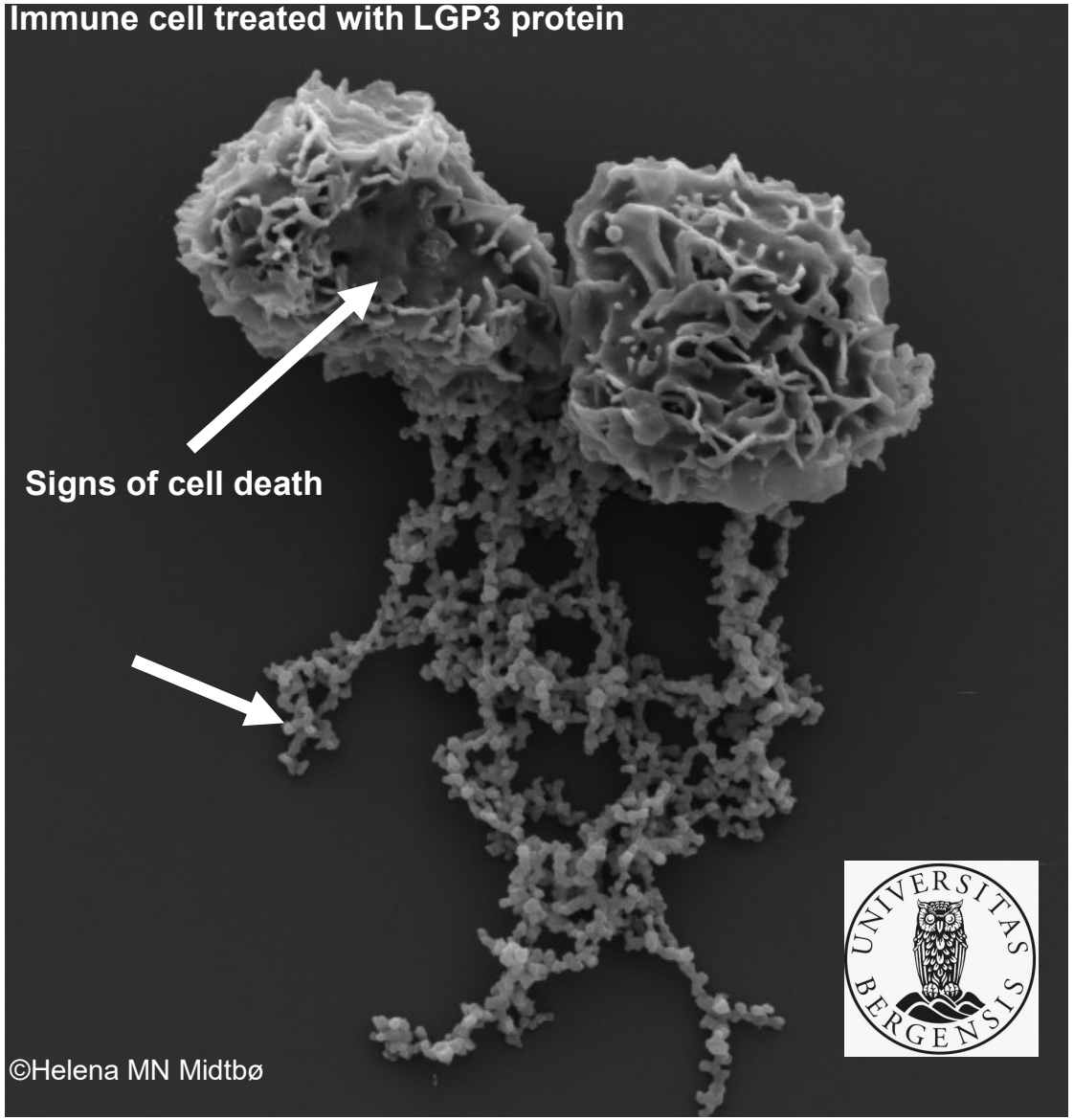


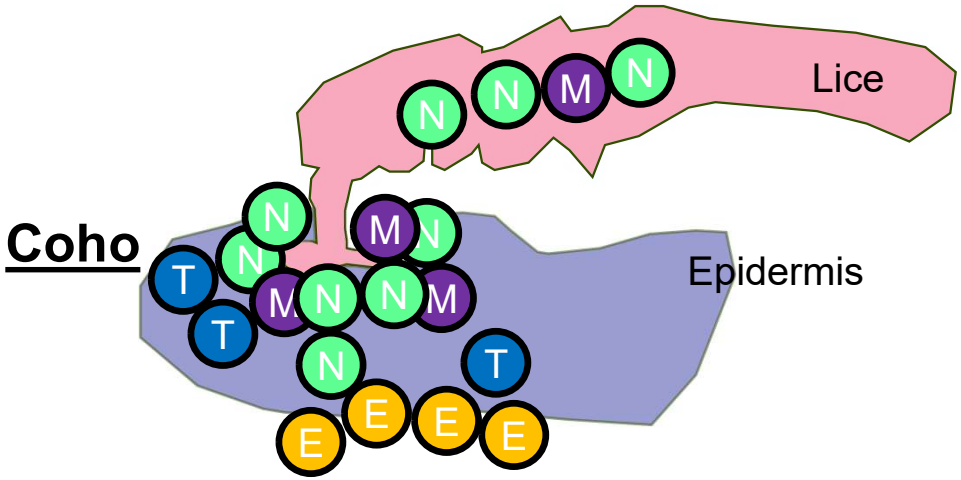
Pictures: Lene Sveen

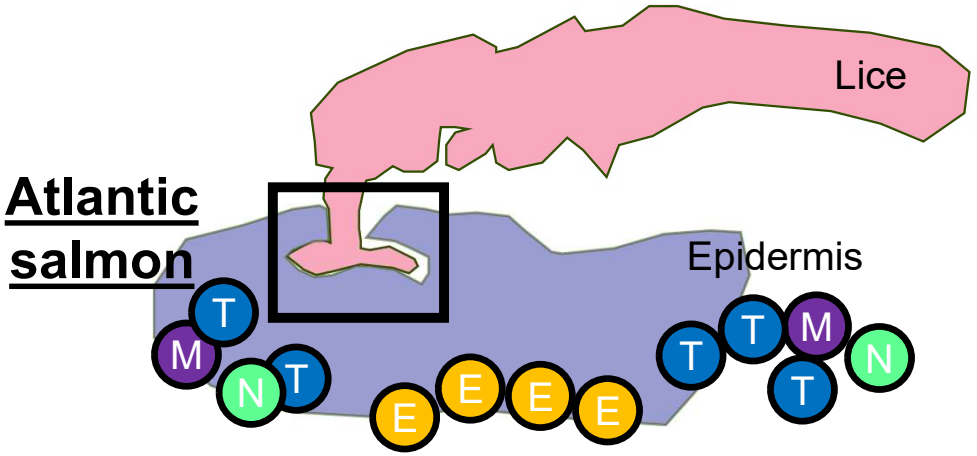
Lice suppression of host-immunity

(FHF projects ModuLus & CrispResist)

Three key immune dampening proteins identified







Immune cells directed toward site of attachment

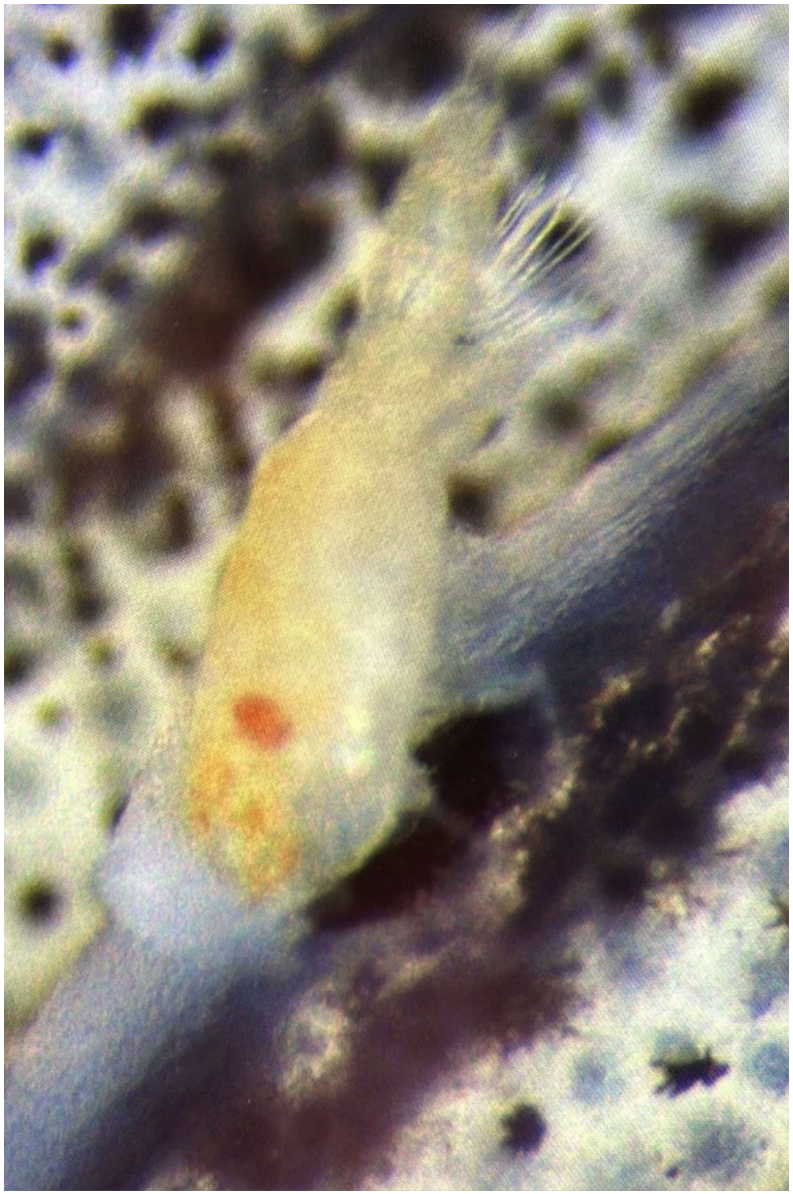
Immune cells lack direction/cleared at site of attachment

Genetic variation in Atlantic immune response?

Biopsy of attachment site to measure difference?

- Cell types & markers responding?
- Coho genes & involvement?

Could we breed to make Atlantics more “coho-like” in response to lice?

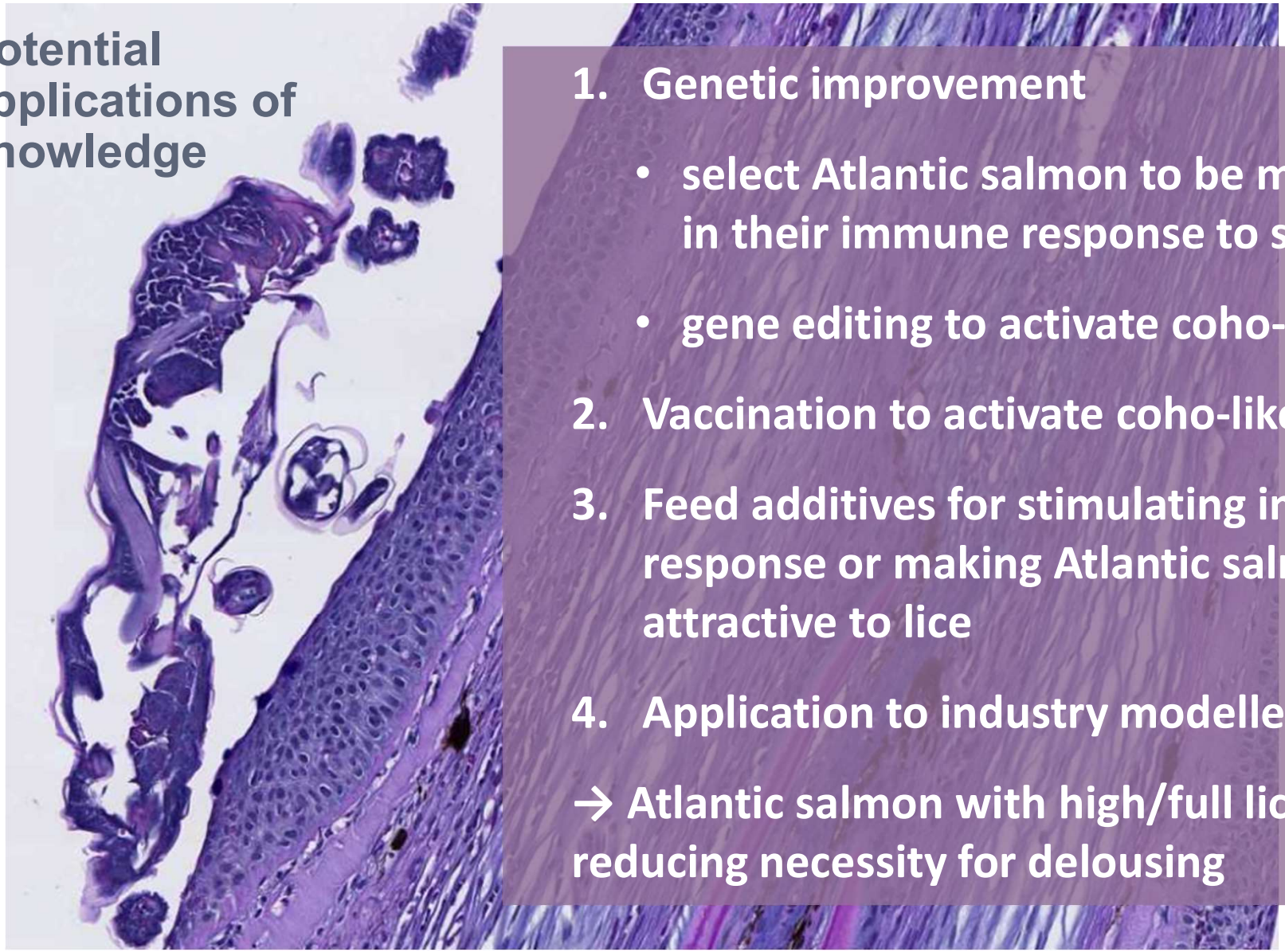


Summary of CrispResist findings

- Complex atlas of spatial & cell specific gene expression in the skin of four salmon species
- Knowledge of genes giving coho ability to destroy/repel lice & semiochemicals affecting lice behaviour
- Some gene edited Atlantic & coho salmon (ins/del) challenge tested

→ Genes making coho & pink more resistant to salmon lice

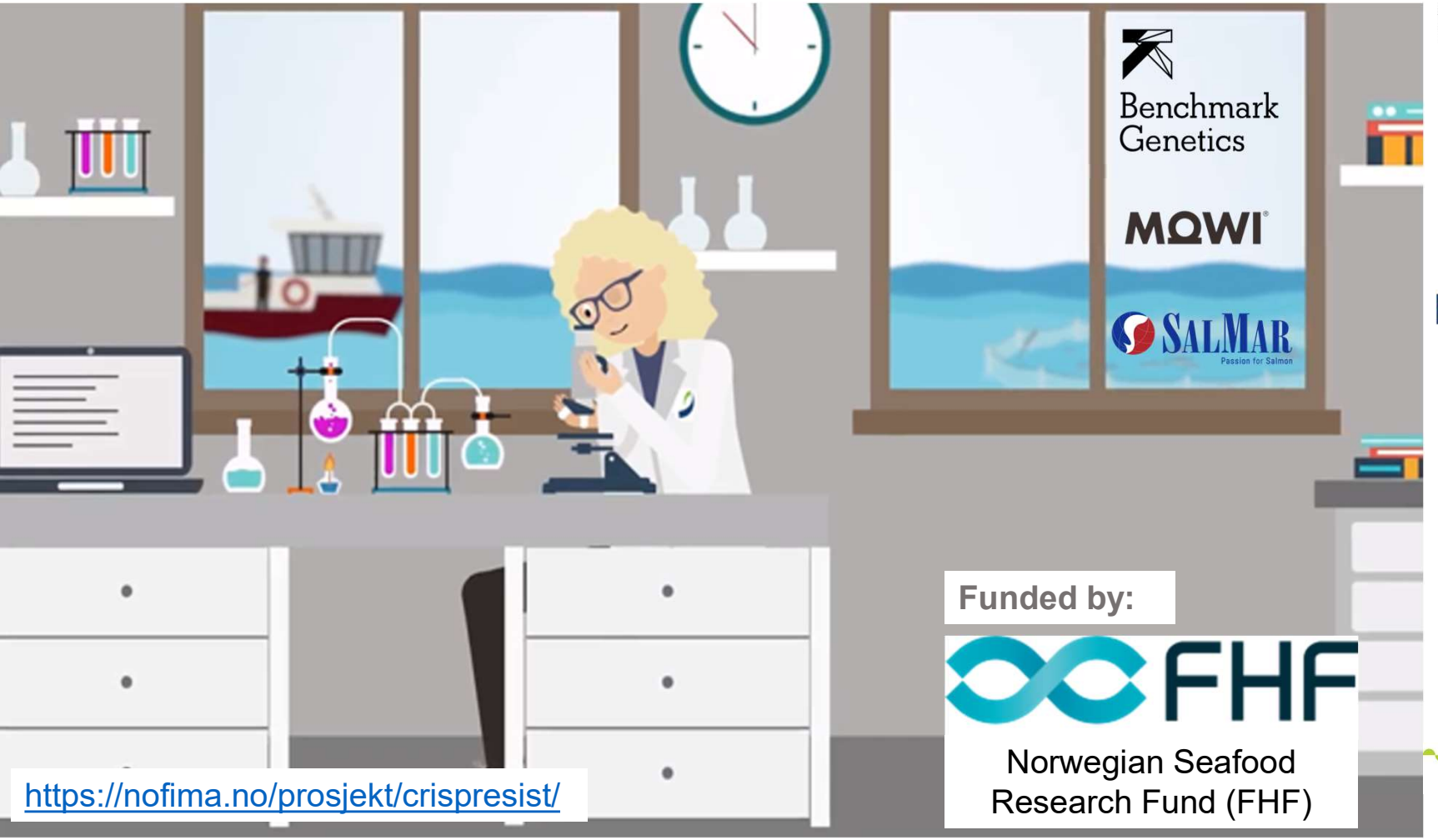
Potential applications of knowledge



1. Genetic improvement
   - select Atlantic salmon to be more coho-like in their immune response to sea lice
   - gene editing to activate coho-like response
2. Vaccination to activate coho-like response
3. Feed additives for stimulating immune response or making Atlantic salmon less attractive to lice
4. Application to industry modelled in project

→ Atlantic salmon with high/full lice resistance reducing necessity for delousing

CrispResist Partners



https://nofima.no/prosjekt/crispresist/

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