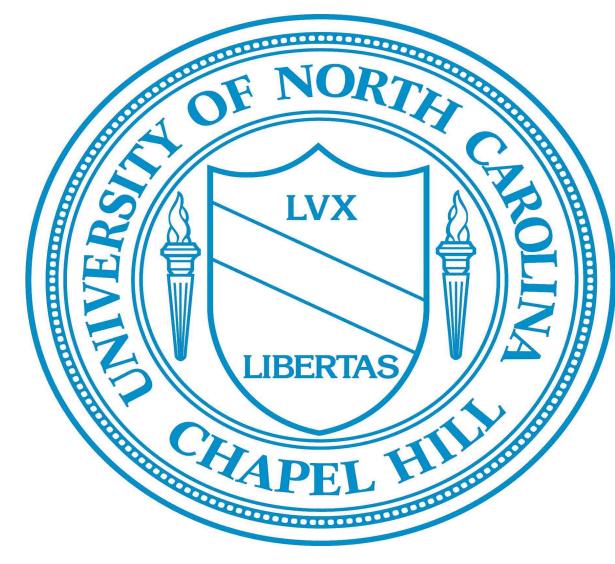


High cellular resolution *in vivo* tracing of macrophage activation due to injury or immune dysregulation



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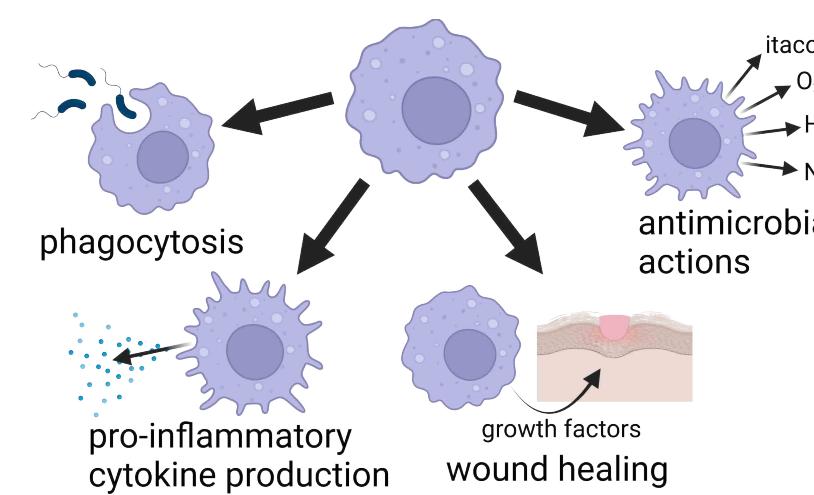
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Abstract

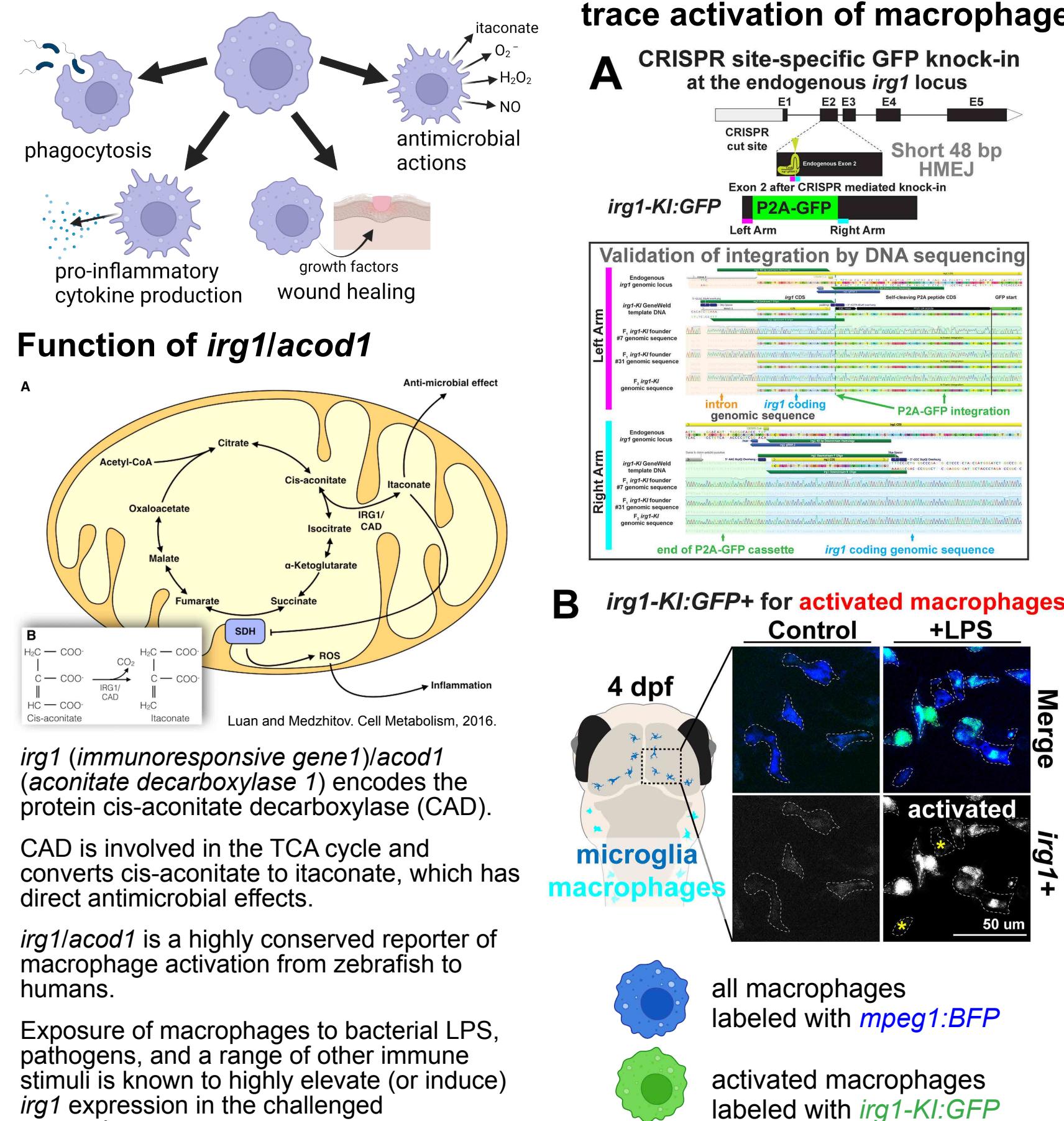
Macrophages and microglia are essential phagocytic cells of the body responsible for detecting and resolving infection and injury. During an immune response, macrophages/microglia become activated as defined by significant changes in cellular behavior and morphology as well as gene expressions, including upregulations of pro-inflammatory cytokines and a conserved activation marker immunoresponsive gene 1 (*irg1*), also known as aconitate decarboxylase 1 (*acod1*). While the importance of macrophages in eliminating tissue damage and infection is clear, the mechanisms and dynamics of the entire course of macrophage response from activation to resolution remain poorly understood. Inappropriate macrophage response can lead to either chronic inflammation or immunodeficiency. To address this critical gap, we leverage a tail wounding paradigm in zebrafish, previously used by others to analyze macrophages in inflammation, wound healing, and tissue regeneration. Most excitingly, we generated a powerful GFP knock-in reporter for *irg1* using CRISPR-Cas9 that allow precise *in vivo* tracing of macrophage activation in real time. This new genetic reporter provides the most faithful and quantitative tool to measure *irg1* transcription at single-cell resolution *in vivo*. To characterize macrophage change in detail, we conducted a long-term longitudinal study of the tail wound response by macrophages. By quantifying macrophage GFP intensity and area of recruited cells over time, we identified the time periods of initiation, peak, and resolution of macrophage activation. This study also reveals the heterogeneity in macrophage response and function that necessitates further investigation. Overall, we demonstrate a novel genetic platform to trace macrophage activation during tissue injury response that can apply to study a variety of biological problems.

Introduction

Macrophage functions



irg1 transcriptional reporter to trace activation of macrophages

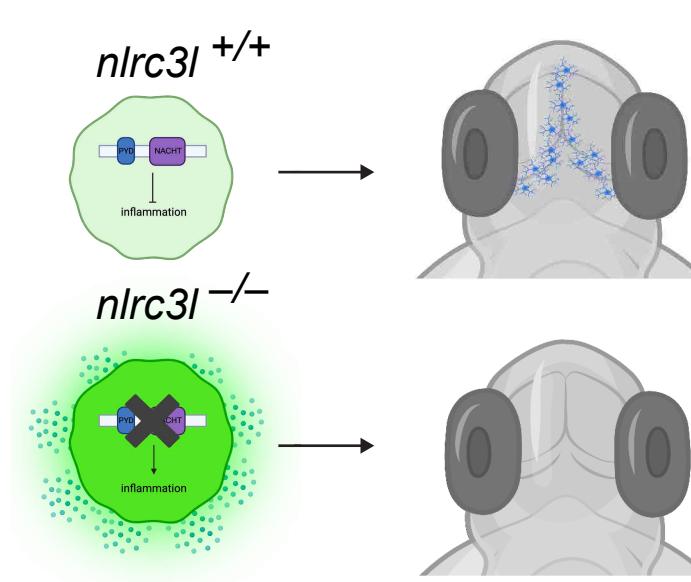


Function of NOD-like receptors

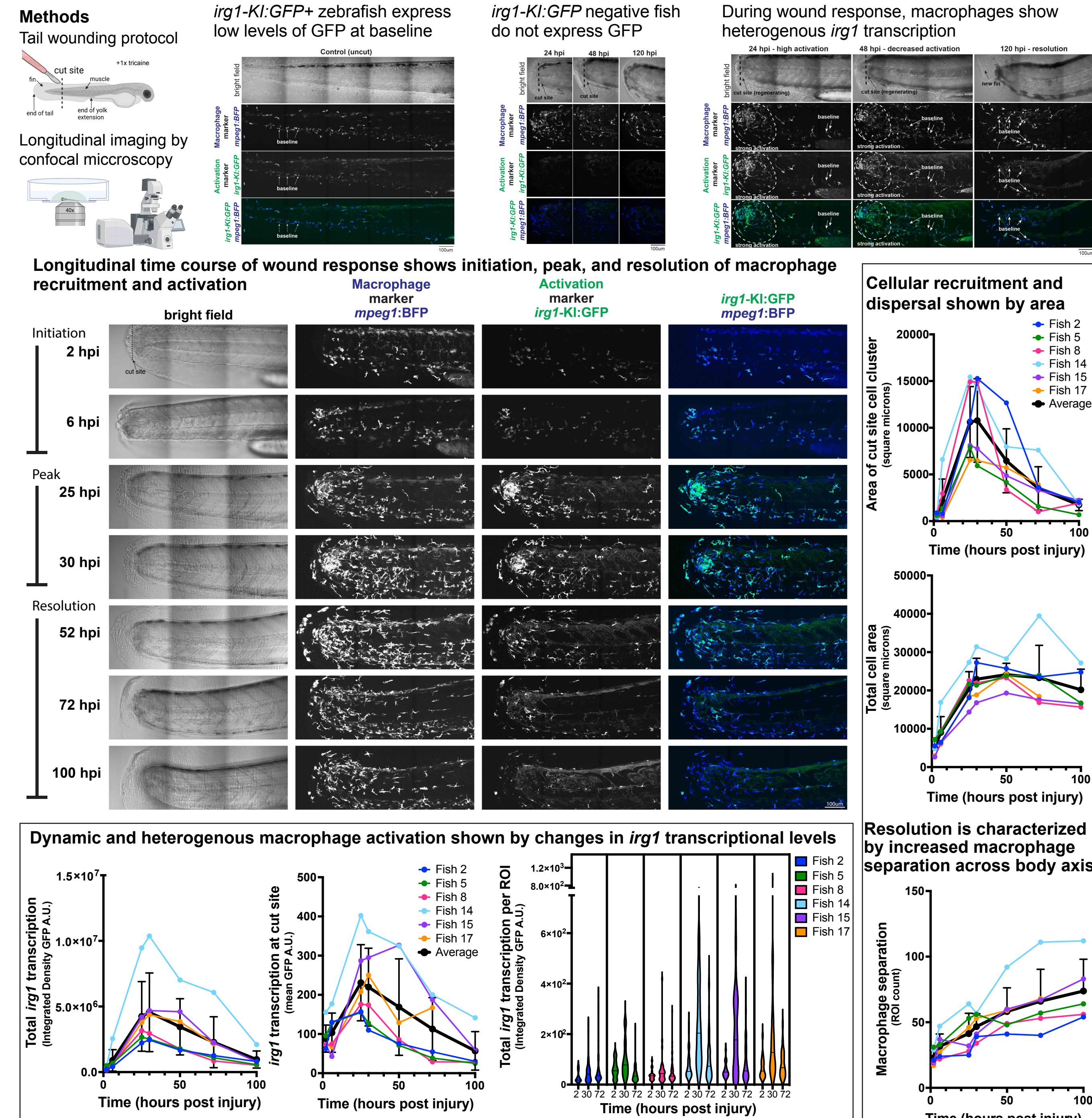
NOD-like receptors (NLRs) are cytosolic pattern recognition receptors important for innate immunity. NLRs are intracellular sentinels for sensing microbes, tissue damage, and cell stress. NLRs are often pro-inflammatory in function.

NLRC3-like is a novel and non-canonical NLR that is essential for microglia formation and also appears to be crucial for suppressing inappropriate activation of immune cells under normal conditions.

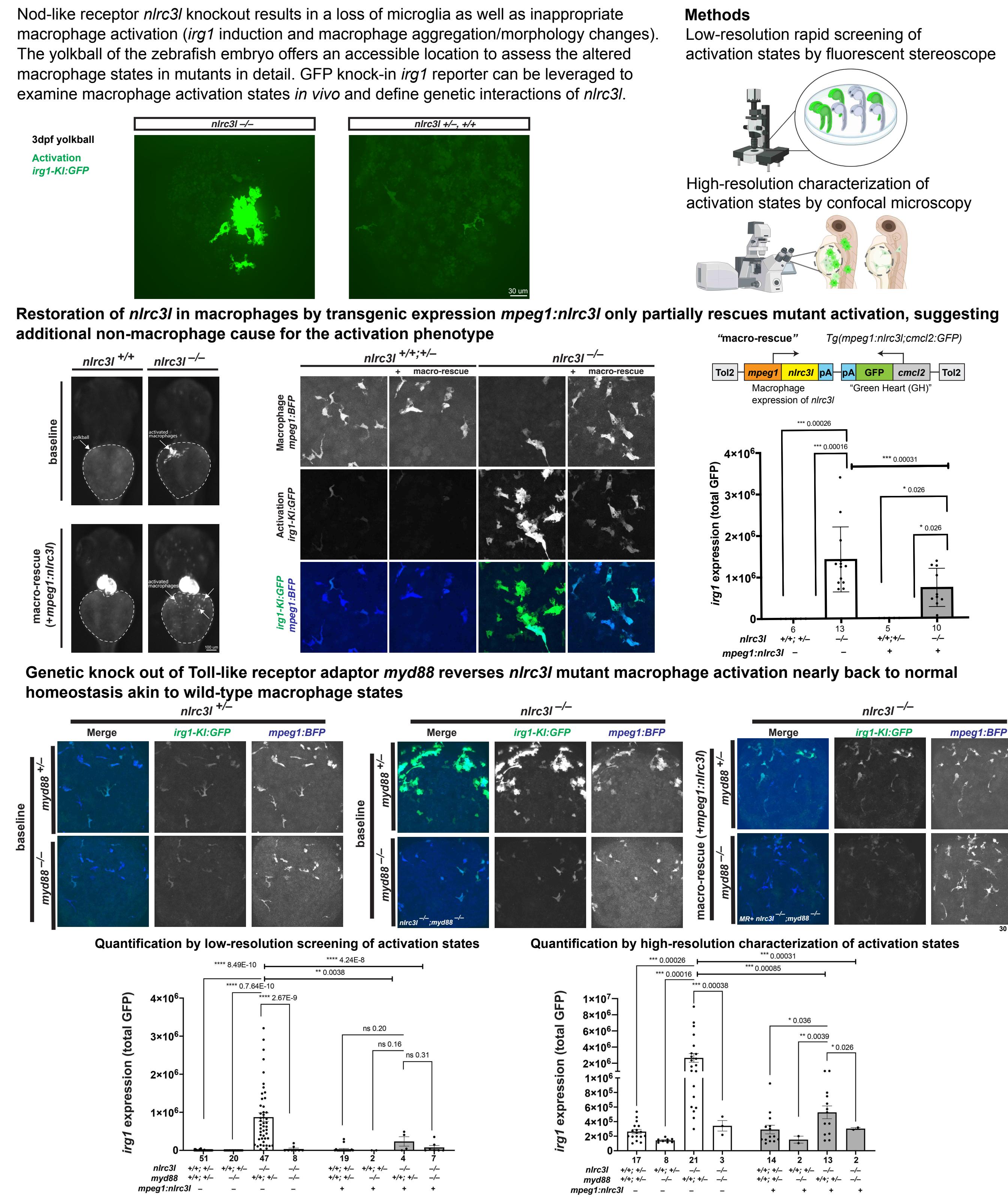
In the absence of *nrc3l*, macrophages are inappropriately activated (shown by *irg1-KI:GFP*) and zebrafish do not develop microglia, the resident immune cells of the brain.



Wound Response Characterization



Mutation Phenotype Characterization



Conclusions

- This study establishes *irg1-KI:GFP* as a powerful genetic tool to trace macrophage activation in different biological contexts in health and disease.
- We determine the general time-course and key stages of macrophage wound response relative to their activation state based on cellular tracing of *irg1* transcription and cellular behaviors. Further time-lapse imaging analysis of these changes will provide a more comprehensive understanding of macrophage dynamics relative to their state changes.
- Our results reveal interesting subsets of macrophages during injury response based on *irg1* expression, cellular behavior, and anatomical location that may be associated with functional differences.
- Use of *irg1-KI:GFP* provides high resolution and quantitative assessment of macrophage activation changes to define important mediators of the immune dysregulation in *nrc3l* mutants.

Acknowledgements

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