Supporting Information

Poy et al. 10.1073/pnas.0810550106

SI Methods

Supplemental Methods. We imported the CEL files from the Affymetrix Mouse Genome 430 2.0 Array into the R software (1) by using the BioConductor affy package (2). The probe set intensities were then background-corrected, adjusted for non-specific binding, and quantile normalized with the GCRMA algorithm (3). Probe sets with more than 2 probes mapping ambiguously (more than 1 match) to the genome were discarded, as were probe sets that mapped to multiple genes. We then collected all probe sets matching a given gene, and we selected for further analysis the Refseq transcript with median 3′UTR length corresponding to that gene. The log-intensities of probe sets mapping to the gene were averaged to obtain the expression level per Refseq transcript. Finally, we used Limma (4) to estimate the fold change and the *P* value of the difference in expression between *375KO* and wild type for each transcript. To investigate whether the transcripts responding to a particular treatment are enriched or depleted in matches to the *miR-375* miRNA, we ranked the transcripts according to their estimated fold change. We labeled the 5% most down-regulated transcripts as "down" and the 5% most up-regulated transcripts as "up." What one means by "miRNA seed" varies to some extent from one study to another. Most commonly this term refers to positions 1–8, 1–7, 2–8, or 2–7 of the miRNA. Because the effect on mRNA stability depends on the extent of miRNA–mRNA sequence complementarity, we generally want to separately analyze putative sites with different degrees of complementarity. *miR-375* has, however, a CG dinucleotide at positions 7–8, leading to a very low number of sites that are complementary to positions 2–8 or 1–8 of this miRNA. Because of the high variance associated with these low numbers of sites, we used for our analysis only sites that are complementary to positions 1–7 (not including those that are also complementary to positions 1–8) of *miR-375*. We call the 1–7 *miR-375* seed complementary sequence with a mismatch at position 8 the "*miR-375* motif." We counted how many times the *miR-375* motif occurred in the 3′UTRs of transcripts labeled as "up" or "down." This number is represented in the plots as a black dot. To assess whether the number of *miR-375* motifs is unusually high or low compared with what would be expected for a "random miRNA," we computed a "background" motif count distribution as follows. We considered all possible "random miRNA seeds." These are all of the possible octamers. For each of these, we determined the number of occurrences of "background motifs" in all of the 3′UTRs of transcripts monitored by the microarray. These are all 3′UTR positions that match perfectly the 1–7, but not the 8th position of the "random miRNA." We then selected the 5% of these background motifs (i.e., 3,277) whose number of occurrences in the entire set of 3′UTRs was closest to that of the miR-375 motif. We computed the expected number of occurrences of these in the 3′UTRs of the "up" and "down" transcripts. This was defined as observed occurrences of the background motifs in the "up" or "down" transcripts 3′UTRs observed occurrences of the *miR-375* motif in the entire 3′UTRs set/observed occurrences of the background motifs in the entire 3′UTRs set. The distribution of the number of occurrences of background motifs is represented as follows: the blue boxes show the interquartile range and the red line the median. The range bounded by the black whiskers indicates the interval that is 1.5 times the interquartile range, and the red dots show all background motifs whose number of occurrences does not fall within this range. The black dot represents the count of *miR-375* motifs. The *P* value of the enrichment is given by the fraction of the background motifs that have at least as many occurrences as the *miR-375* motif in the "up" transcripts, and the significance is represented by the location of the black dot within the box plot representing the distribution of background motifs. Finally, the *P* value of the depletion is given by the fraction of the background motifs that have at most as many occurrences as the *miR-375* motif in the "down" transcripts.

Isolated Islet Secretion and Capacitance Measurements. Islet secretion studies were performed on size-matched islets isolated from 10-week-old animals following collagenase digestion and overnight culture and performed as described (5). Exocytosis of secretory granules was monitored in single α or β cells by capacitance measurements as described previously (5, 6). The measurements were performed in the standard whole-cell configuration of the patch-clamp technique at 32–33 °C and the identity of β cells was subsequently confirmed after the experiment by immunocytochemistry.

1. RDevelopmentCoreTeam (2006) R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna).
2. Gentleman RC, et al. (2004) Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* 5:R80.
3. Wu Z, Irizarry RA, Gentleman R., Martinez-Murillo F, Spencer FA (2004) Model-based background adjustment for oligonucleotide expression arrays. *J Am Stat Assoc* 99:909–917.
4. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3.
5. Poy MN, et al. (2004) A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* 432:226–230.
6. Eliasson L, et al. (2003) SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. *J Gen Physiol* 121:181–197.

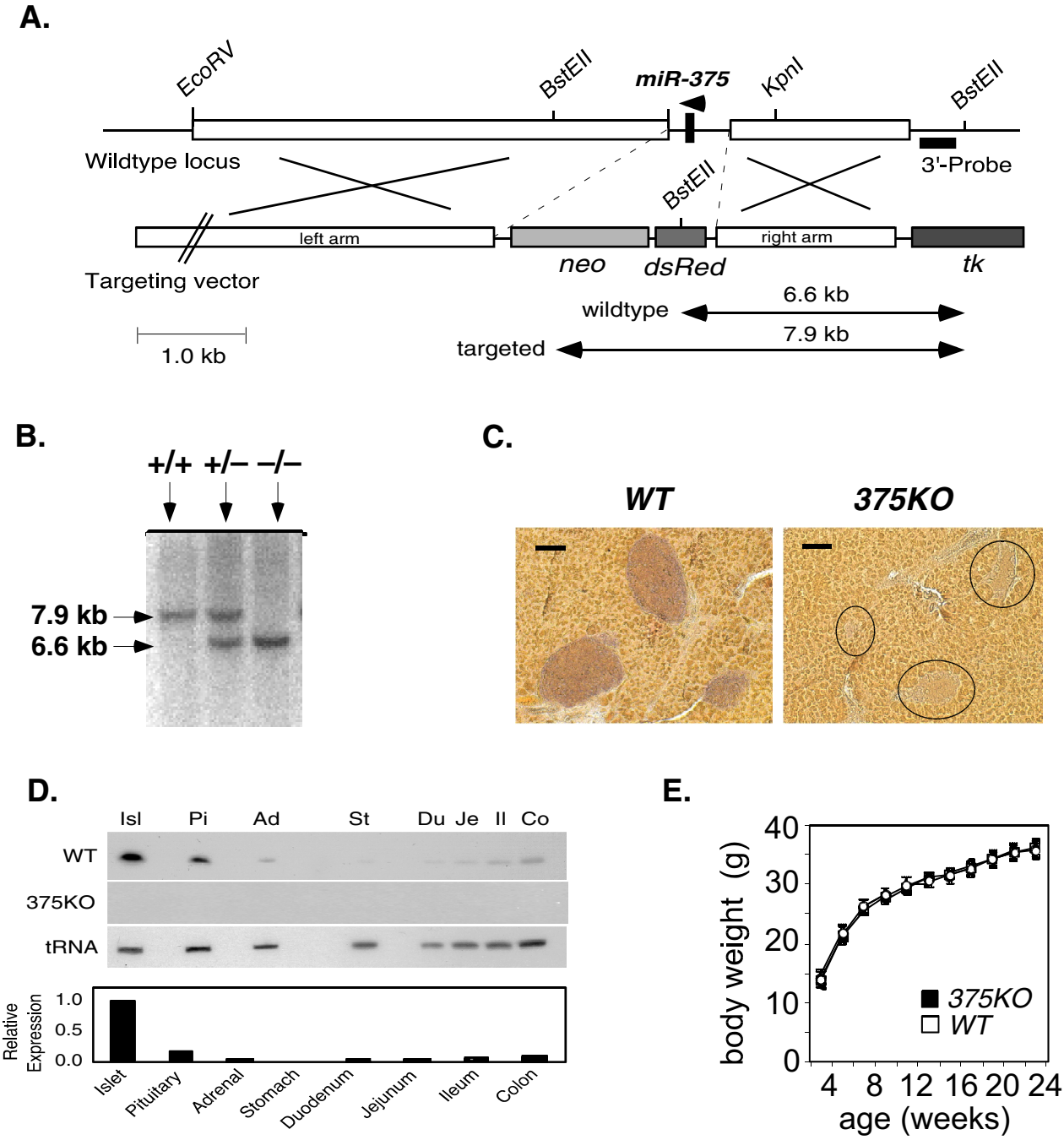

Fig. S1. Deletion of the *miR-375* gene by homologous recombination. (*A*) Targeting strategy for deletion of the *miR-375* locus by replacement with dsRed cDNA and the neomycin (Neo)-resistance cassette by homologous recombination in ES cells. Targeting arms are shown as white boxes, and the probe 3′ to the right targeting arm that was used for Southern blot analysis is shown as a black bar. No fluorescence signal was observed from dsRed of heterozygous or null mice. (*B*) Analysis of genomic DNA from wild-type (+/+), *miR-375* heterozygous (+/−), and *miR-375* homozygous (−/−) mice after digestion with BstEII. (*C*) In situ hybridization in pancreatic sections from wild-type (*WT*) and mutant (*375KO*) mice with a probe for *miR-375*. Black circles indicate islets in *miR-375*-null mice. (Bar, 25 μm.) (*D*) Northern blot of total RNA isolated from 10-week-old *WT* and *375KO* tissues: pancreatic islets (Isl), pituitary gland (Pi), adrenal gland (Ad), stomach (St), duodenum (Du), jejunum (Je), ileum (Il), and colon (Co). Blot was reprobed for tRNA as a loading control and quantified by densitometry. (*E*) Growth curve of *375KO* and wild-type mice.

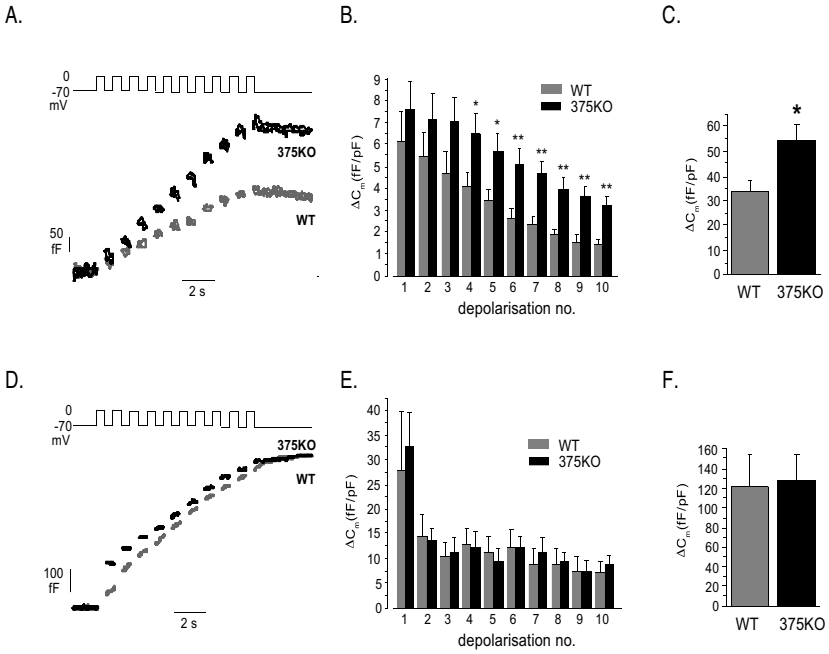

Fig. S2. Single-cell capacitance measurements in pancreatic α and β cells of *375KO* and littermate control mice. (*A*) Secretion was evoked by a train of depolarizations from −70 mV to 0 mV in isolated β cells from 10-week-old male *375KO* (black signals) and wild-type (gray signals) mice. (*B*) Mean increase in membrane capacitance of isolated β cells elicited by individual depolarizations of the train ($\Delta C_{m,n} - \Delta C_{m,n-1}$) displayed against the pulse number (*n*). (*C*) Total mean increase in membrane capacitance elicited by individual depolarizations of pancreatic β cells from *375KO* and wild-type mice. (*D–F*) As in *A–C* but using α cells. *, $P < 0.01$; **, $P < 0.001$.

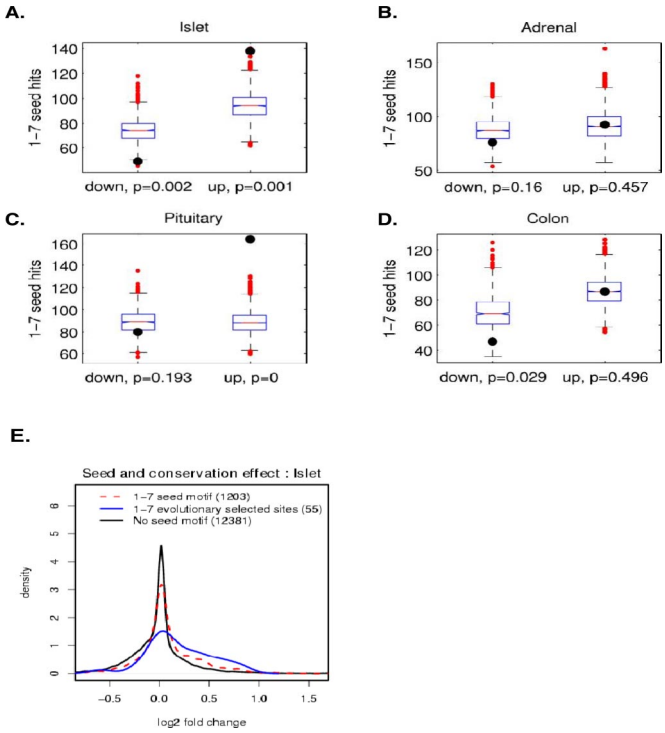

Fig. S3. Identification of miR-375 target genes. (*A–D*) Quantification of the number of occurrences of *miR-375* motifs in the 3′UTRs of both up-regulated and down-regulated transcripts in *375KO* tissues. Black dots indicate the number of occurrences of *miR-375* motifs in the 5% most up- and down-regulated genes (right and left plots, respectively). The distribution of the number of occurrences of motifs complementary to ''random miRNAs'' in these transcripts is represented as a box plot: blue boxes show the interquartile range, the black whiskers indicate the range of 1.5 times the interquartile range, and the red dots represent the motifs whose number of occurrences falls outside of this range. The ''random'' miRNAs are selected to have approximately the same number of complementary motifs as *miR-375* in the entire set of 3′UTRs. (*E*) Density plot showing that transcripts with a *miR-375* motif (dashed red line) or an evolutionarily-conserved *miR-375* motif (solid blue line) are up-regulated in the *375KO* relative to transcripts that do not contain this motif.

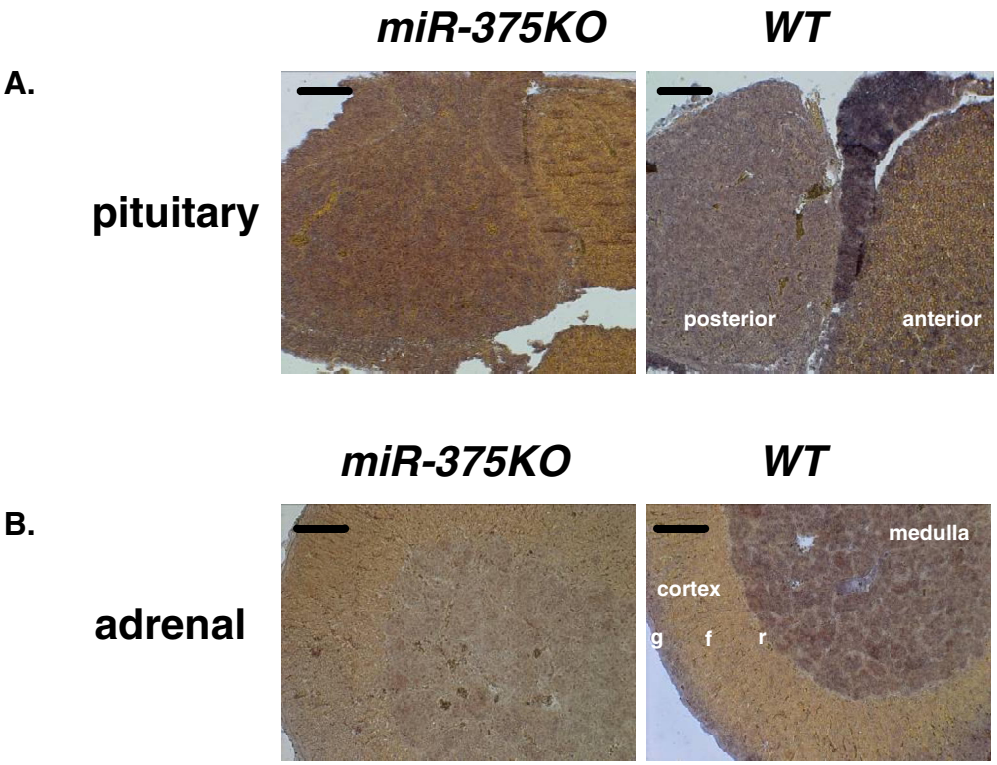

Fig. S4. (*A*) Detection of *miR-375* expression by in situ hybridization in pituitary anterior and posterior regions and (*B*) in adrenal sections from wild-type and *375KO* mice using a sequence specific probe for *miR-375*. *miR-375* is detected in the adrenal medulla and the zona glomerulosa (g) of the cortex and not the fasciculata (f) or reticularis (r). (Bar, 50 μm.)

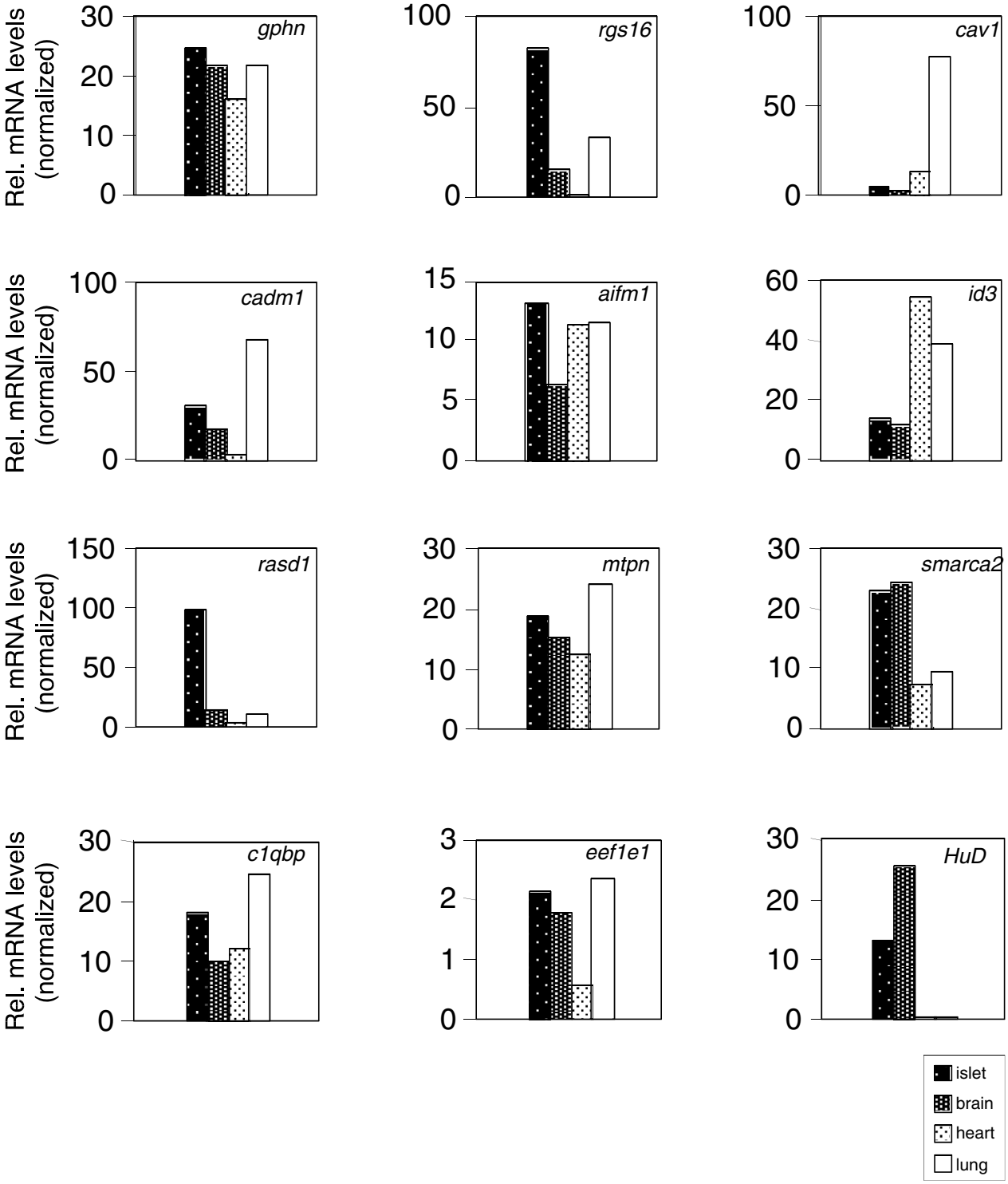

Fig. S5. Real-time PCR analysis of miR-375 targets in islets, brain, heart, and lung. Gene expression in the indicated tissues was normalized to U6 levels.