

Supplementary Materials For:

*Article*

# **A Combined Drug Treatment That Reduces Mitochondrial Iron and Reactive Oxygen Levels Recovers Insulin Secretion in NAF-1-Deficient Pancreatic Cells**

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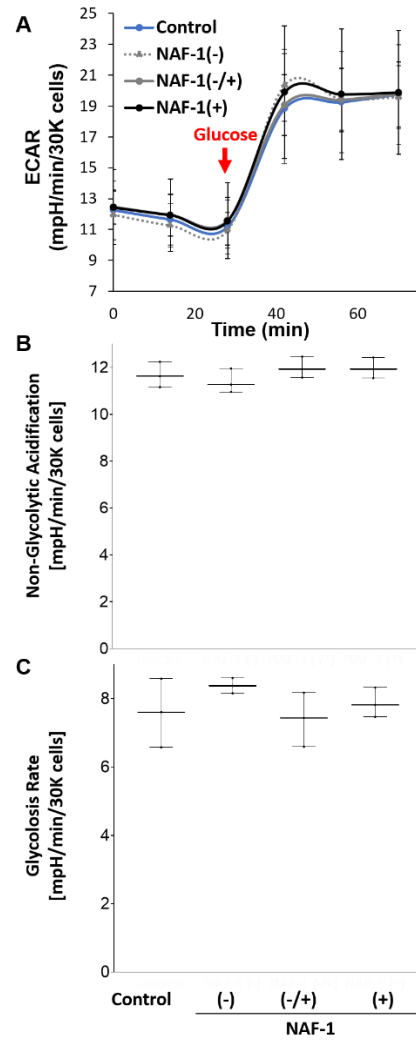
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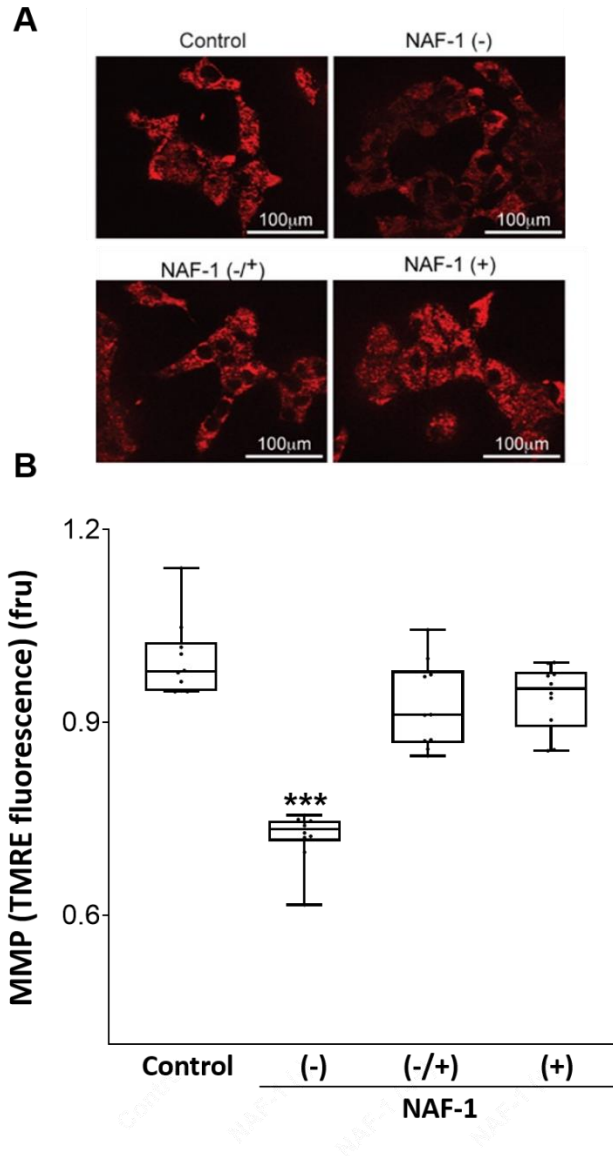
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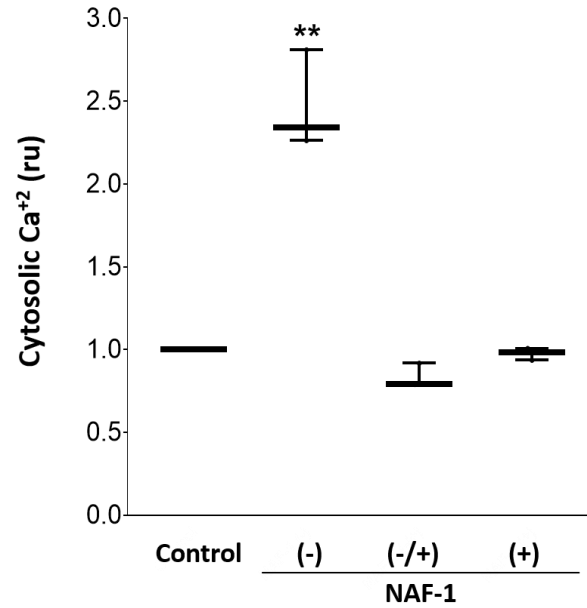
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**Supplementary Figure S1.** Extracellular acidification rate (ECAR) in control and NAF-1 repressed cells. **(A)** Seahorse-generated plots of ECAR obtained for the different lines. Data are presented in normalized values. **(B)** Non-glycolytic basal rate in the different NAF-1 expressing INS-1E cells, calculated from (A). **(C)** Glycolysis rates in the different INS-1E cells calculated from (A). The ECAR results are shown as box-and-whisker plots and include all data points measured from three different experiments, normalized to the number of cells used per experiment (30,000 cells). Abbreviations: ECAR, extracellular acidification rate.



**Supplementary Figure S2.** Mitochondrial membrane potential (MMP) in control and NAF-1 repressed cells. **(A)** Epi- fluorescence images of cells labeled with the MMP probe TMRE. The cell fluorescence is mostly in mitochondria, where the probe accumulates potentiometrically. **(B)** Quantitative analysis of TMRE fluorescence levels of cells expressing different levels of NAF-1. The results are shown as box-and-whisker plots and include all data points measured from three different experiments. \*\*\* $P < 0.001$ , compared to control; Student's t-test,  $N = 150$ .



**Supplementary Figure S3.** Cytosolic  $\text{Ca}^{2+}$  levels in control and NAF-1 repressed cells. Semi-quantitative measurements of cytosolic  $\text{Ca}^{2+}$  levels were performed with Fura Red<sup>TM</sup> preloaded as Fura Red<sup>TM</sup>-AM. Fluorescent microscopy was used to detect the fluorescent signal, by analyzing 5 cells per field, in total 150 cells from 30 fields. The results are shown as box-and-whisker plots and include all data points measured from three different experiments. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared to control; Student's t-test.