



Hypoxia-inducible factor-1 α (HIF-1 α) regulates the immune response via NF- κ B under hypoxia stress in Chinese mitten crab (*Eriocheir sinensis*)

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Introduction

Oxygen is essential for aerobic organisms to maintain normal life processes. Hypoxia exerts a multifaceted influence on the immune system, including physical defenses, innate immune responses, and inflammatory responses. Hypoxia-inducible factor 1-alpha (HIF-1 α), as a master regulator of oxygen homeostasis, plays a central role in immune responses to hypoxic stress.

Antimicrobial peptides (AMPs) are prevalent in nature and possess antimicrobial and immunomodulatory activities. Anti-lipopolysaccharide factors (ALFs) are the primary AMPs of crustaceans involved in defense, which were active in binding to several bacterial strains and inhibiting the proliferation of bacterial. Hypoxia induced a prolonged inflammatory response and involves both innate and adaptive immune systems.

In this study, we investigated the changes in the expression levels of HIF-1 α and ALFs in the hemolymph of *Eriocheir sinensis* under hypoxia stress after stimulation with *Aeromonas hydrophila*, and the expression levels of ALFs and Relish, the phosphorylation level of NF- κ B and the bacterial clearance ability of hemolymph after inhibition or activation of HIF-1 α . On this basis, we explored the immune response mechanism of HIF-1 α in response to pathogen stimulation under hypoxia stress in *Eriocheir sinensis*.

Results and discussion

1. The mRNA expression pattern of *EsHIF-1 α* after *A. hydrophila* stimulation under hypoxia condition

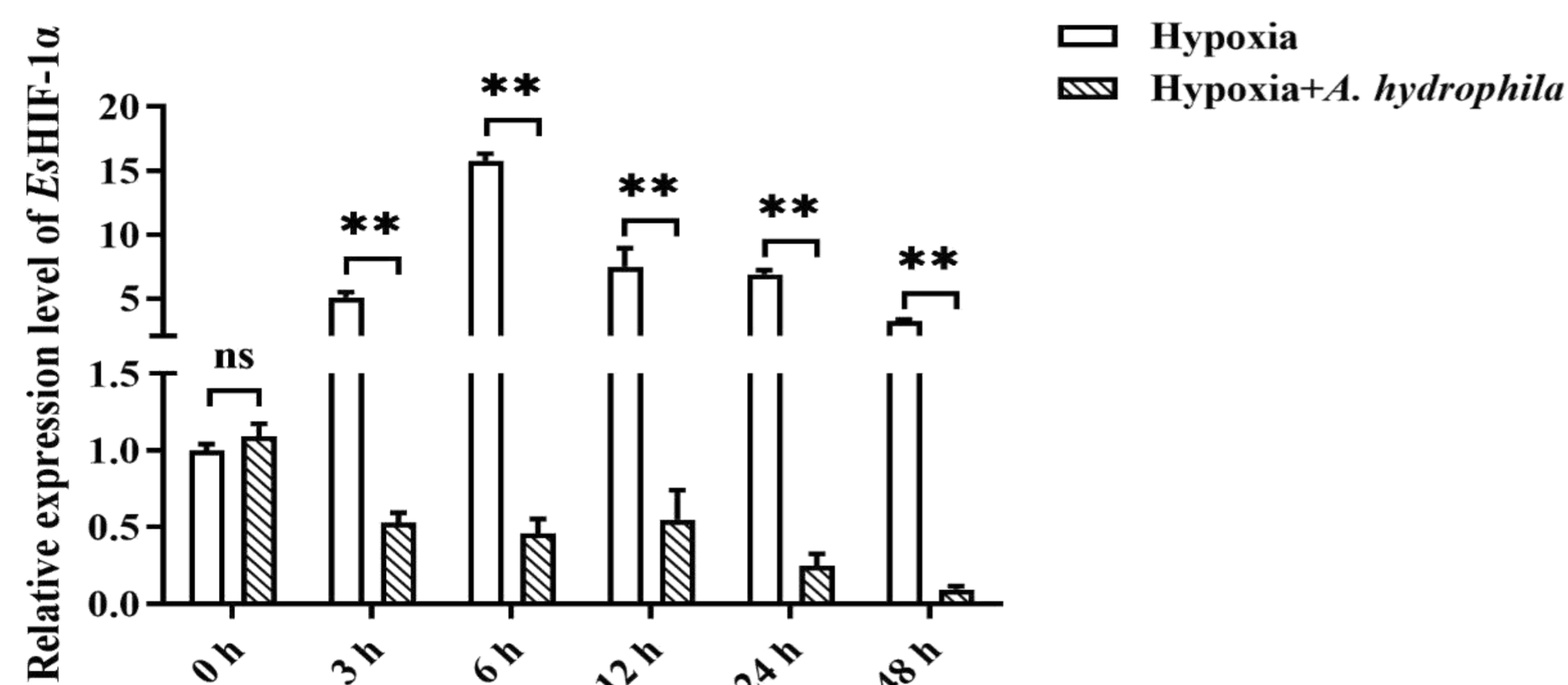


Fig. 1 The effects of bacterial stimulation on the expression levels of *EsHIF-1 α* in haemocytes post hypoxia stress

2. The mRNA expression pattern of *EsALFs* after *A. hydrophila* stimulation under hypoxia condition

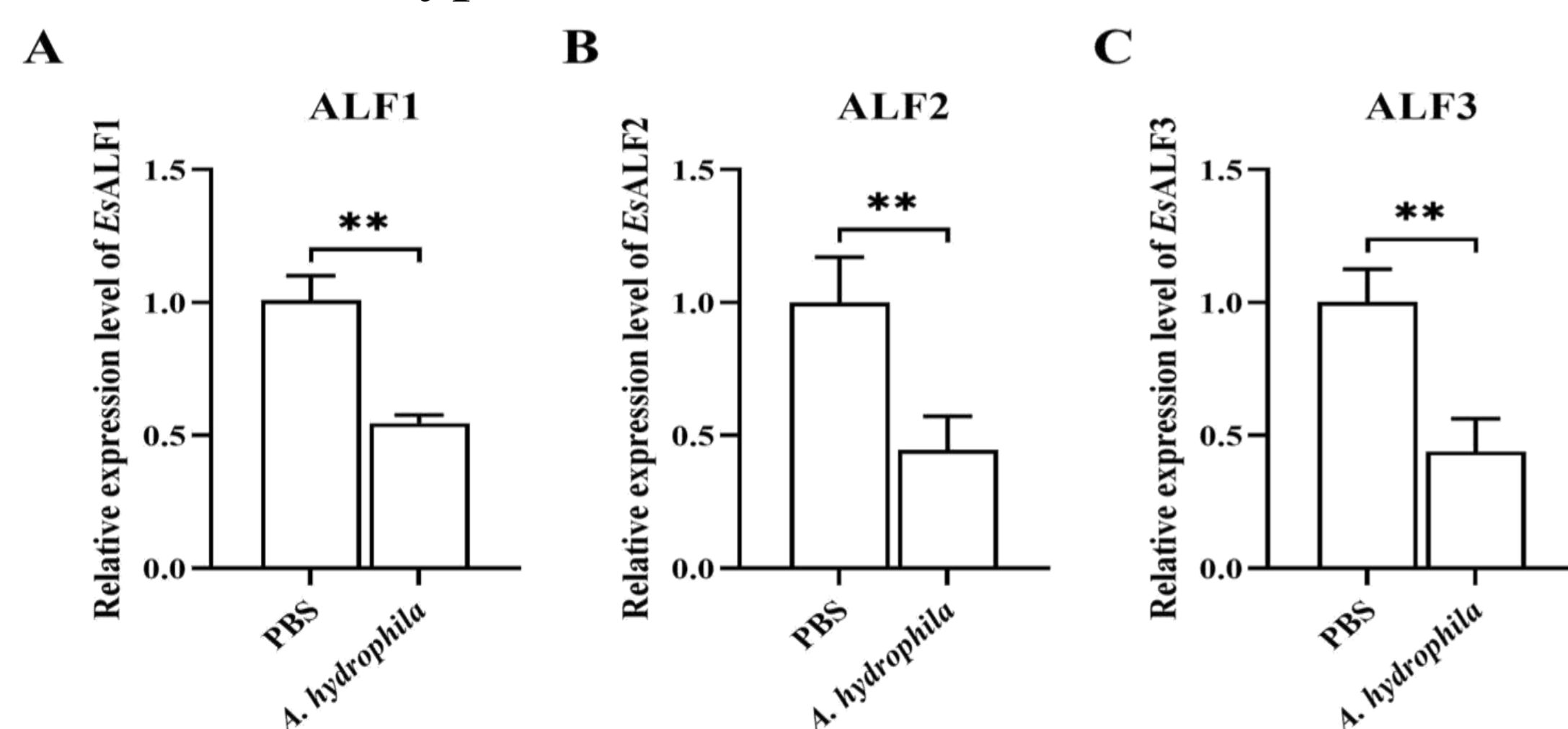


Fig. 2 The effects of bacterial stimulation on the expression levels of *EsALFs* in haemocytes post hypoxia stress

3. The mRNA expression levels of *EsALFs* and *EsRelish* after inhibiting or activating HIF-1 α

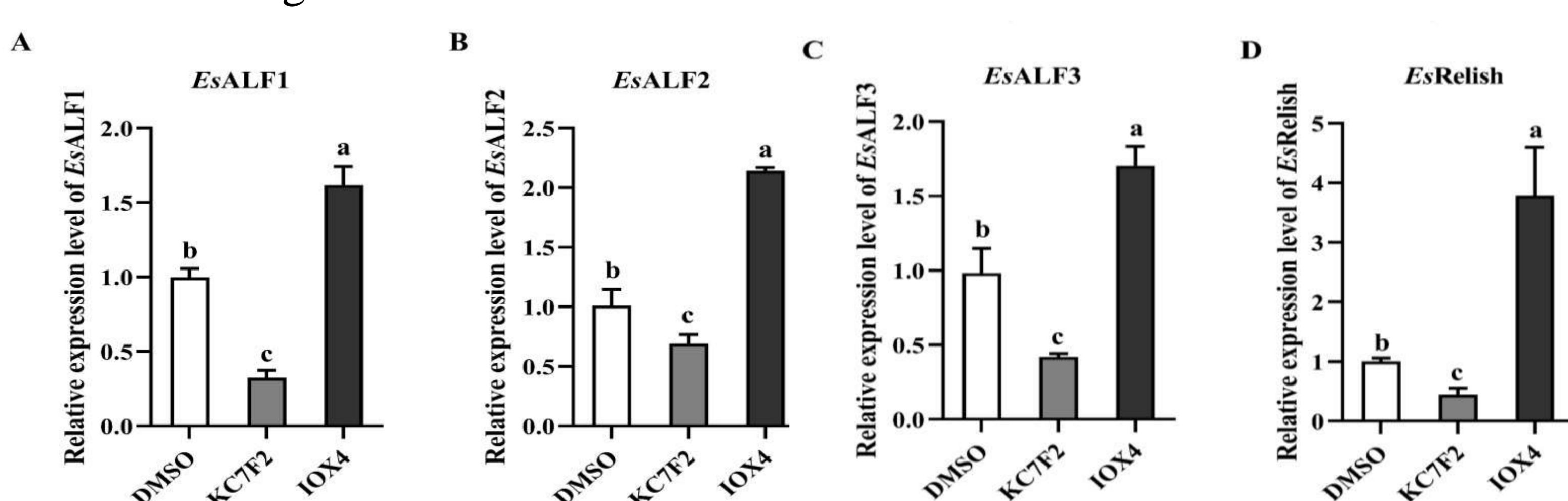


Fig. 3 The relative expression levels of *EsALFs* and *EsRelish* in haemocytes of KC7F2 (HIF-1 α inhibitor) and IOX4 (HIF-1 α activator) injected crabs post hypoxia treatment after bacterial injection

4. The phosphorylation of NF- κ B in HIF-1 α activator or inhibitor injected crabs

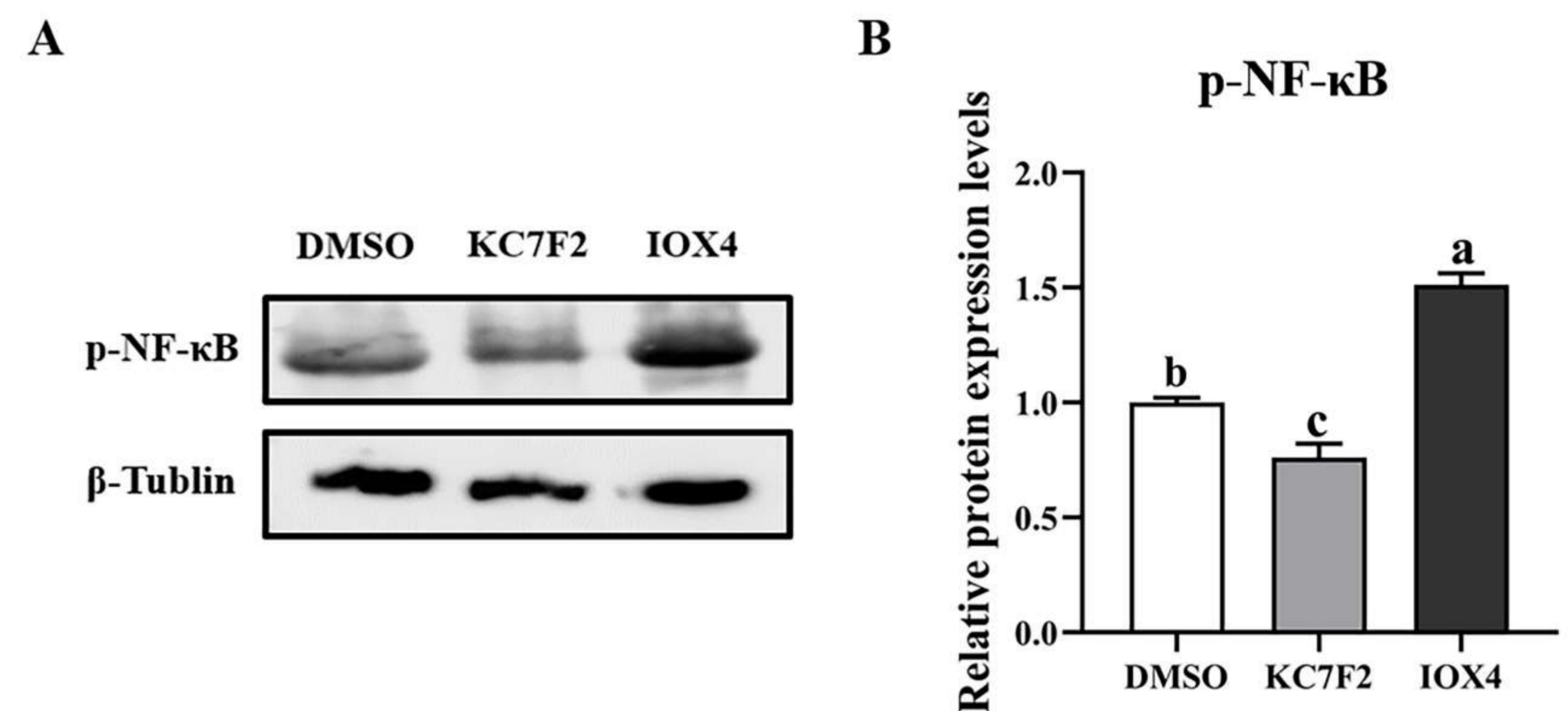


Fig. 4 The phosphorylation of NF- κ B in haemocytes of HIF-1 α activator or inhibitor injected crabs post hypoxia treatment after bacterial injection

5. The bacterial clearance ability of hemolymph in HIF-1 α activator or inhibitor injected crabs post hypoxia treatment after bacterial injection

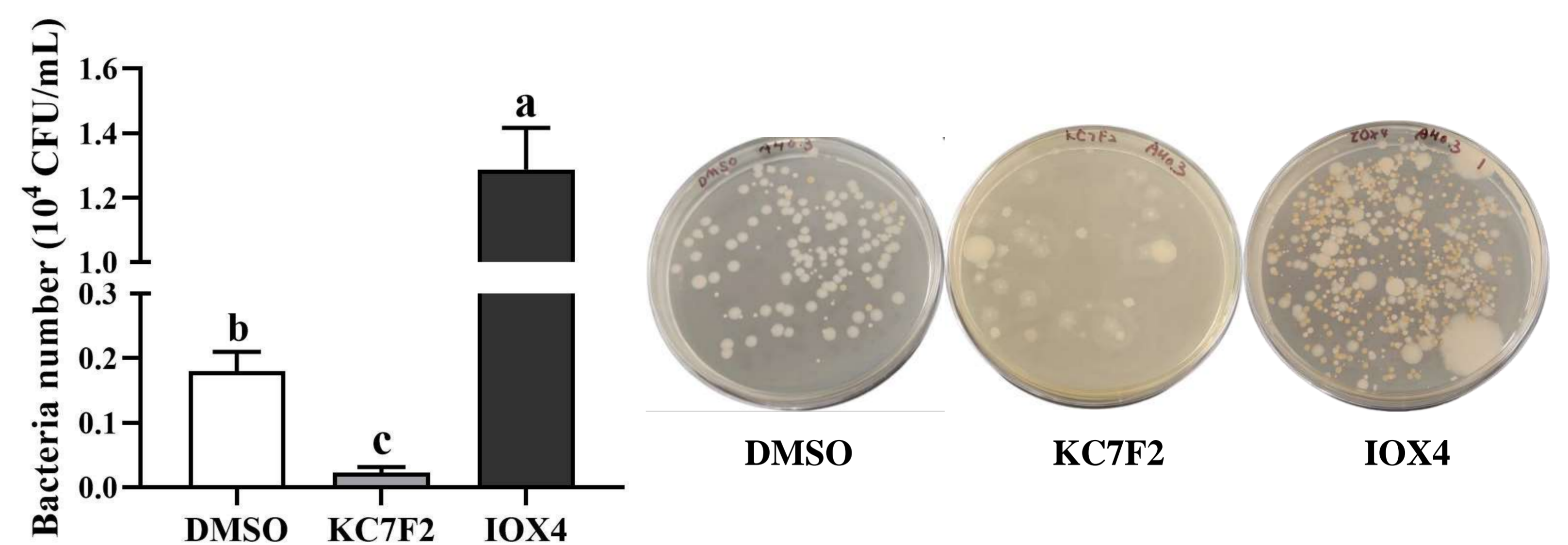
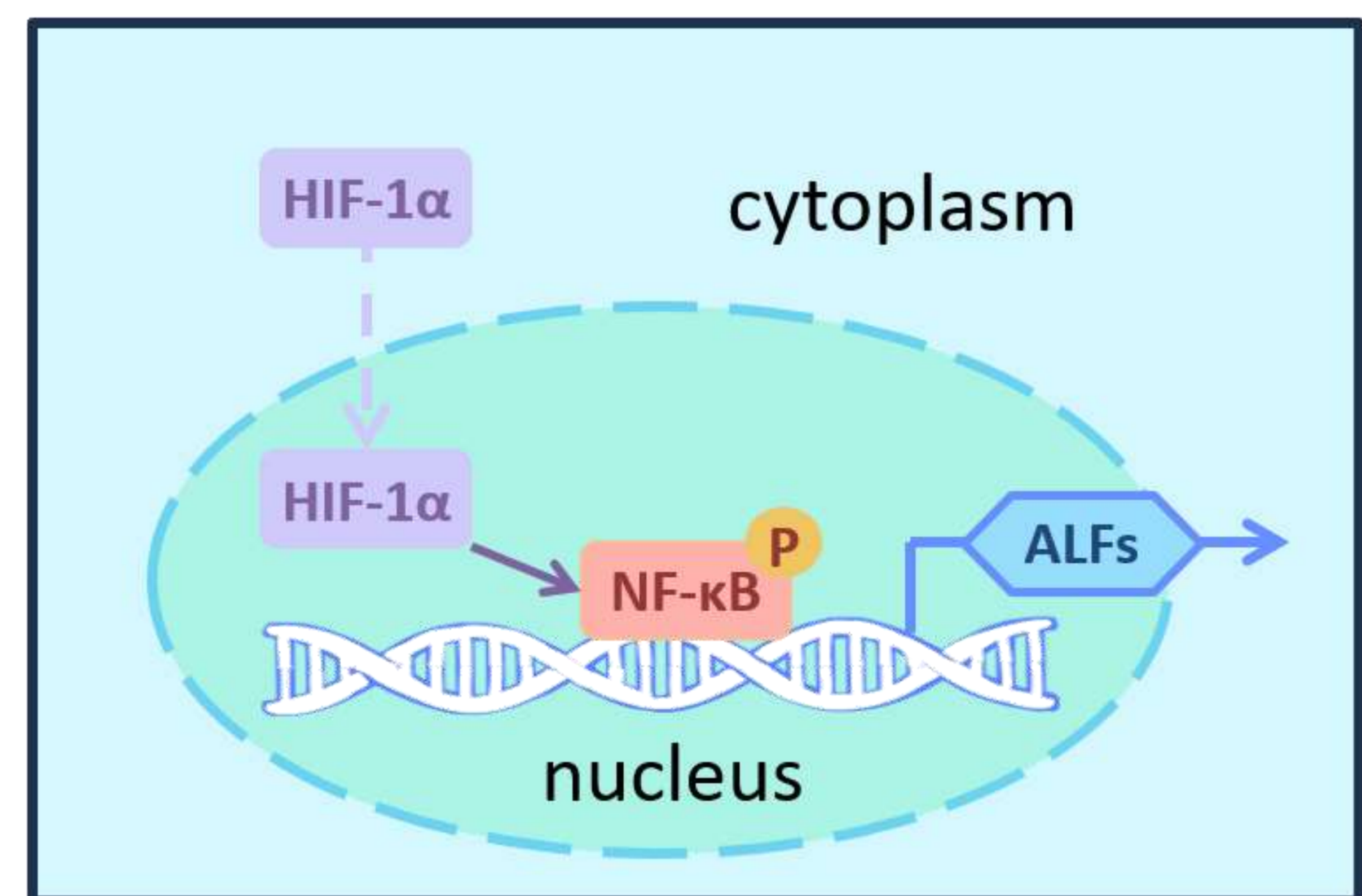


Fig. 5 Bacterial clearance ability of hemolymph in *Eriocheir sinensis* in IOX4 and KC7F2 injected crabs post hypoxia treatment after bacterial injection

6. Overview of the relationship among HIF-1 α , ALFs and NF- κ B in *E. sinensis*



Conclusion

- Under hypoxia stress, the expression levels of HIF-1 α and ALFs in hemolymph were significantly decreased after stimulation with *A. hydrophila*.
- HIF-1 α has a positive regulatory effect on the expression of ALFs and Relish.
- HIF-1 α promotes the phosphorylation level of NF- κ B.
- The bacterial clearance ability of hemolymph in the activated HIF-1 α group was significantly stronger than that in the inhibited HIF-1 α group and the hypoxia group.

HIF-1 α could positively regulates the immune response via NF- κ B under hypoxia stress in *Eriocheir sinensis*