

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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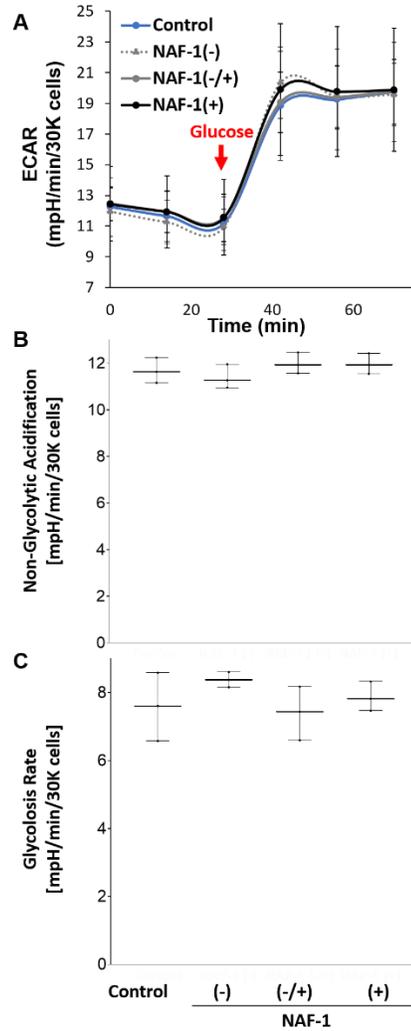
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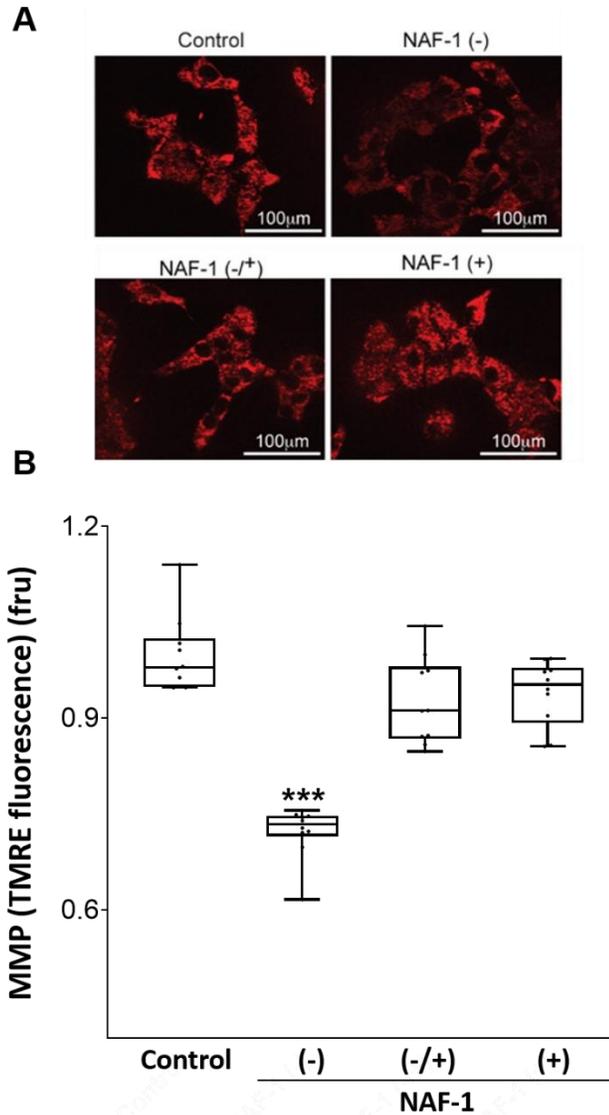
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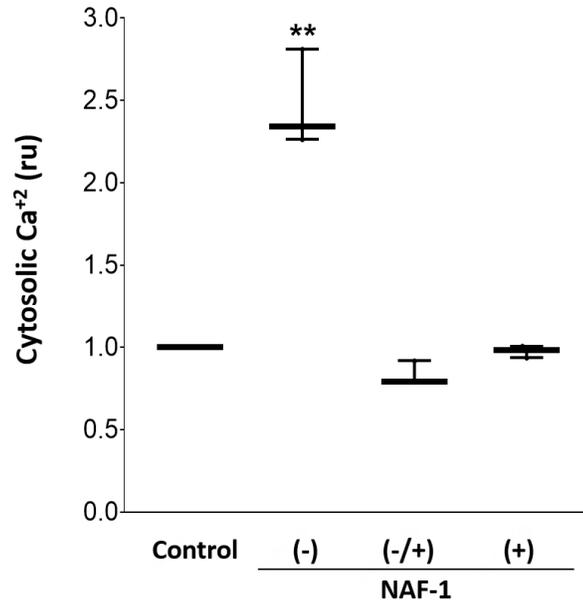
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Supplementary Figure S1. Extracellular acidification rate (ECAR) in control and NAF-1 repressed cells. (A) Seahorse-generated plots of ECAR obtained for the different lines. Data are presented in normalized values. (B) Non-glycolytic basal rate in 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 Ca²⁺ levels in control and NAF-1 repressed cells. Semi-quantitative measurements of cytosolic Ca²⁺ levels were performed with Fura Red™ preloaded as Fura Red™-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.