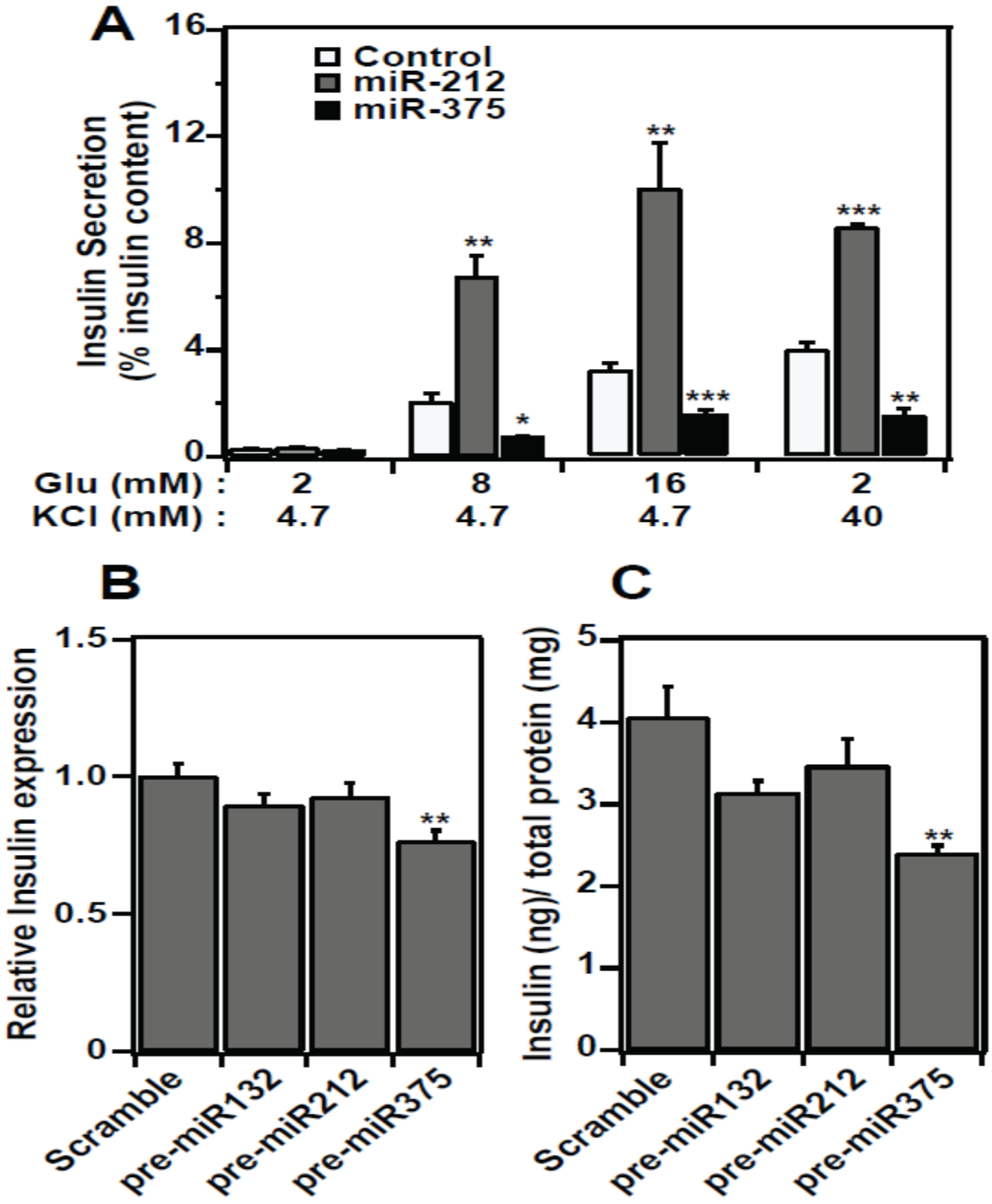
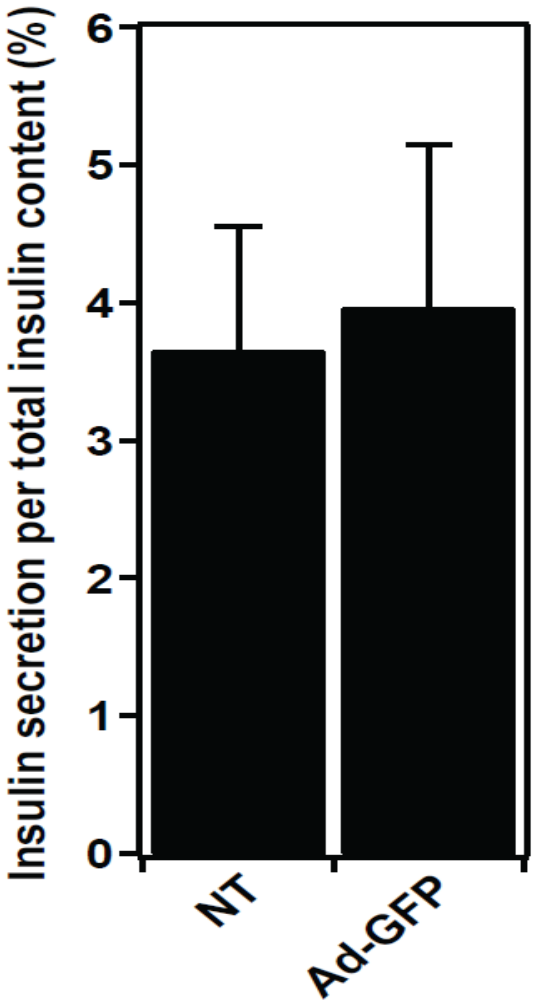
SUPPLEMENTARY DATA

Supplementary Figure 1. (A) MicroRNA 212 enhances IS from pancreatic β-cells. INS-1 832/3 β-cells were transfected with precursors for miRNAs 212, 375, or negative control oligonucleotides. 48 hrs after transfection, IS was measured in response to varying glucose concentrations or elevated KCl. IS into the medium is normalized to total insulin content. Data is representative of 5 independent experiments. Insulin transcript expression and protein content in INS-1 cells did not change after transfection with precursors of miRNAs – 132 and 212. Insulin transcript (B) and insulin protein content (C) were measured from the lysates of cells transfected with pre-miR- 132, pre-miR-212, pre-miR-375 or a Pre-miRTM negative control. Insulin content was normalized to total protein. *p < 0.05; **p < 0.01; ***p < 0.001 as compared to the negative control.



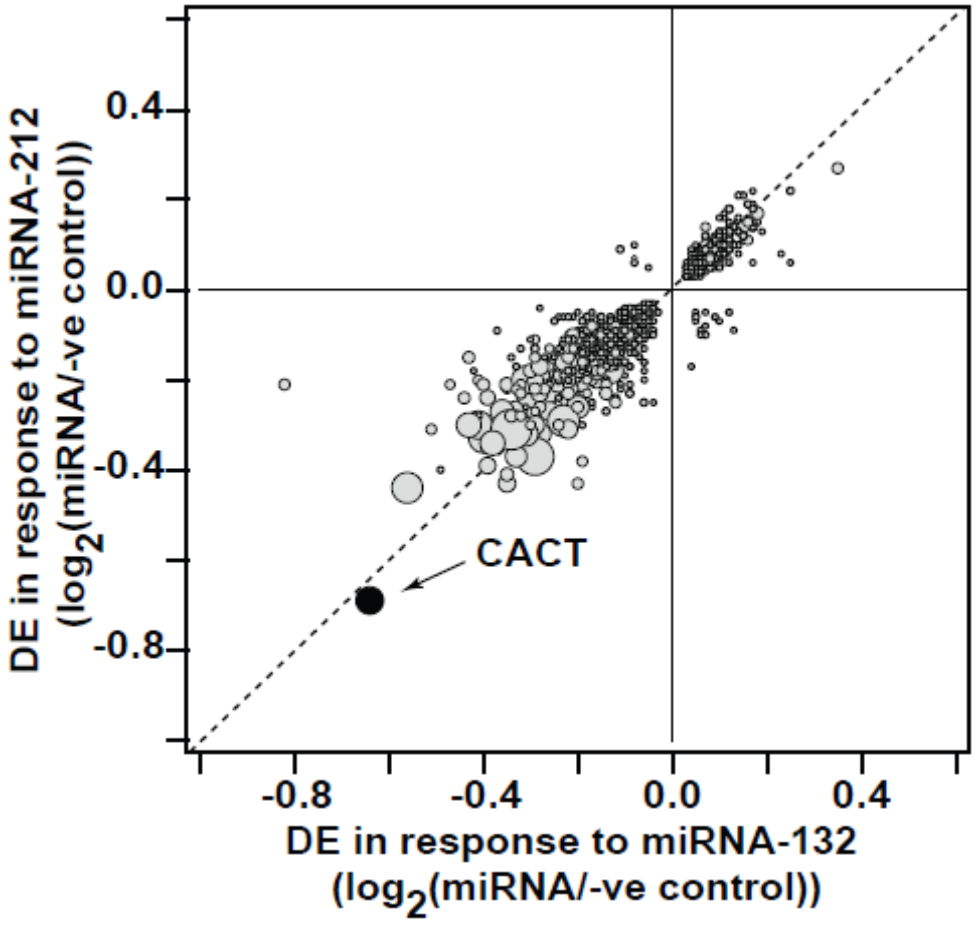
SUPPLEMENTARY DATA

Supplementary Figure 2. Adenovirus-GFP does not affect insulin secretion. INS-1 832/3 cells were either left untreated (NT) or transfected with Ad-GFP. 48 hrs after infection, IS was measured in response to 15 mM Glucose. IS is normalized to total insulin content, and depicted as % of fractional release. Graph is an average of n = 6 experiments.



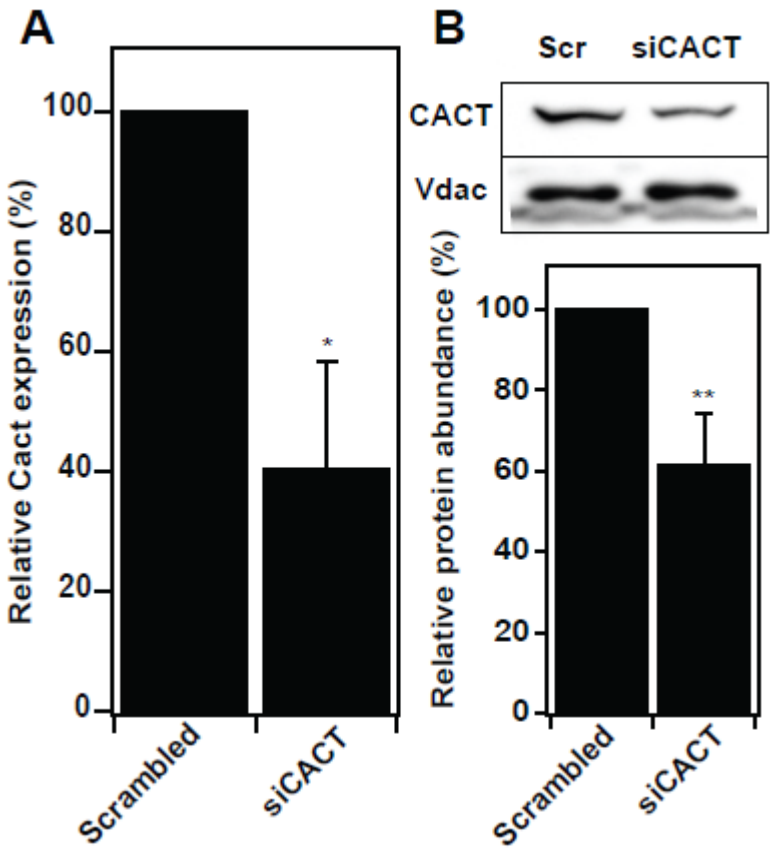
SUPPLEMENTARY DATA

Supplementary Figure 3. CACT is the most downregulated mRNA in response to miRNA 132 and 212 overexpression. Differential expression (DE) of mRNA resulting from miRNA-132 is plotted against DE resulting from miRNA-212, 10hrs following introduction of miRNA oligonucleotides into INS-1 832/3 β-cells ($p<0.05$). Larger symbols indicate greater statistical significance for DE. ~6.7 % of the depicted mRNA has a seed region. Axes show the log10 of the fold-change (miRNA over negative control). Negative values indicate downregulation of expression. CACT is highlighted as the most downregulated gene in response to both miRNAs.



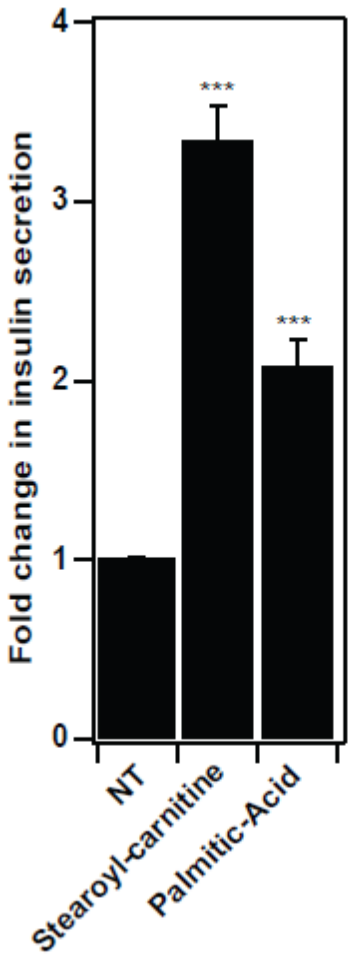
SUPPLEMENTARY DATA

Supplementary Figure 4. CACT (A) mRNA and (B) protein abundance is downregulated in response to siRNA oligonucleotides against CACT. (A) RNA was isolated and CACT expression levels were measured using qPCR methods (described in the methods section) 48 hours post transfection with siCACT oligonucleotides. *p < 0.05 as compared to the negative control. (n =4) (B) siRNA-CACT decreases CACT protein level. Western blot analysis was used to evaluate the effect of CACT siRNA on CACT protein levels in INS-1 832/3 cells. INS-1 832/13 cells were transfected with CACT siRNA or negative control (Scr-siRNA) 832/13 β-cells. Cells were harvested 48 hours after transfection and 25 μg of protein was loaded per lane; Vdac protein was used to normalize loading. Blot is representative of 4 independent experiments.



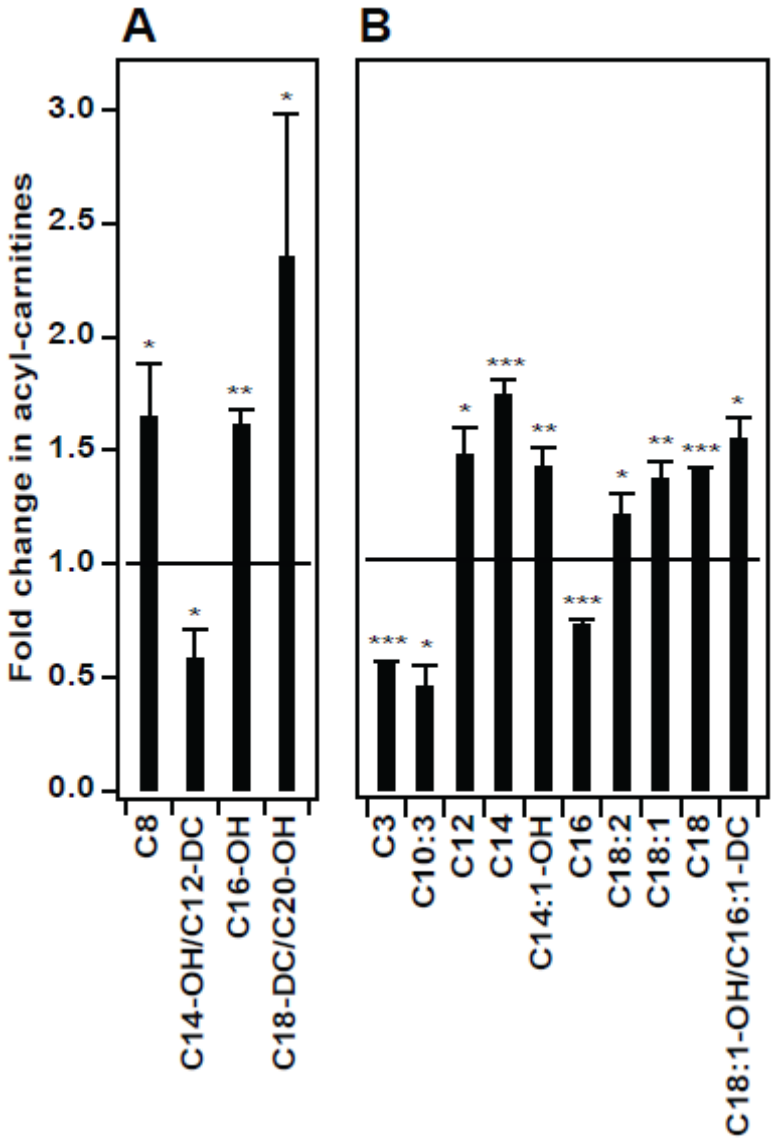
SUPPLEMENTARY DATA

Supplementary Figure 5. Steroyl-L-carnitines enhances IS. 48hrs after plating INS-1 832/3 cells, IS was measured in response to 7 mM glucose alone or in the presence of stearoyl-carnitine (50 μM) or palmitic acid (600 μM). Insulin secreted is normalized to total cellular insulin content and data is expressed relative to 7 mM glucose alone (NT) (2 hrs, n>4). *p < 0.05; **p < 0.01; ***p < 0.001.



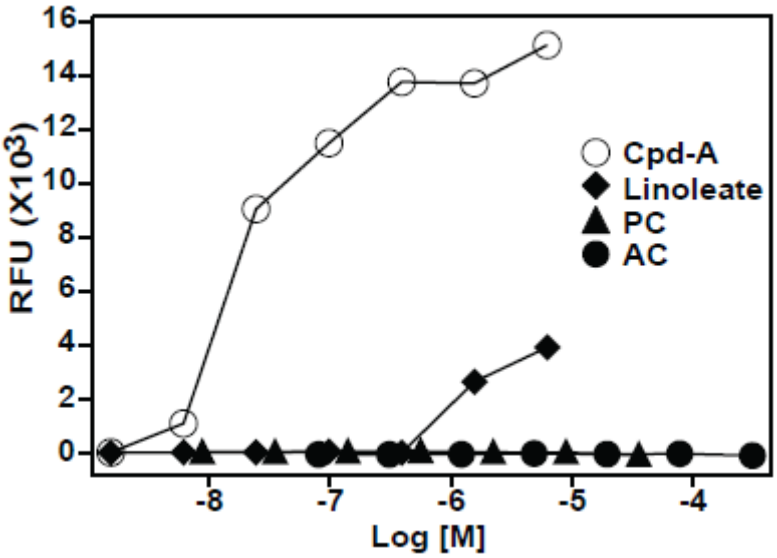
SUPPLEMENTARY DATA

Supplementary Figure 6. (A & B) Metabolic profile demonstrates a subtle increase in long-chain acyl-carnitines in response to CACT down-regulation. INS-1 832/13 cells were transfected with CACT siRNAs or control oligonucleotides (Scr-siRNA) and maintained for 48 hrs in RPMI supplemented without any exogenous L-carnitine. Two hours prior to harvesting the cells for metabolic profiling, the culture medium was refreshed without (A) or with (B) PC (50 μM) and 1.5 mM glucose. All metabolite measurements in response to CACT siRNA is normalized to control measurements. All experiments were performed n = 3 times. *p < 0.05; **p < 0.01; ***p < 0.001.



SUPPLEMENTARY DATA

Supplementary Figure 7. Palmitoyl-L-carnitine cannot activate GPR40. GPR40/CHO nuclear factor of activated T-cells β-lactamase cells were seeded into black-wall clear-bottom 384-well plates (Costar) 1 day before the assay. The cells were incubated with Hanks' buffered salt solution buffer with 0.1% BSA, 2.5 mmol/l probenecid, and 8 μmol/l Fluo-4-AM at room temperature for 100 min. Fluorescence output was measured using a fluorometric imaging plate reader (FLIPR) assay. Compund A, linoleic acid, acetyl-carnitine and palmitoyl-carnitine were added to observe their effect on GPR40 activity. Experiment was repeated twice.



SUPPLEMENTARY DATA

Supplementary Table 1. Acyl-carnitine molecules profiled by Mass spec and their correlation with IS. This table enlists all the acyl-carnitine moelcules that were surveyed by the Mass spec profilfing experiment. Column two of the table shows the coefficient of correlation of each metabolite with the IS effect depicted in Fig. 3B. Column three shows if the metabolite was significantly different between siCACT and Scrambled treated cells in the absence or presence of exogenous PC (50μM). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Acyl-carnitine	Correl Coeff	TTEST
C2	0.899825	**
C3	0.857961	*
C4	0.878836	*
C5:1	0.332214	*
C5	0.947179	**
C4-OH	0.81364	
C6	0.747288	
C5-OH	0.836689	
C3-DC	0.741238	*
C4-DC	0.725612	
C8:1	0.808295	
C8	0.732469	
C5-DC	0.822696	
C8:1-OH/C6:1-DC	0.739477	
C8-OH/C6-DC	0.830778	
C10:3	0.601103	
C10:2	0.78422	*
C10:1	0.867477	
C10	0.895005	**
C7-DC	0.892962	*
C8:1-DC	0.860196	**
C8-DC	0.904018	
C12:1	0.720082	
C12	0.918573	**
C12-OH/C10-DC	0.961403	
C14:2	0.899224	
C14:1	0.901048	*
C14	0.953624	***
C14:1-OH	0.984939	**
C14-OH/C12-DC	0.934851	**
C16:2	0.9788	**
C16:1	0.970214	**
C16	0.84804	**
C16:1-OH/C14:1-DC	0.95452	**
C16-OH	0.93349	**
C18:2	0.993924	**
C18:1	0.905264	***
C18	0.945182	***
C18:2-OH	0.933865	***

C18:1-OH/C16:1-DC	0.936092	***
C18-OH/C16-DC	0.891945	**
C20:4	0.858913	**
C20	0.826204	**
C18:1-DC	0.320051	*
C18-DC/C20-OH	0.463094	**
C22	0.442824	**

Supplementary Table 2. Generation of adenovirus overexpressing miRNA oligonucleotides. An expression cassette of human miRNAs-132, 212 or 375 contained the hairpin stem composed of 22 nt sense and antisense sequence of each miRNAs, fused to a loop (19 nt) and flanking sequence that derived from human miRNA-30. Expression was controlled by the human H1 promoter. To construct the H1-miRNA expression cassette, the forward and reverse oligonucleotides containing the respective miRNA sense and antisense sequence, miRNA30 flanking sequence and loop, BamHI and HindIII overhang were first synthesized as a pair of complementary antiparallel oligonucleotides. The forward and reverse oligonucleotides mentioned above were then annealed and ligated into a Gateway entry plasmid pENTR1A (Invitrogen), containing the human H1 promoter and a GFP expression cassette driven by CMV promoter. H1-miRNACMV- GFP cassette in pENTR1A plasmid was recombined into Gateway-based pAd-Block-iT DEST vector (Invitrogen). Ad-miRNAs were produced in HEK293 cells and purified by CsCl density gradient ultracentrifugation. The purified virus was desalted by dialysis and concentrated over CentriPrepYM-50 column before use.

	Primer Sequences
miRNA-132 forward:	GATCCCCGGGTATATTGCTGTTGACAGTGAGCGAGACCATGGCTG TAGACTGTTATAGTGAAGCCACAGATGTATAACAGTCTACAGCCA TGGTCGTGCCTACTGCCTCGGACTTCAAGGGTTTTTGGA
miRNA-132 reverse:	AGCTTCCAAAAACCCTTGAAGTCCGAGGCAGTAGGCACGACCATG GCTGTAGACTGTTATACATCTGTGGCTTCACTATAACAGTCTACAG CCATGGTCTCGCTCACTGTCAACAGCAATATACCCGGG
miRNA-212 forward:	GATCCCCGGGTATATTGCTGTTGACAGTGAGCGCGGCCGTGACTG GAGACTGTTATAGTGAAGCCACAGATGTATAACAGTCTCCAGTCA CGGCCATGCCTACTGCCTCGGACTTCAAGGGTTTTTGGA
miRNA-212 reverse:	AGCTTCCAAAAACCCTTGAAGTCCGAGGCAGTAGGCATGGCCGTG ACTGGAGACTGTTATACATCTGTGGCTTCACTATAACAGTCTCCA GTCACGGCCGCGCTCACTGTCAACAGCAATATACCCGGG
miRNA-375 forward:	GATCCCCGGGTATATTGCTGTTGACAGTGAGCGCCACGCGAGCCG AACGAACAAATAGTGAAGCCACAGATGTATTTGTTCGTTCGGCTC GCGTGATGCCTACTGCCTCGGACTTCAAGGGTTTTTGGA
miRNA-375 reverse:	AGCTTCCAAAAACCCTTGAAGTCCGAGGCAGTAGGCATCACGCGA GCCGAACGAACAAATACATCTGTGGCTTCACTATTTGTTCGTTCG GCTCGCGTGGCGCTCACTGTCAACAGCAATATACCCGGG