TARGETING OF TRIF SIGNALLING IN VIRAL AND BACTERIAL INFECTION : A STUDY ON TRIF$^{-/-}$ ZEBRAFISH

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ABSTRACT

TRIF serves as a crucial adaptor protein in TLR signaling pathways. TLR3 recruits TRIF directly to initial downstream signaling events leading to the expression of interferon-stimulated genes (ISGs). In aquaculture, it is essential to prevent and control, especially, infections as they can cause high mortality and economic losses in fish. Gene editing using CRISPR/Cas 9 resulting from the 5 bp frameshift mutation in trif ORF renders it non-functional. The *trif* expression was observed from the two-cell stage, which may underscore its significance in early zebrafish developmental events. The detection of *trif* in neuromast signifies additional functions. Disease symptoms and the mortality of both WT and *trif* knockout fish were evaluated following the challenge with VHSV and *E. piscicida*. The results revealed that *trif* KO had increased susceptibility with severe symptoms**,** accompanied by alterations in downstream gene expression. The caudal fin of the 5 dpf zebrafish larvae were amputated and immersed in the fluorescent-tagged VHSV (rVHSV). The *trif* KO had significantly higher infection due to the rapid penetration of VHSV through the caudal fin compared to the WT. The *trif*$^{-/-}$ *Tg (mpeg1:GFP;mpx;mcherry*) was developed for the simultaneous tracking of macrophages and neutrophils in real time**.** The *trif* KO fish showed a reduced number of immune cells at the injury site when stimulated with poly I: C. The findings proved that the intricate mechanisms by which Trif contributes to host defense against both viral and bacterial pathogens hold promising avenues for future research.

Generation of *trif* knockout zebrafish using CRISPR-Cas9

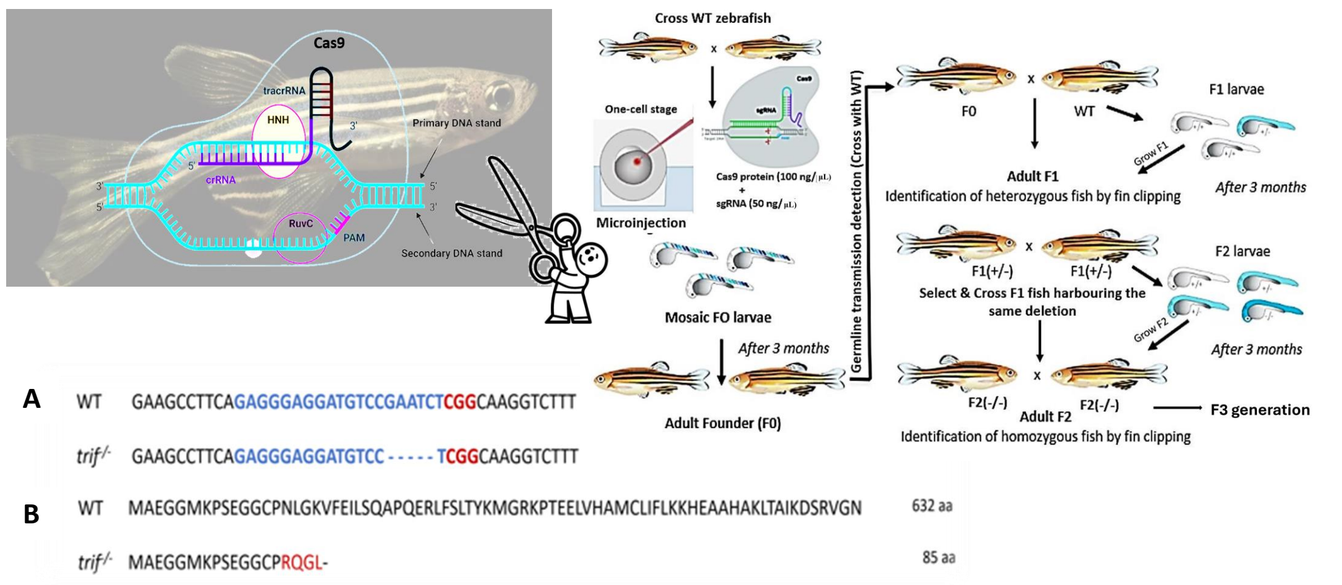
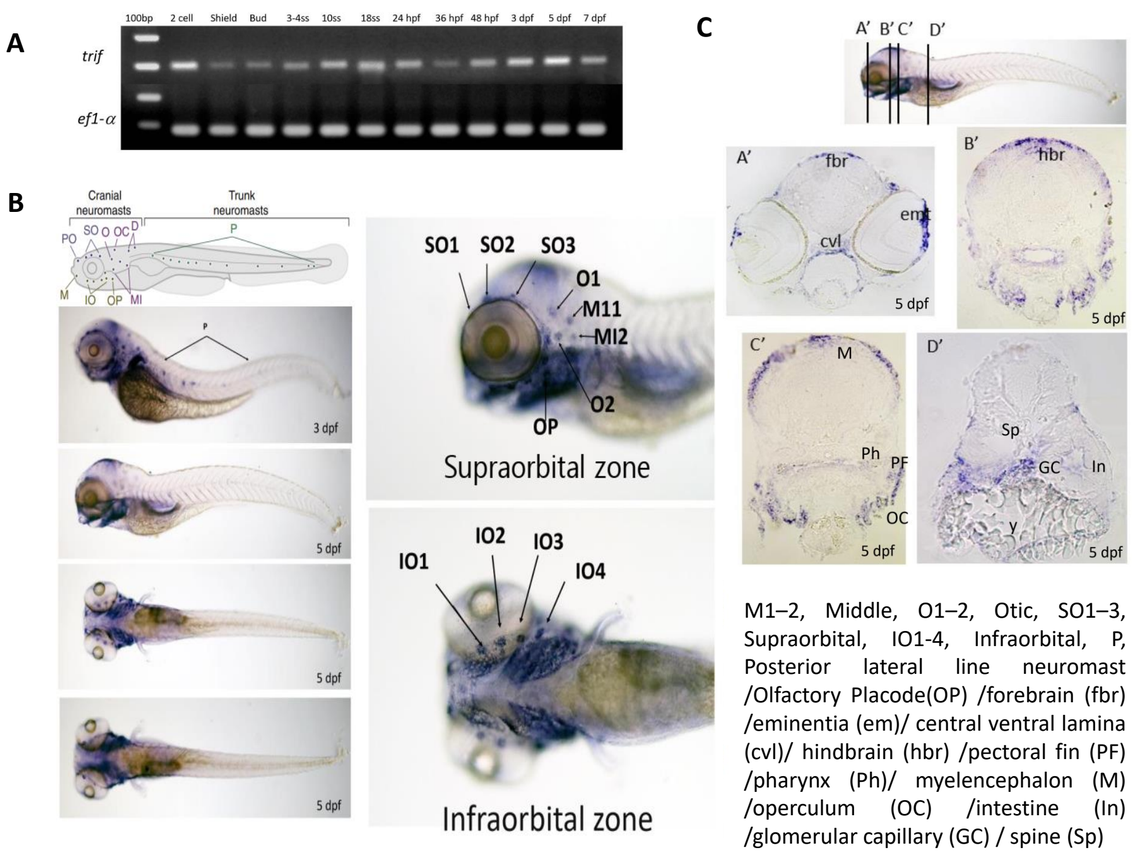


Figure 1. A, Founder fish with 5 bp frameshift deletion **B,** Deduced Trif amino acid sequences obtained from trif KO ORF. **C,** Checking the transcriptome for WT trif transcript. According to the result *trif KO* completely lacks the WT trif transcript

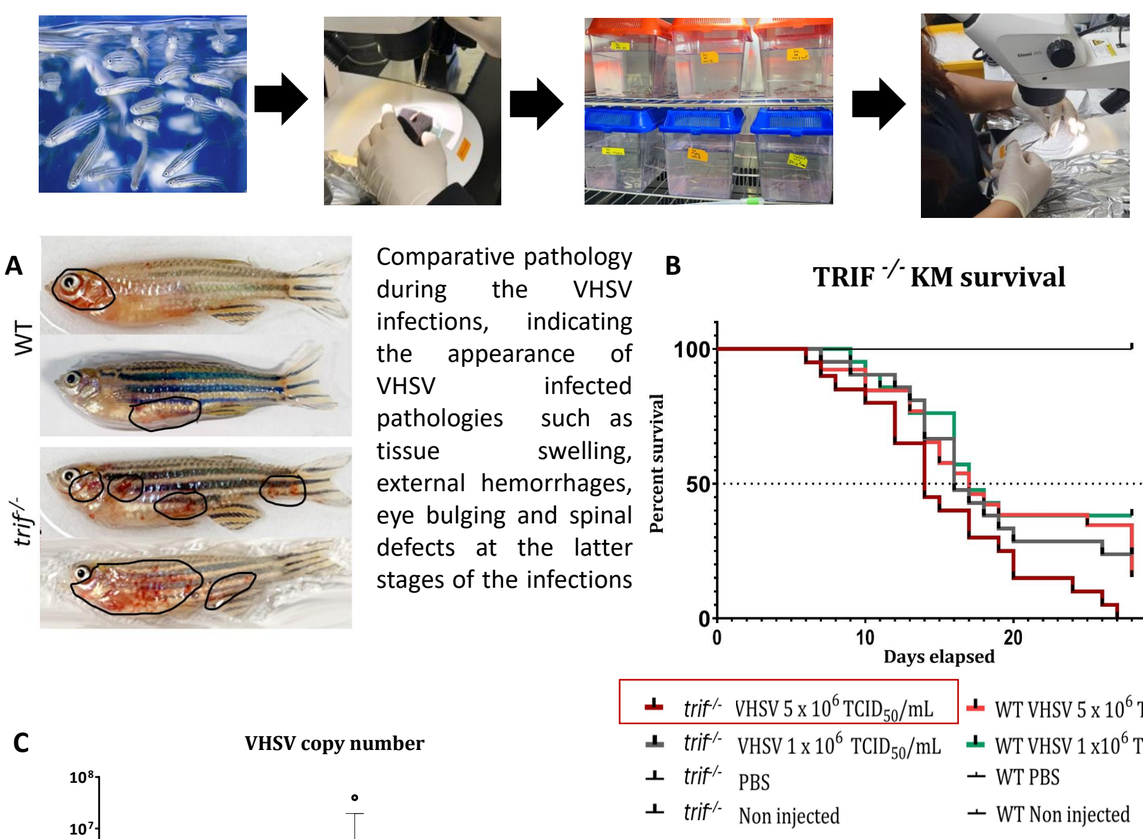
Expression of *trif* during zebrafish embryonic and adult stages



M1–2, Middle, O1–2, Otic, SO1–3, Supraorbital, IO1-4, Infraorbital, P, Posterior lateral line neuromast /Olfactory Placode(OP) /forebrain (fbr) /eminentia (em)/ central ventral lamina (cvl)/ hindbrain (hbr) /pectoral fin (PF) /pharynx (Ph)/ myelencephalon (M) /operculum (OC) /intestine (In) /glomerular capillary (GC) / spine (Sp)

Figure 2. A, *trif* expression was detected in all developmental stages by RT-PCR **B,** Whole-mount images of *in situ* hybridization for *trif* in zebrafish embryos. **C,** Lateral view of the whole embryo showing the planes of sectioning (CI-IV). **D,** Expression of *trif* during zebrafish adult stages.

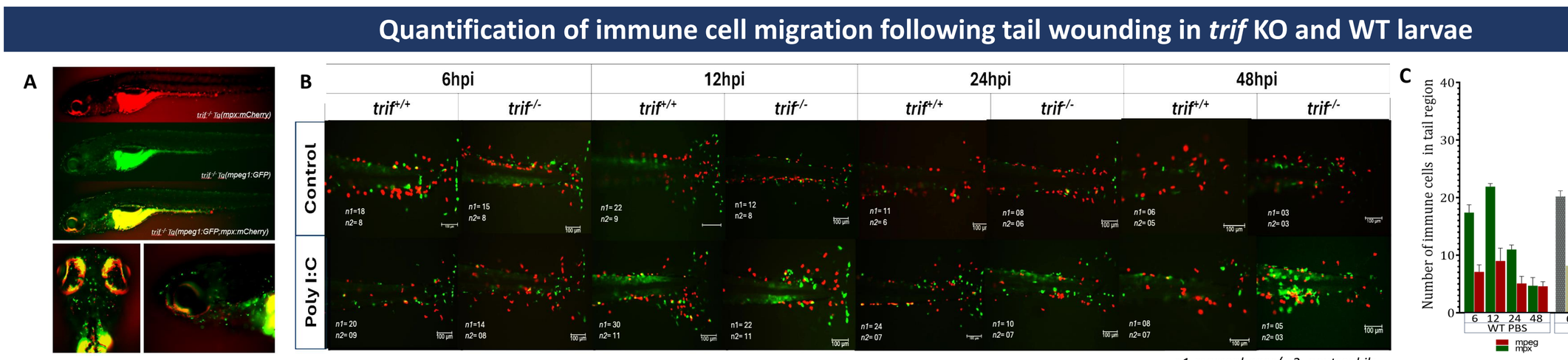
Survival analysis of WT and *trif*-ablated zebrafish during VHSV infection



Comparative pathology during the VHSV infections, indicating the appearance of VHSV infected pathologies such as tissue swelling, external hemorrhages, eye bulging and spinal defects at the latter stages of the infections

Figure 3. A, Macroscopic appearance of WT and *trif* ablated zebrafish infected with 5x10^6 TCID$_{50}$/ml of VHSV. Hemorrhages are marked by circles. **B,** Kaplan-Meier (KM)survival plot for the cumulative survival rate of zebrafish challenged with VHSV.**C,** Relative copy number of VHSV infected WT and *trif* KO zebrafish

Quantification of immune cell migration following tail wounding in *trif* KO and WT larvae



n1-macrophages/ n2- neutrophils

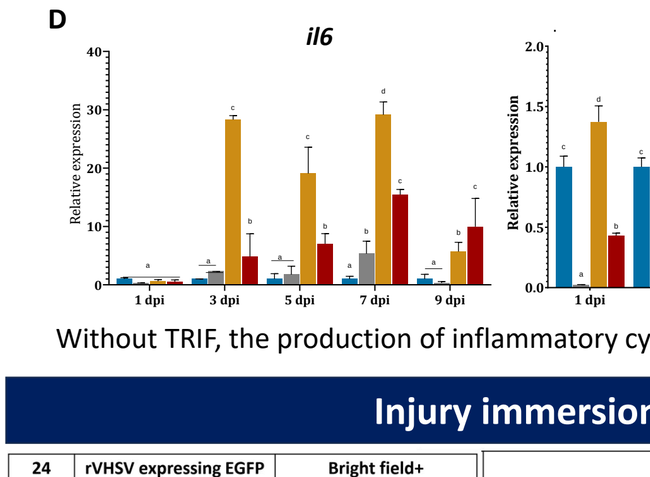
Figure 4. A, Generation of new *trif*$^{-/-}$ *Tg(mpeg1:GFP;mpx:mCherry)* transgenic larvae. **B.** Real time visualization of macrophages and the neutrophils at the injury site of WT and *trif*$^{-/-}$ *Tg(mpeg1:GFP;mpx:mCherry)* transgenic larvae zebrafish. **C.** The number of immune cells over the time at wound age. **D.** Analysis of *il6*, *sod1* and immune cell markers

Without TRIF, the production of inflammatory cytokines antioxidant gene, and immune cell marker expression were reduced, resulting in impaired immune cell recruitment to the site of injury

Injury immersion experiment for larval WT and *trif* KO following VHSV infection -deep wounding method

Figure 5. *trif* ablation resulted in the higher proliferation and rapid migration of the VHSV into zebrafish circulation through the caudal fin

Survival rate and immune gene response of WT and *trif*$^{-/-}$ KO to *E. piscicida* infection



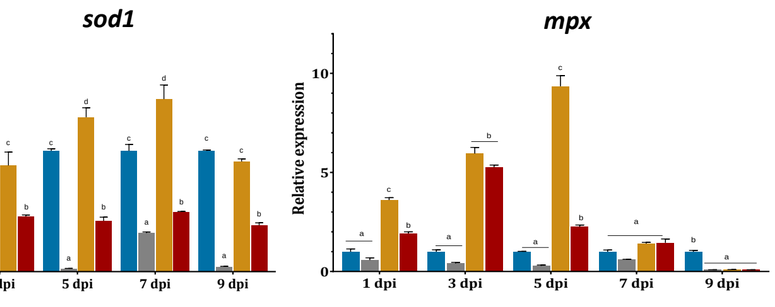


Figure 6. A. KM analysis of survival rates in WT and *trif* larvae following *E. piscicida* infection. **B.** Heat map of temporal gene expression analysis among WT and *trif* KO upon bacterial infection. Blue indicates repressed mRNA levels and red indicates elevated levels

CONCLUSION

Disruption of TRIF function leads to heightened susceptibility to VHSV and *E.piscicida* infections in and a reduction in immune cell recruitment in zebrafish, revealing TRIF's essential role in pathogen defense.