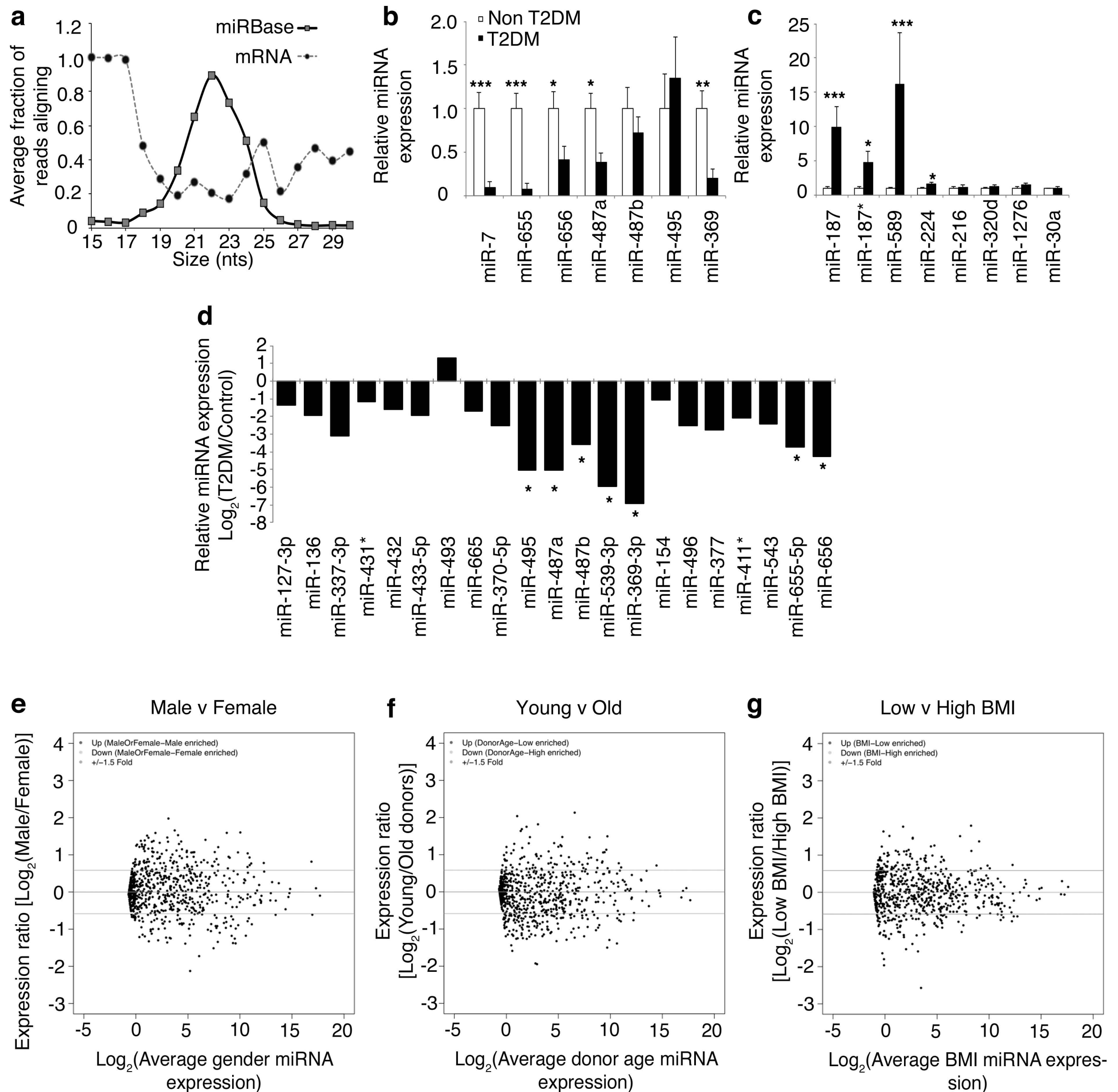
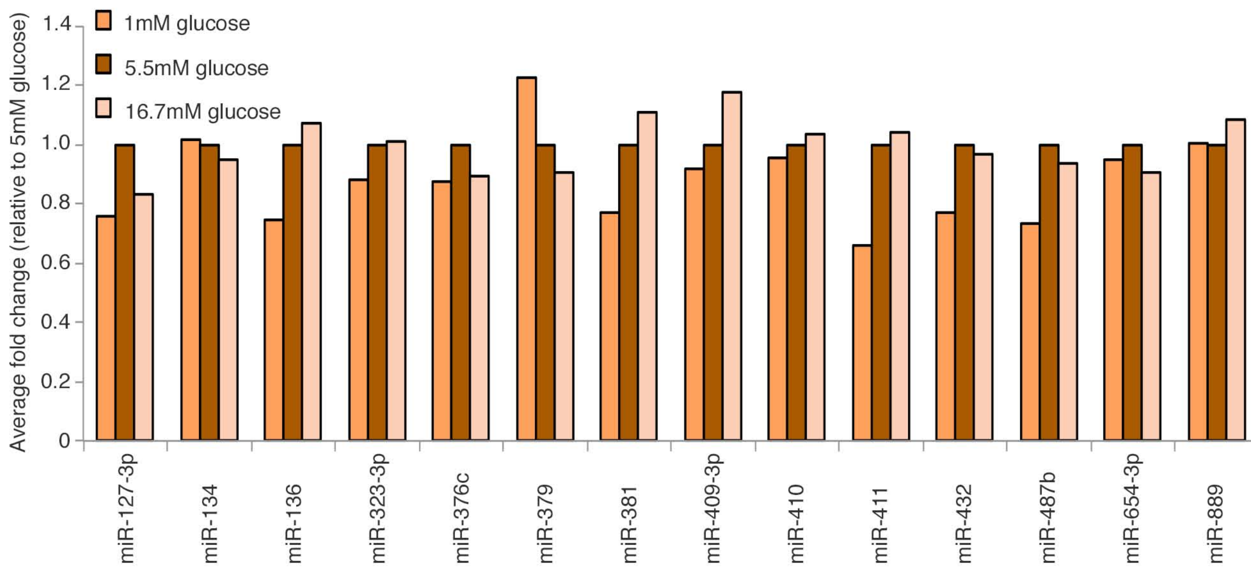
Supp Fig 1.



Supp Fig 2.

Supp Fig 3.

a



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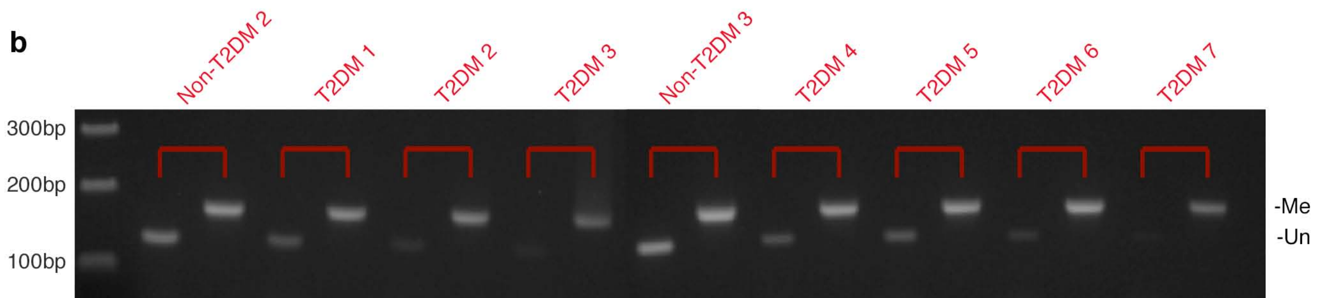
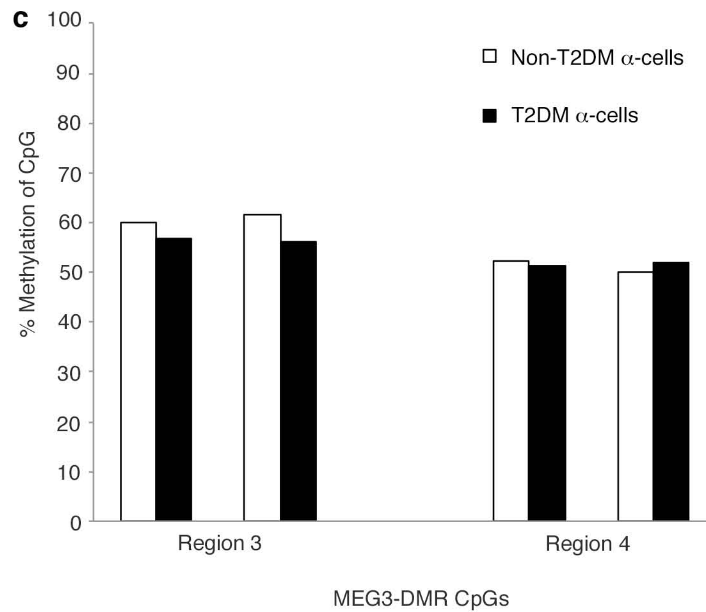
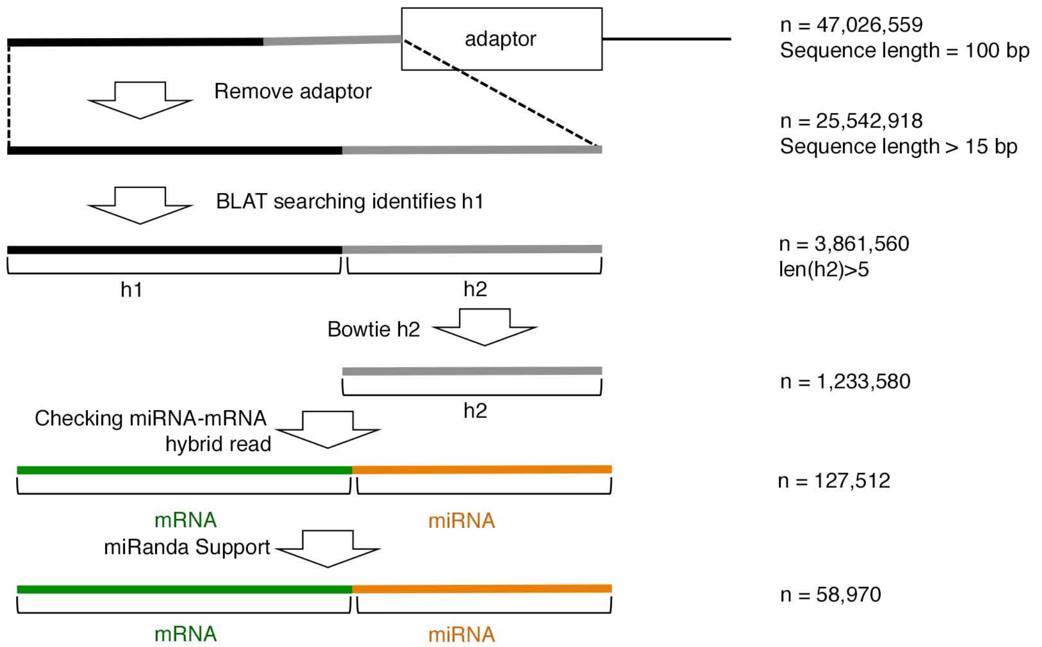


c



Supp Fig 4.



SUPPLEMENTAL FIGURE LEGEND

Supplementary Fig S1. Small RNA Sequencing of human islets. Related to Figure 1. (a) Small RNA sequencing reads alignment to miRBase and Refseq. Taqman qPCR confirmation of (b) down-regulated and (c) up-regulated miRNAs in 16 T2DM and 18 non-T2DM human islets. p-value calculated using Student's T-test. *p<0.05, **p-value<0.01, ***p-value<0.005. +/- SEM. (d) Expression of Chr 14q32 miRNAs as determined by small RNA sequencing of T2DM (n=4) and non-T2DM (n=3) islets. Human islet miRNA correlation with (e) Gender (f) Age (g) Body mass index (BMI). MiRNAs enriched or down-regulated in each category are highlighted in red and green respectively.

Supplementary Fig S2. Related to Figure 2. miRNAs that are differentially expressed between α- and β- cells as determined by small RNA sequencing. MiRNAs enriched in α-cells are highlighted in red, while miRNAs enriched in β-cells are marked in green.

Supplementary Fig S3. Mechanism of MEG3 miRNA down-regulation in T2DM islets. Related to Fig 3. (a) Expression of MEG3 miRNAs in non-diabetic islets (n=1) that were cultured in 1mM, 5.5mM and 16.7mM glucose for 16 hours. After small RNA sequencing, the expression values for each miRNA (for miRNAs >100rpm) was normalized to the 5.5mM value. (b) Methylation specific PCR for MEG3 promoter on two Non-T2DM and 7 T2DM donors' islets. Unmethylated band (Un) is 120bp, Methylated band (Me) is 160 bp. (c) Percent methylation for CpGs in the MEG3-DMR in α-cells sorted from a confirmed T2DM (n=1) and non-T2DM (n=1) donor.

Supplementary Fig S4. Identification of chimeric reads. Related to Figure 5.

Procedure to identify chimeric reads from Argonaute HITS-CLIP sequencing library as described in the Methods section.

SUPPLEMENTAL TABLE LEGEND:

Supplementary Table S1. Donor information. Related to Table 1.

Supplementary Table S2. Small RNA Sequencing of human islets. Related to Figure 1**.**

Supplemental Table S3. Small RNA Sequencing of sorted human α- and β-cells. Related to Figure 2.

Supplemental Table S4. HITS-CLIP on human islets. Related to Fig 4.

Supplemental Table S5. β-cell specific targets of the MEG3 miRNAs. Related to Figure 4.

Supplemental Table S6. Chimeric reads identified in HITS-CLIP of human islets. Related to Figure 5.

Supplemental Table S7. List of all primers used in the study. Related to Experimental Procedures section.

SUPPLEMENTAL METHODS

Processing of miRNA sequencing libraries

After preparation and sequencing of amplified libraries, we trimmed any 3' Illumina adapter sequences from the sequence reads. The resulting unique sequences with lengths between 15-36 base pairs were aligned to the human genome, RefSeq and to mirBase (release 20.0) using Illumina's ELAND program to determine the content of our samples. Insertions/deletions and mismatches were excluded and the counts for all reads with lengths between 19 and 25 (inclusive) were summed to obtain the expression of known miRNAs in each sample. When a trimmed read matched perfectly to two or more mature miRNA forms these forms were grouped together in a single ad hoc family.

Differential analysis consisted of two major components: normalization and statistical evaluation of differences between the groups. Relative read counts (in reads per million) for the expression level of the miRNA (family) genes in each replicate were normalized using quantile normalization using the limma package (normalizeBetweenArrays (Gentleman R, 2005; Smyth GK, 2003)), The resulting normalized intensities were then passed to SAM (Significance Analysis of Microarrays(Tusher VG, 2001)) to determine an FDR significance value. We used a false discovery rate of 20% and a minimum fold change of 1.5x to identify differentially expressed miRNAs.

Glucose induced miRNA expression

15,000 IEQ of human islets obtained from three donors were incubated in 5.56mM glucose for 6 hours. Islets were washed twice in 1x PBS and then divided and re-plated in media containing 1mM, 5.56mM or 16.7mM glucose for 16 hours (Tang et al., 2009) with triplicates for each condition. Total RNA was extracted using the miRvana RNA isolation kit. 1µg of RNA from one donor's islets was used for sequencing library preparation and sequenced to 36bp on an Illumina GAIIx. Sequencing reads were processed as described above.

HITS-CLIP experiment and sequencing data processing

The adapter trimming parameters were set to ≤15% mismatch of ≥8nt alignment to the 3' of the reads; reads mapping parameters were carefully tuned to allow ≤10% or ≤8% total mismatches for miRNA and mRNA library, respectively; only those alignments with ≤4% mismatches compared to the best hits were retained. As a supplement, we also mapped the mRNA reads to RefSeq mRNA sequences (Jul, 2012 release) to recover exon-exon junction mapped reads using the same parameters as above.

To illustrate the global miRNA target preference between CDS and both UTR regions, we calculated the read coverage for each nucleotide of every human islet transcript using all mapped mRNA library reads; Islet transcript was defined as with at least one mapped read from the target library. Then we equally divided islet transcripts into 100 "bins" for 5'UTR, CDS and 3'UTR separately. The average read depth of all nucleotides falling into each bin was used as the "average read coverage", and averaged again across all islet transcripts and shown in Fig 4b.

Identification of Argonaute footprints and their interaction with miRNAs

To identify Argonaute footprints, we first assembled all mapped mRNA reads to consecutive genomic contigs as a starting point. To exclude low-abundance contigs that might come from non-specific binding to the Argonaute protein, we then characterized highly expressed contigs as final Argonaute footprints using a Poisson distribution based statistical model. To be specific, we first calculated the normalized RPKM expression values for every contig, then fitted them to the Poission distribution as

$$f(x;\lambda) = \Pr(RPKM = x) = \frac{\lambda^x e^{-\lambda}}{x!},$$

where the parameter λ was estimated using the mean RPKM value of all contigs. Contigs with significant high RPKM values ($p<0.05$) were defined as Argonaute footprints. As a result, we identified a total of 12,492 Argonaute footprints with an average length of 98.3 nt in human islets.

REFERENCES

Gentleman R, C.V., Huber W, Irizarry R, Dudoit S, ed. (2005). Bioinformatics and Computational Biology Solutions Using R and Bioconductor. (Springer).

Smyth GK, S., T. (2003). Normalization of cDNA microarray data. Methods 4, 265-273.

Tang, X., Muniappan, L., Tang, G., and Ozcan, S. (2009). Identification of glucose-regulated miRNAs from pancreatic {beta} cells reveals a role for miR-30d in insulin transcription. RNA 15, 287-293.

Tusher VG, T.R., Chu G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. Proceedings of the National Academy of Sciences of the United States of America 98, 5116-5121.