

Human iPSC-based modeling reveals coding and non-coding transcriptomic alterations associated with RFX3 deficiency in pancreatic islets

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Research Article

Keywords: RFX3, Pancreatic islets, miRNA, lncRNAs, ceRNA network

Posted Date: September 12th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7435927/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

RFX3 is a transcription factor (TF) critical for pancreatic endocrine development. Its loss impairs β -cell differentiation, promotes enterochromaffin cell (EC) generation, and induces apoptosis. However, the role of non-coding RNAs in mediating these effects remains poorly understood.

Methods

Using CRISPR/Cas9-derived RFX3 knockout (KO) human iPSC-derived pancreatic islets, we performed integrated transcriptomic analyses of coding and non-coding RNAs. Differentially expressed miRNAs (DEmiRs) and lncRNAs (DElncRNAs) were validated by RT-qPCR. Target prediction and competing endogenous RNA (ceRNA) network analyses were conducted to explore potential regulatory interactions affecting key endocrine genes.

Results

RFX3 deficiency induced widespread transcriptomic changes in human iPSC-derived pancreatic islets. Core β -cell markers and key pancreatic TFs were downregulated, alongside genes regulating ion channels, vesicle trafficking, metabolic sensing, and insulin secretion. Conversely, apoptotic and EC genes were upregulated. *RFX3* KO islets exhibited significant alterations in miRNA profiles, including upregulation of *miR-451a*, *miR-215-5p*, *miR-122-5p*, *miR-338-3p*, *miR-194-5p*, *miR-378a-3p*, and the *miR-29* family, which are predicted to target critical pancreatic endocrine genes. Several lncRNAs, including *LINC00461*, *MIAT*, *RMST*, and *AC020916.1*, were downregulated, potentially influencing miRNA activity via ceRNA interactions. Integrated analyses identified core regulatory axes, such as miR-4455/INS, miR-122-5p/ARX, and miR-660-3p/GHRL, while apoptosis-related genes, including *CASP*, *TNFSF10*, and *TXNIP*, were also predicted targets of dysregulated miRNAs.

Conclusions

Our findings reveal widespread alterations in non-coding RNA networks associated with RFX3 loss, highlighting a potential layer of post-transcriptional regulation linked to impaired pancreatic endocrine development. These results provide insights into how RFX3 deficiency may reshape islet transcriptomes, influencing β -cell maturation, function, and survival.

Introduction

Pancreatic development is an intricate tightly regulated process that ensures the proper formation of functional pancreatic islets, essential for the maintenance of glucose homeostasis. This process

involves a series of temporally and spatially controlled events that regulate lineage specification, cell proliferation, and maturation[1]. Central to this progression is the activity of transcription factors (TFs), which coordinates gene regulatory networks to drive endocrine cell fate and ensure their cellular identity [1]. Among these, regulatory factor X 3 (RFX3), a known ciliogenic TF that has been implicated in the development and function of pancreatic β -cells in mice and human [2–4]. Using human induced pluripotent stem cells (iPSCs), we recently showed that RFX3 plays a crucial role in pancreatic development, as its absence impairs the expression of key endocrine genes and decreases the number of hormone-secreting cells, including insulin (INS)- and glucagon (GCG)-producing cells [4]. Additionally, the loss of RFX3 disrupts β -cell function, increases cell death, and abnormally induces enterochromaffin cell (EC) generation [4]. These findings highlight the crucial role of RFX3 in regulating human islet cell differentiation, lineage specification, and potentially influencing diabetes susceptibility.

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have emerged as important regulators of many biological functions at both transcriptional and post-transcriptional levels [5–8]. They play critical roles in pancreatic development, especially in the formation and function of pancreatic islets and β -cells [7–10]. miRNAs regulate gene expression post-transcriptionally by promoting mRNA degradation or inhibiting protein translation [11]. They are essential for pancreatic endocrine cell differentiation[12, 13], the maintenance of β -cell identity [14–16], and the regulation of functions such as insulin secretion, apoptosis, and responses to cytokines [15], all of which are relevant to the pathophysiology of type 2 diabetes (T2D). In contrast, lncRNAs primarily function at the transcriptional level and act as epigenetic regulators, often exhibiting cell-type specific expression patterns [10, 17]. They regulate pancreatic lineage specification and modulate TFs crucial for islet and β -cell differentiation, including *PDX1*, *NKX6.1*, *NKX2.2*, *PAX6*, and *GATA6* [9, 18–24]. Integrative epigenetic analyses have identified more than 1,000 lncRNAs in human pancreatic islets, many of which are differentially expressed under diabetic conditions [9, 10]. lncRNAs can also function as competing endogenous RNAs (ceRNAs), acting as molecular sponges that bind miRNAs and prevent them from targeting specific mRNAs [25]. This sequestration reduces miRNA-mediated repression and restores the expression of their target genes [25]. By analysing mRNAs, miRNAs, and lncRNAs, ceRNA expression profiles, ceRNA networks can be constructed to illustrate how RNAs interact via shared miRNA recognition elements (MREs,) revealing intricate regulatory systems in which RNAs influence each other's expression by competing for common miRNAs [26].

In this study, we sought to understand the complex regulatory network interplay between miRNAs and lncRNAs during pancreatic islet development in the absence of RFX3. Building on our recently established *RFX3*-deficient human iPSC model [4], we performed genome-wide sequencing at the pancreatic islet stage to identify differentially expressed genes (DEGs), miRNAs (DEmiRs), and lncRNAs (DElncRNAs). We integrated DEmiRs with DEGs and DElncRNAs to identify potential miRNA targets, and further combined these datasets to generate ceRNA networks converging on key pancreatic islet genes. Our findings highlight that lack of RFX3 resulted in significant dysregulation of both coding and non-coding RNAs, indicating a complex regulatory network crucial for pancreatic islet development, β -cell homeostasis, and function, while serving as potential biomarkers or therapeutic targets for diabetes.

Materials and Methods

iPSCs culture and differentiation into pancreatic islets

Human iPSC lines with *RFX3* knockout (*RFX3* KO1 and *RFX3* KO2) and their corresponding wild-type (WT) controls were previously generated and validated in our laboratory [4]. All iPSC lines were cultured in mTeSR Plus medium (Stem Cell Technologies, Canada) on dishes coated with Matrigel (Corning, USA) [27, 28]. Differentiation of iPSCs into pancreatic progenitor cells was performed *in vitro* using our previously established protocol [29], while further differentiation into pancreatic islet cells was adapted from Veres et al. version 8 protocol [30] (see details in Table S1).

Immunofluorescence

Cells were rinsed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) for 20–25 minutes at room temperature. Following fixation, they were washed three times for a total of 30 minutes with tris-buffered saline containing 0.5% Tween-20 (TBST). For permeabilization, cells were treated with PBS supplemented with 0.5% Triton X-100 (PBST) for 30 minutes at room temperature. Cells were blocked using 6% bovine serum albumin (BSA) in PBST overnight at 4°C. Primary antibodies, diluted in PBST with 3% BSA, were added and incubated overnight at 4°C. After washing with TBST, Alexa Fluor-conjugated secondary antibodies diluted in PBST were added for 1 hour at room temperature, followed by TBST washes. Nuclei were stained with Hoechst 33258 (1:5000 in PBS) for 5 minutes at room temperature. Images were acquired using a Cell Discoverer 7 imaging system. The antibodies used were anti-RFX3 (NBP1-86301, Novus Biologicals, 1:1000), anti-INS (GN-ID4-s, DSHB, 1:2000), anti-GCG (G2654, Sigma, 1:2000), and anti-SST (MAB354, Millipore, 1:2000).

RNA extraction, non-coding RNA sequencing, and expression analysis

A single well of a 6-well plate of differentiated cells were treated with TRIzol reagent (Life Technologies, California, USA) prior to phase separation using chloroform. Total RNA extraction was performed on the aqueous phase using Direct-zol RNA Miniprep kit (Zymo Research Corporation, California, USA). For miRNA sequencing, 1 µg of RNA input was used for miRNA library preparation using NEBNext Multiplex Small RNA library Prep Set for Illumina (E7560S, New England BioLabs, USA). The amplified cDNA products were subsequently purified with the Monarch PCR Purification Kit (T1130S, New England BioLabs, USA). The FASTQ files were aligned to the miRBase v22 database for mature miRNAs, and miRNA expression was quantified by total read counts using the small RNA analysis workflow in CLC Genomics Workbench 20.2 with default settings, as outlined in [31]. The majority of annotated reads were successfully mapped to the miRBase v22 database. Only mature miRNAs (5p and 3p) were included in the analysis, following miRBase v22 guidelines. To ensure accurate miRNA quantification, read counts were normalized using the TMM (trimmed mean of M values) method, and the resulting log₂-transformed Counts per Million (CPM) values were used for differential expression analysis.

Differentially expressed miRNAs (DEmiRs) in *RFX3* KO pancreatic islets versus WT-islets were identified with a log₂ [fold change] greater than 0.5 or less than - 0.5, and a *p*-value threshold of < 0.05. For lncRNA sequencing, 1 µg of RNA was used to isolate mRNA with the NEBNext Poly(A) mRNA Magnetic Isolation Kit (E7490, New England Biolabs, Massachusetts, USA). RNA-seq libraries were constructed using NEBNext Ultra Directional RNA Library Prep Kit (E7420L, New England Biolabs, Massachusetts, USA) and sequenced on an Illumina HiSeq 4000 platform. FASTQ files were generated with Illumina BCL2Fastq Conversion Software v2.20. The paired-end FASTQ files were aligned to the GRCh38 reference genome using the default settings and built-in module of CLC Genomics Workbench v24.0.2. Transcript expression levels, quantified as Transcripts Per Million (TPM), were imported into AltAnalyze v2.1.3 [32] for differential expression analysis, following the previously established pipeline [33]. Differentially expressed lncRNAs (DElncRNAs) were identified with a cut-off of $-0.5 > \log_2 [\text{fold change}] > 0.5$ and a *p*-value threshold of < 0.05.

Target prediction analysis for DEmiRs and construction of competing endogenous RNA (ceRNA) networks

For miRNA-mRNA target prediction, the lists of DEmiRs and differentially expressed genes (DEGs), reported earlier in [4], were uploaded to Ingenuity Pathway Analysis (IPA) software (QIAGEN, Germany). For miRNA-lncRNA target prediction, the miRNA targets were determined by examining the sequence complementarity between each miRNA and the corresponding lncRNA transcripts (e.g., upregulated miRNAs versus downregulated lncRNAs and vice versa) from the Human Gencode (R) 47 database [34], using the miRanda algorithm (v3.3a) [35]. In this process, miRNA sequences were aligned to lncRNA transcripts with multiple alignment score thresholds, applying both Watson-Crick and GU wobble base pairings, with stringent alignment scores (≥ 140) and thermodynamic parameters (binding energy ≤ -1 kcal/mol) to identify miRNA targets that strongly bind to lncRNA sequences. For competing endogenous RNA (ceRNA) network, the identified miRNA target lists were used to generate a miRNA-mRNA-lncRNA network and visualized using Cytoscape 3.10.3 software (National Resource for Network Biology).

Reverse transcription and RT-qPCR expression validation

For miRNA expression analysis, 5 ng/µl of RNA was used for the miRNA reverse transcription reaction with the miRCURY LNA RT Kit (QIAGEN, Cat. 339340). Quantification of relative miRNA expression was performed using specific miRCURY LNA miRNA PCR Assays and the miRCURY LNA SYBR® Green PCR Kit (QIAGEN, Cat. 339345), with the reverse transcription reaction diluted to 1:15. The relative miRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ fold change method, with SNORD48 serving as the endogenous control for normalization. See Table S2 for list of used miRCURY LNA miRNA PCR Assays. For lncRNA expression analysis, complementary DNA (cDNA) was synthesized from 1 µg of RNA using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following manufacturer's protocol. RT-qPCR was performed for the synthesized cDNA using GoTaq qPCR SYBR Green Master Mix

(Promega, Wisconsin, USA) for specific primers provided in Table S2. GAPDH was used as an endogenous or housekeeping control while fold changes for KO samples were calculated using the 2- $\Delta\Delta C_t$ fold change method, taking WT levels as reference.

Statistical analysis

At least three biological replicates were analysed for each experiment, and *p*-values were calculated using an ANOVA test on GraphPad Prism 8 software (GraphPad Software, Boston, MA, USA; www.graphpad.com). Data are represented as mean $\pm$ SD.

Results

RFX3 deficiency disrupts coding and non-coding RNA expression profiles in iPSC-derived pancreatic islets

To investigate the impact of RFX3 deletion on ncRNA expression during pancreatic islet development, we utilized our previously established *RFX3* KO iPSC lines, engineered via CRISPR/Cas9 genome editing [4]. Two independent KO iPSC lines (*RFX3* KO1 and *RFX3* KO2), alongside their respective WT control, were directed through a stepwise differentiation protocol toward pancreatic islets, as previously described [4] (Fig. 1A; Table S1). While the complete loss of RFX3 in these KO lines had been confirmed in our recent study through multiple approaches, including Sanger sequencing and western blotting [4], immunofluorescence analysis was additionally performed here to visualize the absence of RFX3 in KO-derived islets (Fig. S1). Consistent with our previous findings, RFX3 deficiency was associated with a marked reduction in key endocrine markers, including INS, GCG, and SST (Fig. S1), indicative of impaired islet cell specification.

In addition to these phenotypic alterations, RNA-seq analysis revealed extensive transcriptomic alterations, with 743 significantly dysregulated protein-coding genes (DEGs; $-1 > \log_2$ fold change > 1 , adjusted $p < 0.05$) in *RFX3* KO islets compared to WT controls as we previously reported [4]. While these DEGs highlight the protein-coding disruptions associated with *RFX3* deficiency, we next examined the non-coding transcriptome focusing on miRNAs and lncRNAs to better understand their contribution to the observed developmental defects. To characterize the impact of RFX3 loss on miRNA dynamics, we performed small RNA-seq on the same RNA samples of *RFX3* KO and WT iPSC-derived islets. This analysis uncovered substantial changes in the miRNA expression landscape, with a total of 835 DE miRs identified. Among these, 47 miRNAs were significantly upregulated ($\log_2$ fold change > 0.5 , $p < 0.05$) and 92 were significantly downregulated ($\log_2$ fold change < -0.5 , $p < 0.05$) (Fig. 1B, C; Table S3). The most prominently upregulated miRNAs included *miR-451a*, *miR-215-5p*, *miR-215-3p*, *miR-122-5p*, and *miR-338-3p*, while *miR-9-5p*, *miR-9-3p*, *miR-7-5p*, *miR-1251-5p*, and *miR-135a-2-3p* ranked among the most significantly downregulated DE miRs (Table S3).

We next investigated the effect of RFX3 deficiency on the expression landscape of lncRNAs in the iPSC-derived pancreatic islets. Leveraging the same RNA-seq dataset, we applied a differential lncRNAs

expression analysis with a threshold of $\log_2$ fold change > 0.5 or < -0.5 ($p < 0.05$), which revealed 212 significantly downregulated and 92 significantly upregulated DElncRNAs in *RFX3* KO-derived islets compared to WT controls (Fig. 1D, E; Table S4). The top downregulated DElncRNAs were *UCA1*, *MIR7-3HG*, *RMST*, *LINC01896*, and *LINC01833* while top upregulated DElncRNAs are *AC008760.2*, *SAPCD1-AS1*, *CU634019.1*, *AC023421.1*, and *AC079467.1* (Table S4). To validate the sequencing results, RT-qPCR was performed on a selected subset of DEmiRs and DElncRNAs. This confirmed the significant upregulation of *miR-215-5p*, *miR-122-5p*, *miR-338-3p*, *miR-194-5p*, *miR-29c-3p*, *miR-452-5p*, and *miR-378d* along with significant downregulation of *miR-9-5p*, *miR-9-3p*, *miR-7-5p*, *miR-409-3p*, *miR-139-5p*, *miR-95-3p*, *miR-1-3p*, and *miR-184* (Fig. 2A). For DElncRNAs, RT-qPCR further confirmed the significant downregulation of *FEZF1-AS1*, *MIAT*, *DLX6-AS1*, *LINC01614*, and *LINC00461* as well as the significant upregulation of *LINC00645*, *LINC01133*, *MGAT3-AS1*, *GATA2-AS1*, *LINC00540*, and *LINC02535* (Fig. 2B).

Integrative analysis of miRNA–mRNA networks reveals key regulatory interactions in *RFX3*-deficient iPSC-derived pancreatic islets

To gain insight into how *RFX3* loss during differentiation reshapes the non-coding RNA landscape and its regulatory interactions with mRNAs, we performed integrative analysis of DEmiRs and DEGs in *RFX3* KO iPSC-derived islets. Using IPA miRNA target filter, we focused on upregulated DEmiRs due to their potential repressive effects on genes downregulated as a consequence of *RFX3* deficiency. Among the 47 significantly upregulated DEmiRs, 42 were predicted to target 405 downregulated DEGs. From these, 44 DEGs targeted by 35 DEmiRs were prioritized based on their established roles in islet development and function and were significantly impacted by *RFX3* KO (Fig. 3A; Table 1). For instance, *miR-215-5p*, the most upregulated DEmiR, was predicted to target key genes such as *SLC30A8* and *ERO1B*, while another DEmiR from the same family, *miR-215-3p*, targeted *IAPP*, *KCNA6* and *SYT11*. *miR-122-5p* was predicted to repress *PAX5*, *CNGA3*, *ARX*, *ATP1A2*, and *SYT11*. *miR-338-3p* targeted multiple genes involved in calcium signalling and insulin secretion, including *CACNA1E*, *PDE1A*, *CACNG8*, *KCNJ11*, *NRXN1*, and *NOTCH1*. According to the confidence degree of the predicted analysis, *miR-122-5p* was experimentally validated to target *ATP1A2*, and highly predicted to target *CNGA3*, and *SYT11*. *miR-625-5p* was highly predicted to target *IAPP*, *PAX5*, *ISL1*, and *SLC8A3*. Also, *miR-31-5p* was highly predicted to target *PAX5*, *ISL1*, *KCNN3*, *KCNJ11*, and *SIX2*. *miR-6887-3p* was highly predicted to target *SYT12*, *CACNG8*, and *SYT11*. Moreover, *FOSB*, *KCNMB1*, and *CACNG8* were highly predicted targets of *miR-660-3p* (Table 1). Several critical genes appeared as shared targets of multiple DEmiRs, highlighting key regulatory nodes. For example, *PAX5* was targeted by 11 DEmiRs which are *miR-122-5p*, *miR-4455*, *miR-625-5p**, *miR-509-3p*, *miR-5699-5p**, *miR-378g**, *miR-6842-3p**, *miR-7976*, *miR-4728-3p*, *miR-31-5p**, and *miR-766-5p** (*high prediction confidence). Likewise, *IAPP*, was targeted by *miR-215-3p*, *miR-452-5p*, *miR-676-3p*, *miR-625-5p**, *miR-34b-3p*, *miR-509-5p*, and *miR-660-3p*. *ERO1B* was targeted by *miR-215-5p*, *miR-3131*, *miR-194-3p*, and *miR-627-3p*. *IRX1* was a predicted target of *miR-6887-3p*, *miR-676-3p*, and *miR-625-5p*. *IRX2* was targeted by *miR-4705*, *miR-5699-5p*, *miR-508-3p*, and *miR-378a-5p*. *ISL1* was a target for *miR-29c-3p*, *miR-224-5p*, *miR-625-5p**, *miR-378a-5p*, and *miR-31-5p**. Hormone associated genes were also targeted by several DEmiRs such as *miR-4455* which targeted *INS* and *SST*. Moreover,

SST was targeted by *miR-625-5p* and *miR-766-5p*. Also, *GHRL* was targeted by *miR-660-3p*. *ARX* was targeted by *miR-122-5p*, *miR-766-5p**, and *miR-130a-3p*. KCN family members were target for several DEmiRs, such as *KCNJ11*, a predicted target for *miR-338-3p*, *miR-4705*, *miR-6842-3p*, and *miR-31-5p**. *KCNK17* was targeted by *miR-194-3p*, *miR-3131* and *miR-625-5p*. In addition, solute carrier family members such as *SLC30A8* was targeted by *miR-215-5p*, *miR-194-5p*, *miR-452-5p*, *miR-676-3p*, *miR-625-5p*, *miR-378a-5p*, and *miR-502-3p*. Ultimately, this integrative analysis identified a panel of upregulated DEmiRs with predicted regulatory interactions to key pancreatic islet genes (Fig. 3B). Similarly, we employed the integration analysis between the downregulated DEmiRs ($\log_2$ fold change < -0.5 , $p < 0.05$) and the upregulated DEGs ($\log_2$ fold change > 1 , adjusted $p < 0.05$) where it revealed 83 out of 92 downregulated DEmiRs predicted to target 226 upregulated DEGs. Several upregulated DEGs were found to be linked to lipid metabolism and enterochromaffin cell (EC) markers that were targeted by different downregulated DEmiRs (Fig. S2A, B; Table S5). For instance, *APOB* was a highly predicted target of *miR-2682-5p** while *APOD* was targeted by *miR-409-5p*. Meanwhile, *APOA2* was a predicted target for *miR-1251-5p*, *miR-1468-5p*, *miR-7974**, and *miR-7-5p**. Also, the members of CYP family were predicted targets for several DEmiRs such as *miR-132-3p*, *miR-135a-5p*, *miR-216b-3p*, *miR-329-3p*, *miR-1224-3p*, *miR-504-5p*, *miR-485-5p*, *miR-3938*, and *miR-887-5p* (Fig. S2B; Table S5). Interestingly *TPH1*, an EC specific gene, was a predicted target of *miR-212-5p* and *miR-7156-5p**. Our previous study highlighted the effect of RFX3 deletion on the viability of the derived pancreatic islets. In the current analysis, several downregulated DEmiRs were found to target DEGs associated with apoptosis such as Caspase (*CASP*) and Tumour Necrosis Factor (*TNF*) family members. For instance, *CASP5* was found to be targeted by 9 DEmiRs, including *let-7i-5p*, *miR-1197*, *miR-216a-3p*, *miR-143-3p*, *miR-216b-5p*, *miR-2682-5p*, *miR-3663-5p*, *miR-491-3p*, and *miR-7974**. *CASP1* was targeted by *miR-216a-3p*, *miR-135a-5p**, and *miR-887-5p*. *TNFRSF1B* was targeted by *let-7i-5p*, *miR-1251-5p*, *miR-212-5p**, *miR-329-3p*, and *miR-495-3p**. In addition, *TNFSF10* was targeted by 10 downregulated DEmiRs such as *let-7i-5p**, *miR-133a-3p*, *miR-135a-3p*, *miR-139-5p**, *miR-154-5p*, *miR-3938*, *miR-494-3p*, *miR-654-3p*, *miR-6740-3p*, and *miR-887-5p*. More importantly, *TXNIP*, a pro-apoptotic marker, and previously validated to be upregulated in our RFX3 deficient pancreatic islets [4], was experimentally validated as a target of *miR-216a-3p* and *miR-411-5p* and a predicted target for *miR-135a-5p* and *miR-5006-3p*. (Fig. S2C; Table S5).

Table 1

Upregulated DEmiRs ($\log_2$ fold change > 0.5, $p < 0.05$) predicted to target key downregulated DEGs involved in pancreatic development and insulin secretion in *RFX3* KO islets compared to WT-islets.

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
miR-215-5p	1.386	0.0237	SLC30A8	-4.957	5.04E-33
miR-194-5p	1.263	0.0138			
miR-378a-5p	0.658	0.0098			
miR-452-5p	0.911	0.0100			
miR-502-3p	0.527	0.0171			
miR-625-5p	0.834	0.0026			
miR-676-3p	0.899	0.0079			
miR-215-3p	1.364	0.0286	IAPP	-4.763	1.73E-19
miR-34b-3p	0.624	0.0069			
miR-452-5p	0.911	0.0100			
miR-509-5p	0.624	0.0316			
miR-625-5p*	0.834	0.0026			
miR-660-3p	0.527	0.0287			
miR-676-3p	0.899	0.0079			
miR-4455	1.164	0.0003	INS	-4.611	2.19E-10
miR-4455	1.164	0.0003	SST	-4.323	4.55E-45
miR-625-5p	0.834	0.0026			
miR-766-5p	0.512	0.0130			
miR-4455	1.164	0.0003	KCNK16	-3.183	1.17E-22
miR-766-5p	0.512	0.0130			
miR-122-5p	1.362	0.0014	PAX5	-3.123	1.66E-02
miR-31-5p*	0.538	0.0014			
miR-378g*	0.664	0.0007			
miR-4455	1.164	0.0003			
* Highly confident predicted targets					
** Experimentally validated targets					

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
<i>miR-4728-3p</i>	0.545	0.0467			
<i>miR-509-3p</i>	0.802	0.0219			
<i>miR-5699-5p*</i>	0.800	0.0034			
<i>miR-625-5p*</i>	0.834	0.0026			
<i>miR-6842-3p*</i>	0.573	0.0081			
<i>miR-766-5p*</i>	0.512	0.0130			
<i>miR-7976</i>	0.546	0.0471			
<i>miR-130a-3p</i>	0.501	0.0245	<i>PAX6</i>	-2.868	1.85E-12
<i>miR-4455</i>	1.164	0.0003	<i>GCK</i>	-2.798	7.32E-10
<i>miR-6887-3p</i>	0.972	0.0217	<i>CNGA3</i>	-2.751	4.71E-27
<i>miR-122-5p*</i>	1.362	0.0014			
<i>miR-31-5p</i>	0.538	0.0014			
<i>miR-766-5p*</i>	0.512	0.0130	<i>CACNA1E</i>	-2.557	8.00E-05
<i>miR-338-3p</i>	1.320	0.0386			
<i>miR-378a-5p</i>	0.658	0.0098			
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-660-3p</i>	0.527	0.0287			
<i>miR-766-5p</i>	0.512	0.0130			
<i>miR-7976</i>	0.546	0.0471			
<i>miR-224-5p</i>	0.914	0.0002	<i>ISL1</i>	-2.385	1.22E-10
<i>miR-29c-3p</i>	0.946	0.0155			
<i>miR-31-5p*</i>	0.538	0.0014			
<i>miR-378a-5p</i>	0.658	0.0098			
<i>miR-625-5p*</i>	0.834	0.0026			
<i>miR-215-5p</i>	1.386	0.0237			
<i>miR-194-3p</i>	1.054	0.0086	<i>ERO1B</i>	-2.364	2.64E-33
* Highly confident predicted targets					
** Experimentally validated targets					

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
miR-3131	1.140	0.0004	SYT12	-2.334	6.01E-05
miR-627-3p	0.620	0.0217			
miR-194-3p	1.054	0.0086			
miR-378g*	0.664	0.0007			
miR-5699-5p*	0.800	0.0034			
miR-6887-3p*	0.972	0.0217	ARX	-2.201	7.06E-04
miR-122-5p	1.362	0.0014			
miR-130a-3p	0.501	0.0245			
miR-766-5p*	0.512	0.0130			
miR-625-5p	0.834	0.0026	NEUROG3	-2.128	3.96E-05
miR-766-5p	0.512	0.0130			
miR-130a-3p	0.501	0.0245	PDE1A	-2.047	2.78E-04
miR-338-3p	1.320	0.0386			
miR-509-3p	0.802	0.0219	EPHA5	-2.032	1.33E-04
miR-625-5p	0.834	0.0026	DPP6	-1.962	4.57E-08
miR-6842-3p	0.573	0.0081			
miR-194-3p	1.054	0.0086	KCNK17	-1.950	1.31E-06
miR-3131	1.140	0.0004			
miR-625-5p	0.834	0.0026			
miR-215-3p	1.364	0.0286	KCNA6	-1.915	4.46E-08
miR-31-5p	0.538	0.0014			
miR-508-3p	0.677	0.0271			
miR-625-5p	0.834	0.0026			
miR-660-3p	0.527	0.0287			
miR-766-5p	0.512	0.0130	IRX1	-1.898	2.43E-03
miR-625-5p	0.834	0.0026			
* Highly confident predicted targets					
** Experimentally validated targets					

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
miR-676-3p	0.899	0.0079	SLC8A3	-1.828	2.53E-06
miR-6887-3p	0.972	0.0217			
miR-3934-5p	0.535	0.0029			
miR-625-5p*	0.834	0.0026	IRX2	-1.826	3.25E-03
miR-378a-5p	0.658	0.0098			
miR-4705	0.904	0.0233			
miR-508-3p	0.677	0.0271	FOSB	-1.800	3.15E-04
miR-5699-5p	0.800	0.0034			
miR-4455	1.164	0.0003			
miR-4521	0.709	0.0020	GPM6A	-1.722	3.10E-02
miR-625-5p	0.834	0.0026			
miR-660-3p*	0.527	0.0287			
miR-6887-3p	0.972	0.0217	KCNN3	-1.676	1.34E-03
miR-766-5p	0.512	0.0130			
miR-766-5p	0.512	0.0130			
miR-31-5p*	0.538	0.0014	SYT5	-1.544	1.33E-06
miR-766-5p*	0.512	0.0130			
miR-29b-2-5p	0.556	0.0080			
miR-4455	1.164	0.0003	TRPC4	-1.508	2.16E-03
miR-194-5p	1.263	0.0138			
miR-194-3p	1.054	0.0086			
miR-194-5p	1.263	0.0138	KCNMB1	-1.431	1.84E-02
miR-508-3p	0.677	0.0271			
miR-660-3p*	0.527	0.0287			
miR-6842-3p	0.573	0.0081			
miR-6887-3p	0.972	0.0217			
* Highly confident predicted targets					
** Experimentally validated targets					

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
<i>miR-7976</i>	0.546	0.0471			
<i>miR-122-5p**</i>	1.362	0.0014	<i>ATP1A2</i>	-1.422	1.63E-02
<i>miR-4705</i>	0.904	0.0233	<i>ADGRV1</i>	-1.303	1.62E-06
<i>miR-31-5p</i>	0.538	0.0014	<i>NNAT</i>	-1.290	4.17E-07
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-6842-3p</i>	0.573	0.0081			
<i>miR-6887-3p</i>	0.972	0.0217			
<i>miR-766-5p</i>	0.512	0.0130			
<i>miR-338-3p</i>	1.320	0.0386			
<i>miR-378c</i>	1.007	0.0254			
<i>miR-5699-5p</i>	0.800	0.0034	<i>CACNG8</i>	-1.288	1.99E-03
<i>miR-660-3p*</i>	0.527	0.0287			
<i>miR-6842-3p*</i>	0.573	0.0081			
<i>miR-6887-3p*</i>	0.972	0.0217			
<i>miR-122-5p*</i>	1.362	0.0014			
<i>miR-215-3p</i>	1.364	0.0286			
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-6887-3p*</i>	0.972	0.0217	<i>SYT11</i>	-1.262	1.60E-05
<i>miR-31-5p*</i>	0.538	0.0014			
<i>miR-338-3p</i>	1.320	0.0386			
<i>miR-4705</i>	0.904	0.0233			
<i>miR-6842-3p</i>	0.573	0.0081			
<i>miR-29c-3p</i>	0.946	0.0155			
<i>miR-502-3p</i>	0.527	0.0171			
<i>miR-627-3p</i>	0.620	0.0217	<i>FOS</i>	-1.197	7.44E-04
<i>miR-194-3p</i>	1.054	0.0086			
<i>miR-194-3p</i>	1.054	0.0086	<i>FXVD2</i>	-1.175	4.46E-07
* Highly confident predicted targets					
** Experimentally validated targets					

Upregulated miRNA	log ₂ fold change	p-value	Target gene	log ₂ fold change	Adj p-value
<i>miR-502-3p</i>	0.527	0.0171			
<i>miR-660-3p</i>	0.527	0.0287	<i>GHRL</i>	-1.174	7.29E-03
<i>miR-29c-3p</i>	0.946	0.0155	<i>SYT2</i>	-1.170	5.18E-04
<i>miR-5699-5p</i>	0.800	0.0034			
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-6842-3p</i>	0.573	0.0081			
<i>miR-6887-3p</i>	0.972	0.0217			
<i>miR-7976*</i>	0.546	0.0471			
<i>miR-338-3p</i>	1.320	0.0386	<i>NRXN1</i>	-1.134	8.03E-07
<i>miR-4705</i>	0.904	0.0233			
<i>miR-29c-3p</i>	0.946	0.0155	<i>HCN1</i>	-1.088	2.90E-04
<i>miR-31-5p</i>	0.538	0.0014			
<i>miR-509-3p</i>	0.802	0.0219			
<i>miR-29b-2-5p</i>	0.556	0.0080	<i>SIX2</i>	-1.045	3.05E-04
<i>miR-31-5p*</i>	0.538	0.0014			
<i>miR-5699-5p</i>	0.800	0.0034			
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-194-5p</i>	1.263	0.0138	<i>SYT4</i>	-1.042	2.33E-05
<i>miR-29b-2-5p</i>	0.556	0.0080			
<i>miR-4705</i>	0.904	0.0233			
<i>miR-625-5p</i>	0.834	0.0026			
<i>miR-338-3p</i>	1.320	0.0386	<i>NOTCH1</i>	-1.006	5.51E-05
* Highly confident predicted targets					
** Experimentally validated targets					

Disrupted lncRNA–miRNA regulatory interactions in pancreatic islets lacking RFX3

To uncover additional regulatory mechanisms affected by RFX3 deficiency, we performed an integrative analysis linking DE miRNAs and DE lncRNAs in iPSC-derived pancreatic islets. Since DE lncRNAs can act as ceRNAs that modulate miRNAs activity and stabilize target gene expression, we specifically focused on upregulated DE miRNAs and their correlated downregulated DE lncRNAs targets. This approach aimed to identify functional lncRNA-miRNA networks potentially contributing to the disrupted transcriptomic landscape observed in RFX3 KO islets. 47 significantly upregulated DE miRNAs were aligned against the sequences of 196 significantly downregulated DE lncRNAs using default miRanda algorithm parameters with high complementarity interaction score ≥ 140 and favourable minimum free energy ≤ -1 kcal/mol (Fig. S3; Table S6). The most upregulated miRNA, *miR-215-5p* was predicted to be correlated to 23 downregulated DE lncRNAs with highest interaction scores of 327 and 305 with *LINC00461* and *MIR100HG*, respectively. *miR-215-3p* was correlated to 71 DE lncRNAs with 725 and 588 interaction scores for *DLX6-AS1* and *AL732314.6*. Also, there were 69 DE lncRNAs linked to *miR-122-5p* with highest scores for *AC020916.1* (748) and *AC134312.5* (428). *miR-338-3p* was correlated to 58 DE lncRNAs with highest score for *AC020916.1* (1138) and *MIAT* (737). Among 38 DE lncRNAs, the highest interaction scores with *miR-194-5p* were for *LINC00461* (433) and *AC020916.1* (429). Among overall pairs of correlations, the highest score was for the correlation between *miR-378g* and *LINC00115* with 3069 interaction score followed by the score of 1520 between *miR-6887-3p* and *MIAT*. Then the correlation between *miR-378g* and *AC020916.1* with score of 1495. The highest interaction scores also include the link between *AC020916.1* and *miR-6887-3p*, *miR-6842-3p* and *miR-660-3p*. The top 10 strongly correlated DE lncRNAs were *LINC00115*, *MIAT*, *AC020916.1*, *UCA1*, *DNAAF4-CCPG1*, *DLX6-AS1*, *UNC5B-AS1*, *AP006284.1*, *STAG3L5P-PVRIG2P-PILRB*, and *LINC00461* (Table S6). For deep investigation of the most correlated downregulated DE lncRNAs, we restricted the selection to be $\log_2$ fold change < 1 , $p < 0.05$ which revealed 31 lncRNAs (Fig. 4A; Table 2) with their correlated upregulated DE miRNAs. The most downregulated DE lncRNA, *UCA1*, was correlated with 29 DE miRNAs with highest correlation to *miR-766-5p*, *miR-6842-3p*, *miR-378g*, *miR-224-5p*, and *miR-6842-3p*. *MIR7-3HG* was predicted to be linked to 18 miRNAs with highest correlation to *miR-6887-3p*, *miR-224-5p*, *miR-378g*, *miR-676-3p*, and *miR-338-5p*. *RMST* was linked to 38 miRNAs and its highest interaction scores were with *miR-627-3p*, *miR-224-5p*, *miR-6887-3p*, and *miR-338-3p*, and *miR-34b-3p*. Also, *LINC01896* was correlated to 15 miRNAs which are *miR-616-5p*, *miR-6887-3p*, *miR-378c*, *miR-378a-3p*, *miR-660-3p*, *miR-378f*, *miR-378d*, *miR-378e*, *miR-378a-5p*, *miR-452-5p*, *miR-627-3p*, *miR-6842-3p*, *miR-766-5p*, *miR-338-5p*, and *miR-378g*. Moreover, *LINC01833* was correlated to 27 miRNAs with highest correlation with *miR-6887-3p*, and *miR-29b-2-5p*. *MIAT* lncRNA was correlated to 41 miRNAs with highest scores for *miR-6887-3p*, *miR-338-3p*, *miR-378c*, *miR-378a-3p*, and *miR-378d* (Fig. 4B; Table 2).

Table 2

Top downregulated DElncRNAs and their associated upregulated DEmiRs correlated with DEG targets in *RFX3* KO-derived pancreatic islets compared with WT (DEGs: $\log_2$ fold change < -1 , adjusted $p < 0.05$; DEmiRs: $\log_2$ fold change > 0.5 , $p < 0.05$; DElncRNAs: $\log_2$ fold change < -1 , $p < 0.05$).

lncRNA	$\log_2$ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
<i>UCA1</i>	-3.442	<i>miR-7976, miR-31-5p, miR-224-5p, miR-6842-3p, miR-378c, miR-509-3p, miR-3934-5p, miR-29b-2-5p, miR-122-5p, miR-194-3p, miR-378g, miR-766-5p, miR-194-5p, miR-509-5p, miR-3131, miR-508-3p, miR-627-3p, miR-215-3p, miR-452-5p, miR-338-3p, miR-676-3p, miR-338-5p, miR-4521, miR-224-3p, miR-4455, miR-625-5p, miR-660-3p</i>	<i>PAX5, SLC6A17, CACNA1E, SLC1A2, KCNMB1, ADCYAP1R1, SYT2, ATCAY, CENPF, CNGA3, ISL1, KCNA6, ZIC4, KCNN3, KCNJ11, HCN1, SIX2, VCAM1, DLX2, DPP6, VEGFC, SIX3, CACNG8, RSPH4A, ZIC3, EPHA5, SLC8A3, SYT5, SYT4, ARX, ATP1A2, SYT11, ERO1B, SYT12, KCNK17, LOXL2, FXYD2, SOD3, DNAAF3, SST, KCNK16, NEUROG3, PCDH15, FOSB, SLC30A8, SIX6, TRPC4, IAPP, IRX2, DLX1, FOS, SLC17A8, SPAG6, PDE1A, CXCR4, NRXN1, NOTCH1, IRX1, POSTN, INS, GCK, DNAAF1, ZIC1, PTCH2, GHRL, EGR1</i>
<i>MIR7-3HG</i>	-2.646	<i>miR-224-5p, miR-676-3p, miR-338-5p, miR-378c, miR-224-3p, miR-6758-3p, miR-7976, miR-3934-5p, miR-6887-3p, miR-378a-5p, miR-378g, miR-194-3p, miR-194-5p, miR-6842-3p</i>	<i>ISL1, VCAM1, DLX2, SLC30A8, IAPP, IRX1, SIX3, CXCR4, VEGFC, CACNG8, RSPH4A, POSTN, PAX5, SLC6A17, CACNA1E, SLC1A2, KCNMB1, ADCYAP1R1, SYT2, ATCAY, CENPF, SLC8A3, GCK, SYT12, DLX1, ZIC1, SLC17A8, FOSB, SYT11, ANGPTL4, IRX2, DNAAF3, ERO1B, KCNK17, LOXL2, FXYD2, SOD3, SIX6, PCDH15, TRPC4, SYT4, DPP6, KCNJ11</i>
<i>RMST</i>	-1.792	<i>miR-338-3p, miR-636, miR-194-3p, miR-3934-5p, miR-6887-3p, miR-378a-5p, miR-5699-5p, miR-224-3p, miR-502-3p, miR-29b-2-5p, miR-4705, miR-509-5p, miR-31-5p, miR-4521, miR-338-5p, miR-660-3p, miR-122-5p, miR-130a-3p, miR-215-3p, miR-378g, miR-509-3p, miR-224-5p, miR-4455, miR-4728-3p, miR-34b-3p, miR-378c, miR-625-5p, miR-766-5p, miR-29c-3p, miR-3131, miR-452-5p, miR-627-3p, miR-6758-3p</i>	<i>CACNA1E, PDE1A, CXCR4, CACNG8, KCNJ11, NRXN1, NOTCH1, DLX1, SLC1A2, LOXL2, CENPF, SLC6A17, ERO1B, SYT12, KCNK17, SIX3, KCNMB1, DLX2, ADCYAP1R1, FXYD2, ATCAY, SOD3, SLC8A3, GCK, ZIC1, SLC17A8, IRX1, FOSB, SYT11, ANGPTL4, SYT2, SLC30A8, ISL1, IRX2, PAX5, SIX2, POSTN, ZIC4, FOS, SYT5, SYT4, ADGRV1, IAPP, CNGA3, KCNA6, KCNN3, HCN1, VEGFC, PTCH2, GHRL, EGR1, ARX, ATP1A2, PAX6, SIX6, ZIC5, DNAAF3, ZIC3, EPHA5, VCAM1, INS, SST, KCNK16, DNAAF1, RSPH4A, NEUROG3, DPP6, PCDH15, SPAG6</i>
<i>LINC01896</i>	-1.763	<i>miR-378c, miR-660-3p, miR-378a-5p, miR-452-5p, miR-627-3p, miR-6887-3p, miR-6842-3p, miR-766-5p, miR-338-5p, miR-378g</i>	<i>CACNG8, RSPH4A, IAPP, CACNA1E, DLX1, KCNA6, FOSB, PTCH2, KCNMB1, LOXL2, GHRL, EGR1, SLC30A8, ISL1, IRX2, SLC17A8, SPAG6, ERO1B, FOS, SLC6A17, GCK,</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
			<i>SYT12, ZIC1, IRX1, SYT11, ANGPTL4, SYT2, PAX5, DPP6, VEGFC, SIX3, ADCYAP1R1, KCNJ11, SST, KCNK16, CNGA3, ARX, ZIC3, NEUROG3, PCDH15, ZIC4, KCNN3, SOD3, CXCR4, DNAAF3</i>
<i>LINC01833</i>	-1.653	<i>miR-6887-3p, miR-766-5p, miR-215-5p, miR-660-3p, miR-5699-5p, miR-215-3p, miR-224-5p, miR-29b-2-5p, miR-378c, miR-29c-3p, miR-378g, miR-627-3p, miR-452-5p, miR-6842-3p, miR-31-5p, miR-4455, miR-508-3p, miR-130a-3p, miR-194-3p, miR-34b-3p, miR-378a-5p</i>	<i>SLC6A17, GCK, SYT12, DLX1, ZIC1, SLC17A8, IRX1, FOSB, KCNMB1, CACNG8, SYT11, ANGPTL4, SYT2, SST, KCNK16, PAX5, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, KCNN3, SIX3, RSPH4A, SOD3, SLC30A8, ERO1B, LOXL2, ADCYAP1R1, IAPP, PTCH2, GHRL, EGR1, IRX2, SIX2, ATCAY, ISL1, VCAM1, DLX2, SYT5, SYT4, SIX6, FOS, HCN1, DNAAF3, SPAG6, DPP6, VEGFC, KCNJ11, SLC1A2, INS, DNAAF1, PAX6, PDE1A, ZIC5, KCNK17, FXYD2</i>
<i>MIR9-3HG</i>	-1.590	<i>miR-378g, miR-6887-3p, miR-627-3p, miR-509-5p, miR-3934-5p, miR-766-5p, miR-378a-5p, miR-31-5p, miR-338-3p, miR-4728-3p, miR-660-3p, miR-4521, miR-508-3p, miR-5699-5p, miR-29b-2-5p, miR-378c, miR-4455, miR-676-3p, miR-224-3p, miR-3131, miR-130a-3p, miR-215-5p, miR-7976, miR-122-5p, miR-6842-3p, miR-224-5p, miR-625-5p, miR-6758-3p, miR-194-5p, miR-215-3p, miR-29c-3p</i>	<i>PAX5, SYT12, DNAAF3, SLC6A17, GCK, DLX1, ZIC1, SLC17A8, IRX1, FOSB, KCNMB1, CACNG8, SYT11, ANGPTL4, SYT2, ERO1B, FOS, IAPP, SLC1A2, DLX2, SLC8A3, SST, KCNK16, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, KCNN3, SIX3, RSPH4A, SOD3, SLC30A8, ISL1, IRX2, KCNJ11, HCN1, SIX2, PDE1A, CXCR4, NRXN1, NOTCH1, PTCH2, LOXL2, GHRL, EGR1, CENPF, FXYD2, SYT5, SYT4, INS, DNAAF1, ATCAY, POSTN, KCNK17, PAX6, SIX6, ZIC5, ADCYAP1R1, ATP1A2, DPP6, VEGFC, VCAM1, TRPC4</i>
<i>FEZF1-AS1</i>	-1.473	<i>miR-224-3p, miR-625-5p, miR-31-5p, miR-5699-5p, miR-224-5p, miR-766-5p, miR-627-3p, miR-3131, miR-29b-2-5p, miR-378c, miR-508-3p, miR-502-3p, miR-122-5p, miR-509-3p, miR-378g</i>	<i>POSTN, SLC30A8, IAPP, SST, PAX5, SLC6A17, CACNA1E, ISL1, NEUROG3, ZIC1, DPP6, KCNK17, KCNA6, IRX1, SLC8A3, ZIC4, FOSB, SIX3, DLX2, SYT11, ADCYAP1R1, SYT2, ATCAY, SIX2, SYT4, SOD3, CNGA3, SLC1A2, KCNN3, KCNJ11, HCN1, SYT12, SLC17A8, IRX2, CACNG8, VCAM1, KCNK16, ARX, ZIC3, PCDH15, RSPH4A, ERO1B, DLX1, FOS, SYT5, KCNMB1, FXYD2, ATP1A2, EPHA5, DNAAF3</i>
<i>AC011139.1</i>	-1.473	<i>miR-627-3p, miR-122-5p, miR-338-5p, miR-6758-3p, miR-378g, miR-4728-3p</i>	<i>ERO1B, DLX1, FOS, PAX5, CNGA3, ARX, ZIC4, SIX3, ATP1A2, SYT11, CXCR4, VEGFC, POSTN, SYT12, DNAAF3</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
<i>MIAT</i>	-1.450	<i>miR-378a-5p, miR-6758-3p, miR-660-3p, miR-378g, miR-766-5p, miR-378c, miR-3934-5p, miR-215-5p, miR-502-3p, miR-6887-3p, miR-3131, miR-452-5p, miR-130a-3p, miR-224-3p, miR-34b-3p, miR-4705, miR-122-5p, miR-4521, miR-5699-5p, miR-194-3p, miR-508-3p, miR-509-3p, miR-224-5p, miR-338-3p, miR-4728-3p, miR-625-5p, miR-29c-3p, miR-29b-2-5p, miR-4455, miR-509-5p, miR-215-3p, miR-676-3p, miR-6842-3p</i>	<i>SLC30A8, CACNA1E, ISL1, IRX2, POSTN, IAPP, DLX1, KCNA6, FOSB, PTCH2, KCNMB1, CACNG8, LOXL2, GHRL, EGR1, PAX5, SYT12, DNAAF3, SST, KCNK16, CNGA3, ARX, ZIC3, NEUROG3, PCDH15, ZIC4, KCNN3, SIX3, RSPH4A, SOD3, SLC8A3, ERO1B, ZIC1, ADCYAP1R1, FOS, FXRD2, SLC6A17, GCK, SLC17A8, IRX1, SYT11, ANGPTL4, SYT2, KCNK17, SPAG6, PAX6, SIX6, PDE1A, ZIC5, ADGRV1, KCNJ11, NRXN1, SYT4, ATP1A2, CENPF, SIX2, DLX2, ATCAY, EPHA5, HCN1, VCAM1, CXCR4, NOTCH1, DPP6, SYT5, INS, DNAAF1, SLC1A2, VEGFC</i>
<i>AL391069.3</i>	-1.414	<i>miR-509-5p, miR-625-5p, miR-508-3p, miR-31-5p, miR-29b-2-5p, miR-6758-3p, miR-4521, miR-6887-3p</i>	<i>IAPP, SLC6A17, SLC1A2, DLX2, SLC30A8, SST, PAX5, CACNA1E, ISL1, NEUROG3, ZIC1, DPP6, KCNK17, KCNA6, IRX1, SLC8A3, ZIC4, FOSB, SIX3, SYT11, ADCYAP1R1, SYT2, ATCAY, SIX2, SYT4, SOD3, IRX2, KCNMB1, CNGA3, KCNN3, KCNJ11, HCN1, SYT5, POSTN, CENPF, GCK, SYT12, DLX1, SLC17A8, CACNG8, ANGPTL4</i>
<i>AL138930.1</i>	-1.403	<i>miR-215-3p, miR-122-5p, miR-509-5p, miR-31-5p, miR-378a-5p, miR-636, miR-627-3p, miR-6758-3p</i>	<i>IAPP, KCNA6, SYT11, ATCAY, PAX5, CNGA3, ARX, ZIC4, SIX3, ATP1A2, SLC6A17, SLC1A2, DLX2, ISL1, KCNN3, KCNJ11, HCN1, SIX2, SLC30A8, CACNA1E, IRX2, DLX1, LOXL2, CENPF, ERO1B, FOS, POSTN</i>
<i>LINC02106</i>	-1.236	<i>miR-4728-3p, miR-338-5p, miR-378g, miR-378a-5p, miR-29b-2-5p, miR-122-5p, miR-6887-3p, miR-224-3p, miR-3934-5p, miR-627-3p, miR-636, miR-660-3p, miR-509-5p, miR-625-5p</i>	<i>PAX5, CXCR4, VEGFC, SYT12, DNAAF3, SLC30A8, CACNA1E, ISL1, IRX2, SYT5, SIX2, SYT4, CNGA3, ARX, ZIC4, SIX3, ATP1A2, SYT11, SLC6A17, GCK, DLX1, ZIC1, SLC17A8, IRX1, FOSB, KCNMB1, CACNG8, ANGPTL4, SYT2, POSTN, SLC8A3, ERO1B, FOS, SLC1A2, LOXL2, CENPF, IAPP, KCNA6, PTCH2, GHRL, EGR1, DLX2, SST, NEUROG3, DPP6, KCNK17, ADCYAP1R1, ATCAY, SOD3</i>
<i>AL353150.1</i>	-1.167	<i>miR-766-5p, miR-130a-3p, miR-338-5p, miR-4728-3p, miR-627-3p</i>	<i>SST, KCNK16, PAX5, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, SIX3, RSPH4A, SOD3, PAX6, SIX6, PDE1A, ZIC5, CXCR4, VEGFC, ERO1B, DLX1, FOS</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
<i>LINC01614</i>	-1.166	<i>miR-378g, miR-766-5p, miR-627-3p, miR-660-3p, miR-215-3p, miR-338-3p, miR-4705, miR-6887-3p, miR-5699-5p, miR-122-5p, miR-224-3p, miR-194-5p, miR-29b-2-5p</i>	<i>PAX5, SYT12, DNAAF3, SST, KCNK16, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, SIX3, RSPH4A, SOD3, ERO1B, DLX1, FOS, IAPP, PTCH2, KCNMB1, CACNG8, LOXL2, GHRL, EGR1, SYT11, ATCAY, PDE1A, CXCR4, KCNJ11, NRXN1, NOTCH1, IRX2, ADGRV1, SYT4, SLC6A17, GCK, ZIC1, SLC17A8, IRX1, ANGPTL4, SYT2, SIX2, ATP1A2, POSTN, SLC30A8, SIX6, TRPC4, SYT5</i>
<i>DLX6-AS1</i>	-1.157	<i>miR-766-5p, miR-627-3p, miR-625-5p, miR-452-5p, miR-215-3p, miR-194-3p, miR-3934-5p, miR-4521, miR-224-3p, miR-378a-5p, miR-338-5p, miR-224-5p, miR-378g, miR-660-3p, miR-6758-3p, miR-6842-3p, miR-509-5p, miR-34b-3p, miR-31-5p, miR-215-5p, miR-29b-2-5p, miR-3131, miR-508-3p, miR-378c, miR-6887-3p, miR-122-5p, miR-130a-3p, miR-338-3p, miR-4705, miR-29c-3p, miR-7976, miR-676-3p, miR-509-3p, miR-502-3p, miR-636, miR-4728-3p, miR-5699-5p</i>	<i>SST, KCNK16, PAX5, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, SIX3, RSPH4A, SOD3, ERO1B, DLX1, FOS, SLC30A8, IAPP, SLC6A17, ISL1, ZIC1, DPP6, KCNK17, IRX1, SLC8A3, DLX2, SYT11, ADCYAP1R1, SYT2, ATCAY, SIX2, SYT4, SLC17A8, SPAG6, SYT12, KCNMB1, LOXL2, FXYD2, CENPF, POSTN, IRX2, CXCR4, VEGFC, VCAM1, DNAAF3, PTCH2, CACNG8, GHRL, EGR1, KCNJ11, SLC1A2, HCN1, SYT5, GCK, ANGPTL4, ATP1A2, PAX6, SIX6, PDE1A, ZIC5, NRXN1, NOTCH1, ADGRV1, EPHA5</i>
<i>AL133415.1</i>	-1.155	<i>miR-627-3p, miR-6758-3p, miR-766-5p, miR-378g, miR-660-3p</i>	<i>ERO1B, DLX1, FOS, POSTN, SST, KCNK16, PAX5, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, SIX3, RSPH4A, SOD3, SYT12, DNAAF3, IAPP, PTCH2, KCNMB1, CACNG8, LOXL2, GHRL, EGR1</i>
<i>DNAAF4-CCPG1</i>	-1.144	<i>miR-627-3p, miR-224-5p, miR-29b-2-5p, miR-4705, miR-194-3p, miR-338-5p, miR-502-3p, miR-4521, miR-224-3p, miR-34b-3p, miR-452-5p, miR-625-5p, miR-766-5p, miR-3934-5p, miR-194-5p, miR-29c-3p, miR-378g, miR-4728-3p, miR-5699-5p, miR-636</i>	<i>ERO1B, DLX1, FOS, ISL1, VCAM1, DLX2, SYT5, SIX2, SYT4, IRX2, ZIC4, ADGRV1, KCNJ11, NRXN1, SLC6A17, SYT12, KCNK17, SIX3, KCNMB1, LOXL2, ADCYAP1R1, FXYD2, ATCAY, SOD3, CXCR4, VEGFC, SLC30A8, FOSB, CENPF, POSTN, IAPP, SLC17A8, SPAG6, SST, PAX5, CACNA1E, NEUROG3, ZIC1, DPP6, KCNA6, IRX1, SLC8A3, SYT11, SYT2, KCNK16, CNGA3, ARX, ZIC3, PCDH15, KCNN3, RSPH4A, SIX6, TRPC4, ANGPTL4, HCN1, DNAAF3, CACNG8, SLC1A2</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
<i>C1orf61</i>	-1.122	<i>miR-338-5p, miR-215-5p, miR-5699-5p, miR-6758-3p, miR-6887-3p, miR-378a-5p, miR-29b-2-5p, miR-378g, miR-452-5p, miR-4705, miR-194-3p, miR-660-3p, miR-34b-3p, miR-766-5p, miR-627-3p, miR-31-5p, miR-7976, miR-224-3p, miR-3131, miR-509-3p, miR-122-5p, miR-4521, miR-625-5p, miR-130a-3p, miR-194-5p, miR-215-3p, miR-224-5p, miR-3934-5p, miR-4455, miR-502-3p, miR-636, miR-338-3p, miR-378c, miR-4728-3p, miR-509-5p, miR-6842-3p</i>	<i>CXCR4, VEGFC, SLC30A8, ERO1B, ZIC1, LOXL2, ADCYAP1R1, PAX5, SYT12, SLC17A8, IRX2, CACNG8, SYT2, SIX2, POSTN, SLC6A17, GCK, DLX1, IRX1, FOSB, KCNMB1, SYT11, ANGPTL4, CACNA1E, ISL1, SYT5, SYT4, DNAAF3, IAPP, SPAG6, ZIC4, ADGRV1, KCNJ11, NRXN1, KCNK17, SIX3, DLX2, FXYD2, ATCAY, SOD3, KCNA6, PTCH2, GHRL, EGR1, SST, KCNK16, CNGA3, ARX, ZIC3, NEUROG3, PCDH15, KCNN3, RSPH4A, FOS, SLC1A2, HCN1, CENPF, EPHA5, ATP1A2, DPP6, SLC8A3, PAX6, SIX6, PDE1A, ZIC5, TRPC4, VCAM1, INS, DNAAF1, NOTCH1</i>
<i>C5orf38</i>	-1.122	<i>miR-4455, miR-625-5p, miR-502-3p, miR-4728-3p, miR-6842-3p, miR-130a-3p, miR-378c, miR-194-3p, miR-378a-5p, miR-5699-5p, miR-766-5p, miR-509-5p, miR-194-5p, miR-627-3p, miR-636, miR-660-3p</i>	<i>INS, SST, KCNK16, PAX5, GCK, FOSB, SYT5, DNAAF1, ATCAY, RSPH4A, SLC30A8, IAPP, SLC6A17, CACNA1E, ISL1, NEUROG3, ZIC1, DPP6, KCNK17, KCNA6, IRX1, SLC8A3, ZIC4, SIX3, DLX2, SYT11, ADCYAP1R1, SYT2, SIX2, SYT4, SOD3, FOS, FXYD2, VEGFC, KCNMB1, CACNG8, KCNJ11, PAX6, SIX6, ARX, PDE1A, ZIC5, ERO1B, SYT12, LOXL2, IRX2, SLC17A8, CNGA3, ZIC3, PCDH15, KCNN3, SLC1A2, TRPC4, DLX1, CENPF, PTCH2, GHRL, EGR1</i>
<i>AC009955.4</i>	-1.105	<i>miR-122-5p, miR-194-3p, miR-3131, miR-4728-3p, miR-6842-3p, miR-636, miR-4455, miR-452-5p</i>	<i>PAX5, CNGA3, ARX, ZIC4, SIX3, ATP1A2, SYT11, SLC6A17, ERO1B, SYT12, KCNK17, KCNMB1, DLX2, LOXL2, ADCYAP1R1, FXYD2, ATCAY, SOD3, DPP6, VEGFC, CACNG8, KCNJ11, SYT2, DLX1, SLC1A2, CENPF, INS, SST, KCNK16, GCK, FOSB, SYT5, DNAAF1, RSPH4A, SLC30A8, IAPP, SLC17A8, SPAG6</i>
<i>AP006284.1</i>	-1.097	<i>miR-6887-3p, miR-378g, miR-194-3p, miR-3131, miR-660-3p, miR-766-5p, miR-4728-3p, miR-4455, miR-215-3p, miR-636, miR-224-5p, miR-627-3p, miR-7976, miR-194-5p, miR-509-3p, miR-31-5p, miR-29b-2-5p</i>	<i>SLC6A17, GCK, SYT12, DLX1, ZIC1, SLC17A8, IRX1, FOSB, KCNMB1, CACNG8, SYT11, ANGPTL4, SYT2, PAX5, DNAAF3, ERO1B, KCNK17, SIX3, DLX2, LOXL2, ADCYAP1R1, FXYD2, ATCAY, SOD3, IAPP, CACNA1E, KCNA6, PTCH2, GHRL, EGR1, SST, KCNK16, CNGA3, ARX, ZIC3, NEUROG3, PCDH15, ZIC4, KCNN3, RSPH4A, INS, SYT5, DNAAF1, SLC1A2, CENPF, ISL1, VCAM1, FOS, SLC30A8, SIX6,</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
			<i>TRPC4, SYT4, EPHA5, HCN1, KCNJ11, SIX2</i>
<i>LINC00461</i>	-1.095	<i>miR-625-5p, miR-627-3p, miR-4455, miR-130a-3p, miR-224-5p, miR-3934-5p, miR-6887-3p, miR-338-5p, miR-636, miR-215-3p, miR-4521, miR-6842-3p, miR-676-3p, miR-766-5p, miR-31-5p, miR-29c-3p, miR-4728-3p, miR-502-3p, miR-378c, miR-378g, miR-508-3p, miR-5699-5p, miR-660-3p, miR-224-3p, miR-378a-5p, miR-6758-3p, miR-452-5p, miR-29b-2-5p, miR-215-5p, miR-4705, miR-509-5p, miR-122-5p, miR-194-3p, miR-194-5p, miR-338-3p, miR-34b-3p, miR-7976</i>	<i>SLC30A8, IAPP, SST, PAX5, SLC6A17, CACNA1E, ISL1, NEUROG3, ZIC1, DPP6, KCNK17, KCNA6, IRX1, SLC8A3, ZIC4, FOSB, SIX3, DLX2, SYT11, ADCYAP1R1, SYT2, ATCAY, SIX2, SYT4, SOD3, ERO1B, DLX1, FOS, INS, KCNK16, GCK, SYT5, DNAAF1, RSPH4A, PAX6, SIX6, ARX, PDE1A, ZIC5, VCAM1, SYT12, SLC17A8, KCNMB1, CACNG8, ANGPTL4, CXCR4, VEGFC, SLC1A2, LOXL2, CENPF, KCNJ11, CNGA3, ZIC3, PCDH15, KCNN3, HCN1, FXYD2, DNAAF3, IRX2, PTCH2, GHRL, EGR1, POSTN, SPAG6, ADGRV1, NRXN1, ATP1A2, TRPC4, NOTCH1</i>
<i>AL133523.1</i>	-1.089	<i>miR-660-3p, miR-122-5p, miR-627-3p, miR-4728-3p, miR-29c-3p, miR-766-5p, miR-3131, miR-6842-3p, miR-34b-3p, miR-4705, miR-625-5p, miR-378c, miR-378g, miR-509-5p, miR-194-3p, miR-29b-2-5p, miR-636, miR-3934-5p, miR-6758-3p</i>	<i>IAPP, CACNA1E, DLX1, KCNA6, FOSB, PTCH2, KCNMB1, CACNG8, LOXL2, GHRL, EGR1, PAX5, CNGA3, ARX, ZIC4, SIX3, ATP1A2, SYT11, ERO1B, FOS, SIX6, ISL1, PCDH15, ADCYAP1R1, ANGPTL4, SYT2, HCN1, SST, KCNK16, ZIC3, NEUROG3, KCNN3, RSPH4A, SOD3, KCNK17, DPP6, VEGFC, KCNJ11, IRX2, ADGRV1, NRXN1, SYT4, SLC30A8, SLC6A17, ZIC1, IRX1, SLC8A3, DLX2, ATCAY, SIX2, SYT12, DNAAF3, SLC1A2, FXYD2, SYT5, CENPF, POSTN</i>
<i>AC020916.1</i>	-1.075	<i>miR-378g, miR-6842-3p, miR-6887-3p, miR-660-3p, miR-3934-5p, miR-338-3p, miR-378a-5p, miR-29b-2-5p, miR-4455, miR-224-5p, miR-7976, miR-122-5p, miR-31-5p, miR-34b-3p, miR-627-3p, miR-194-3p, miR-29c-3p, miR-5699-5p, miR-509-5p, miR-4705, miR-509-3p, miR-194-5p, miR-6758-3p, miR-378c, miR-130a-3p, miR-3131, miR-625-5p, miR-766-5p, miR-636, miR-215-3p, miR-338-5p, miR-4521, miR-502-3p</i>	<i>PAX5, SYT12, DNAAF3, DPP6, VEGFC, SIX3, KCNMB1, CACNG8, ADCYAP1R1, KCNJ11, SYT2, SLC6A17, GCK, DLX1, ZIC1, SLC17A8, IRX1, FOSB, SYT11, ANGPTL4, IAPP, CACNA1E, KCNA6, PTCH2, LOXL2, GHRL, EGR1, SLC8A3, PDE1A, CXCR4, NRXN1, NOTCH1, SLC30A8, ISL1, IRX2, SYT5, SIX2, SYT4, INS, SST, KCNK16, DNAAF1, ATCAY, RSPH4A, VCAM1, DLX2, SLC1A2, CENPF, CNGA3, ARX, ZIC4, ATP1A2, KCNN3, HCN1, ERO1B, FOS, KCNK17, FXYD2, SOD3, SIX6, PCDH15, ADGRV1, ZIC3, EPHA5, TRPC4, POSTN, PAX6, ZIC5, NEUROG3</i>
<i>MIR4458HG</i>	-1.059	<i>miR-338-3p, miR-766-5p, miR-6887-3p, miR-224-5p, miR-</i>	<i>CACNA1E, PDE1A, CXCR4, CACNG8, KCNJ11, NRXN1, NOTCH1, SST,</i>

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
		627-3p, miR-660-3p, miR-194-3p, miR-31-5p, miR-224-3p, miR-7976, miR-636, miR-6758-3p, miR-215-3p, miR-4521, miR-4705, miR-34b-3p, miR-4455, miR-29b-2-5p, miR-29c-3p, miR-338-5p	KCNK16, PAX5, CNGA3, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, SIX3, RSPH4A, SOD3, SLC6A17, GCK, SYT12, DLX1, ZIC1, SLC17A8, IRX1, KCNMB1, SYT11, ANGPTL4, SYT2, ISL1, VCAM1, DLX2, ERO1B, FOS, IAPP, PTCH2, LOXL2, GHRL, EGR1, KCNK17, ADCYAP1R1, FXYD2, ATCAY, SLC1A2, HCN1, SIX2, POSTN, CENPF, IRX2, ADGRV1, SYT4, INS, SYT5, DNAAF1, SIX6, VEGFC
AC137630.3	-1.035	miR-676-3p, miR-378g, miR-122-5p, miR-378a-5p, miR-378c, miR-7976	SLC30A8, IAPP, IRX1, SIX3, ERO1B, DLX1, FOS, PAX5, SYT12, DNAAF3, CNGA3, ARX, ZIC4, ATP1A2, SYT11, CACNA1E, ISL1, IRX2, CACNG8, RSPH4A, SLC6A17, SLC1A2, KCNMB1, ADCYAP1R1, SYT2, ATCAY, CENPF
AL138976.2	-1.0164775	miR-627-3p, miR-4728-3p	ERO1B, DLX1, FOS, PAX5
LINC01311	-1.007	miR-3934-5p, miR-194-3p, miR-224-5p, miR-509-5p, miR-378a-5p, miR-766-5p, miR-4728-3p, miR-6887-3p, miR-378g, miR-6758-3p, miR-215-3p, miR-4455, miR-122-5p, miR-378c, miR-625-5p, miR-636, miR-29b-2-5p, miR-502-3p, miR-6842-3p	SLC8A3, SLC6A17, ERO1B, SYT12, KCNK17, SIX3, KCNMB1, DLX2, LOXL2, ADCYAP1R1, FXYD2, ATCAY, SOD3, ISL1, VCAM1, IAPP, SLC1A2, SLC30A8, CACNA1E, IRX2, SST, KCNK16, CNGA3, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, ZIC4, FOSB, KCNN3, RSPH4A, GCK, DLX1, ZIC1, SLC17A8, IRX1, CACNG8, SYT11, ANGPTL4, SYT2, DNAAF3, POSTN, INS, SYT5, DNAAF1, ATP1A2, DPP6, SIX2, SYT4, CENPF, FOS, VEGFC, KCNJ11
LINC00865	-1.007	miR-509-5p, miR-338-3p, miR-509-3p, miR-676-3p, miR-215-3p, miR-627-3p, miR-660-3p, miR-3131, miR-6758-3p, miR-4728-3p, miR-3934-5p, miR-5699-5p, miR-378g, miR-130a-3p, miR-378c, miR-4455, miR-625-5p, miR-31-5p, miR-224-3p, miR-29c-3p, miR-224-5p, miR-452-5p, miR-194-3p, miR-6887-3p, miR-29b-2-5p, miR-338-5p, miR-4521, miR-766-5p	IAPP, SLC6A17, SLC1A2, DLX2, CACNA1E, PDE1A, CXCR4, CACNG8, KCNJ11, NRXN1, NOTCH1, PAX5, ZIC3, EPHA5, HCN1, SLC30A8, IRX1, SIX3, KCNA6, SYT11, ATCAY, ERO1B, DLX1, FOS, FOSB, PTCH2, KCNMB1, LOXL2, GHRL, EGR1, KCNK17, POSTN, SLC8A3, SYT12, SLC17A8, IRX2, SYT2, SIX2, DNAAF3, PAX6, SIX6, ARX, ZIC5, RSPH4A, INS, SST, KCNK16, GCK, SYT5, DNAAF1, ISL1, NEUROG3, ZIC1, DPP6, ZIC4, ADCYAP1R1, SYT4, SOD3, CNGA3, KCNN3, PCDH15, ANGPTL4, VCAM1, SPAG6, FXYD2, VEGFC, CENPF
SOX1-OT	-1.004	miR-509-5p, miR-29b-2-5p, miR-4705, miR-4728-3p, miR-	IAPP, SLC6A17, SLC1A2, DLX2, SYT5, SIX2, SYT4, IRX2, ZIC4,

lncRNA	log ₂ fold change	Correlated upregulated miRNAs	Correlated downregulated genes
		766-5p, miR-378c, miR-7976, miR-338-5p, miR-660-3p, miR-338-3p, miR-625-5p, miR-4521, miR-502-3p, miR-627-3p, miR-194-5p, miR-224-3p, miR-4455, miR-31-5p, miR-6887-3p, miR-3934-5p, miR-34b-3p, miR-636, miR-29c-3p, miR-5699-5p, miR-6758-3p, miR-224-5p, miR-452-5p, miR-122-5p, miR-3131, miR-378g	ADGRV1, KCNJ11, NRXN1, PAX5, SST, KCNK16, CNGA3, CACNA1E, ARX, ZIC3, NEUROG3, KCNA6, PCDH15, FOSB, KCNN3, SIX3, RSPH4A, SOD3, CACNG8, KCNMB1, ADCYAP1R1, SYT2, ATCAY, CENPF, CXCR4, VEGFC, DLX1, PTCH2, LOXL2, GHRL, EGR1, PDE1A, NOTCH1, SLC30A8, ISL1, ZIC1, DPP6, KCNK17, IRX1, SLC8A3, SYT11, FOS, FXRD2, ERO1B, SIX6, TRPC4, POSTN, INS, GCK, DNAAF1, HCN1, SYT12, SLC17A8, ANGPTL4, VCAM1, SPAG6, ATP1A2, DNAAF3

Integrative analysis of mRNA–miRNA–lncRNA networks reveals key regulatory interactions in RFX3-deficient pancreatic islets

To obtain a comprehensive understanding of the epigenetic regulatory architecture disrupted by RFX3 deficiency, we performed an integrative analysis combining DE miRNAs, DE lncRNAs, and DEGs. This three-tiered network aimed to uncover key regulatory interactions wherein upregulated miRNAs concurrently target downregulated lncRNAs and mRNAs associated with pancreatic islet development and function. Through this integrative approach, we identified putative ceRNA axes and critical regulatory hubs that may contribute to the widespread transcriptomic dysregulation observed in *RFX3* KO-derived islets while focusing mainly on upregulated DE miRNAs and downregulated DEGs and DE lncRNAs. These included key regulators such as *INS*, *SST*, *GHRL*, *ARX*, *ERO1B*, *GCK*, *NEUROG3*, and *ISL1*. *INS* was shown to be strictly regulated by one miRNA, *miR-4455*, which is linked to 71 DE lncRNAs with highest interaction scores with *AC020916.1*, *UNC5B-AS1*, *LINC00461*, *SOX1-OT*, and *MIR9-3HG* (Fig. 5A; Table 3). *SST* was shown to be targeted by 3 DE miRNAs: *miR-4455*, *miR-625-5p*, and *miR-766-5p* with a total of 132 DE lncRNAs targeted by these three DE miRNAs with highest interaction scores with *UCA1*, *DLX6-AS1*, *AC020916.1*, *UNC5B-AS1*, and *LINC00461* (Fig. 5A; Table 3). *GHRL* was only targeted by *miR-660-3p* which was linked to 97 DE lncRNAs with *AC020916.1*, *LINC00982*, *AP006623.1*, *LINC00461*, and *AC005838.2* having the highest correlation scores (Fig. 5A; Table 3). We noticed 122 DE lncRNAs correlated to 3 miRNAs; *miR-122-5p*, *miR-130a-3p*, and *miR-766-5p* that were predicted to target *ARX*. The correlation scores of *UCA1*, *DLX6-AS1*, *LINC00461*, *AC020916.1*, *AL731571.1*, *AC098936.1*, and *MIAT* were the highest among the DE lncRNA linked to *ARX* (Fig. 5A; Table 3). Through 4 DE miRNAs; *miR-194-3p*, *miR-215-5p*, *miR-3131*, and *miR-627-3p*, *ERO1B* correlated to 140 DE lncRNAs. Meanwhile 117 DE lncRNAs were correlated to *GCK* through *miR-4455* and *miR-6887-3p*. In addition, we noticed 123 and 143 DE lncRNAs were linked to *NEUROG3* and *ISL1*, respectively through their predicted targeting DE miRNAs (Fig. S4; Table 3). From the upregulated genes, we were interested with *TPH1* which showed to be linked with 50 upregulated DE lncRNAs through two miRNAs; *miR-7156-5p* and *miR-212-5p*. *LINC00494*, *AC019117.1*, *LINC01291*, *LINC00494*, and

AC007663.2 were the most upregulated DElncRNAs linked with *TPH1* (Fig. 5B). Also, *TXNIP* gene was correlated with 61 DElncRNAs through 4 downregulated DEmiRs and the top linked DElncRNAs were *AC107398.3*, *LINC02035*, *AC022400.3*, *LINC02541*, and *LINC01852* (Fig. 5C, Table S7).

Table 3

Selected mRNA-miRNA-lncRNA correlation networks targeting key pancreatic genes in RFX3-deficient islets compared with WT (DEGs: log₂ fold change < - 1, adjusted $p < 0.05$; DEmiRs: log₂ fold change > 0.5, $p < 0.05$; DElncRNAs: log₂ fold change < - 0.5, $p < 0.05$).

mRNA	miRNA	lncRNA
<i>INS</i>	<i>miR-4455</i>	<i>LINC00115</i> , <i>NFYC-AS1</i> , <i>AL358215.3</i> , <i>CYMP-AS1</i> , <i>AC234582.1</i> , <i>C1orf61</i> , <i>AC098936.1</i> , <i>LINC01139</i> , <i>LINC01833</i> , <i>AC010884.1</i> , <i>AC013275.1</i> , <i>AC011893.1</i> , <i>AC009955.4</i> , <i>AC141928.1</i> , <i>EPHA5-AS1</i> , <i>AC124016.1</i> , <i>AP002026.1</i> , <i>AC098864.1</i> , <i>SLC9A3-AS1</i> , <i>C5orf38</i> , <i>MIR4458HG</i> , <i>AC010478.1</i> , <i>LINC00461</i> , <i>AC099509.1</i> , <i>AL353759.1</i> , <i>CAHM</i> , <i>AC215522.2</i> , <i>AC092171.3</i> , <i>STAG3L5P-PVRIG2P-PILRB</i> , <i>KMT2E-AS1</i> , <i>MIR124-2HG</i> , <i>AC067773.1</i> , <i>AL772307.1</i> , <i>AL158152.1</i> , <i>SFTA1P</i> , <i>CASC10</i> , <i>UNC5B-AS1</i> , <i>DLG5-AS1</i> , <i>LINC00865</i> , <i>AP006284.1</i> , <i>AP006623.1</i> , <i>AC068580.3</i> , <i>AP001160.1</i> , <i>AP000759.1</i> , <i>MIR100HG</i> , <i>ITFG2-AS1</i> , <i>AGAP2-AS1</i> , <i>RMST</i> , <i>AC084018.2</i> , <i>SOX1-OT</i> , <i>MIR9-3HG</i> , <i>AC009041.2</i> , <i>AC134312.5</i> , <i>AC006435.2</i> , <i>AC027796.4</i> , <i>SNHG25</i> , <i>AC110285.2</i> , <i>AC020916.1</i> , <i>UCA1</i> , <i>AC123912.4</i> , <i>PTOV1-AS2</i> , <i>LINC00654</i> , <i>AL110115.1</i> , <i>MIR1-1HG-AS1</i> , <i>LINC01311</i> , <i>MIAT</i> , <i>AL022238.3</i> , <i>AL118516.1</i> , <i>AL732314.6</i> , <i>AC244197.2</i> , <i>AJ271736.1</i>
<i>SST</i>	<i>miR-4455</i> <i>miR-625-5p</i> <i>miR-766-5p</i>	<i>LINC00115</i> , <i>NFYC-AS1</i> , <i>AL358215.3</i> , <i>CYMP-AS1</i> , <i>AC234582.1</i> , <i>C1orf61</i> , <i>AC098936.1</i> , <i>LINC01139</i> , <i>LINC01833</i> , <i>AC010884.1</i> , <i>AC013275.1</i> , <i>AC011893.1</i> , <i>AC009955.4</i> , <i>AC141928.1</i> , <i>EPHA5-AS1</i> , <i>AC124016.1</i> , <i>AP002026.1</i> , <i>AC098864.1</i> , <i>SLC9A3-AS1</i> , <i>C5orf38</i> , <i>MIR4458HG</i> , <i>AC010478.1</i> , <i>LINC00461</i> , <i>AC099509.1</i> , <i>AL353759.1</i> , <i>CAHM</i> , <i>AC215522.2</i> , <i>AC092171.3</i> , <i>STAG3L5P-PVRIG2P-PILRB</i> , <i>KMT2E-AS1</i> , <i>MIR124-2HG</i> , <i>AC067773.1</i> , <i>AL772307.1</i> , <i>AL158152.1</i> , <i>SFTA1P</i> , <i>CASC10</i> , <i>UNC5B-AS1</i> , <i>DLG5-AS1</i> , <i>LINC00865</i> , <i>AP006284.1</i> , <i>AP006623.1</i> , <i>AC068580.3</i> , <i>AP001160.1</i> , <i>AP000759.1</i> , <i>MIR100HG</i> , <i>ITFG2-AS1</i> , <i>AGAP2-AS1</i> , <i>RMST</i> , <i>AC084018.2</i> , <i>SOX1-OT</i> , <i>MIR9-3HG</i> , <i>AC009041.2</i> , <i>AC134312.5</i> , <i>AC006435.2</i> , <i>AC027796.4</i> , <i>SNHG25</i> , <i>AC110285.2</i> , <i>AC020916.1</i> , <i>UCA1</i> , <i>AC123912.4</i> , <i>PTOV1-AS2</i> , <i>LINC00654</i> , <i>AL110115.1</i> , <i>MIR1-1HG-AS1</i> , <i>LINC01311</i> , <i>MIAT</i> , <i>AL022238.3</i> , <i>AL118516.1</i> , <i>AL732314.6</i> , <i>AC244197.2</i> , <i>AJ271736.1</i> , <i>DLEU2L</i> , <i>AL391069.3</i> , <i>AC104653.1</i> , <i>AC007319.1</i> , <i>MIR4453HG</i> , <i>LINC02106</i> , <i>AL662797.1</i> , <i>AL133375.1</i> , <i>AC004906.1</i> , <i>DLX6-AS1</i> , <i>FEZF1-AS1</i> , <i>AL356489.2</i> , <i>FAM201A</i> , <i>AL590399.1</i> , <i>AL354733.3</i> , <i>C9orf163</i> , <i>AL450311.2</i> , <i>AC087239.1</i> , <i>PCBP2-OT1</i> , <i>AL139089.1</i> , <i>AL135744.1</i> , <i>AL133523.1</i> , <i>DNAAF4-CCPG1</i> , <i>SLX1A-SULT1A3</i> , <i>FOXC2-AS1</i> , <i>AC009005.1</i> , <i>AC010331.1</i> , <i>AL133325.3</i> , <i>LINC01370</i> , <i>LINC00479</i> , <i>LINC01315</i> , <i>AL022316.1</i> , <i>MIR503HG</i> , <i>KC877982.1</i> , <i>AL390719.2</i> , <i>LINC00982</i> , <i>AL592166.1</i> , <i>AL606534.1</i> , <i>AC012360.3</i> , <i>LINC01614</i> , <i>AC107464.1</i> , <i>AC106881.1</i> , <i>AC034236.2</i> , <i>JAKMIP2-AS1</i> , <i>AC003986.1</i> , <i>AC004837.2</i> , <i>ELN-AS1</i> , <i>ST7-AS1</i> , <i>AL353150.1</i> , <i>ACTA2-AS1</i> <i>AL121928.1</i> , <i>AL731571.1</i> , <i>AP006621.1</i> , <i>AP000757.2</i> , <i>AL137058.2</i> , <i>AC124312.3</i> , <i>AL031714.1</i> , <i>AC080038.1</i> , <i>AC022211.1</i> , <i>LINC01896</i> , <i>AC012615.1</i> , <i>AC245884.9</i> , <i>MIF-AS1</i>
<i>GHRL</i>	<i>miR-660-3p</i>	<i>LINC00115</i> , <i>LINC00982</i> , <i>AL592166.1</i> , <i>AC104170.2</i> , <i>DLEU2L</i> , <i>AL358215.3</i> , <i>CYMP-AS1</i> , <i>AC234582.1</i> , <i>C1orf61</i> , <i>AC098936.1</i> , <i>LINC01139</i> , <i>AC019129.2</i> , <i>LINC01833</i> , <i>AC012360.3</i> , <i>AC104653.1</i> , <i>AC013275.1</i> , <i>LINC01614</i> , <i>AC046143.1</i> , <i>AC107464.1</i> , <i>AC141928.1</i> , <i>AC124016.1</i> , <i>AP002026.1</i> , <i>MIR4453HG</i> , <i>AC098864.1</i> , <i>SLC9A3-AS1</i> , <i>C5orf38</i> , <i>MIR4458HG</i> , <i>LINC02106</i> , <i>AC010478.1</i> , <i>LINC00461</i> , <i>AC008522.1</i> , <i>JAKMIP2-AS1</i> , <i>AL353759.1</i> , <i>AL133375.1</i> , <i>AL139393.2</i> , <i>CAHM</i> , <i>AC215522.2</i> , <i>AC092171.3</i> , <i>HOTAIRM1</i> , <i>AC004837.2</i> , <i>DLX6-AS1</i> , <i>STAG3L5P-PVRIG2P-PILRB</i> , <i>KMT2E-AS1</i> , <i>AC090150.1</i> , <i>MIR124-2HG</i> , <i>AC103706.1</i> , <i>AL356489.2</i> , <i>FAM201A</i> , <i>AL772307.1</i> , <i>AL590399.1</i> , <i>AL354733.3</i> , <i>AL158152.1</i> , <i>SFTA1P</i> , <i>CASC10</i> , <i>AL450311.2</i> , <i>DLG5-AS1</i> , <i>LINC00865</i> , <i>AL121928.1</i> , <i>AL731571.1</i> , <i>AP006284.1</i> , <i>AP006621.1</i> , <i>AP006623.1</i> , <i>AC068580.3</i> , <i>AP001160.1</i> , <i>MIR100HG</i> , <i>ITFG2-</i>

mRNA	miRNA	lncRNA
		AS1, RMST, AC084018.2, AL137058.2, FGF14-AS2, SOX1-OT, AL160313.1, AL133523.1, AC013356.2, MIR9-3HG, AC009041.2, SLX1A-SULT1A3, AC134312.5, AC027796.4, AC005224.4, AC005838.2, AC080038.1, AC110285.2, LINC01896, AC125437.1, AC020916.1, UCA1, AC010331.1, AC018766.1, LINC00654, AL133325.3, MIR1-1HG-AS1, LINC00479, MIAT, LINC01315, AL732314.6, AJ271736.1
ARX	miR-122-5p miR-130a-3p miR-766-5p	LINC00115, LINC00982, AC104170.2, DLEU2L, CYMP-AS1, AC234582.1, C1orf61, AL138930.1, LINC01139, AC012360.3, AC104653.1, LINC01614, AC009955.4, AC137630.3, AC046143.1, AC107464.1, AC141928.1, AC124016.1, AC106881.1, AP002026.1, LINC02106, LINC00461, AC034236.2, AL358852.1, CAHM, AC003986.1, HOTAIRM1, AC004837.2, ELN-AS1, DLX6-AS1, KMT2E-AS1, ST7-AS1, FEZF1-AS1, MIR124-2HG, AL772307.1, AL590399.1, SFTA1P, UNC5B-AS1, AL135925.1, AP006623.1, AP000757.2, MIR100HG, ITFG2-AS1, AGAP2-AS1, RMST, FGF14-AS2, SOX1-OT, AL133523.1, MIR9-3HG, AC009041.2, AL031714.1, AC134312.5, AC006435.2, SNHG25, AC110285.2, AC011139.1, AC016586.1, AC020916.1, UCA1, LINC00654, LINC01370, AJ011932.1, LINC01311, MIF-AS1, MIAT, AL118516.1, AL732314.6, MIR503HG, AJ271736.1, AC098936.1, LINC01833, LINC01014, AC098864.1, C5orf38, AC010478.1, AC099509.1, AKMIP2-AS1, AC215522.2, FAM201A, AL354733.3, AL353150.1, AL450311.2, DLG5-AS1, LINC00865, AL731571.1, AL137058.2, AC007497.1, FOXC2-AS1, AL133325.3, MIR1-1HG-AS1, AL022316.1, AL390719.2, NFYC-AS1, AL592166.1, AL358215.3, AL606534.1, AC010884.1, AC013275.1, AC011893.1, MIR4453HG, MIR4458HG, JAKMIP2-AS1, AL133375.1, STAG3L5P-PVRIG2P-PILRB, AC067773.1, AL356489.2, AL158152.1, CASC10, ACTA2-AS1, AL121928.1, AP006284.1, AP006621.1, AC068580.3, AP000759.1, AL139089.1, AC124312.3, DNAAF4-CCPG1, AC027796.4, AC080038.1, AC022211.1, LINC01896, AC009005.1, AC012615.1, AC123912.4, AC245884.9, LINC00479, KC877982.1, AJ271736.1
ERO1B	miR-194-3p miR-215-5p miR-3131 miR-627-3p	LINC00115, AL391244.2, LINC00982, NFYC-AS1, AC234582.1, C1orf61, AC019129.2, LINC01833, AC104653.1, AC007319.1, AC009955.4, FGF12-AS2, AC046143.1, AC107464.1, AC141928.1, EPHA5-AS1, AC124016.1, AP002026.1, AC095055.1, MIR4453HG, AC098864.1, SLC9A3-AS1, C5orf38, MIR4458HG, AC010478.1, LINC00461, JAKMIP2-AS1, CAHM, AC215522.2, AC004837.2, DLX6-AS1, STAG3L5P-PVRIG2P-PILRB, SLC12A9-AS1, KMT2E-AS1, AL356489.2, AL772307.1, AL590399.1, AL354733.3, AL158152.1, C9orf163, CASC10, AL135925.1, ACTA2-AS1, LINC00865, AL731571.1, AP006284.1, AP006623.1, AC068580.3, AP001160.1, AP000759.1, MIR100HG, ITFG2-AS1, RMST, AC084018.2, AL137058.2, AL135744.1, AL133523.1, DNAAF4-CCPG1, AC009041.2, AL031714.1, SLX1A-SULT1A3, AC006435.2, AC005224.4, AC080038.1, AC110285.2, AC016586.1, MIR7-3HG, AC020916.1, UCA1, AC123912.4, AC010331.1, AC018766.1, AC245884.9, LINC00654, AL133325.3, LINC01370, MIR1-1HG-AS1, LINC00479, LINC01311, MIAT, AC010884.1, AC012360.3, AC011893.1, AL662797.1, AL450311.2, AL160313.1, MIR9-3HG, LINC01670, AL358215.3, AC098936.1, LINC01943, AC013275.1, LINC00992, FEZF1-AS1, AC090150.1, MIR124-2HG, FAM201A, DLG5-AS1, AP006621.1, AC087239.1, AGAP2-AS1, SOX1-OT, PTOV1-AS2, MIF-AS1, AL732314.6, KC877982.1, AC104170.2, DLEU2L, CYMP-AS1, AL354760.1, AL138930.1, LINC01139, AC074091.1, LINC01614, AC137630.3, LINC02106, AC099509.1, AL136162.1, AL139393.2, AC004906.1, AC003986.1, AC067773.1, AC103706.1, AL353150.1, SFTA1P, AL133415.1, UNC5B-AS1, U47924.2, FGF14-AS2, AL138976.2, AC013356.2, AC007497.1, FOXC2-AS1,

mRNA	miRNA	lncRNA
		AC134312.5, AC011139.1, LINC01896, AC125437.1, AL110115.1, LINC01315, AL022316.1, MIR503HG
GCK	miR-4455 miR-6887-3p	LINC00115, NFYC-AS1, AL358215.3, CYMP-AS1, AC234582.1, C1orf61, AC098936.1, LINC01139, LINC01833, AC010884.1, AC013275.1, AC011893.1, AC009955.4, AC141928.1, EPHA5-AS1, AC124016.1, AP002026.1, AC098864.1, SLC9A3-AS1, C5orf38, MIR4458HG, AC010478.1, LINC00461, AC099509.1, AL353759.1, CAHM, AC215522.2, AC092171.3, STAG3L5P-PVRIG2P-PILRB, KMT2E-AS1, MIR124-2HG, AC067773.1, AL772307.1, AL158152.1, SFTA1P, CASC10, UNC5B-AS1, DLG5-AS1, LINC00865, AP006284.1, AP006623.1, AC068580.3, AP001160.1, AP000759.1, MIR100HG, ITFG2-AS1, AGAP2-AS1, RMST, AC084018.2, SOX1-OT, MIR9-3HG, AC009041.2, AC134312.5, AC006435.2, AC027796.4, SNHG25, AC110285.2, AC020916.1, UCA1, AC123912.4, PTOV1-AS2, LINC00654, AL110115.1, MIR1-1HG-AS1, LINC01311, MIAT, AL022238.3, AL118516.1, AL732314.6, AC244197.2, AJ271736.1, AL391244.2, AL139246.5, LINC00982, AL391069.3, AC019129.2, AC012360.3, AC104653.1, LINC01614, AC107464.1, AC106881.1, MIR4453HG, LINC02106, AC034236.2, JAKMIP2-AS1, AL031963.3, AL358852.1, AL139393.2, AC003986.1, HOTAIRM1, DLX6-AS1, ST7-AS1, AC004908.2, AC103706.1, AL354733.3, C9orf163, AL450311.2, AL135925.1, ACTA2-AS1, AL731571.1, AP003043.1, AC087239.1, AL135744.1, AL031714.1, SLX1A-SULT1A3, AC005224.4, AC080038.1, LINC01896, MIR7-3HG, AC010331.1, AC018766.1, LINC01670, LINC00479, AJ011932.1, AL022316.1, CR559946.2, MIR503HG
NEUROG3	miR-625-5p miR-766-5p	LINC00115, NFYC-AS1, DLEU2L, CYMP-AS1, AL391069.3, AC234582.1, C1orf61, LINC01139, AC010884.1, AC104653.1, AC007319.1, AC141928.1, AP002026.1, MIR4453HG, AC098864.1, SLC9A3-AS1, C5orf38, LINC02106, AC010478.1, LINC00461, AC099509.1, AL662797.1, AL133375.1, AC215522.2, AC004906.1, DLX6-AS1, STAG3L5P-PVRIG2P-PILRB, KMT2E-AS1, FEZF1-AS1, MIR124-2HG, AL356489.2, FAM201A, AL772307.1, AL590399.1, AL354733.3, AL158152.1, C9orf163, SFTA1P, CASC10, AL450311.2, UNC5B-AS1, DLG5-AS1, LINC00865, AC068580.3, MIR100HG, TFG2-AS1, AC087239.1, PCBP2-OT1, RMST, AC084018.2, AL139089.1, SOX1-OT, AL135744.1, AL133523.1, DNAAF4-CCPG1, MIR9-3HG, AC009041.2, SLX1A-SULT1A3, FOXC2-AS1, AC134312.5, AC027796.4, AC110285.2, AC009005.1, AC020916.1 UCA1, AC010331.1, LINC00654, AL133325.3, LINC01370, MIR1-1HG-AS1, LINC00479, LINC01311, MIAT, LINC01315, MIR503HG, KC877982.1, AL390719.2, LINC00982, AL592166.1, AL358215.3, AL606534.1, LINC01833, AC012360.3, AC013275.1, AC011893.1, LINC01614, AC107464.1, AC124016.1, AC106881.1, MIR4458HG, AC034236.2, JAKMIP2-AS1, CAHM, AC003986.1, AC004837.2, ELN-AS1, ST7-AS1, AC067773.1, AL353150.1, ACTA2-AS1, AL121928.1, AL731571.1, AP006284.1, AP006621.1, AP000759.1, AP000757.2, ITFG2-AS1, AGAP2-AS1, AL137058.2, AC124312.3, AL031714.1, AC006435.2, AC080038.1, AC022211.1, LINC01896, AC012615.1, AC020916.1, AC123912.4, AC245884.9, MIF-AS1, AL022316.1, AL732314.6, AJ271736.1
ISL1	miR-224-5p miR-29c-3p	LINC00115, LINC00982, AC104170.2, C1orf61, LINC01139, AL606534.1, LINC01833, AC012360.3, AC104653.1, AC013275.1, AC046143.1, AC107464.1, AC141928.1, EPHA5-AS1, AC106881.1, AP002026.1, AC095055.1, AC098864.1, MIR4458HG, AC010478.1, LINC00461, AC008522.1, CAHM, AC215522.2, AC211476.2, DLX6-AS1, STAG3L5P-PVRIG2P-PILRB, KMT2E-AS1, ST7-AS1, FEZF1-AS1, MIR124-2HG, AC067773.1, AL356489.2, AL772307.1, AL158152.1, SFTA1P, CASC10,

mRNA	miRNA	lncRNA
	<i>miR-31-5p</i>	<i>AL450311.2, DLG5-AS1, LINC00865, AL731571.1, AP006284.1, MIR100HG, ITFG2-AS1, RMST, AC084018.2, AL137058.2, SOX1-OT, AL135744.1, AC124312.3, AC013356.2, DNAAF4-CCPG1, MIR9-3HG, AC009041.2, SNHG19, MIR7-3HG, AC020916.1, UCA1, AC018766.1, LINC00654, AL133325.3, AL110115.1, LINC01370, MIR1-1HG-AS1, LINC01311, MIAT, CR559946.2, AL732314.6, MIR503HG, AJ271736.1, DLEU2L, AL358215.3, AC007319.1, JAKMIP2-AS1, C9orf163, AL135925.1, ACTA2-AS1, AP000759.1, AP000757.2, PCBP2-OT1, AL133523.1, AL031714.1, SLX1A-SULT1A3, AC134312.5, AC027796.4, AC123912.4, PTOV1-AS2, LINC01670, MIF-AS1, KC877982.1, CYMP-AS1, AL391069.3, AC234582.1, AL138930.1, AC074091.1, AC019129.2, AC010884.1, AC124016.1, AL358852.1, AC003986.1, SLC12A9-AS1, AC103706.1, AL590399.1, AL354733.3, AL121929.2, AL139089.1, SNHG9, FOXC2-AS1, AC093484.3, SNHG25, LINC01315, AL391244.2, AC098936.1, AC011893.1, AC137630.3, SEC62-AS1, SLC9A3-AS1, C5orf38, LINC02106, AL662797.1, AL139393.2, AC044849.1, AP006621.1, AP006623.1, AC068580.3, AC005224.4, AC080038.1, AC110285.2, LINC01896, AC012615.1, LINC00479, AC244197.2, NFYC-AS1, MIR4453HG, AC099509.1, AL133375.1, AC004906.1, FAM201A, UNC5B-AS1, AC087239.1, AC009005.1, AC010331.1, AL022316.1</i>

Discussion

Our findings demonstrate that *RFX3* deficiency profoundly alters both coding and non-coding RNA landscapes in iPSC-derived pancreatic islets, revealing multilayered regulatory disruptions that likely contribute to impaired islet development and function. Consistent with our previous observations of reduced endocrine markers in *RFX3* KO islets [4], transcriptomic profiling uncovered widespread dysregulation of protein-coding genes, as well as extensive changes in miRNA and lncRNA expression. Notably, integrative analyses highlighted that upregulated miRNAs in *RFX3*-deficient islets target a broad set of downregulated mRNAs and lncRNAs, establishing key regulatory interactions that include critical genes involved in insulin secretion, calcium signaling, and endocrine differentiation. Conversely, downregulated miRNAs were predicted to regulate upregulated genes associated with lipid metabolism, enterochromaffin cell markers, and apoptosis, underscoring the potential contribution of non-coding RNA dysregulation to both functional and survival defects in *RFX3* KO islets. These results collectively reveal a complex miRNA–lncRNA–mRNA network architecture disrupted by *RFX3* loss, providing mechanistic insight into how transcription factor deficiency reshapes the epigenetic and post-transcriptional regulatory landscape during human pancreatic islet differentiation.

RFX TFs have been increasingly recognized as regulators of non-coding RNA expression across diverse tissues. For example, *RFX3* and *RFX5* directly regulate *miR-150* in human memory T cells, modulating PDAP1 and proliferation [36], while *RFX6* influence networks of miRNAs including *miR-199a-3p*, *miR-125b-5p*, and *miR-199-5p* in gastric cancer [37]. Importantly, our previous work confirmed that *RFX6* also plays a critical role in human pancreatic islet development and function, highlighting its broader importance in endocrine lineage specification [38]. Although many of these studies were performed in non-pancreatic contexts, they underscore the potential of *RFX* TFs to orchestrate non-coding RNA

transcription. In line with this, our data demonstrate that RFX3 plays a central role in human pancreatic islet development by coordinating multilayered regulation of both coding and non-coding transcripts. Using *RFX3* KO iPSC models, we show that RFX3 loss disrupts key transcriptional programs and reshapes the epigenetic landscape governing islet development. Core pancreatic endocrine markers such as *INS*, *GCG*, *SST*, *IAPP*, and *GHRL* were significantly downregulated, reflecting impaired β - and α -cell identity and hormone production. Similarly, critical TFs for endocrine lineage commitment, including *ISL1*, *ARX*, *NEUROG3*, and *PAX6*, were downregulated, indicating broader disruption of differentiation programs. RFX3 deficiency also impaired islet functional genes, including ion channels (*KCNJ11*, *ABCC8*, *KCNK16*, *KCNA17*), transporters (*SLC8A3*), metabolic sensors (*GCK*, *FFAR1*), and vesicle docking/exocytosis regulators (*SYT2*, *SYT4*, *SYT11*, *TRPC4*, *FOS*), suggesting compromised electrophysiology, insulin secretion, and metabolic responsiveness [4].

Our integrative analysis revealed that 47 upregulated miRNAs in RFX3-deficient islets were likely responsible for repressing key target genes involved in islet differentiation and function. Notably, *miR-451a*, primarily expressed in exocrine cells, was elevated, suggesting disruption of endocrine identity. Other miRNAs, including *miR-122-5p*, *miR-338-3p*, and *miR-194-5p*, were predicted to target genes critical for β -cell function, insulin secretion, and calcium signaling. *miR-338-3p*, for instance, has previously been shown to suppress glucose-stimulated insulin secretion by downregulating PDX1, whereas its inhibition enhances β -cell survival and proliferation [39–42]. Similarly, *miR-378a-3p*, *miR-29c-3p*, and *miR-452-5p* target genes essential for insulin granule exocytosis, membrane excitability, and β -cell survival, and have been linked to metabolic stress, obesity, and diabetes pathogenesis [43–52]. Other miRNAs, such as *miR-502-3p*, *miR-766*, and *miR-130a-3p*, are associated with insulin resistance and T2D, suggesting that RFX3 deficiency may recapitulate aspects of metabolic dysfunction through dysregulated miRNA networks [53–56].

In parallel, RFX3 loss promoted the upregulation of non-endocrine lineage genes, including lipid metabolism and EC markers, accompanied by elevated apoptotic markers. Several downregulated miRNAs normally maintain β -cell identity and survival, including *miR-127-3p*, *miR-143-3p*, and miRNAs targeting apoptotic genes such as CASPs and TNF family members. Reduced expression of *let-7i-5p*, *miR-216a-3p*, and *miR-135a-5p* likely contributes to increased cellular stress, proliferation defects, and apoptosis [57–60] [75–78]. Consistently, we observed upregulation of TXNIP, a pro-apoptotic gene implicated in β -cell loss and diabetes pathogenesis, and identified several downregulated miRNAs (*miR-411-5p*, *miR-135a-5p*, *miR-216a-3p*, *miR-5006-3p*) predicted to target *TXNIP*, revealing a novel mechanism linking RFX3 deficiency to apoptosis [61–63].

Collectively, these findings highlight RFX3 as a master regulator of pancreatic islet development, coordinating transcriptional and post-transcriptional networks to ensure proper endocrine lineage specification, β -cell functional maturation, and cell survival. Loss of RFX3 disrupts this multilayered regulatory network, resulting in impaired hormone production, functional deficits, lineage drift, and increased apoptosis.

Our study also revealed that RFX3 deficiency leads to the downregulation of multiple lncRNAs critical for β -cell identity, function, and survival. For example, *MIR7-3HG*, which encodes a protective peptide activating the PI3K/AKT pathway, is essential for β -cell protection against dysfunction and has been previously linked to FOXA2-deficient islets [24, 83]. *RMST*, expressed specifically in α - and β -cells, likely contributes to lineage-specific gene regulation [82, 84]. Other lncRNAs, such as *FEZF1-AS1* are downregulated in T2D and regulate pathways including EMT, STAT3, and Wnt/ β -catenin, potentially affecting islet architecture and function [85, 86]. *MIAT* modulates the MIAT/Nrf2 signalling pathway, and its altered expression is associated with cell viability and diabetic complications, underscoring a context-dependent role in diabetes pathophysiology [87, 88]. Similarly, *DNAAF4-CCPG1* was downregulated in RFX3-deficient islets, correlating with TXNIP elevation, and may regulate PIK3R1 expression through miRNAs like miR-29a/b/c and miR-221, implicating it in apoptosis and β -cell survival [89, 90]. Downregulation of *LINC00461*, which normally sponges *miR-411-5p* to enhance BNIP3 expression and promote cell survival, further contributes to the increased apoptosis observed in our model by impairing MAPK/ERK and PI3K/AKT signalling [91, 92].

To dissect the mechanistic underpinnings, we constructed an integrated mRNA–miRNA–lncRNA (ceRNA) network. This analysis revealed that several downregulated lncRNAs act as miRNA sponges, indirectly protecting key pancreatic endocrine transcripts. Notably, *AC020916.1* and *LINC00461* emerged as central nodes, strongly interacting with miRNAs targeting critical genes such as *INS*, *GHRL*, and *SST*. For *ARX*, 24 downregulated lncRNAs, including *AC020916.1*, *LINC00461*, *MIAT*, and *LINC00115*, were predicted to sequester its targeting miRNAs. Across multiple networks, a core set of 13 lncRNAs—including *AC020916.1*, *LINC00461*, *MIAT*, *LINC00115*, *RMST*, *MIR100HG*, and *MIR9-3HG*—consistently sponged miRNAs predicted to repress *INS*, *SST*, *GHRL*, *ARX*, *NEUROG3*, and *GCK*, highlighting a convergent regulatory axis that may be modulated in the context of RFX3 deficiency. Moreover, in networks associated with *TPH1* and *TXNIP*, several upregulated lncRNAs (e.g., *LINC01214*, *AL031123.2*, *RPARP-AS1*, and *HNF1A-AS1*) were linked to miRNAs targeting these genes, potentially explaining their aberrant expression in RFX3-deficient islets. Collectively, these findings indicate that RFX3 preserves endocrine identity and function by maintaining a balanced ceRNA landscape, and disruption of this network may underlie the impaired islet specification, hormone production, and survival observed in the absence of RFX3 expression.

In conclusion, our study highlights that RFX3 deficiency profoundly impacts human pancreatic islet development, leading to impaired β - and α -cell identity, altered hormone gene expression, and increased apoptosis via TXNIP upregulation, consistent with our previous findings [4]. While we do not provide direct evidence that all observed non-coding RNA changes are transcriptionally controlled by RFX3, the upregulation of specific miRNAs and downregulation of lncRNAs may be associated with the dramatic transcriptional and functional alterations seen upon RFX3 loss. Notably, key endocrine genes and pathways involved in ion channel function, metabolic sensing, and vesicle trafficking are predicted targets of these non-coding RNAs. These findings suggest that the effects of RFX3 deficiency may be mediated, at least in part, through changes in post-transcriptional regulatory networks, providing new insights into the molecular mechanisms underlying impaired islet maturation and survival.

Declarations

Acknowledgment

We thank the Genomic Core members at Sidra Medicine for their assistance for technical support in RNA and miRNA sequencing.

Author contributions

A.K.E and A.A. performed the experiments, analyzed the data, prepared the figures, and wrote the manuscript. N.A. contributed to performing some experiments and writing the manuscript. B.Y.A. and N.M.A. analyzed the miRNA and lncRNA sequencing data. E.M.A. conceived and designed the study, supervised the project, secured research funding, and revised the manuscript. All authors critically reviewed the article and approved the final version of the manuscript.

Conflict of interests

The authors declare no conflict of interests in this manuscript.

Funding

This work was funded by grants from Sidra Medicine (SDR400217). Aldana Alnesf and Noura Aldous, authors of this article, are PhD students supported by QREDI scholarships (QRLP10-G-1803010 and GSRA9-L-1-0511-22008, respectively).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval and consent to participate

Not applicable

Competing Interest

The authors declare no competing interests in this manuscript.

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Figures

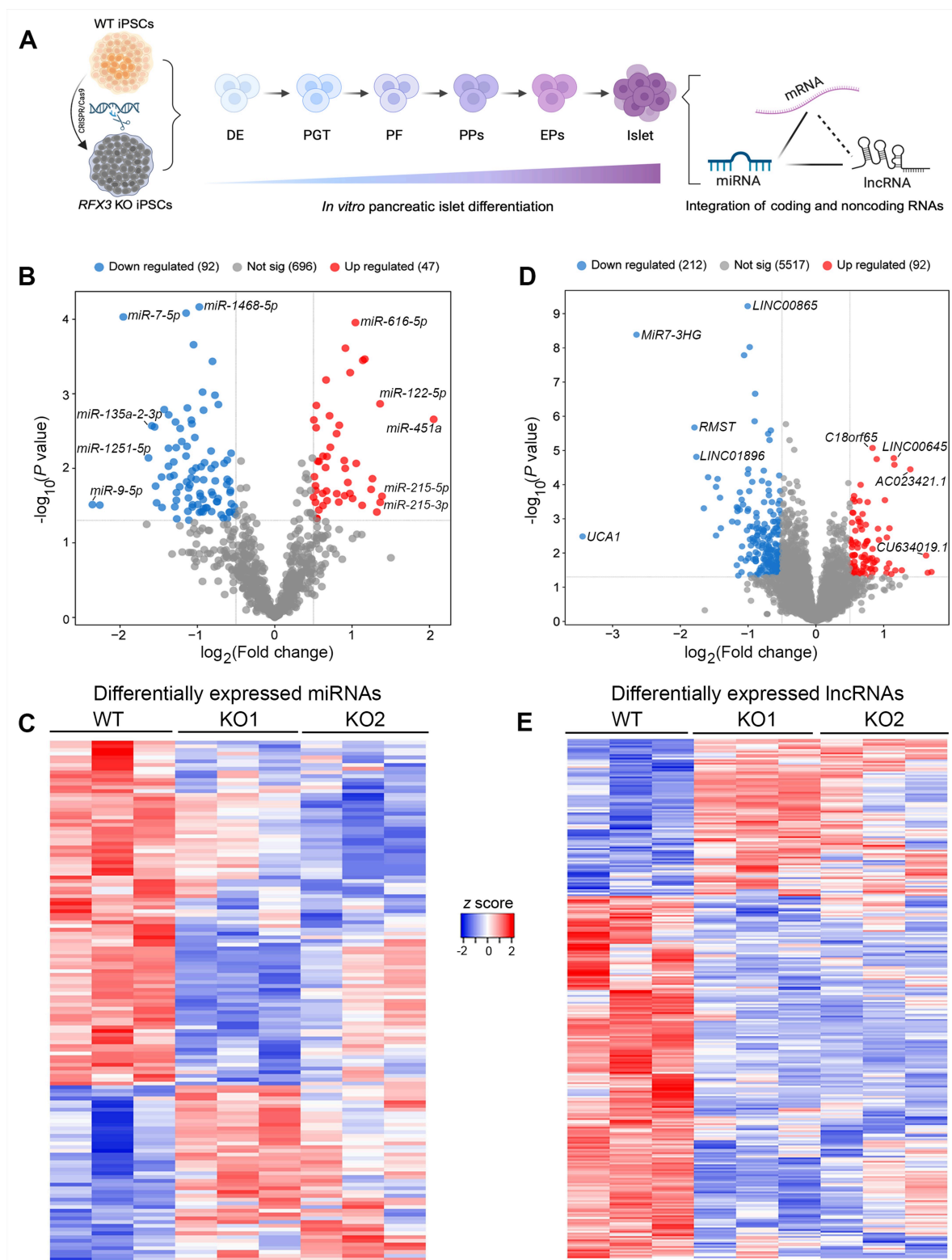


Figure 1

***RFX3* deficiency disrupts non-coding RNA expression profiles in iPSC-derived pancreatic islets.** (A) Schematic representation of the experimental workflow, including iPSC differentiation into pancreatic islets. (B) Volcano plot showing significantly downregulated DEmiRs (blue), significantly upregulated (red), and non-significantly dysregulated DEmiRs (grey) in *RFX3* KO islets. (C) Heatmap expression profile of the significantly dysregulated DEmiRs in *RFX3*KO islets ($n=3$). (D) Volcano plot showing

significantly downregulated DEIncRNAs (blue), significantly upregulated DEIncRNAs (red), and non-significantly dysregulated DEIncRNAs (grey) in *RFX3* KO islets. (E) Heatmap expression profile of the significantly dysregulated DEIncRNAs in *RFX3* KO islets ($n=3$). DE; definitive endoderm, PGT; primitive gut tube, PF; posterior foregut, PPs; pancreatic progenitors, EPs; endocrine progenitors.

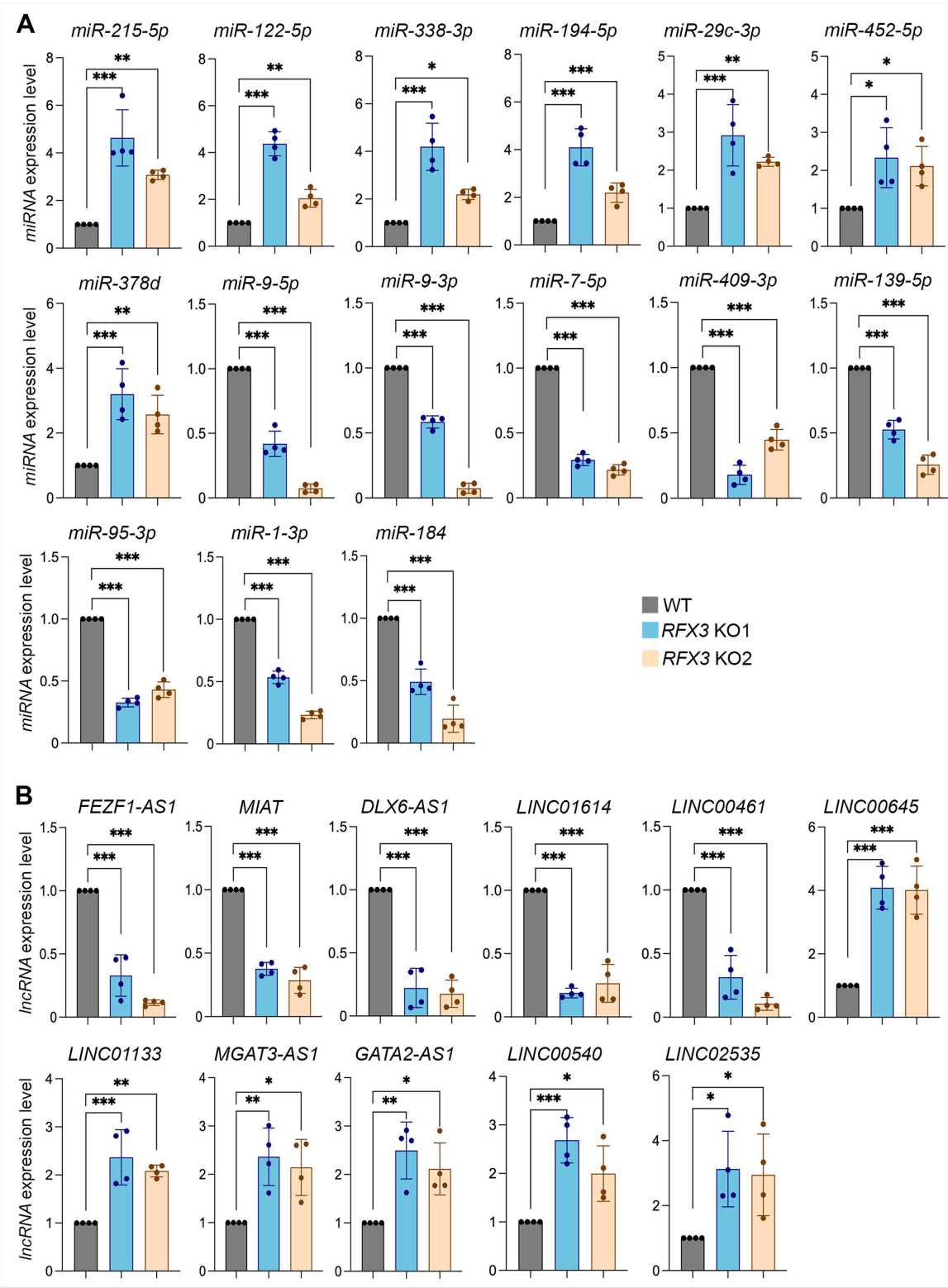


Figure 2

Validation of differentially expressed miRNAs and lncRNAs in RFX3-deficient islets. (A) RT-qPCR validation of selected upregulated and downregulated DEmiRs ($n=4$) in RFX3 KO islets compared to WT. (B) RT-qPCR validation of selected upregulated and downregulated DElncRNAs in RFX3 KO islets compared to WT ($n=4$). Data are represented as mean $\pm$ SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

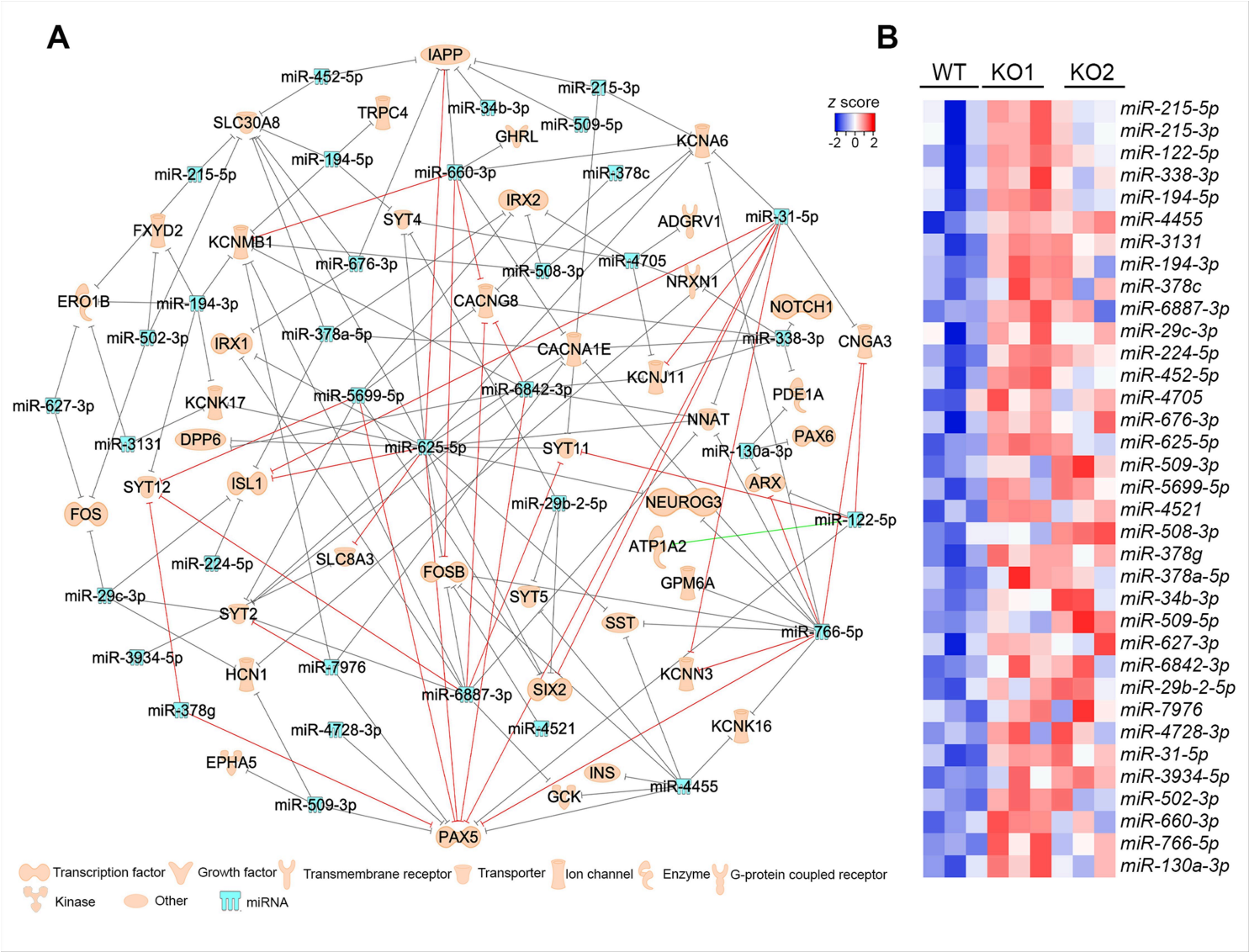


Figure 3

Predicted interactions between upregulated miRNAs and downregulated pancreatic islet genes in RFX3-deficient islets. (A) Target prediction analysis of the selected upregulated DEmiRs targeting key downregulated pancreatic islets DEGs. The interaction network between upregulated DEmiRs and downregulated DEGs was conducted using Ingenuity Pathway Analysis (IPA) tool. Green lines indicate the experimentally validated miRNAs, red lines indicate a high prediction confidence level, while grey lines indicate a moderate prediction confidence level. (B) Heatmap of the significantly upregulated DEmiRs targeting the selected DEGs ($n=3$).

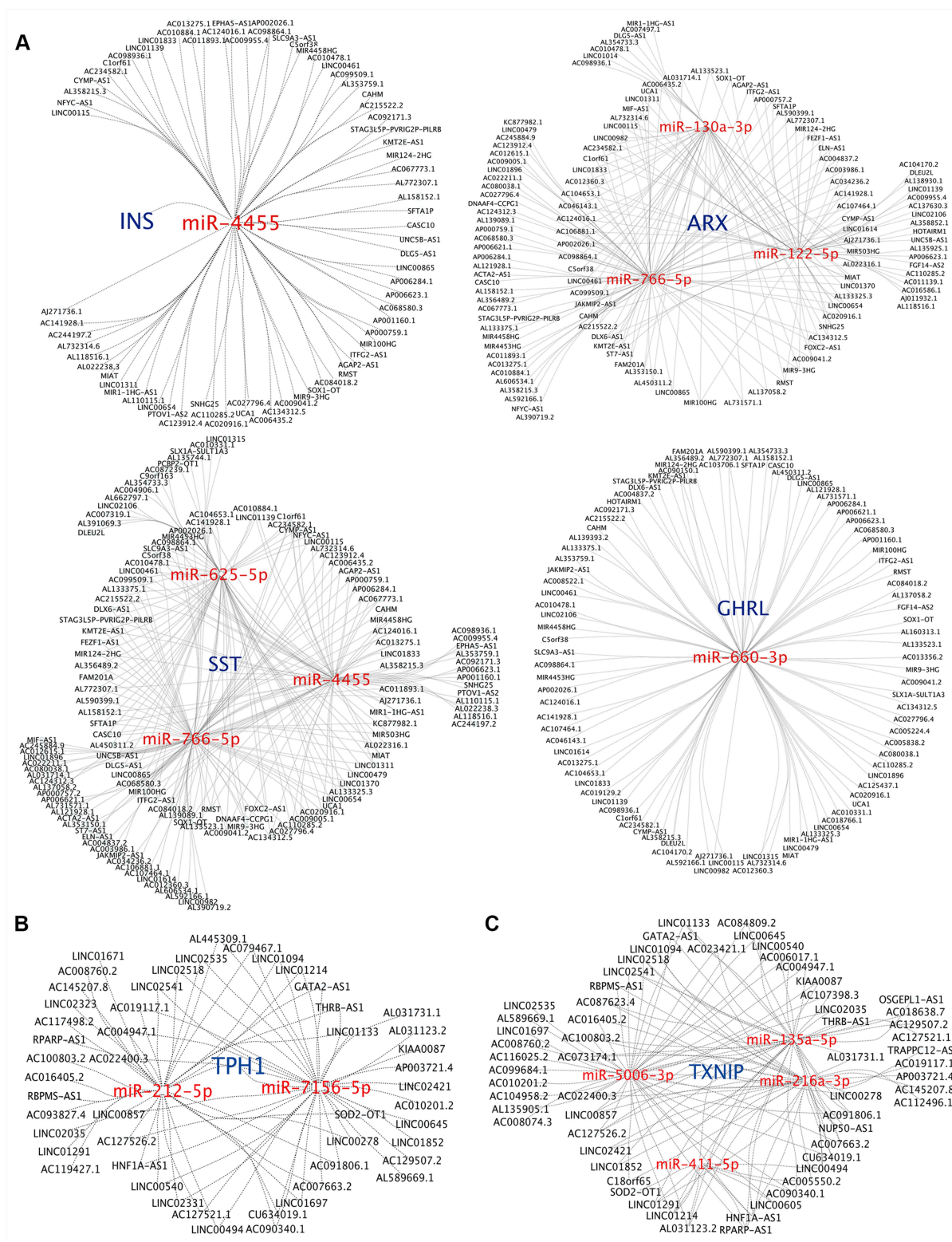


Figure 5

Representative miRNA-mRNA-lncRNA correlation networks in RFX3-deficient pancreatic islets. The networks illustrate predicted interactions between DEmiRs, DElncRNAs, and DEGs. Selected pancreatic islet (A), EC marker (B), and apoptosis (C) markers were used to construct ceRNA networks using Cytoscape software.

Supplementary Files

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