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Differential impact of glucose administered intravenously and orally on circulating miR-375 levels in human subjects

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Short Title:
Oral glucose impacts circulating miR-375 in humans

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The authors declare no competing interests.

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Abstract

Background

To date, numerous nucleic acid species have been detected in the systemic circulation including microRNAs (miRNAs); however their functional role in this compartment remains unclear.

Objective

The aim of this study was to determine whether systemic levels of miRNAs abundant in blood, including the neuroendocrine tissue-enriched miR-375, are altered in response to a glucose challenge.

Design

Twelve healthy males were recruited for an acute cross-over study which consisted of two tests each following an eight-hour fasting period. An oral glucose tolerance test (OGTT) was performed and blood samples were collected over a 3-hour period. Following a period of at least one week, the same participants were administered an isoglycemic intravenous glucose infusion (IIGI) with the same blood collection protocol.

Results

The glucose response curve following the IIGI mimicked that obtained after the OGTT, but as expected systemic insulin levels were lower during the IIGI compared to the OGTT ($P<0.05$). MiR-375 levels in circulation were increased only in response to an OGTT and not during an IIGI. In addition, the response to the OGTT also coincided with the transient increase of circulating glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and glucose-dependent insulinotropic polypeptide (GIP).

Conclusions

The present findings show levels of miR-375 increase following administration of an OGTT and in light of its enrichment in cells of the gut, suggest that the gastrointestinal tract may play a significant role to the abundance and function of this microRNA in the blood.

Précis

Here we show using a clinical cohort the impact of glucose administered orally on miR-375 in the blood. This result suggests a role for the gut in regulating miR-375 levels in systemic circulation.

Abbreviations:

IIGI	Isoglycemic intravenous glucose infusion
GLP-1	Glucagon-like peptide-1
GLP-2	Glucagon-like peptide-2
GIP	Glucose-dependent insulinotropic polypeptide
miRNA	microRNA
OGTT	Oral glucose tolerance test

Introduction

It is now widely accepted that miRNAs are present in the circulation; however their precise function in this compartment is not completely understood (1)(2)(3). MiR-375 is an abundant miRNA that has been identified in several tissues including pancreatic islets, the pituitary, adrenal glands, and the gastrointestinal tract (4). Whereas miR-375 has been established as a regulator of pancreatic β-cell function, its expression profile beyond this one cell type suggests a prominent role in other metabolic processes (5)(6)(7). Previous reports have identified miR-375 in the blood indicating that endocrine cells may contribute to its presence in circulation (1). As studies also now show alterations in systemic levels of miRNAs during disease states, it is still unclear how the kinetics of the miRNAs in circulation reflect changes in metabolism and physiology (8). Whether the miRNAs are actively released in response to changes in systemic glucose or other factors present in blood or whether they are constitutively released has also not been described.

In response to dietary intake, several hormones including insulin and the incretins, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released into the blood to facilitate several physiological processes and ultimately to lower blood glucose levels by promoting insulin release (9). GLP-1 is released by the intestinal endocrine L-cells and performs an array of functions in addition to its direct action on the β-cell. GLP-1 acts to slow gastric emptying while indirectly inhibiting glucagon secretion via the release of somatostatin. Meanwhile, GIP present in the K-cells of the small intestine, will facilitate energy storage via direct action on adipose tissue, and promote bone formation by increasing osteoblast proliferation (9). Studies primarily in rodent models have begun to explore the role of GLP-1 and GIP in other tissues including the brain and together these observations highlight the complex nature of incretin action in the context of energy homeostasis.

In this study we sought to determine whether miRNAs are released into the systemic circulation following a glucose challenge in human subjects and whether the presence of specific miRNAs coincide with established factors regulating glucose homeostasis. To date, extensive miRNA profiling has been performed on human tissues including blood. Here we show the systemic level of miR-375 is increased following oral ingestion of glucose implicating a role for the gut in the release of this small RNA into

124 circulation. We also observe that miR-375 is the most highly abundant and enriched
125 miRNA in Sox9-High cells of the intestine, and robustly expressed and enriched in Sox9-
126 Low cells, suggesting that the enteroendocrine cell population, and possibly the
127 proliferating cells of the intestinal crypt, may contribute to systemic levels of this
128 miRNA.
129

130 **Materials and Methods**

131 **Human Patient Samples**

132 As previously reported, approval was obtained from the Danish Data Protection Agency
133 (2007-58-0010) and the Ethics Committee of the Central Denmark Region (1-16-02-377-
134 13) and informed consent was obtained from all patients for being included in the study
135 (10). Twelve healthy Caucasian males, aged 20 to 50 years, participated in the study
136 which was registered at ClinicalTrials.gov (NCT02213276). During the OGTT and IGII
137 experiments, blood samples were collected at 0, 15, 30, 60, 120, and 180 minutes from
138 receiving the glucose load which lasted 5 minutes. For the 3-hour fasting control
139 experiment, blood samples were collected after 0, 1, 2 and 3 hours. Blood samples for
140 plasma analysis of insulin, GIP, GLP-1 and GLP-2 were prepared and quantified at the
141 Department of Clinical Biochemistry at Aarhus University Hospital accredited in
142 accordance with ISO15189 (10). After collection, blood samples were centrifuged at
143 2000 x g for 10 minutes and then stored at -80°C until analysis. Plasma insulin was
144 measured by ELISA (cat. no. K6219; Dako, Glostrup, Denmark). All samples were
145 extracted in a final concentration of 70% ethanol before GIP and GLP-1 measurements
146 and 75% before GLP-2 measurements. Total GIP was measured using a
147 radioimmunoassay with a C-terminally directed antibody (antiserum. no. 80867), which
148 reacts fully with intact GIP and N-terminally truncated forms (11). The standard was
149 human GIP (Bachem, cat no. H-5645) and the tracer was ^{125}I-labeled human GIP (Perkin
150 Elmer, cat no. Nex402). Total GLP-1 was measured using a radioimmunoassay with an
151 antibody (antiserum. no. 89390) specific for the C-terminal of the GLP-1 molecule and
152 reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite
153 (12). Intact GLP-2 was measured using a radioimmunoassay as originally described (13).
154 The antiserum (code no. 92160) is directed against the N-terminus of GLP-2 and

155 therefore measures only fully processed GLP-2 of intestinal origin. For standards, we
156 used recombinant human GLP-2 and the tracer was ^{125}I-labeled rat GLP-2 with an Asp33
157 -> Tyr33 substitution. Sensitivity for all the radioimmunoassays was below 5 pmol/l, and
158 intra-assay coefficient of variation below 10 %.

159 **Gene Expression Analysis**

160 Total RNA was isolated from plasma using Trizol LS (Invitrogen) with glycogen as a
161 carrier. MiRNAs were quantified by TaqMan Assays using the TaqMan MicroRNA
162 Reverse Transcription Kit and miRNA specific primer sets (Thermo Scientific). Plasma
163 miR-375, miR-148a, and miR-27b levels were normalized to miR-16 expression in each
164 respective sample.

165 **Small RNA Sequencing Analysis**

166 Raw miRNA counts were retrieved from Supplementary Table 4 of Kang et al. 2017 (14).
167 The publically available data was downloaded from Sequence Read Archive FTP site and
168 NCBI dbGaP on 12/08/2015 resulting in 823 data sets from human tissue samples from
169 and 395 data sets originating from serum and CSF. The precise details of the analysis can
170 be retrieved from the Material and Methods section of Kang et al. 2017 (14).

171 Tissue samples were normalized to estimated number of miRNA molecules per
172 cell and we assumed the total amount of miRNA per cell to be ~100,000 (15). Counts for
173 individual miRNA were divided by the sum of all miRNA reads in the sample and
174 multiplied with the assumed total miRNA abundance. The same normalization was
175 applied to body fluid samples but as the total abundance of miRNA in those fluids is
176 unknown, the values do not represent copies per cell and are indicated as 'arbitrary units'.
177 In total, 1787 miRNA were detected and analyzed; independent sample sizes were blood
178 cells (n=197), liver cells (n=93), brain cells (n=102), serum samples (n=212), exosome
179 samples (n=37) and cerebrospinal fluid (CSF) (n=180). In summary, the number of times
180 a given miRNA is detected in sequencing depends on its abundance and the overall
181 sequencing depth (16).

182 For small RNA sequencing from gut tissue of Sox9-EGFP mice, IEC isolation
183 and FACS was performed with the UNC Flow Cytometry Core Facility as described
184 previously (17). RNA was isolated from sorted subpopulations using the Total RNA
185 Purification Kit (Norgen Biotek), and small RNA libraries were prepared with a range of

186 ~60ng to ~850ng of total RNA using the CleanTag Small RNA Ligation Kit (TriLink
187 Biotechnologies, CA) at the sequencing facility at the University of Texas Health
188 Sciences Center, San Antonio. Libraries were sequenced on the Illumina HiSeq2500
189 platform, yielding an average of ~40 million total reads per sample. Raw sequencing data
190 and miRNA quantification tables for all samples can be accessed through the NCBI GEO
191 accession number GSE XXXX.

192 **Animal Studies.**

193 All animal studies were approved by the Institutional Animal Care and Use Committee at
194 the University of North Carolina, where the mouse experiments were performed. Chow-
195 fed male Sox9-EGFP mice were housed in cages with ¼-inch Bed-O'cobs laboratory
196 animal bedding. At 24-28 weeks of age, the mice were anesthetized using isofluorane,
197 then euthanized by cervical dislocation.

198 **Statistical analysis**

199 Comparisons between data sets with two groups were evaluated using an unpaired
200 Student's t-test. Repeated measures ANOVA was performed using GraphPad Prism
201 Software 6.07 to compare levels of miR-375, glucose, insulin and gastrointestinal
202 hormones between OGTT, IIGI and the 3-hour fasting control (Supplemental Table 1).
203 Post hoc statistics were performed using Sidak's multiple comparison test. A *P*-value of
204 less than or equal to 0.05 was considered statistically significant.

205

206 **Results**

207 **Quantification of miR-375 in human blood**

208 Surveying miRNA abundances in >800 human samples from six different tissues and
209 body fluids we observed high abundances of miR-16 in all analyzed tissues including
210 blood cells, liver, brain, serum, exosome and CSF samples (Figure 1a and 1b). In
211 contrast, miR-375 was only abundant in serum, exosomes, liver and CSF, while it was
212 moderately expressed in the brain and virtually absent in blood cells. This lack of
213 expression in blood cells suggests that miR-375 may be exported into the bloodstream by
214 adjoining tissues. In contrast, miR-124 was found to be only moderately expressed in the
215 brain and was at low levels in all other analyzed tissues (Figure 1b). This further
216 underlines the differential tissue expression pattern and function of miRNAs.

217 **Systemic miR-375 increases during OGTT**

218 In light of its abundance in circulation, we quantified systemic miR-375 levels in
219 the plasma of healthy human subjects in response to either an OGTT or an isoglycemic
220 IIGI. Blood glucose levels were comparable between the OGTT and IIGI experiments
221 (Figure 2a). The plasma miR-375 level significantly increased during the OGTT where it
222 reached a maximum after 60 and 120 min (~4.8 and ~5.3-fold increase, respectively) and
223 returned to baseline level after 180 min. In contrast, during the IIGI expression of this
224 microRNA remained indistinguishable from steady state levels (Figure 2a, Supplemental
225 Figure 1a, and Supplemental Table 1).

226 The oral glucose administration resulted in a pronounced increase in circulating
227 gut hormones GIP, GLP-1, GLP-2 (at time 30 minutes, ~3.9, ~2.2, and ~3.5-fold
228 increase, respectively) as well as plasma insulin (~8.5-fold) as recently published (Figure
229 2b-f) (10). During the intravenous glucose infusion plasma levels of GIP, GLP-1 and
230 GLP-2 remained at baseline levels, while insulin showed only a small increase (Figure
231 2c-f). Together these results indicate the temporal rise in miR-375 correlates with
232 increased levels of the gut hormones and this is further supported with both area-under-
233 curve calculations and analyzing miR-375 levels using analysis of covariance
234 (ANCOVA), as plotted in relation to GIP or GLP-1 (Supplemental Figure 1b-e).
235 Meanwhile, we also addressed the expression of two additional miRNAs abundant in
236 human serum, miR-148a amd 27b, and observed no changes in systemic levels during the
237 OGTT or IIGI (Suppementary Figure 2a and 2b) (14).

238 **MiR-375 is enriched in enteroendocrine cells**

239 We next performed small RNA-sequencing on intestinal epithelial subpopulations
240 isolated from 24-28-week-old male Sox9-EGFP reporter mice to identify cell types
241 enriched for miR-375 (Figure 3) (18)(19). As described previously, FACS sorting
242 resulted in four independent cell populations: the Sox9-High group is enriched for
243 enteroendocrine cells and reserve/quiscent intestinal stem cells (Figure 3a), Sox9-Low is
244 enriched for actively-cycling intestinal stem cells (Figure 3b), Sox9-Sublow is enriched
245 for transit amplifying cells (Figure 3c), and Sox9-Neg is enriched for enterocytes (Figure
246 3d) (17). Together, these results show that miR-375 is the most highly expressed and

247 highly enriched miRNA in Sox9-High cells and is also robustly expressed and enriched
248 in Sox9-Low cells (though not as high as in Sox9-High cells).
249

250 **Discussion**

251 Our knowledge is incomplete with regards to the dynamics and function of the majority
252 of circulating factors in human blood. It has long been known that administration of
253 glucose orally versus intravenously differentially affects the release of insulin due to the
254 role of the incretins in islet cell function; however the full extent to the interplay of these
255 endocrine factors in the context of glucose and energy metabolism remains to be
256 determined (20)(21). In the present study, we observed the presence of miRNA sequences
257 known to be enriched in tissues central to the regulation of glucose and energy
258 homeostasis including miR-375. Interestingly, higher levels of miR-375 were observed in
259 the plasma of human subjects following an oral glucose challenge in contrast to an
260 intravenous glucose infusion. Levels of miR-375 peaked 30 minutes after the peaks of
261 insulin and incretins, establishing that systemic increase of miR-375 is temporally
262 associated the release of these hormones. The differential response of miR-375 in blood
263 may yet identify an additional physiologic function of the gut or its secreted incretin
264 hormones. Consistent with previous observations, both methods of glucose
265 administration achieved similar blood glucose levels while the insulin secretory response
266 was diminished during the IIGI (22)(23). The robust increase in plasma insulin levels
267 quantified during the OGTT coincided with a concomitant increase in plasma GLP-1,
268 GLP-2, and GIP. Hence, these results present correlative evidence that the gut may play
269 either a direct or indirect role in the regulation of the circulating levels of miR-375 in
270 response to oral glucose.

271 It is also unclear whether our results are related to previous studies measuring
272 miR-375 in blood after pharmacological destruction of beta cell mass; previous studies
273 have reported an alteration in the cellular distribution within the gastrointestinal tract
274 after administration of streptozotocin to mice (24)(25)(26). Latreille *et al.* reported that
275 the pancreatic β-cell, the cell type expressing the highest levels of miR-375, contributes
276 to ~1% of the systemic miR-375 levels, indicating that the amount of miRNAs released
277 by the β-cell is either negligible in comparison to other tissues or they are rapidly taken

278 up or they are degraded once in circulation (25). It is also possible that several endocrine
279 cell types release miR-375 in response to incretin signaling in line with their role as
280 paracrine effectors and insulin secretagogues.
281 Our observations here showing a rise in miR-375 levels in blood following the
282 oral intake of glucose suggests several possibilities regarding the role of the gut with
283 regard to the presence miRNAs in circulation. First, circulating levels of this miRNA
284 may either originate from the gut or respond to signaling molecules released by this
285 tissue. Our profiling results from sorted cells of the gut confirm miR-375 to be highly
286 abundant in subpopulations enriched for both enteroendocrine cells and intestinal stem
287 cells (and also present albeit at lower levels in transit-amplifying cells and enterocytes)
288 (27). In light of the heterogeneity of L-cells, another possibility is that L-cells of the
289 upper gut act primarily in nutrient sensing while in the lower gut, these cells contribute to
290 the release of miR-375 (28). The highest levels of miR-375 were measured between 30
291 and 120 minutes post-administration; therefore the presence of this miRNA in the
292 secretory granules storing either insulin or GLP-1 is unlikely. It is unclear how many
293 additional miRNAs the differential impact of oral glucose versus intravenous on systemic
294 levels will be shown to affect as abundant sequences such as miR-148a and miR-27b
295 were observed not altered.
296 A recent study by Kahn and colleagues has shown evidence that circulating
297 miRNAs are derived from adipose tissue and are able to target genes of the liver (29).
298 Using a tissue-specific knockout of the miRNA-processing enzyme Dicer, they observed
299 a decrease in circulating miRNAs present in exosomes and that over-expression of miR-
300 99b was able to suppress activity of a reporter construct containing the 3'UTR of the
301 gene Fgf21, a circulating factor expressed by the liver and other tissues (29). MiR-375 is
302 also detected in liver and while expression of miR-375 in this tissue is several magnitudes
303 lower than the levels measured in the pancreatic islet, miR-122 is detected in blood
304 suggesting the hepatocyte is also a site of miRNA export (6)(30)(31). However, it would
305 be prudent for future investigations to determine whether the measured amounts of miR-
306 375 are endogenous expression from hepatocytes. Given the volume of blood transported
307 throughout the liver, it is likely a significant fraction of miR-375 expression quantified in
308 this tissue is derived from its presence in the systemic circulation. Therefore the

309 functional relevance of miR-375 in the liver at steady state conditions is unclear at this
310 time. Given the dozens of other more abundant miRNA sequences present, most notably
311 miR-122, the function of low-expressed miRNAs is not known based on the absence of
312 strong evidence showing target regulation in vivo. While dysregulation of miR-375 target
313 genes in the liver cannot be ruled out, none of the targets of this miRNA that were
314 experimentally-validated using knockout tissues, have been studied in the context of
315 glucose or lipid metabolism in the liver (4). The mild hyperglycemia observed in miR-
316 375 knockout mice appears to result from increased glucagon levels stemming from
317 increased alpha cell mass in the islet (4).

318 In summary, our observations on miR-375 in circulation continue to reflect the
319 exceedingly complex and dynamic state of human blood following glucose uptake. In
320 light of the temporal relationship between miR-375 and incretin hormones, future studies
321 should explore the physiological relevance of miRNA in circulation and whether
322 functional overlap exists between non-coding RNA and signaling hormones.

323

324 **Declarations**

325 **Ethics Approval and Consent to Participate**

326 As previously reported, approval was obtained from the Danish Data Protection Agency
327 (2007-58-0010) and the Ethics Committee of the Central Denmark Region (1-16-02-377-
328 13) and informed consent was obtained from all patients for being included in the study
329 (10).

330 **Consent for publication**

331 Not applicable.

332 **Availability of data and material**

333 All primary data supporting the findings of this study are available on reasonable request.

334 **Author's contributions**

335 X.Y., Z.W., S.R., M.T., T.R., S.G.T., B.C.E.P., M.K., C.O., J.F., P.S., M.F., J.S., S.G.,
336 and M.P. contributed to the conception and design of the study, and wrote the manuscript.
337 All authors approved the final version of this manuscript.

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455

456 **Figure Legends**

457 **Figure 1.** Abundances of miR-375, miR-16 and miR-124 in three human tissues and
458 three body fluids. (**a**) Abundances of the three miRNAs in blood cells, liver and brain.
459 The vertical axis shows estimated miRNA molecules per cell, logarithmic scale. (**b**)
460 Abundances in serum, exosome and cerebrospinal fluid. The vertical axis indicates
461 arbitrary units for cell-free samples. In total, 197 blood cell, 93 liver, 102 brain, 212
462 serum, 37 exosome and 180 cerebrospinal samples were profiled.

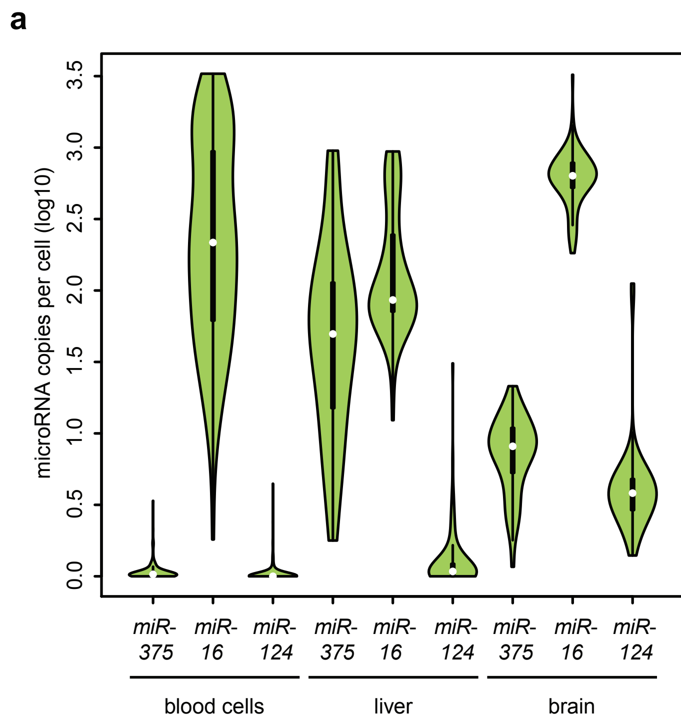
463

464 **Figure 2.** Measurements of miR-375, glucose, insulin, GIP, GLP-1, and GLP-2 in the
465 plasma of healthy human individuals during an oral glucose tolerance test (OGTT),
466 intravenous isoglycemic glucose infusion (IIGI), and a 3-hour fasting control (open
467 circles (OGTT), filled squares (IIGI), and open squares (control)). (**a**) Plasma miR-375
468 levels during the OGTT (open circles), the IIGI (filled squares), and during the control
469 (open squares). (**b**) Plasma glucose concentrations during the OGTT (open circles), the
470 IIGI (filled squares), and during the control (open squares). (**c**) Plasma insulin
471 concentrations during the OGTT (open circles), the IIGI (filled squares), and during the
472 control (open squares). (**d**) Plasma GIP concentrations during the OGTT (open circles),
473 the IIGI (filled squares)), and during the control (open squares). (**e**) Plasma GLP-1
474 concentrations during the OGTT (open circle), the IIGI (filled squares), and during the
475 control (open squares). (**f**) Plasma GLP-2 concentrations during the OGTT (open circle),
476 the IIGI (filled squares), and during the control (open squares). Results are presented as
477 mean ± SEM. Data on plasma glucose, insulin, GLP-1, GLP-2, and GIP have recently
478 been published and are reproduced with permission (10). The summary of statistical
479 analyses for these datasets is compiled in Supplemental Table 1.

480

481 **Figure 3.** MiR-375 is highly expressed and significantly enriched in independent
482 intestinal epithelial subpopulations. (**a**) Bar graph showing normalized levels of
483 expression for miR-375 (y-axis) from small RNA-sequencing across four different
484 intestinal epithelial subpopulations (x-axis) in 24-week-old male Sox9-EGFP reporter
485 mice (n=3). Sox9-High (enriched for enteroendocrine cells), Sox9-Low (enriched for
486 intestinal stem cells), Sox9-Sublow (enriched for transit-amplifying cells), and Sox9-Neg
487 (enriched for enterocytes). RPMMM, reads per million mapped to microRNAs. (**b**)
488 Scatter plot showing average expression of microRNAs from small RNA-sequencing in
489 Sox9-High cells (y-axis) and fold-enrichment in Sox9-High cells relative to unsorted
490 intestinal epithelial cells (x-axis) from 24-week-old male Sox9-EGFP reporter mice
491 (n=3). Only microRNAs with average RPMMM > 1000 are displayed. Each data point
492 represents a microRNA, miR-375 is labeled. (**c**) Scatter plot showing average expression
493 of microRNAs from small RNA-sequencing in Sox9-Low cells (y-axis) and fold-
494 enrichment in Sox9-Low cells relative to unsorted intestinal epithelial cells (x-axis) from
495 24-week-old male Sox9-EGFP reporter mice (n=3). Only microRNAs with average
496 RPMMM > 1000 are displayed. Each data point represents one single microRNA, miR-
497 375 is labeled. (**d**) Scatter plot showing average expression of microRNAs from small
498 RNA-sequencing in Sox9-Sublow cells (y-axis) and fold-enrichment in Sox9-Sublow
499 cells relative to unsorted intestinal epithelial cells (x-axis) from 24-week-old male Sox9-
500 EGFP reporter mice (n=3). Only microRNAs with average RPMMM > 1000 are
501 displayed. Each data point represents a microRNA, miR-375 is labeled. (**e**) Scatter plot
502 showing average expression of microRNAs from small RNA-sequencing in Sox9-Neg
503 cells (y-axis) and fold-enrichment in Sox9-Neg cells relative to unsorted intestinal
504 epithelial cells (x-axis) from 24-week-old male Sox9-EGFP reporter mice (n=3). Only
505 microRNAs with average RPMMM > 1000 are displayed. Each data point represents a
506 microRNA.
507
508 **Supplemental Figure Legends**
509 **Supplemental Figure 1.** Analysis of plasma levels of miR-375, GIP, and GLP-1, during
510 an oral glucose tolerance test (OGTT), an intravenous isoglycemic glucose infusion
511 (IIGI), and during the fasting control (Control). (**a**) Comparison of area under the curve

512 analyses for miR-375 during the OGTT, IIGI, and fasting control. (**b**) Comparison of area
513 under the curve analyses for GIP during the OGTT, IIGI, and fasting control. (**c**)
514 Comparison of area under the curve analyses for GLP-1 during the OGTT, IIGI, and
515 fasting control. (**d**) Plasma levels of total GIP plotted against plasma levels of miR-375
516 from each individual patient during the OGTT and IIGI. (**e**) Plasma levels of total GLP-1
517 plotted against plasma levels of miR-375 from each individual patient during the OGTT
518 and IIGI. The summary of statistical analyses for these datasets is compiled in
519 Supplemental Table 1.
520
521 **Supplemental Figure 2.** Quantification of miR-148a and miR-27b in the plasma of
522 healthy human individuals during an oral glucose tolerance test (OGTT), an intravenous
523 isoglycemic glucose infusion (IIGI), or during a 3-hour fasting control. (**a**) Plasma miR-
524 148a levels during the OGTT (open circles), the IIGI (filled squares), and the fasting
525 control (open squares). (**b**) Plasma miR-27b levels during the OGTT (open circles), the
526 IIGI (filled squares), and the fasting control (open squares). Results are presented as
527 mean ± SEM.
528



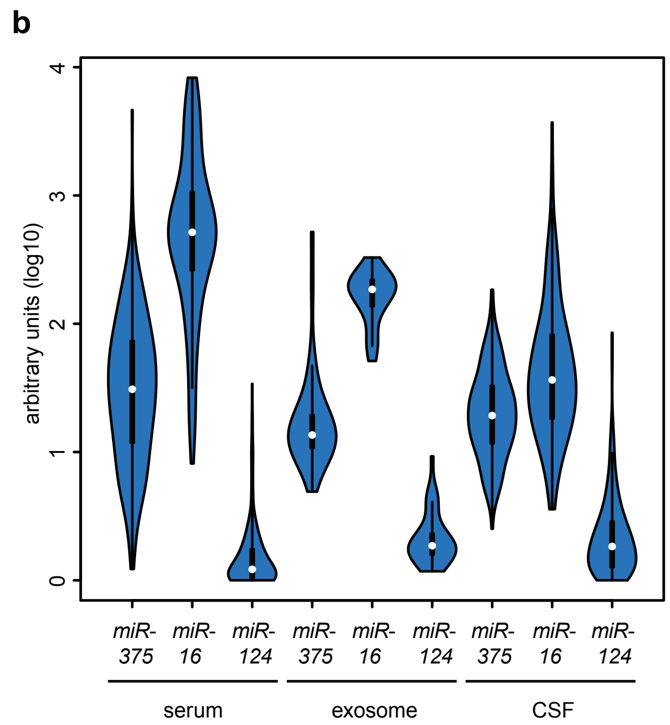


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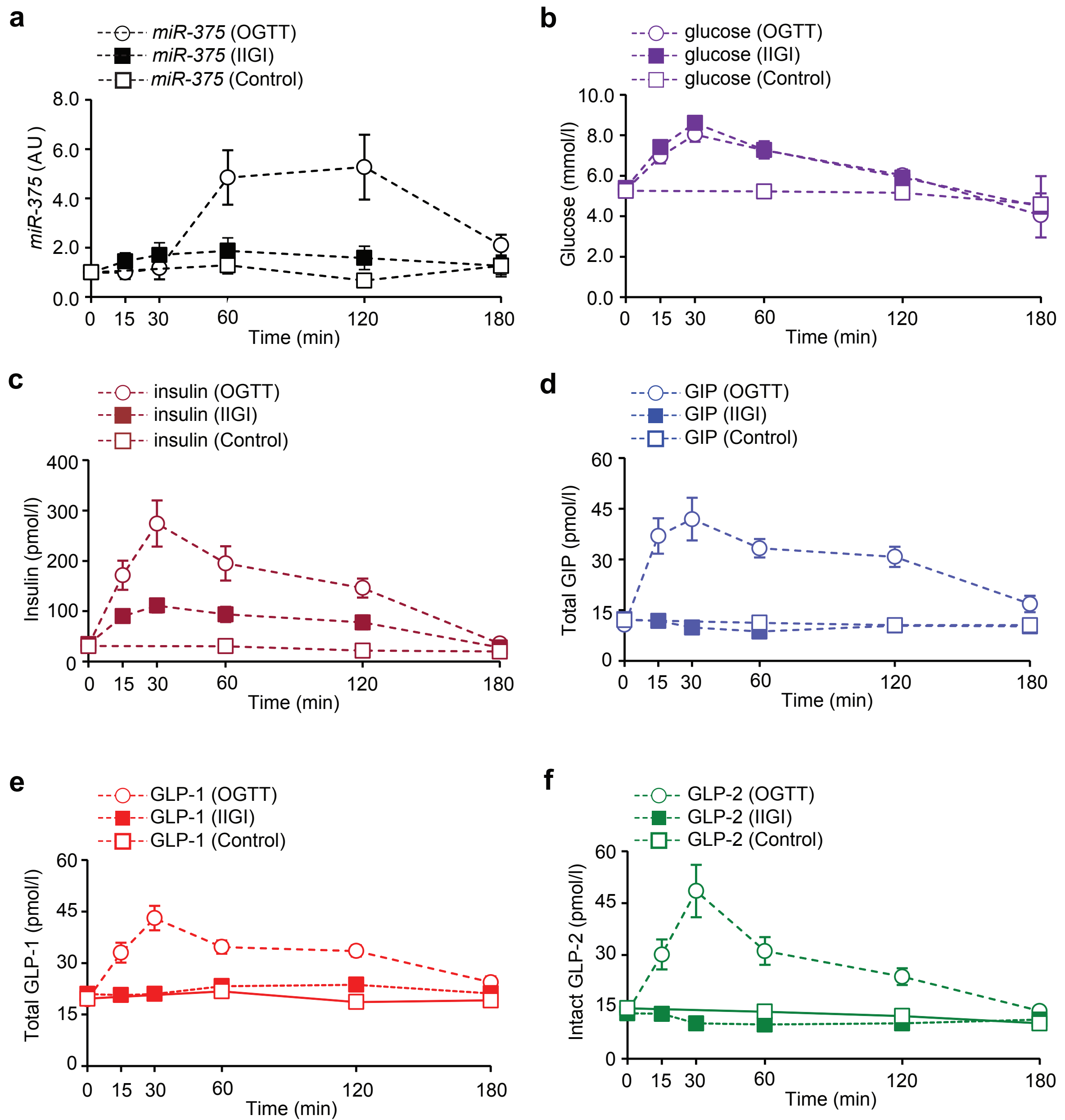
Figure 1



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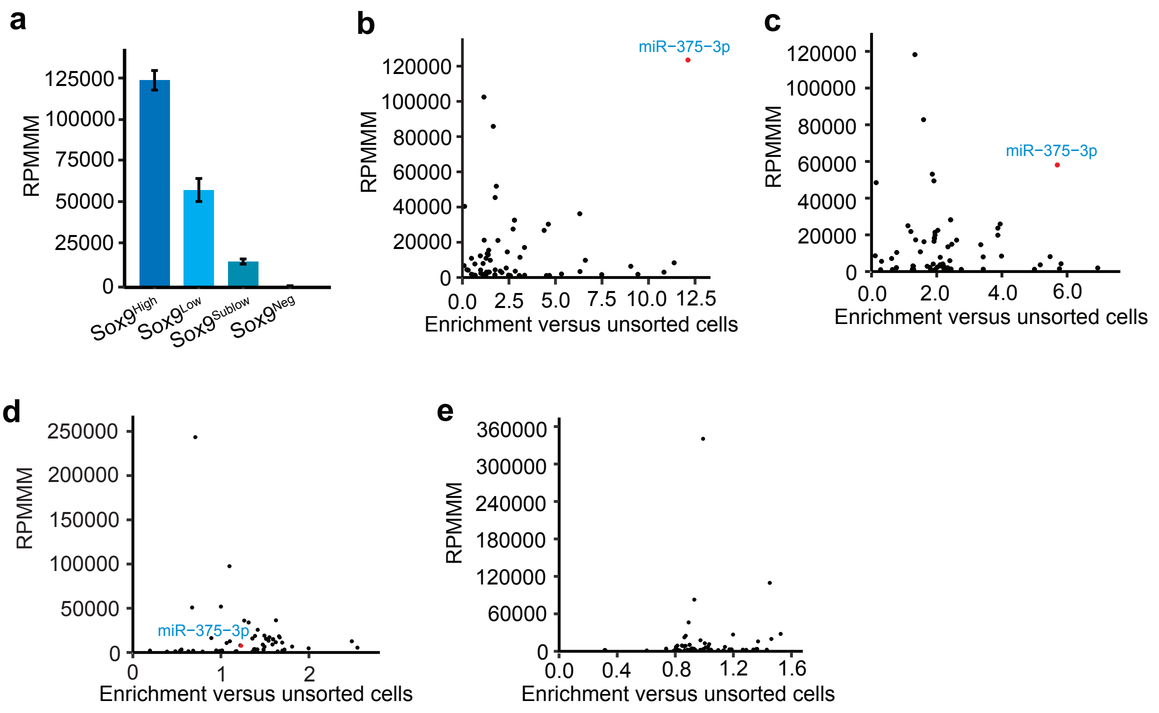

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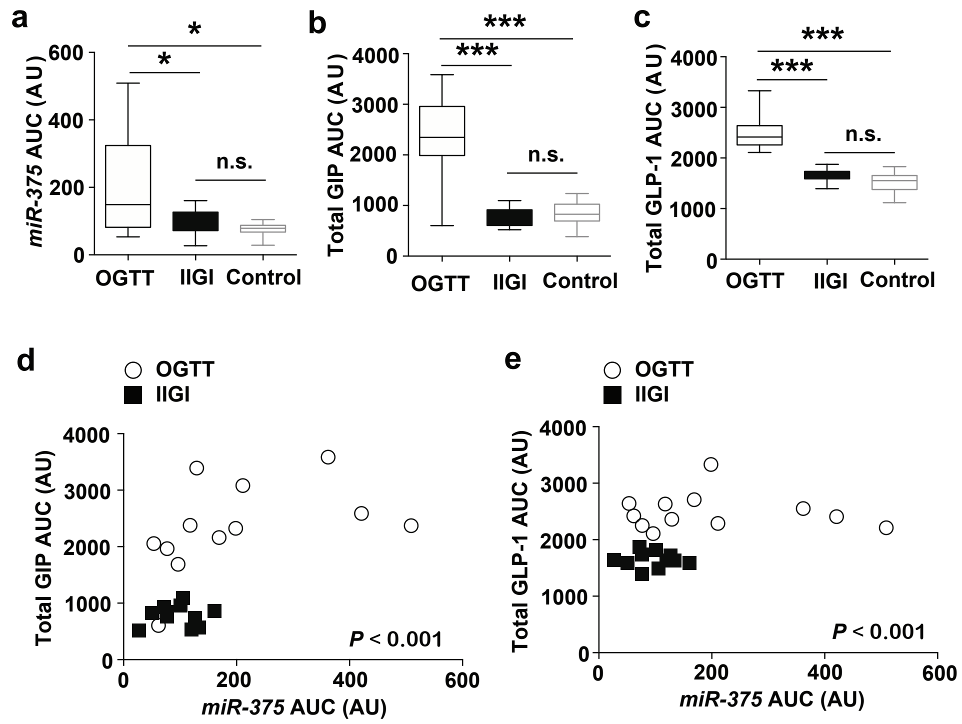
Figure 3



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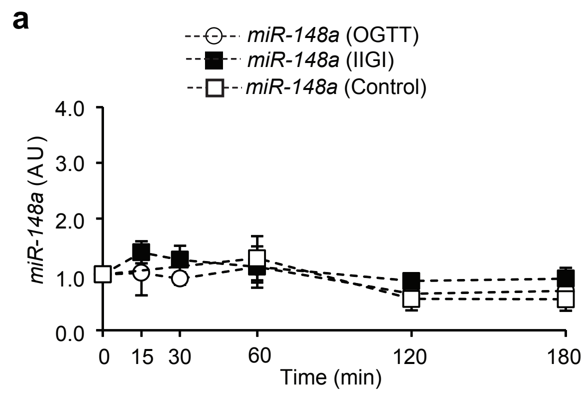
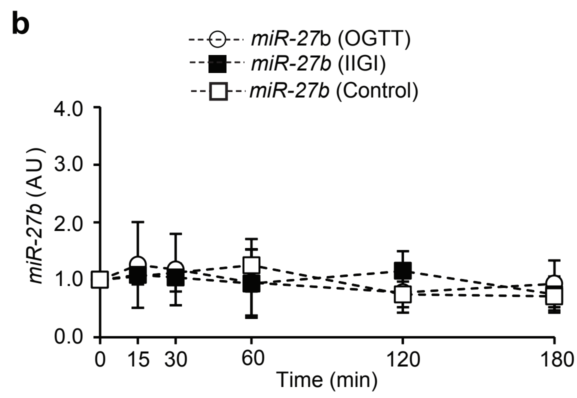
Supplementary Figure 1





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Supplementary Figure 2