

SUPPLEMENTARY DATA

Supplementary Table 1. Comparison of miRNA expression in islets of 4- versus 8-week old NOD mice. Total RNAs from freshly isolated islets from three 4-week and three 8-week old NOD mice were analyzed with the Agilent miRNA microarray platform (Release 12.0) to assess the level of 627 mouse miRNAs. The table shows the list of the miRNAs displaying changes of more than 1.3 fold and with a corrected p-value <0.05 (unpaired t-test corrected with the Benjamini and Hochberg False Discovery Rate method). The fluorescence intensity of the miRNAs at 4 and 8 weeks is given in a Log2 scale.

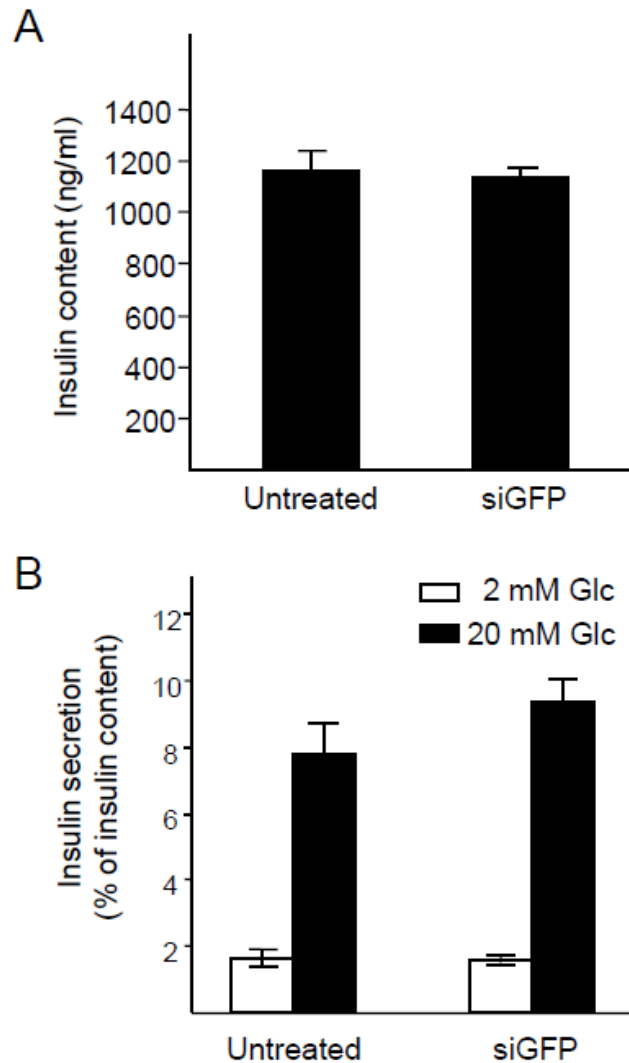
| microRNA | Fold change | Regulation | p-value | Mean 4 weeks | Mean 8 weeks | 4 weeks 1 | 4 weeks 2 | 4 weeks 3 | 8 weeks 1 | 8 weeks 2 | 8 weeks 3 |
|----------------|-------------|------------|------------|--------------|--------------|------------|------------|-----------|------------|-----------|-----------|
| mmu-miR-142-5p | 13.882584 | up | 0.02394782 | 1.604805 | 5.400009 | 0.06843123 | 2.0834262 | 2.662557 | 5.01978 | 6.806515 | 4.37373 |
| mmu-miR-216a | 10.793439 | up | 0.00234757 | 0.030428 | 3.462510 | 0.06843123 | 0.02285154 | 0.000001 | 2.718083 | 3.2592092 | 4.4102397 |
| mmu-miR-155 | 8.709191 | up | 0.01313969 | 0.032936 | 3.196962 | 0.07666883 | 0.02213872 | 0.000001 | 2.8995519 | 4.607994 | 2.08334 |
| mmu-miR-142-3p | 7.269941 | up | 0.02206676 | 5.011021 | 7.872964 | 4.0675364 | 5.2419505 | 5.7235756 | 7.473005 | 9.08008 | 7.065807 |
| mmu-miR-150 | 5.292377 | up | 0.03464681 | 2.184940 | 4.136881 | 2.094386 | 2.08334 | 2.3770947 | 3.5496035 | 5.362443 | 3.4985964 |
| mmu-miR-223 | 2.412870 | up | 0.01170051 | 4.462517 | 5.733267 | 4.320565 | 4.320565 | 4.74642 | 5.7235756 | 6.1737995 | 5.302425 |
| mmu-miR-146b | 2.366180 | up | 0.00623746 | 2.629138 | 3.871697 | 2.4500315 | 2.602168 | 2.8352134 | 3.7434604 | 4.278139 | 3.5934913 |
| mmu-miR-383 | 2.231925 | up | 0.00141464 | 2.791266 | 3.949554 | 2.5592728 | 2.9624636 | 2.8520608 | 4.11744 | 3.841741 | 3.8894796 |
| mmu-miR-146a | 2.099150 | up | 0.01500742 | 4.208928 | 5.278733 | 4.037517 | 4.179026 | 4.4102397 | 5.1354575 | 5.7437124 | 4.9570293 |
| mmu-miR-21 | 1.975061 | up | 0.01871874 | 8.925387 | 9.907285 | 8.633223 | 9.038778 | 9.10416 | 9.8018465 | 10.312709 | 9.607301 |
| mmu-miR-29b | 1.891797 | up | 0.00150424 | 11.123570 | 12.043327 | 10.926149 | 11.119513 | 11.325051 | 12.072881 | 12.072881 | 11.984222 |
| mmu-miR-29c | 1.854613 | up | 6.48E-04 | 10.733589 | 11.624707 | 10.595323 | 10.802723 | 10.802723 | 11.7355385 | 11.523515 | 11.615068 |
| mmu-miR-152 | 1.854181 | up | 1.61E-05 | 7.046236 | 7.937018 | 7.065807 | 7.03645 | 7.03645 | 8.00344 | 7.8858 | 7.921813 |
| mmu-miR-34c | 1.765560 | down | 0.00225326 | 6.788988 | 5.968862 | 6.6745453 | 6.8859034 | 6.806515 | 6.0417967 | 5.769911 | 6.094879 |
| mmu-miR-15b | 1.747932 | down | 0.00204910 | 9.365298 | 8.559649 | 9.360682 | 9.308219 | 9.426996 | 8.550387 | 8.750633 | 8.377925 |
| mmu-miR-210 | 1.733338 | up | 0.01168990 | 4.149811 | 4.943364 | 4.055395 | 4.4584146 | 3.9356236 | 5.1153927 | 4.8382072 | 4.8764915 |
| mmu-miR-132 | 1.646140 | down | 0.00149849 | 6.931026 | 6.211939 | 6.8859034 | 6.9997053 | 6.907469 | 6.2744894 | 6.3195324 | 6.0417967 |
| mmu-let-7d* | 1.644523 | down | 0.04754141 | 3.236759 | 2.519090 | 3.48402 | 3.1735573 | 3.0526998 | 2.9195096 | 2.1648386 | 2.472923 |
| mmu-miR-296-5p | 1.606803 | down | 0.02837747 | 6.054087 | 5.369894 | 6.3195324 | 5.837525 | 6.0052056 | 5.605481 | 5.1001196 | 5.404082 |
| mmu-miR-96 | 1.596440 | up | 0.00395209 | 8.018975 | 8.693833 | 7.8498735 | 8.068594 | 8.13846 | 8.780481 | 8.550387 | 8.750633 |
| mmu-miR-93 | 1.576287 | down | 0.02725296 | 5.929945 | 5.273415 | 6.0417967 | 6.1425543 | 5.605481 | 5.162821 | 5.474063 | 5.1833606 |
| mmu-miR-184 | 1.567421 | down | 0.01283729 | 7.352545 | 6.704153 | 7.319355 | 7.4189262 | 7.319355 | 6.556377 | 6.556377 | 6.9997053 |
| mmu-miR-101a | 1.552175 | up | 0.00210844 | 7.635762 | 8.270053 | 7.506613 | 7.6027293 | 7.7979426 | 8.217271 | 8.296444 | 8.296444 |
| mmu-miR-29c* | 1.550103 | up | 0.00277211 | 5.590926 | 6.223290 | 5.4587326 | 5.6469727 | 5.6670732 | 6.334292 | 6.094879 | 6.240699 |
| mmu-miR-212 | 1.492809 | down | 0.00630452 | 8.682609 | 8.104579 | 8.864214 | 8.633223 | 8.550387 | 8.068594 | 8.027871 | 8.217271 |
| mmu-miR-676 | 1.481741 | up | 0.02950124 | 3.462455 | 4.029748 | 3.1254241 | 3.6432207 | 3.6187181 | 3.9918106 | 4.011225 | 4.086208 |
| mmu-miR-29a | 1.478413 | up | 0.03060832 | 11.890838 | 12.454887 | 11.844145 | 11.984222 | 11.844145 | 12.289177 | 12.786307 | 12.289177 |
| mmu-miR-702 | 1.463597 | down | 0.04173794 | 4.363165 | 3.813647 | 4.474355 | 4.2414107 | 4.37373 | 4.105703 | 3.506112 | 3.829125 |
| mmu-miR-205 | 1.458529 | down | 0.00515639 | 4.567444 | 4.022930 | 4.6294827 | 4.6492486 | 4.4236007 | 4.1534348 | 3.9356236 | 3.9797308 |

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|----------------|----------|------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| mmu-miR-195 | 1.436867 | up | 0.01753156 | 7.170496 | 7.693422 | 7.03645 | 7.2806964 | 7.1943417 | 7.757388 | 7.8498735 | 7.473005 |
| mmu-miR-720 | 1.420869 | down | 0.04934545 | 14.382652 | 13.875878 | 14.382652 | 14.382652 | 14.382652 | 14.057561 | 13.512513 | 14.057561 |
| mmu-miR-25 | 1.419201 | down | 0.00187379 | 7.418440 | 6.913361 | 7.4189262 | 7.473005 | 7.363391 | 6.8518167 | 7.03645 | 6.8518167 |
| mmu-miR-34b-5p | 1.414908 | down | 0.00740253 | 8.057221 | 7.556513 | 7.8858 | 8.217271 | 8.068594 | 7.6027293 | 7.560197 | 7.506613 |
| mmu-miR-541 | 1.414843 | down | 0.00406071 | 5.942362 | 5.441721 | 5.94888 | 5.9694195 | 5.9087877 | 5.378303 | 5.3413754 | 5.605481 |
| mmu-miR-34a | 1.407731 | up | 0.00486145 | 7.500717 | 7.994089 | 7.448228 | 7.6349974 | 7.4189262 | 8.027871 | 8.068594 | 7.8858 |
| mmu-miR-709 | 1.405744 | down | 0.01223927 | 11.418794 | 10.92746 | 11.407817 | 11.325051 | 11.523515 | 10.860141 | 10.802723 | 11.119513 |
| mmu-miR-181a | 1.395049 | down | 0.02789521 | 4.320825 | 3.840509 | 4.2884574 | 4.556578 | 4.11744 | 3.9157236 | 3.8894796 | 3.716324 |
| mmu-miR-484 | 1.385215 | down | 0.01105737 | 4.937784 | 4.467674 | 5.1354575 | 4.801403 | 4.8764915 | 4.5210075 | 4.4584146 | 4.4236007 |
| mmu-miR-23b | 1.384536 | down | 0.00590534 | 11.438339 | 10.968937 | 11.523515 | 11.523515 | 11.26799 | 10.990331 | 10.990331 | 10.926149 |
| mmu-miR-365 | 1.380477 | down | 0.01170148 | 5.534460 | 5.069294 | 5.7056932 | 5.404082 | 5.493605 | 4.9570293 | 5.1153927 | 5.1354575 |
| mmu-miR-101b | 1.371451 | up | 0.00586726 | 5.398455 | 5.854158 | 5.2647257 | 5.5036182 | 5.427022 | 5.7958765 | 5.94888 | 5.817716 |
| mmu-miR-98 | 1.352772 | up | 0.01228271 | 7.386749 | 7.822668 | 7.2806964 | 7.319355 | 7.560197 | 7.7730956 | 7.921813 | 7.7730956 |
| mmu-miR-204 | 1.325126 | up | 0.03297585 | 7.624664 | 8.030794 | 7.5784845 | 7.5381217 | 7.757388 | 8.13846 | 7.815462 | 8.13846 |
| mmu-miR-185 | 1.324663 | up | 0.00751312 | 5.081466 | 5.487091 | 4.9507103 | 5.2294726 | 5.0642147 | 5.474063 | 5.493605 | 5.493605 |
| mmu-miR-301a | 1.314922 | down | 6.16E-04 | 7.569429 | 7.174451 | 7.560197 | 7.506613 | 7.6414757 | 7.164506 | 7.1943417 | 7.164506 |
| mmu-miR-690 | 1.309947 | down | 0.03804172 | 9.007348 | 8.61784 | 9.14512 | 8.864214 | 9.012711 | 8.4398155 | 8.633223 | 8.780481 |
| mmu-miR-129-3p | 1.303504 | up | 0.00263255 | 9.510615 | 9.89301 | 9.510615 | 9.510615 | 9.510615 | 9.923798 | 9.78189 | 9.973344 |

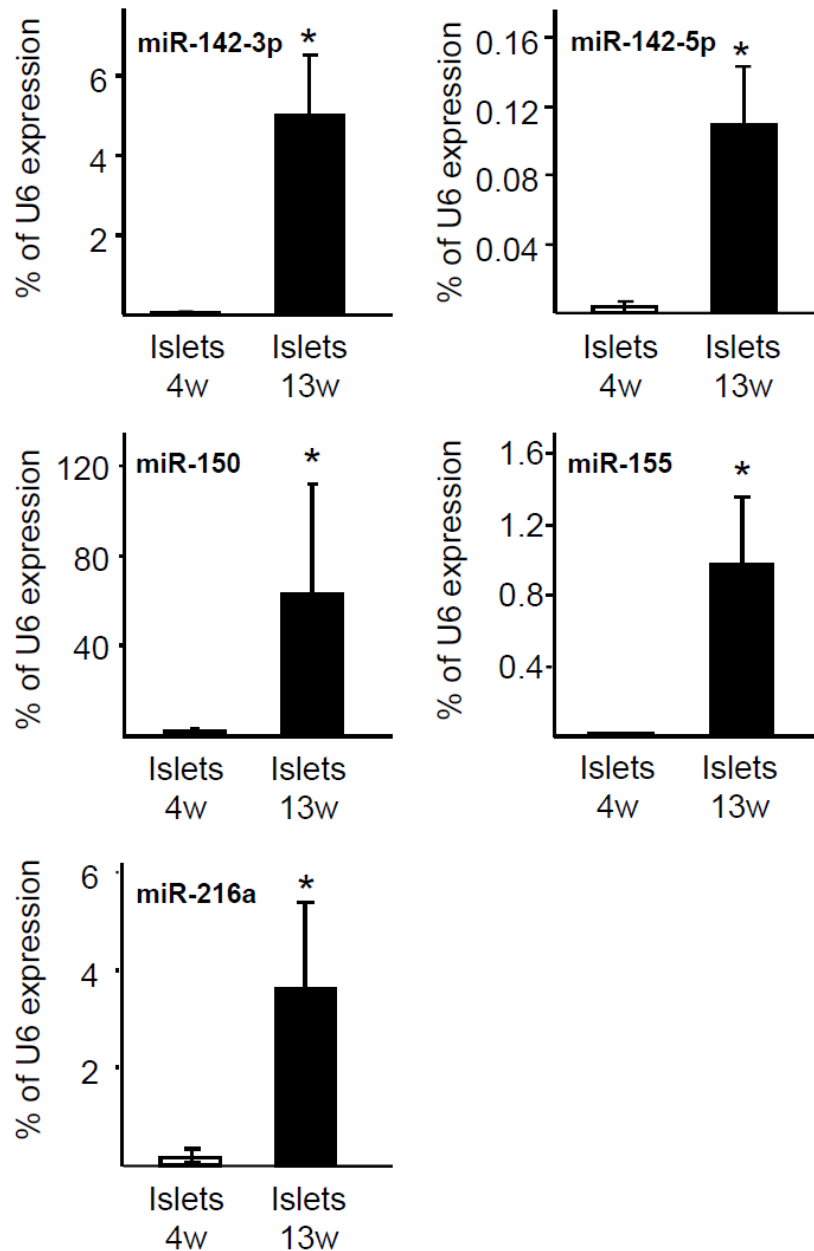
SUPPLEMENTARY DATA

Supplementary Figure 1. Transfection of b-cells with a control oligonucleotide duplex against GFP does not affect insulin content and insulin secretion. A) MIN6 cells were left untreated or transfected with an siRNA directed against siGFP. Two days later they were disrupted and the total insulin content was assessed by ELISA. The results are means \pm SD of 3 independent experiments. B) MIN6 cells were left untreated or transfected with an siRNA directed against siGFP. Two days later the cells were incubated in the presence of 2 mM (open bars) or 20 mM glucose (black bars) for 45 min. The amount of insulin secreted during the incubation period was measured by ELISA and was divided by total insulin content. The results represent the mean \pm SD of 3 independent experiments.



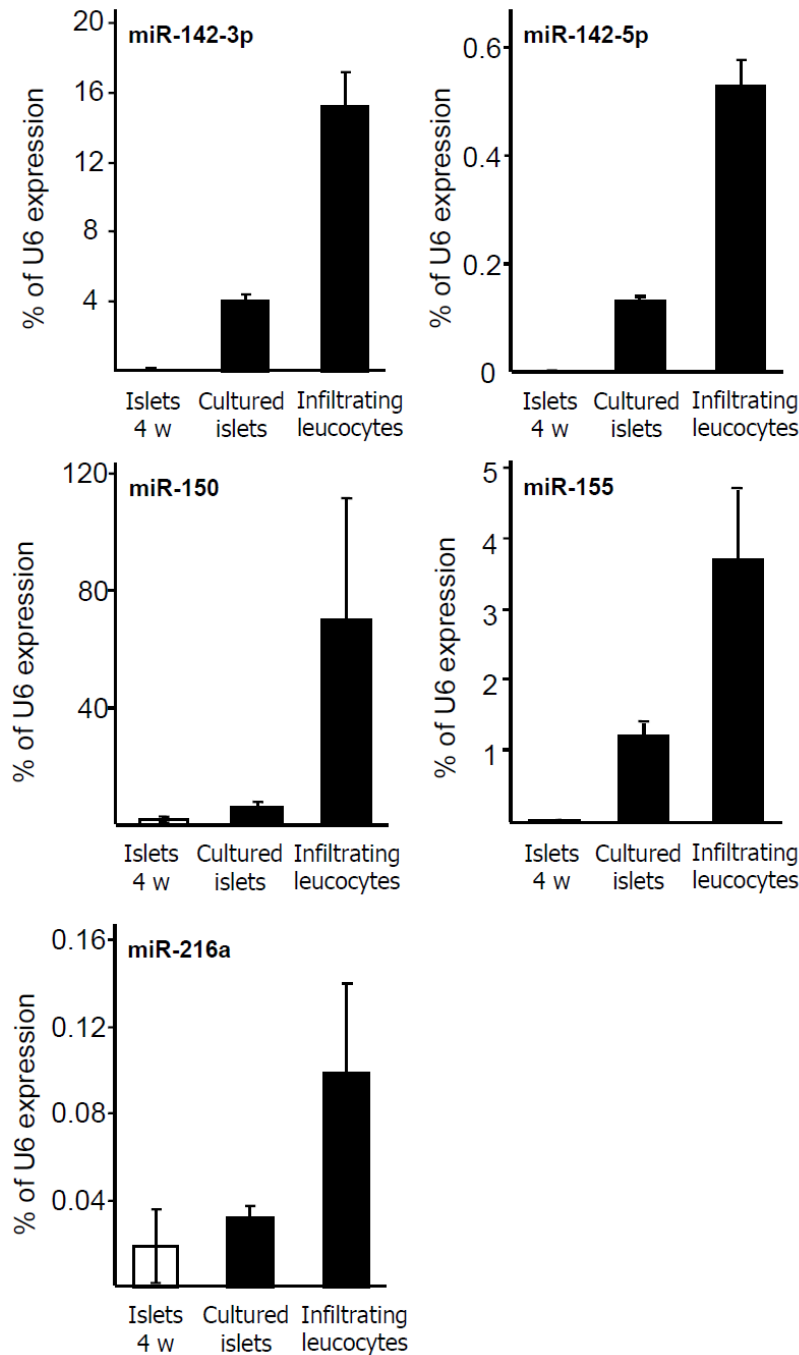
SUPPLEMENTARY DATA

Supplementary Figure 2. The level of miRNAs expressed by immune cells increases with aging in the islets of NOD mice. The levels of miR-142-3p, -5p, miR-150, miR-155 and miR-216a were measured by quantitative RT-PCR in 4- and 13-week old NOD mice. The data were normalized to U6 expression, and shown as mean \pm SD of 3 independent experiments. The asterisks indicate the conditions that are significantly different from control ($p < 0.05$).



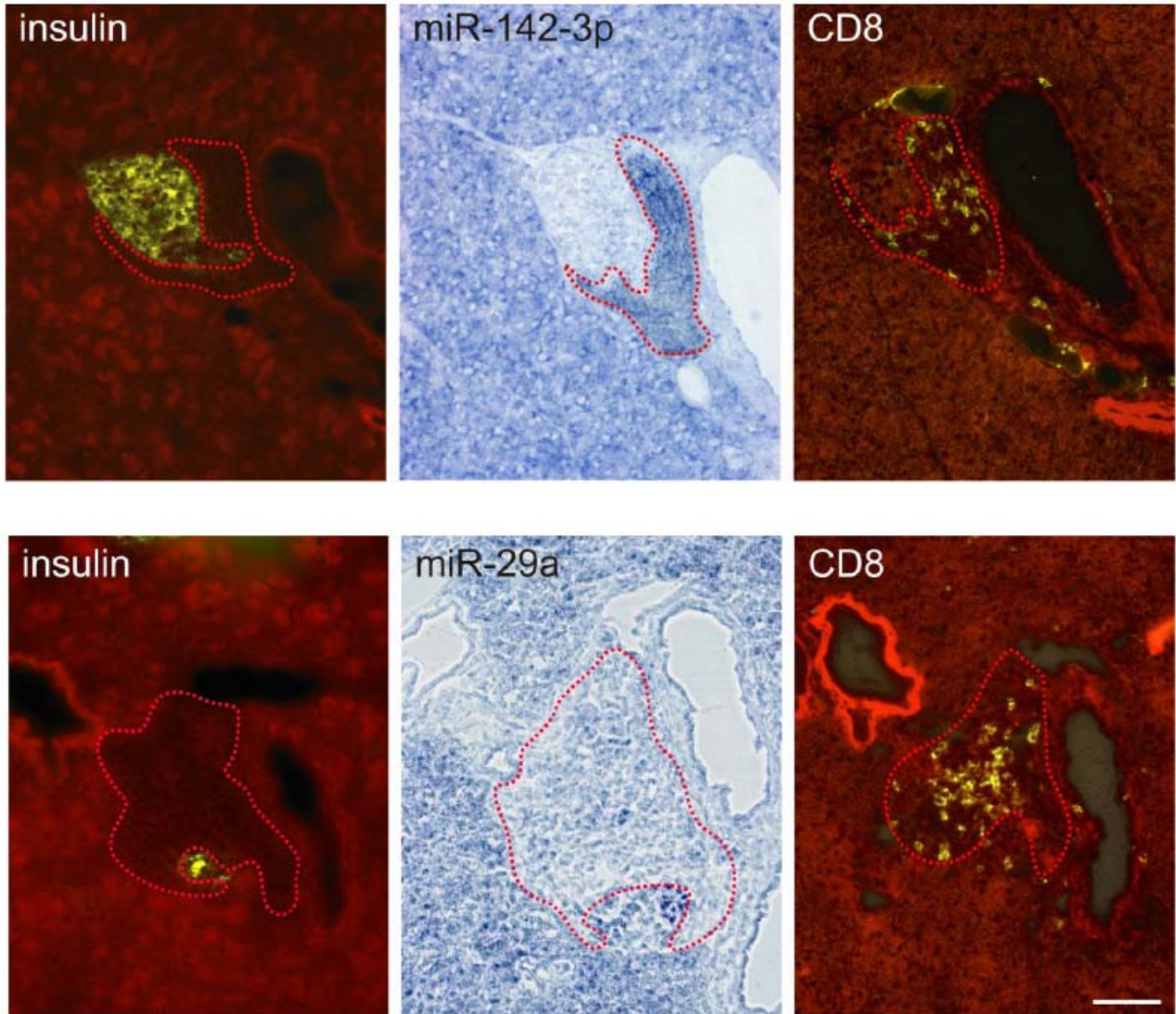
SUPPLEMENTARY DATA

Supplementary Figure 3. miR-142-3p, miR-142-5p, miR-150, miR-155 and miR-216a are highly expressed in leukocytes escaping from the islets of NOD mice. Pancreatic islets were isolated from 4- and 14-week old NOD mice. Islets of 14-weeks old mice were cultured for 16 hours to allow infiltrating lymphocytes to escape in the culture medium. The levels of the indicated miRNAs were measured by quantitative RT-PCR, expressed as % of U6 expression, and shown as mean +/- SD of 4 independent experiments.



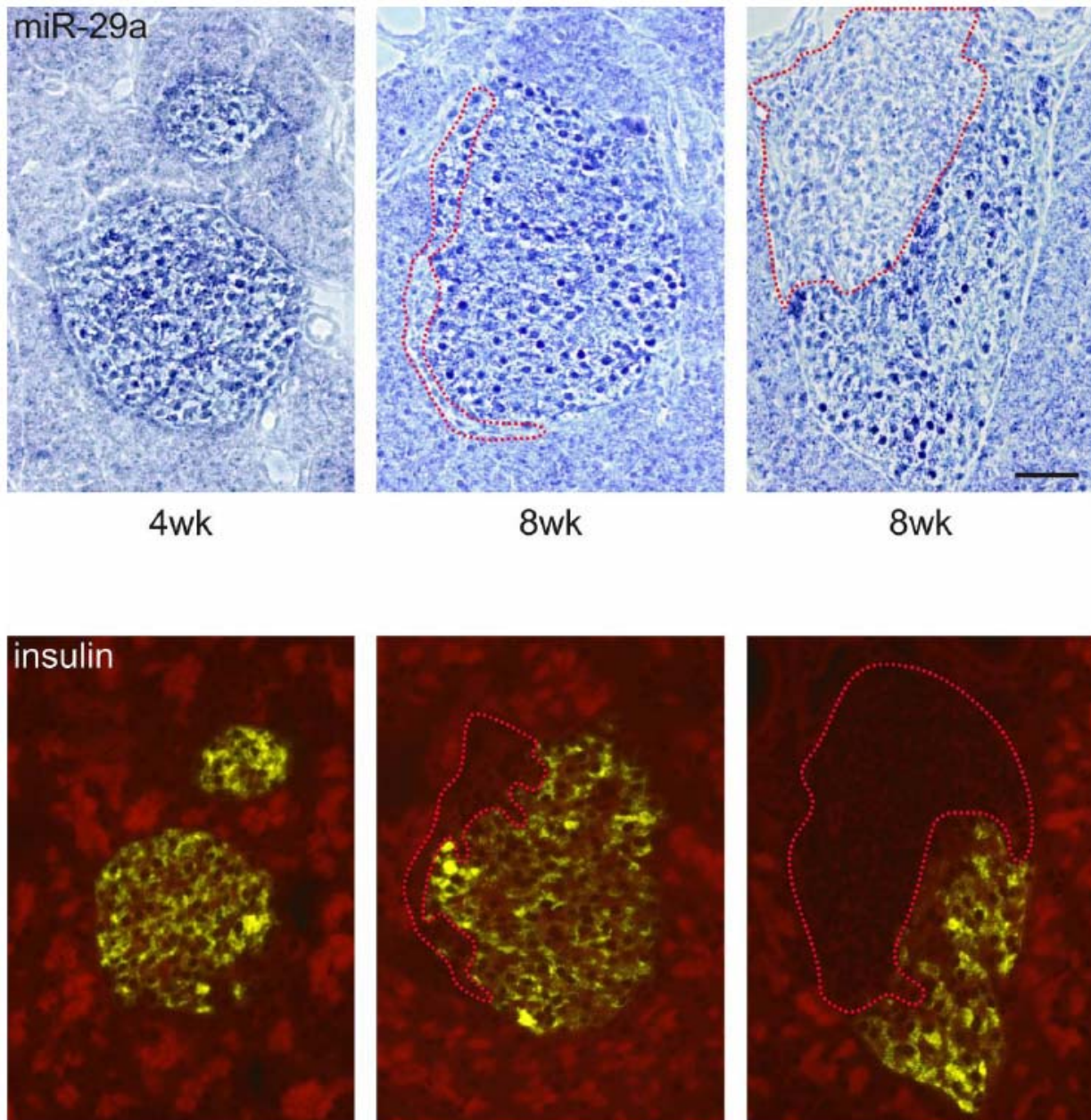
SUPPLEMENTARY DATA

Supplementary Figure 4. miR-142-3p and miR-29a are differentially distributed in the pancreas of 13 week-old NOD mice. Top panels) In situ hybridization reveals that most dark blue-stained cells expressing miR-142-3p (center panel) are immune cells forming the peri-insulitis halo (limited by the dotted line), several of which are positive for the lymphocyte marker CD8 (right panel), and not insulin-containing β -cells (left panel). Bottom panels) Dark blue-stained cells expressing the highest level of miR-29a (center panel) are residual, insulin-containing β -cells (left panel). No miR-29a was seen in the large number of immune cells, several of which were positive for the lymphocyte marker CD8 (right panel), that formed an extensive peri-insular halo (limited by the dotted line). Bar, 50 μ m.



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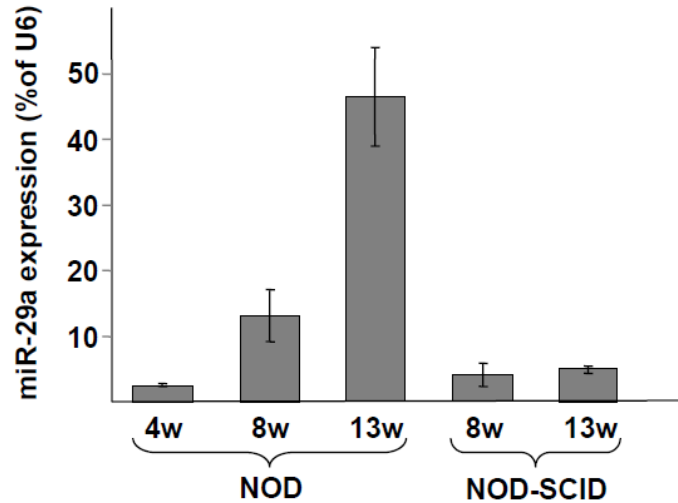
Supplementary Figure 5. *In situ* hybridization detects miR-29a in b-cells but not in peri-insular lymphocytes of NOD mice. Top panels) In the pancreas of a 4 week old NOD mouse (left panel), the miR-29a signal, as revealed by an intense blue staining, is detected in several islet cells. The proportion of intensively stained islet cells increased in the islets of 8 (middle panel) and 13-week-old mice (right panel), that displayed variable levels of peri-insulinitis (limited by the dotted lines). Bottom panels) Consecutive sections of the very same islets were processed for *in situ* hybridization and immunostained for insulin. Comparison of top and bottom panels reveals that most of the miR-29 is expressed by insulin-containing b-cells. Bar, 40 μ m.



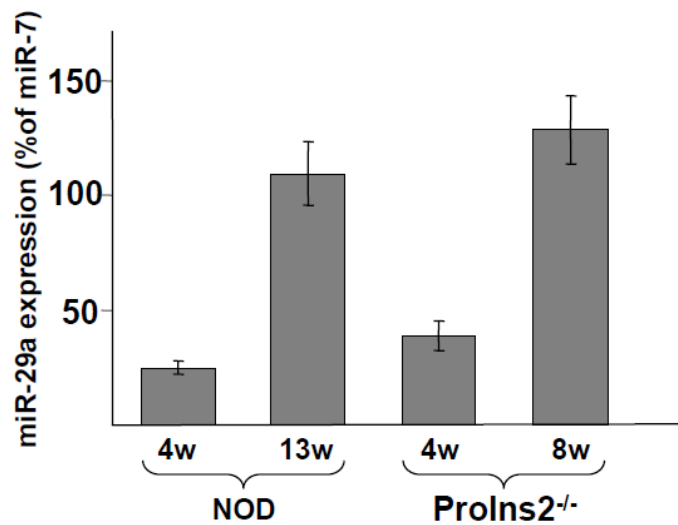
SUPPLEMENTARY DATA

Supplementary Figure 6. miR-29a expression in the islets of NOD-SCID and Proinsulin 2-deficient NOD mice. A) miR-29a expression in pancreatic islets isolated from three female NOD-SCID mice of 8 and of 13 weeks of age was analyzed by qRT-PCR and compared to the level in female NOD mice of 4, 8 and 13 weeks. The results have been normalized to U6 expression. B) miR-29a expression in the islets of three female NOD ProIns2^{-/-} mice of 4 and of 8 weeks of age was analyzed by qRT-PCR and compared to the expression in female NOD mice of 4 and 13 weeks. The results have been normalized to miR-7 expression, an miRNA specifically expressed in islet cells.

A

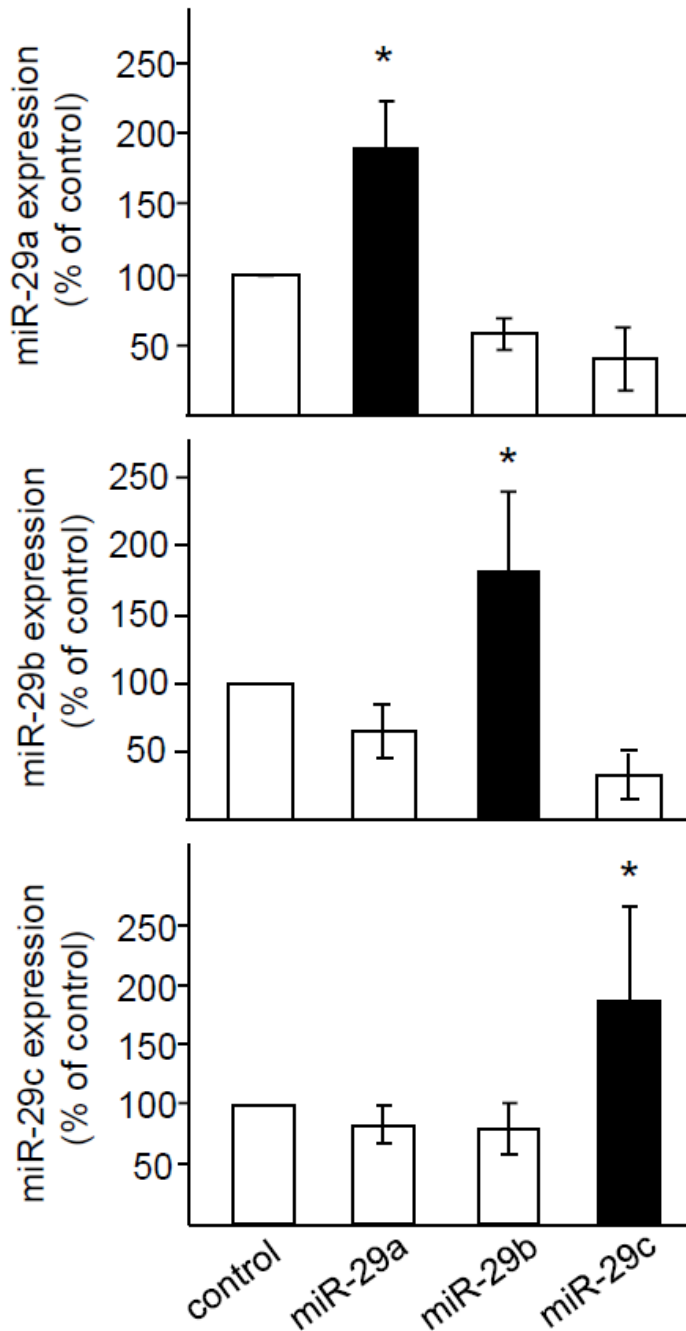


B



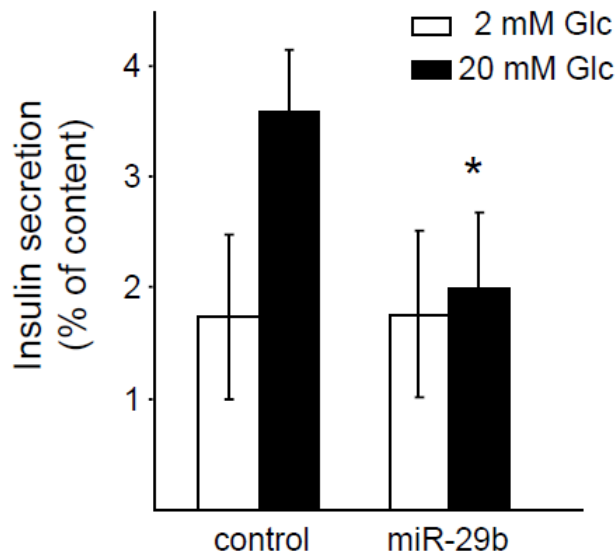
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Supplementary Figure 7. The levels of miR-29a, -29b and -29c increased upon transfection of MIN6 cells with specific oligonucleotides. Real Time PCR performed on MIN6 cells transiently transfected with control duplex or with duplexes corresponding to the mature forms of miR-29a, miR-29b and miR-29c. The value obtained under control condition in each experiment was set to 100%. Values are shown as mean \pm SD of 3-4 independent experiments. * Significantly different from control condition, $p < 0.05$.

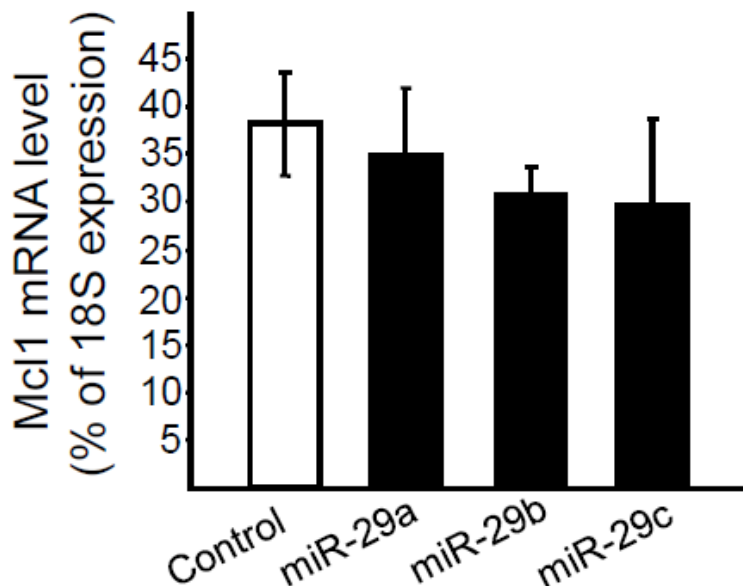


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Supplementary Figure 8. Overexpression of miR-29 decreases glucose-induced insulin secretion of human islet cells. Human islet cells were dispersed, plated on 24 multiwells and transfected with either a control siRNA or a duplex leading to miR-29b overexpression. Two days later the cells were incubated in the presence of 2 mM (open bars) or 20 mM glucose (black bars) for 45 min. The amount of insulin secreted during the incubation period was assessed by ELISA. The results are the mean \pm SD of 3 independent experiments (3 isolations from 3 distinct donors). * Significantly different from stimulated control condition, $p < 0.05$.



Supplementary Figure 9. Overexpression of miR-29 does not significantly affect MCL1 mRNA levels. MIN6 cells were transiently transfected with a control oligonucleotide or with oligonucleotides corresponding to the mature sequence of miR-29a/b/c. MCL1 mRNA levels were assessed by qPCR. The results are the mean \pm SD of 5 independent experiments.



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Supplementary Figure 10. Protection of the miR-29 binding site prevents the decrease of Mcl1 expression mediated by miR-29 and cytokines. A) MIN6 cells were transfected with either a control RNA duplex or oligonucleotide duplexes leading to miR-29c overexpression, together with miScript Target Protectors. When indicated MIN6 cells were treated for 24 hours with a mix of cytokines (Cyt mix) including IL-1b (0.1ng/ml), TNFa (10ng/ml) and IFNg (30ng/ml). Expression of Mcl1 and Actin were assessed by western blotting. The figure shows a representative experiment out of 3. B) Quantification showed that protection of the miR-29 binding site in the 3'UTR of Mcl1 prevents the reduction of Mcl1 levels induced by miR-29c overexpression (grey bars) and by the cytokine treatment (black bars), both reduced the levels of Mcl1. The results are shown as means \pm SD of 3-6 independent experiments. * Significantly different from control condition ($p < 0.05$).

