Characterizing the effect of the deletion of the oxygen sensor FIH on H9c2 cardiomyoblasts under ischemic conditions

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Introduction:

Cells must be able to adapt to changes in their micro-environment to guarantee survival, including conditions of reduced oxygen availability (hypoxia). Hypoxia plays a critical role in the pathophysiology of ischemia. Cellular adaptation to hypoxia is mediated by cellular oxygen sensors. One such sensor, factor inhibiting HIF (FIH), hydroxylates Hypoxia-inducible factor (HIF) α subunits, thereby reducing their transactivation activity. Under hypoxic conditions, hydroxylation cannot occur due to the lack of oxygen as a co-substrate, leading to activation of the hypoxic response. In mice, deletion of FIH results in a hypermetabolic phenotype characterized by increased energy expenditure, elevated oxygen consumption, and enhanced insulin sensitivity. These findings indicate that FIH plays an important role in energy metabolism. However, it remains unknown whether this hypermetabolic phenotype is present in all cell types and how FIH specifically affects cellular metabolism, especially under pathophysiological conditions. The effect of FIH knockout in cardiac cells, as well as its potential protective role under ischemic conditions, is also unclear. To address these questions, FIH knockout was introduced into H9c2 cardiomyoblasts. H9c2 FIH knockout (KO) cells were characterized under normoxic conditions. Subsequently, an *in vitro* ischemia model was established, and cells were exposed to hypoxia (1 % O_2) in nutrient-deprived medium at defined time points to evaluate metabolic activity and proliferation.

Results:

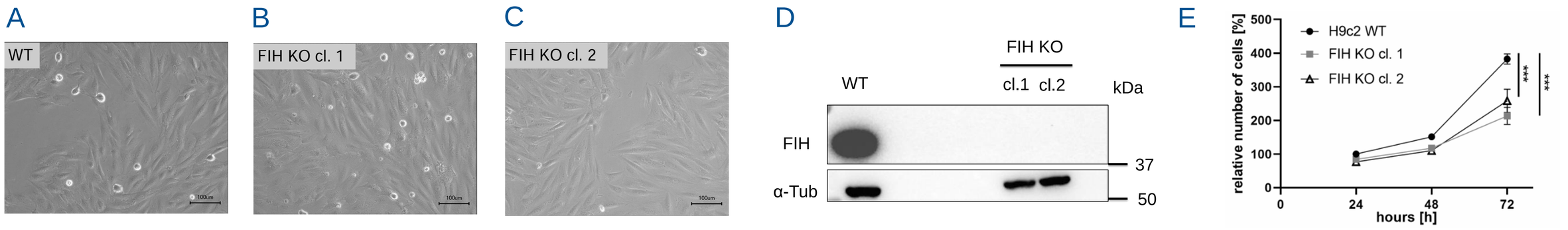

Fig. 1: H9c2 FIH KO characterization: (A) Images of H9c2 wild-type (WT), **(B)** H9c2 FIH KO clone 1 (KO cl. 1) and **(C)** H9c2 FIH KO clone 2 (KO cl. 2), **(D)** FIH detection in Immunoblot analysis of H9c2 WT and KO cells (cl. 1 and cl. 2), **(E)** cellular growth of H9c2 wild-type WT, FIH KO cl. 1 and FIH KO cl. 2 24, 48 and 72 hours after seeding, significance tested with two way ANOVA with Dunnett's multiple comparison test; *** p<0.001, n=3

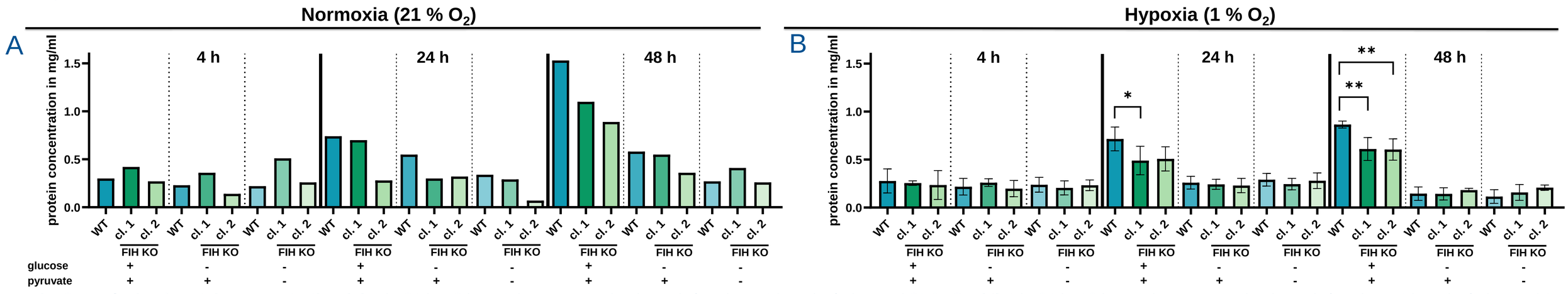

Fig. 2: Analysis of protein concentration (A) 4 h, 24 h, 48 h protein concentration in mg/ml under nutrient depletion and 21 % O_2, **(B)** 4 h, 24 h, 48 h protein concentration in mg/ml under nutrient depletion and 1 % O_2, significance tested with two way ANOVA with Dunnett's multiple comparison test; * p<0.05, ** p<0.01, *** p<0.001 indicate statistical significance relative to WT in control condition, 21 % O_2 n=1, 1 % O_2 n=4

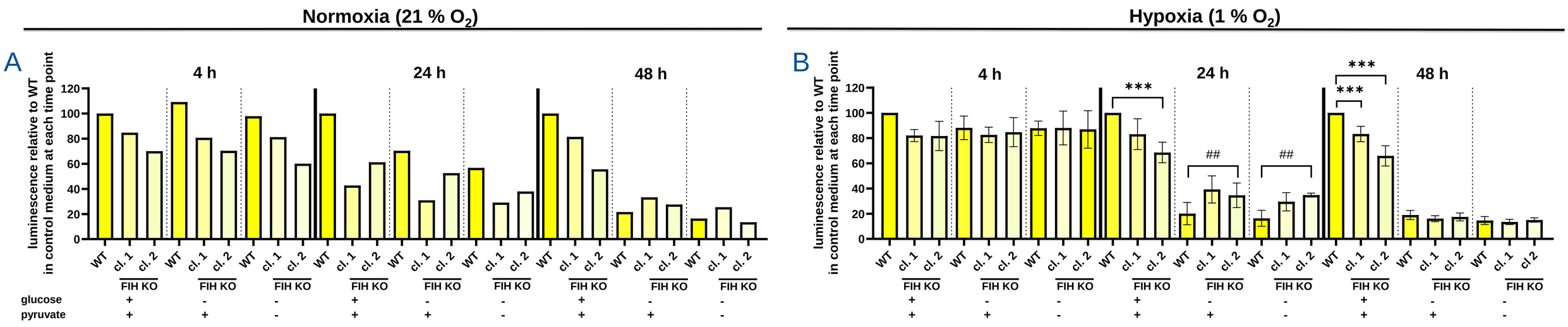

Fig. 3: Effect of FIH KO on ATP concentration (A) 4 h, 24 h, 48 h ATP concentration normalized to WT in control medium under nutrient depletion and 21 % O_2, **(B)** 4 h, 24 h, 48 h ATP concentration normalized to WT in control medium under nutrient depletion and 1 % O_2, significance tested with two way ANOVA with Dunnett's multiple comparison test; * p<0.05, ** p<0.01, *** p<0.001 indicate statistical significance relative to WT in control condition, # p<0.05, ## p<0.01 indicate statistical significance relative to WT in each condition, 21 % O_2 n=1, 1 % O_2 n=4

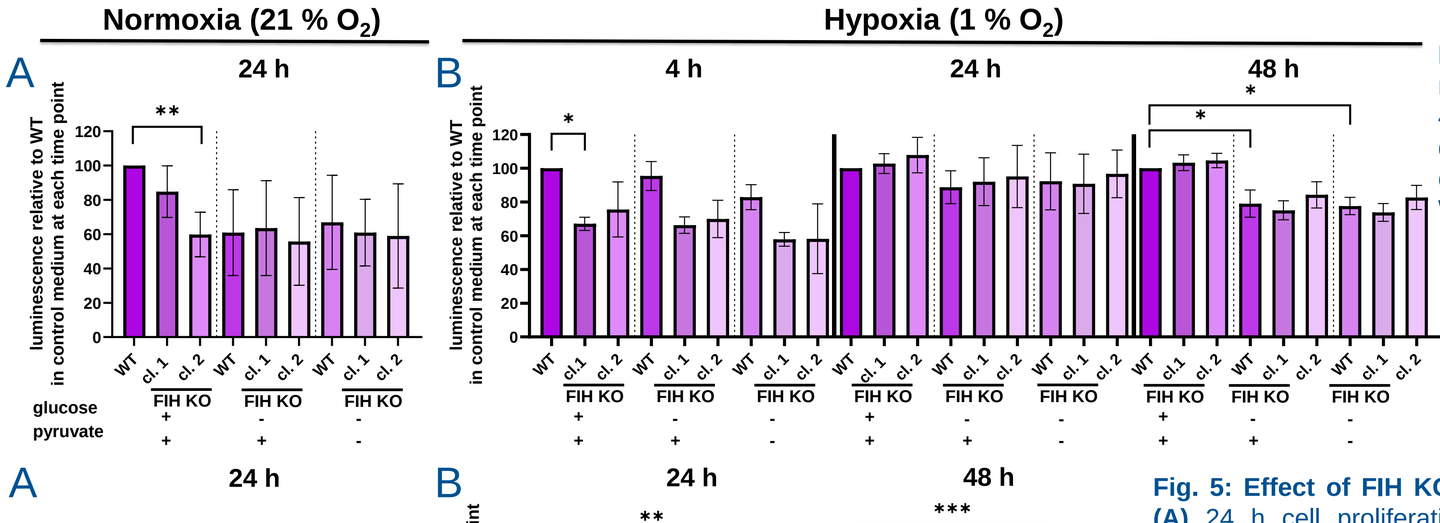

Fig. 4: Effect of FIH KO on metabolism (A) 24 h metabolic activity of H9c2 cells normalized to WT in control medium under nutrient depletion and 21 % O_2, **(B)** 4 h, 24 h, 48 h metabolic activity of H9c2 cells normalized to WT in control medium under nutrient depletion and 1 % O_2, significance tested with two way ANOVA with Dunnett's multiple comparison test; * p<0.05, ** p<0.01, *** p<0.001 indicate statistical significance relative to WT in control condition, 21 % O_2 n=3, 1 % O_2 n=4

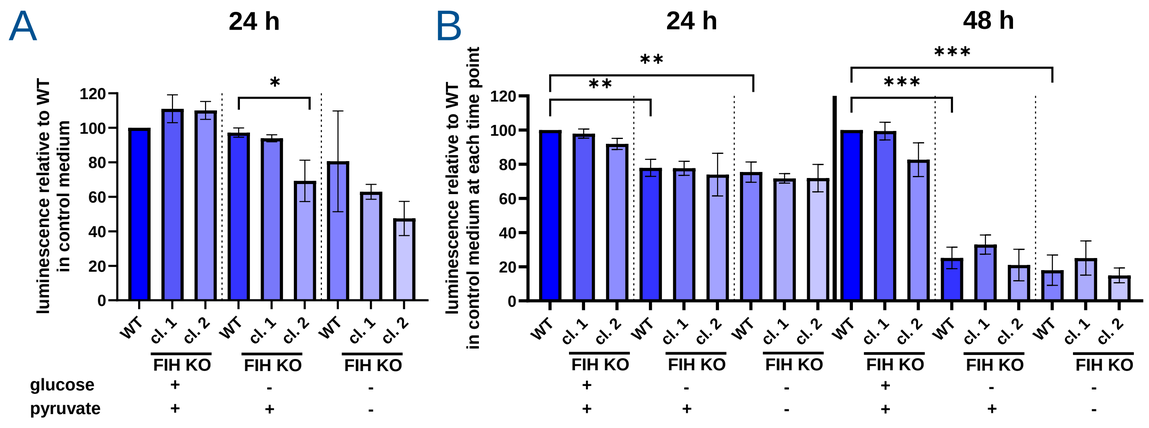

Fig. 5: Effect of FIH KO on proliferation (A) 24 h cell proliferation of H9c2 cells normalized to WT in control medium under nutrient depletion and 21 % O_2, **(B)** 24 h, 48 h cell proliferation of H9c2 cells normalized to WT in control medium under nutrient depletion and 1 % O_2 Significance tested with two way ANOVA with Dunnett's multiple comparison test; * p<0.05, ** p<0.01, *** p<0.001 indicate statistical significance relative to WT in control condition, 21 % O_2 n=3, 1 % O_2 n=4

Summary:

Successful establishment of an *in vitro* ischemia model

H9c2 FIH KO characterization:

- decreases cell growth

H9c2 FIH KO nutrient depletion and hypoxia:

- likely leads to in higher ATP levels compared to WT under ischemia

Conclusion:

➡ FIH may protect against ischemia by increasing available ATP levels.