Effect of FIH knockdown and knockout on IL-1β-mediated NF-κB activity in THP-1 monocytes

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Introduction

Lack of oxygen (hypoxia) occurs both under physiological and pathophysiological conditions. During inflammatory processes, local tissue hypoxia can develop as a result of damaged blood vessels and increased oxygen consumption. Under these conditions, the ability of cells to detect and respond to changes in oxygen levels is essential for maintaining cellular functions. Oxygen-sensing hydroxylases play a central role in the adaptation to hypoxic conditions by regulating the activity of the hypoxia-inducible factor (HIF). One of these enzymes is the asparagine hydroxylase factor inhibiting HIF (FIH), which regulates the transcriptional activity of HIF. FIH has been associated with the modulation of inflammatory signaling pathways *in vitro* and *in vivo* in the past, however the underlying molecular mechanisms linking FIH activity to inflammatory signaling remain poorly understood. The pro-inflammatory cytokine interleukin-1β (IL-1β) is a key mediator of inflammatory processes and activates the canonical NF-κB signaling pathway. This study investigates how FIH modulates the IL-1β-induced NF-κB signaling pathway in THP-1 monocytes and thereby influences the expression of inflammatory target genes. For this purpose, THP-1 monocytes with FIH knockdown (shFIH), FIH knockout (FIH K.O.) or corresponding control cells (shCtrl; WT) were stimulated with 1 ng/mL IL-1β at the indicated time points.

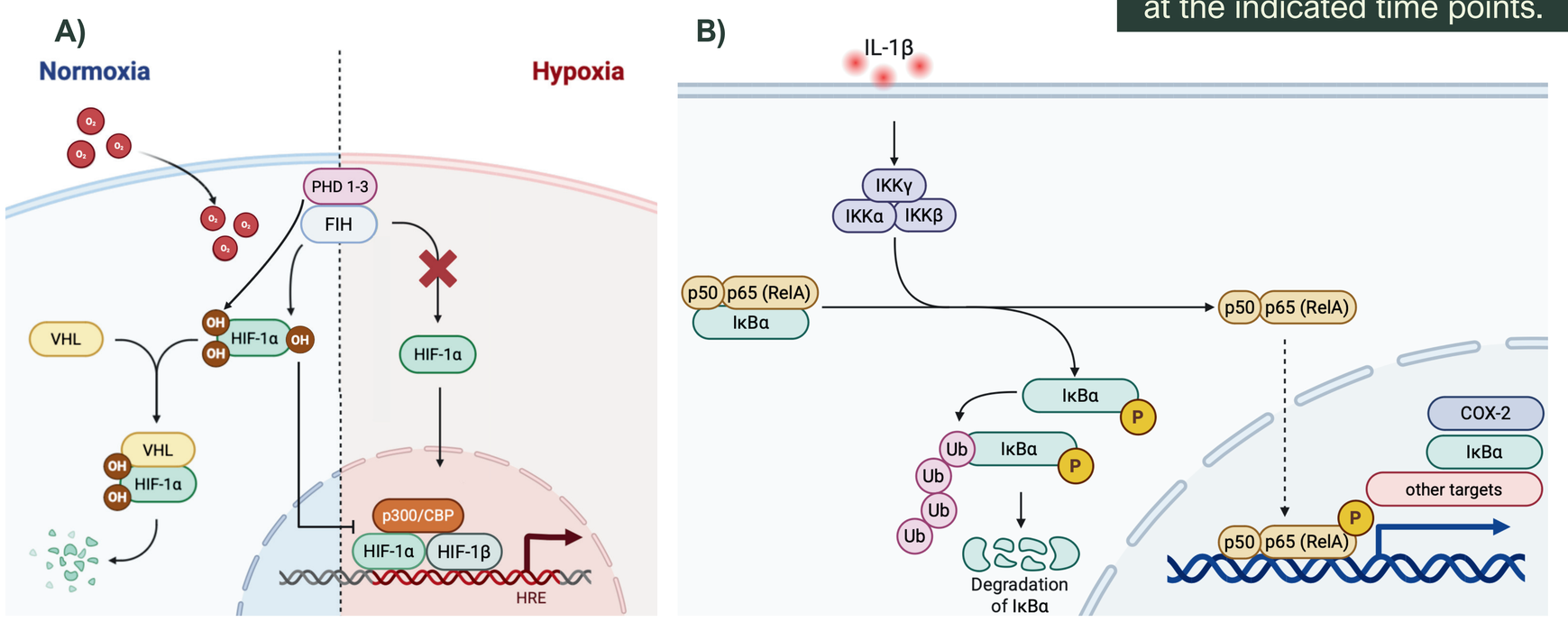
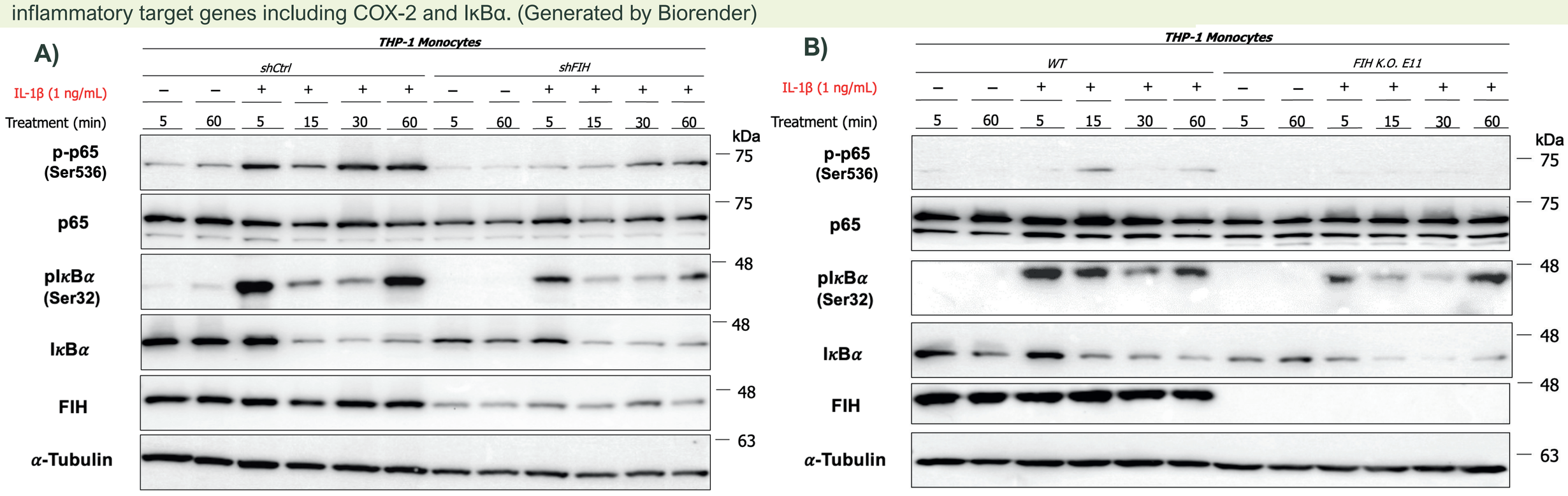


Fig. 1: The HIF and the NF-κB signaling pathway:

(A) Under normoxic conditions, FIH-dependent hydroxylation of an asparagine residue on HIF-1α prevents its interaction with the transcriptional coactivators p300/CBP, thereby inhibiting HIF transcriptional activity. Under hypoxic conditions, the availability of molecular oxygen is significantly reduced, leading to decreased FIH activity. Consequently, HIF-1α is not hydroxylated and can translocate into the cell nucleus, where it dimerizes with HIF-1β to form the active HIF complex, binds to hypoxia-response elements (HREs) and enhances the expression of specific target genes e.g. EPO or VEGF to enable adaptation to hypoxia. (B) IL-1β activates a pro-inflammatory NF-κB signaling pathway. This leads to the phosphorylation of the inhibitor protein IκBα and its proteasomal degradation. As a result, the NF-κB dimer, which most commonly consists of p65 and p50, is released and migrates into the cell nucleus. Additionally, p65 is phosphorylated at Ser536, which is associated with increased NF-κB transcriptional activity, regulating the expression of numerous pro-inflammatory target genes including COX-2 and IκBα. (Generated by Biorender)



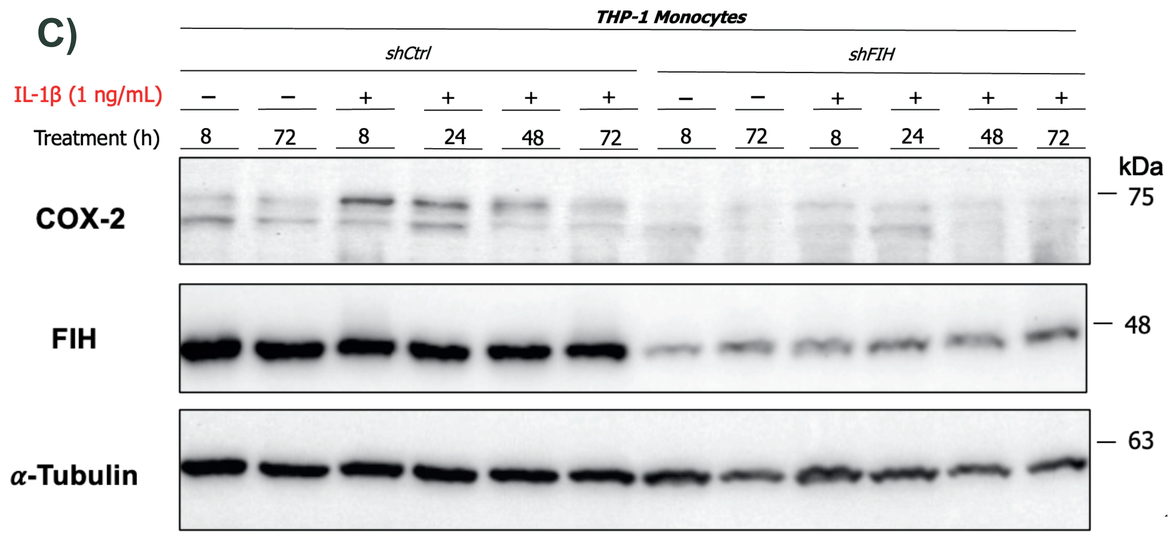


Fig. 2: FIH knockdown and knockout on IL-1β mediated signaling in THP-1 monocytes: (A, B)

To investigate the effect of FIH on IL-1β-induced signal transduction, THP-1 monocytes without (shCtrl) or with FIH knockdown (shFIH), as well as wildtype (WT) or knockout cells (FIH K.O. E11), were stimulated with 1 ng/mL IL-1β for 5-60 minutes. Activation of the NF-κB signaling pathway was assessed by analyzing the phosphorylation of p65 and IκBα in THP-1 monocytes with the indicated **(A)** knockdown and **(B)** knockout by immunoblot. **(C)** The expression of the downstream target COX-2 was analyzed over a stiumulation period of 8–72 hours by immunoblot. Successful FIH knockdown and knockout was confirmed in the corresponding samples. n = 2-5

Summary & Conclusions

- FIH knockdown leads to
  - decreased phosphorylation of IκBα and p65
  - reduced COX-2 expression

→ ***reduced IL-1β-mediated NF-κB activity***

Outlook

- Proliferation analyses for further characterization of the effect of FIH knockdown and knockout
- futher validation of the results using FIH knockout cells
- Extension of the experiments to macrophages
- Establishment of experiments under hypoxic conditions (hypoxia chamber)
- Establishment of transfection methods in THP-1 cells to rescue FIH knockdown and knockout