FUNCTIONAL CHARACTERIZATION OF INTERFERON REGULATORY FACTOR 5 (IRF5) IN ANTIVIRAL IMMUNE RESPONSES OF RED-SPOTTED GROUPER (*Epinephelus akaara*)

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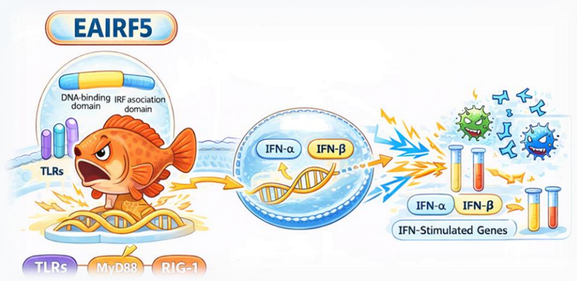
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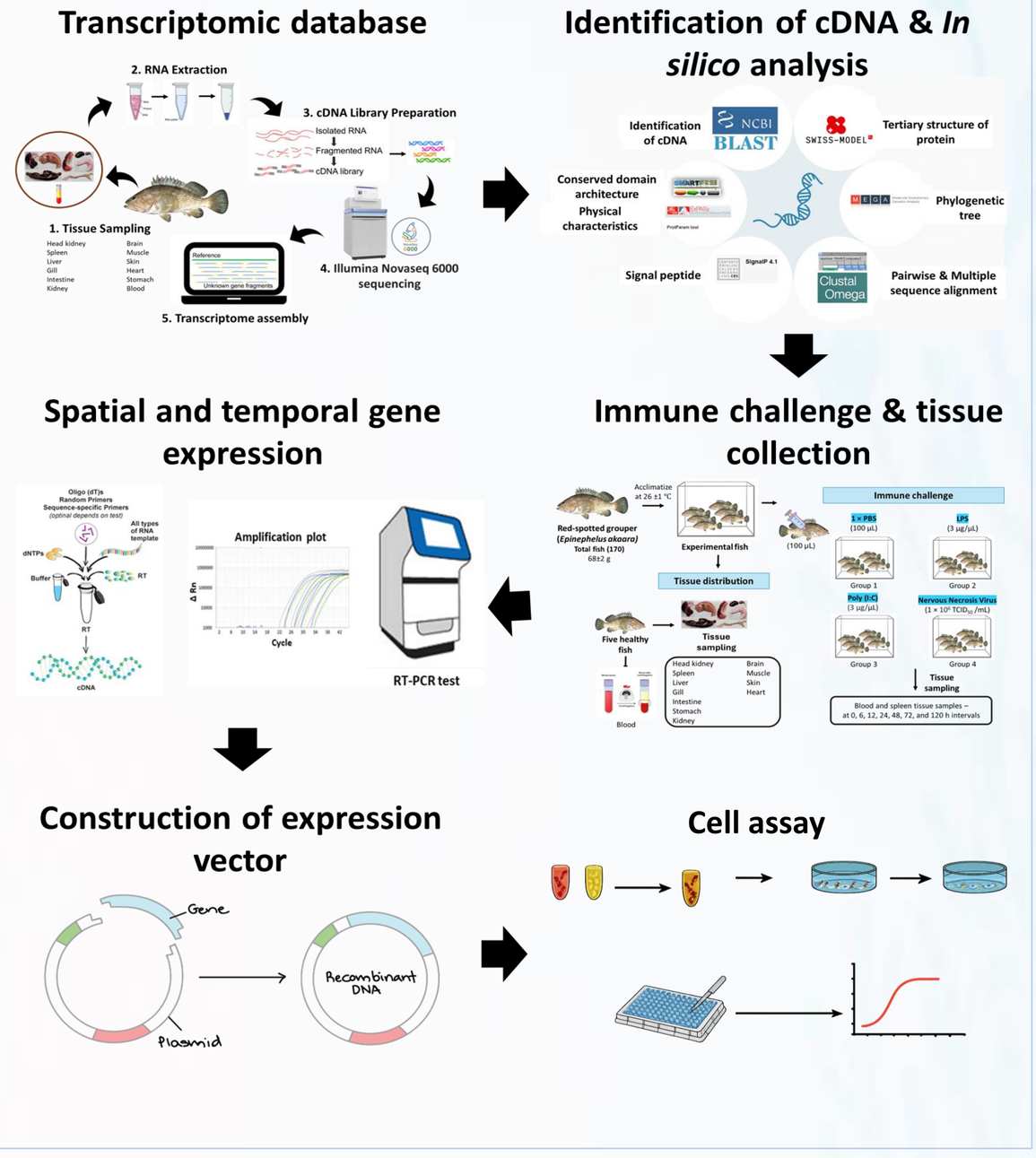
1 Abstract

Red-spotted grouper (*Epinephelus akaara*) is a highly valuable species in marine aquaculture, but its production is severely affected by viral diseases such as nervous necrosis virus (NNV) and viral hemorrhagic septicemia virus (VHSV).



Understanding the mechanisms of the fish immune system is essential for developing effective disease control strategies. Interferon regulatory factors (IRFs) are key transcription factors involved in antiviral immunity, particularly in regulating type I interferon (IFN) responses and innate immune signaling pathways. Among them, IRF5 plays an important role in activating immune responses against viral infections. However, the function of IRF5 in red-spotted grouper remains largely unexplored. Therefore, this study aims to characterize IRF5 (*EAIRF5*) and investigate its role in antiviral immune responses, providing insights that may contribute to improved disease management in grouper aquaculture.

2 Methodology



3 Results

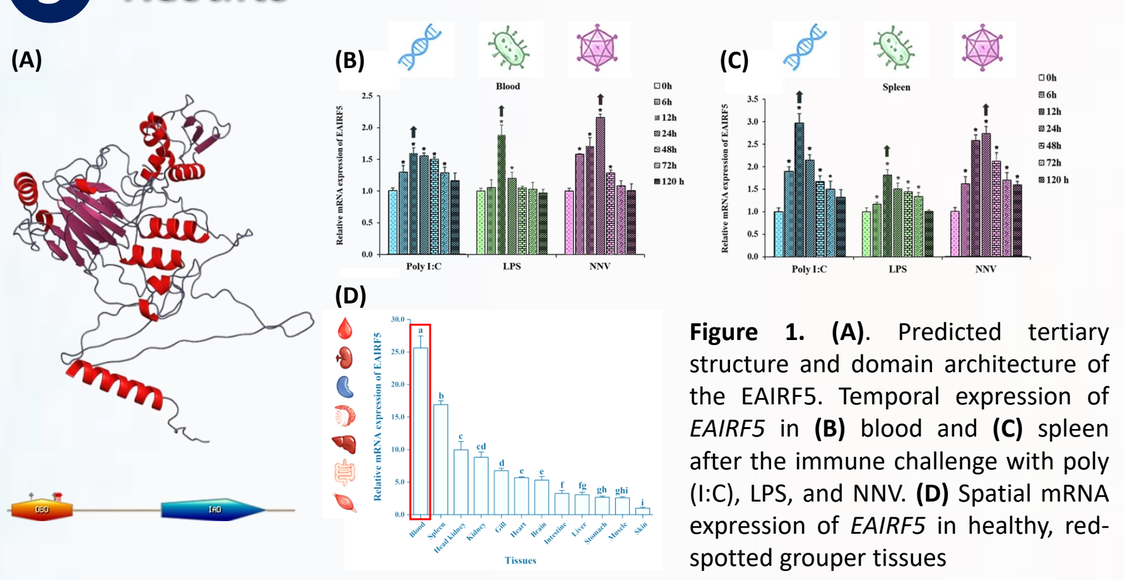


Figure 1. (A). Predicted tertiary structure and domain architecture of the EAIRF5. Temporal expression of *EAIRF5* in **(B)** blood and **(C)** spleen after the immune challenge with poly (I:C), LPS, and NNV. **(D)** Spatial mRNA expression of *EAIRF5* in healthy, red-spotted grouper tissues

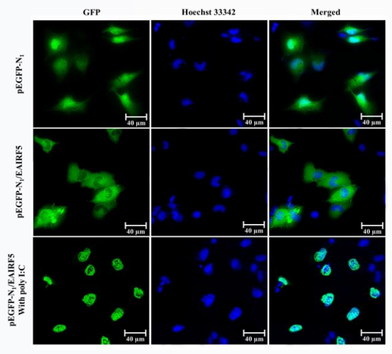


Figure 2: Subcellular localization and nuclear translocation of EAIRF5. Fluorescence images of FHM cells transfected with pEGFP-N1 control vector (First row), transfected with pEGFP-N1/EAIRF5(Second row), and pEGFP-N1/EAIRF5with poly I:C at 24 (Third row).

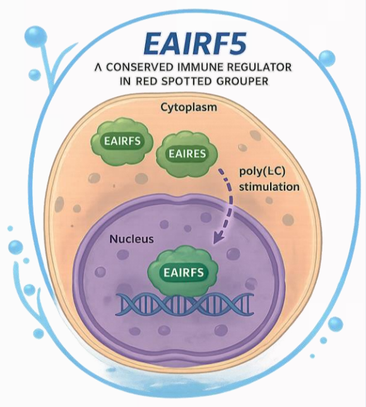
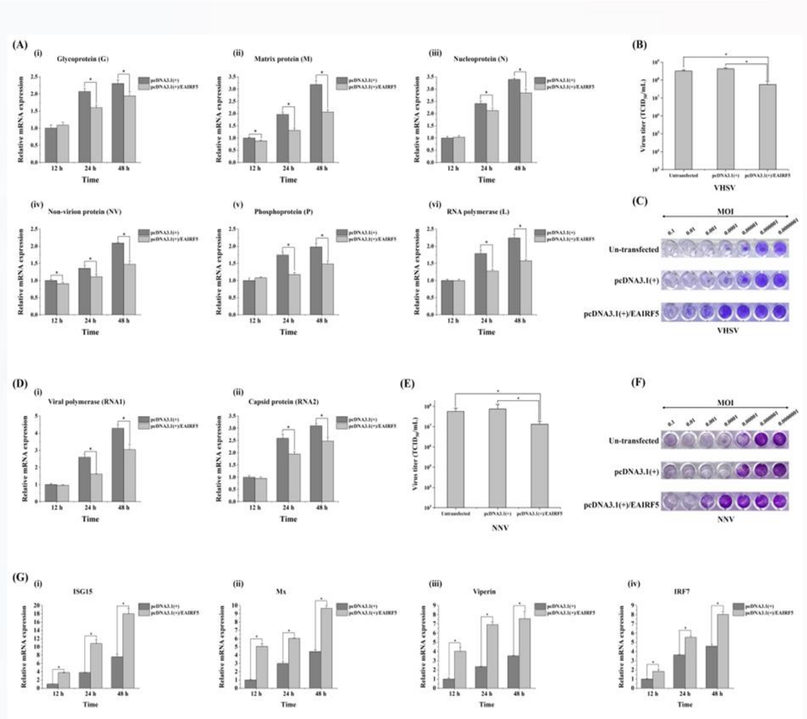




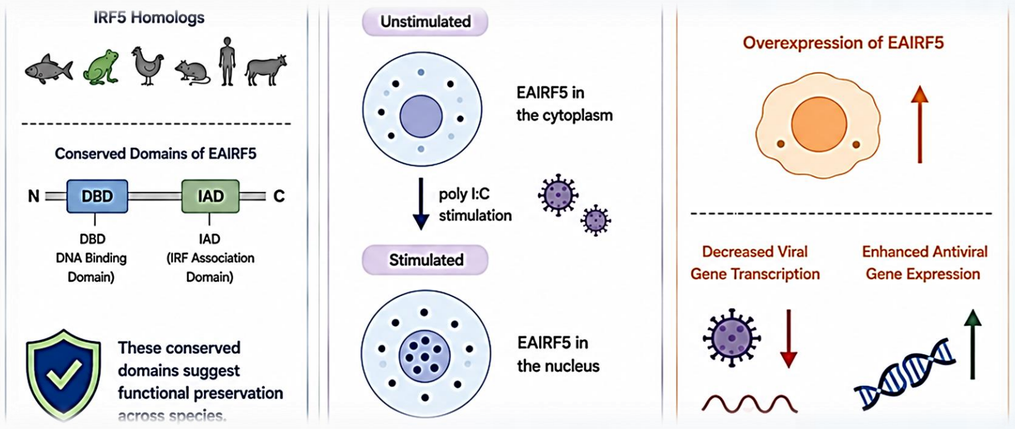
Figure 3: Antiviral properties of EAIRF5. **(A)** The mRNA expression levels of six VHSV genes at 12 h, 24 h, and 48 h post-infection. **(B)** Effect of *EAIRF5* overexpression on VHSV titer and **(C)** cell survival under different MOI levels. **(D)** The mRNA expression levels in two NNV genes, **(E)** NNV titer and **(F)** cell survival under different MOI levels. **(G)** The impact of *EAIRF5* on downstream genes involved in the IFN/IRF signaling pathway.



4 Discussion



- EAIRF5 showed a well conserved structural properties among various IRF 5 homologs.
- Tissue distribution and temporal expression analysis highlighted its vital engagement in host immune response, specifically in antiviral activity.
- EAIRF5 protein was localized in the cytoplasm under unstimulated state and was demonstrated its nuclear translocation following the poly I:C stimulation, emphasizing its crucial role against viral antigens in cellular state.
- At the functional level, cells overexpressing EAIRF5 demonstrated robust antiviral properties which was accompanied by a decreasing viral gene transcription and an enhancement in the expression of antiviral genes.



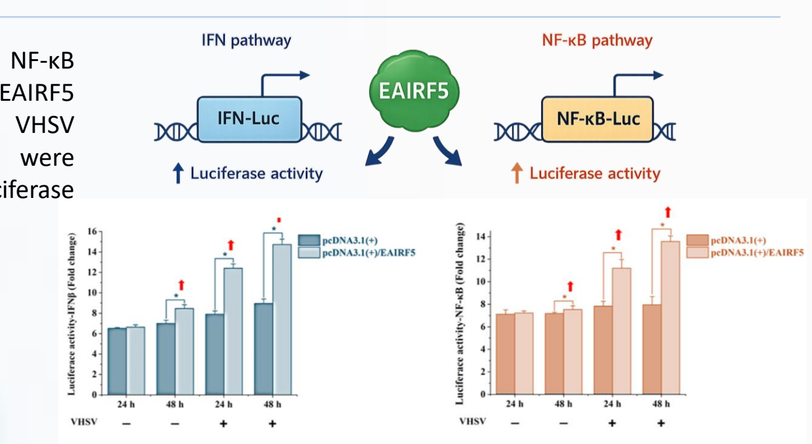
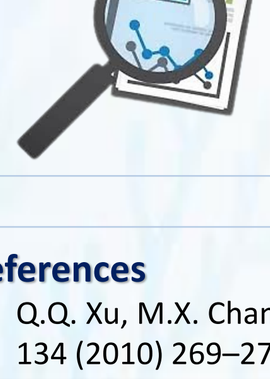


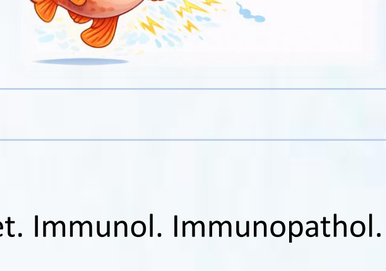
Figure 4: The IFN β and NF-κB induction upon EAIRF5 overexpression with VHSV infection (MOI 0.005) were evaluated by dual luciferase assay.



5 Conclusion



- ✓ These insights contribute to a deeper understanding of fish immunology and the intricate mechanisms governing immune responses in aquatic organisms



References

- Q.Q. Xu, M.X. Chang, F.S. Xiao, B. Huang, P. Nie, The gene and virus-induced expression of IRF-5 in grass carp Ctenopharyngodon idella, Vet. Immunol. Immunopathol. 134 (2010) 269–278. https://doi.org/https://doi.org/10.1016/j.vetimm.2009.10.020.

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