Supplemental Material to:

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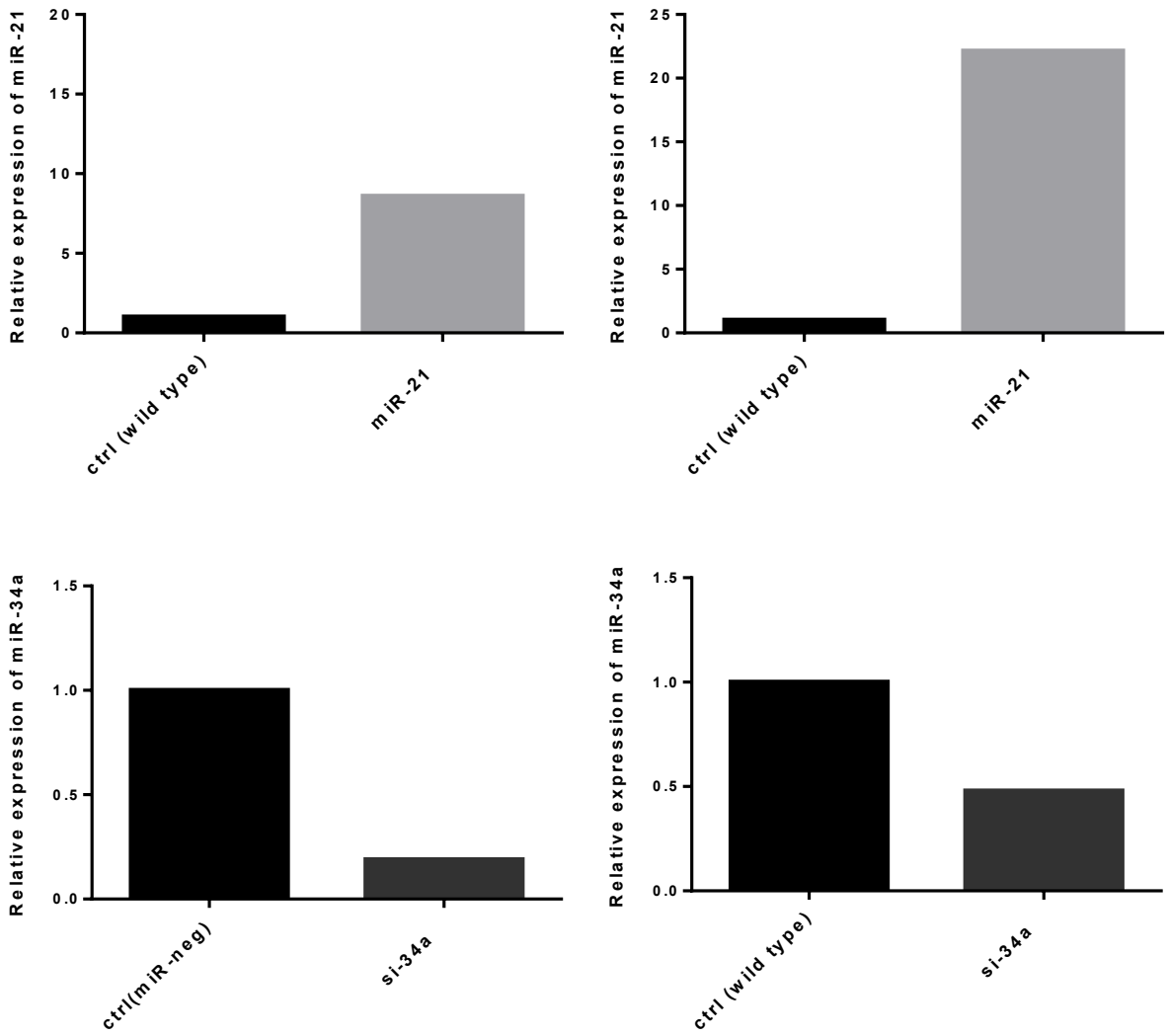
Altering β-cell number through stable alteration of miR-21 and miR-34a expression

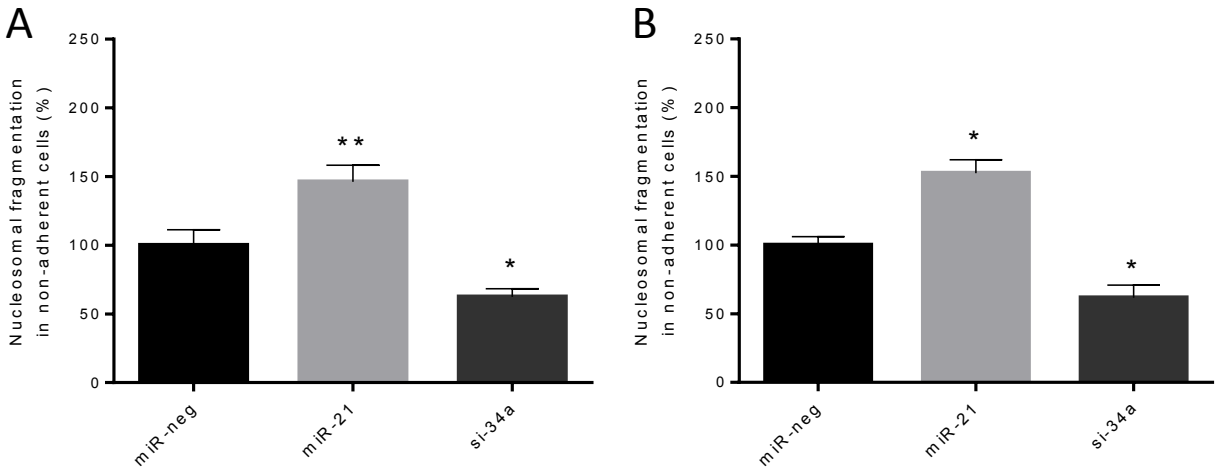
Islets 2013; 6(1)
http://dx.doi.org/10.4161/isl.27754

http://www.landesbioscience.com/journals/islets/article/27754/



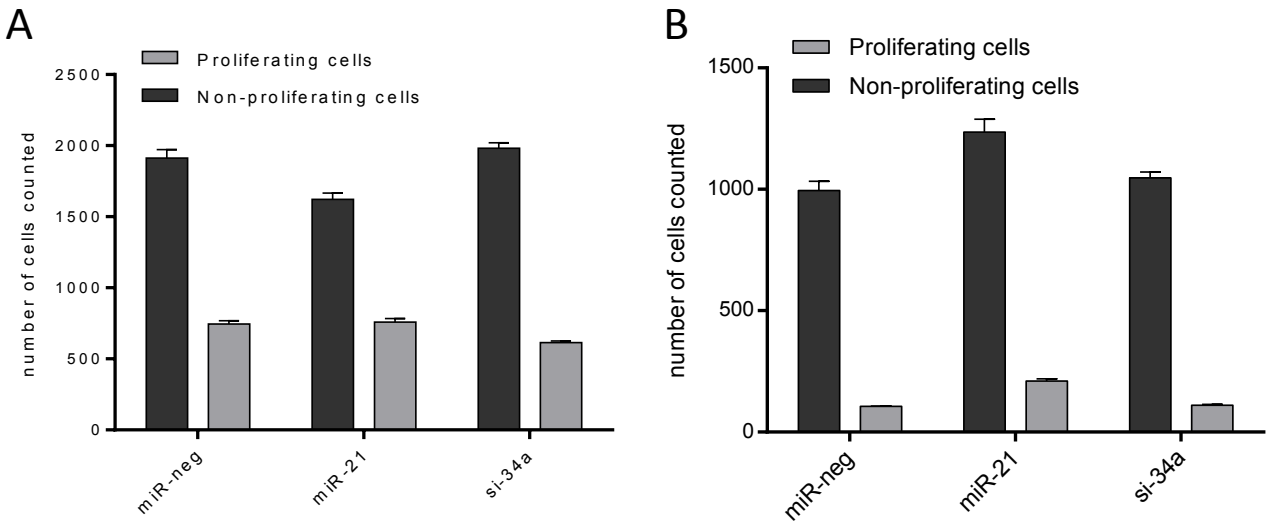
Suppl. Figure 1. Verification of overexpression of miR-21 and knockdown of miR-34a.

Expression of miR-21 and miR-34a was increased and reduced, respectively, compared to controls. Total RNA was extracted, converted to cDNA and quantified using qPCR. Data was normalized using the reference gene U6. Alterations of miRNA expression were investigated compared to two controls: wild type cells ('wild type'), and cells used for control of transduction ('miR-neg'). Four individual experiments are shown.



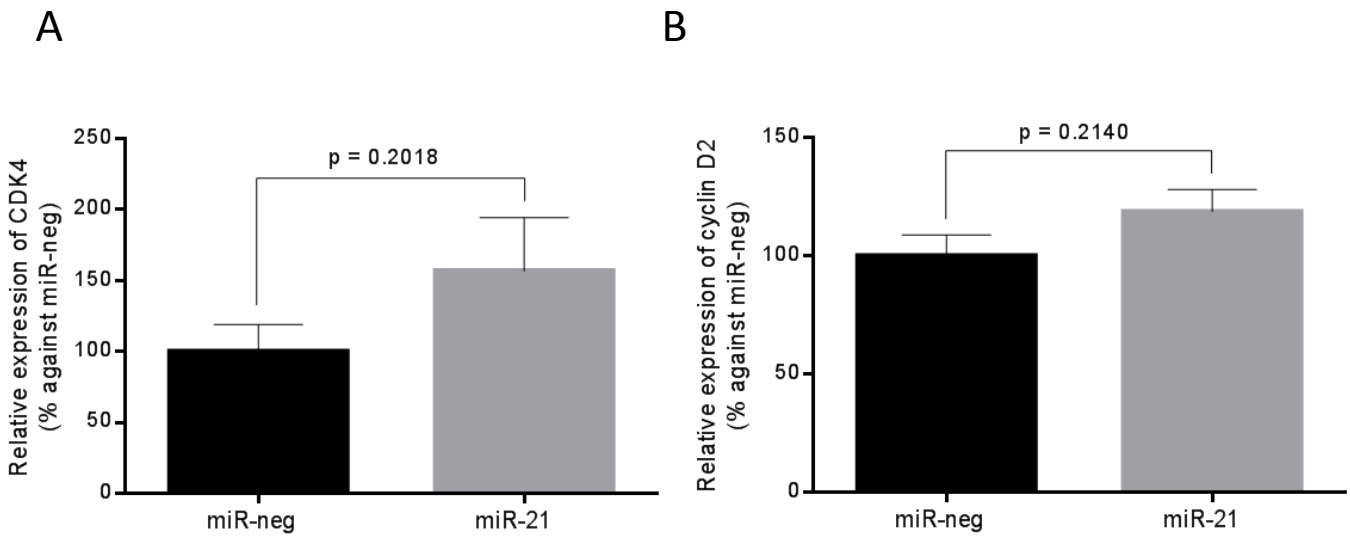
Suppl. Figure 2. Late β-cell apoptosis is potentiated by overexpression of miR-21, but reduced by knockdown of miR-34a both in absence and presence of cytokines.

Cells (50,000 cells/0.5 ml) were left untreated **(A)** or were exposed to IL-1β and IFN-γ for 24 hours **(B)**. Nucleosomal fragmentation from non-adherent cells was detected by ELISA (Cell Death Detection ELISA). Results of four independent experiments are shown as means + SEM. * $p < 0.05$ vs miR-neg, ** $p < 0.01$ vs. miR-neg. Statistical significance was determined using a two-way paired t-test.



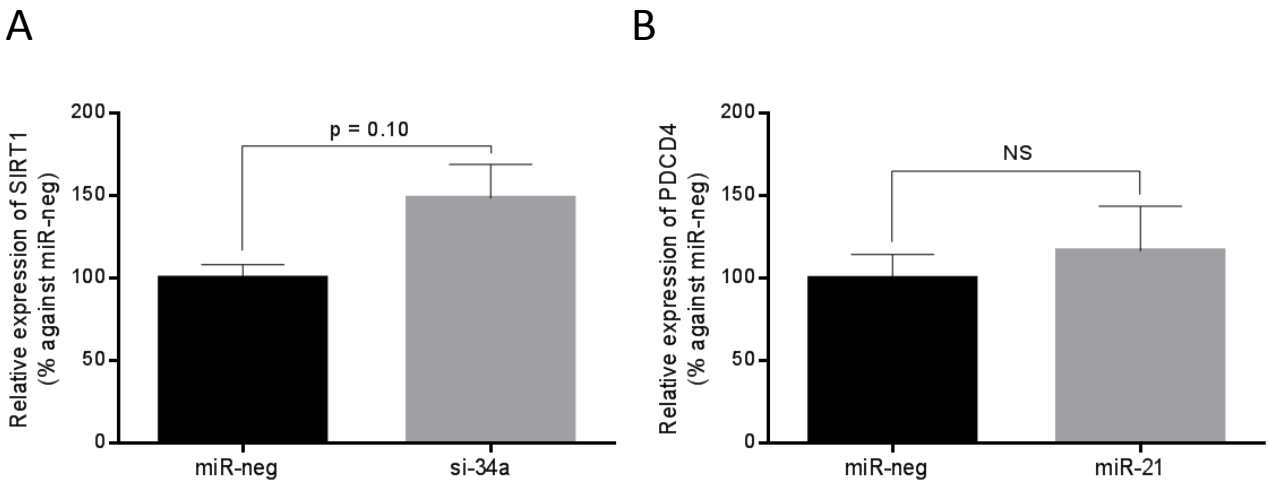
Suppl. Figure 3. miR-21 overexpression and miR-34a knockdown affect β-cell proliferation.

Cells (25,000 cells) were left untreated **(A)** or exposed to IL-1β and IFN-γ for 24 hours **(B)**. Nuclei were stained using EdU and HCD Blue, and fixed with mounting reagent. Nuclei were counted by two blinded observers as total number of cells (blue nuclei) and proliferating cells (green nuclei). Counted cells are shown as non-proliferating cells (EdU-positive cells subtracted from HCS-stained cells) and proliferative cells (Edu-positive cells). Results of four independent experiments are shown as means + SEM.



Suppl. Figure 4. Overexpression of miR-21 alters the cell cycle profile in β-cells.

Expression of CDK4 (A) and cyclin D2 (B) compared to miR-neg. Total RNA was extracted, converted to cDNA and quantified using qPCR. Data was normalized using the reference gene HPRT. Results of four and three independent experiments are shown for miR-neg and miR-21, respectively, as means + SEM. Statistical significance was determined using a two-way unpaired t-test.



Suppl. Figure 5. Knockdown of miR-34a leads to upregulation of SIRT1, while overexpression of miR-21 does not affect PDCD4.

Knockdown of miR-34a leads to a slight upregulation of expression of SIRT1, while PDCD4 seems unchanged when miR-21 is overexpressed, compared to miR-neg. Total RNA was extracted, converted to cDNA and quantified using qPCR. Data was normalized using the reference gene HPRT. Results of four and three independent experiments are shown for miR-neg and si-34a/miR-21, respectively, as means + SEM. Statistical significance was determined using a one-way unpaired t-test.