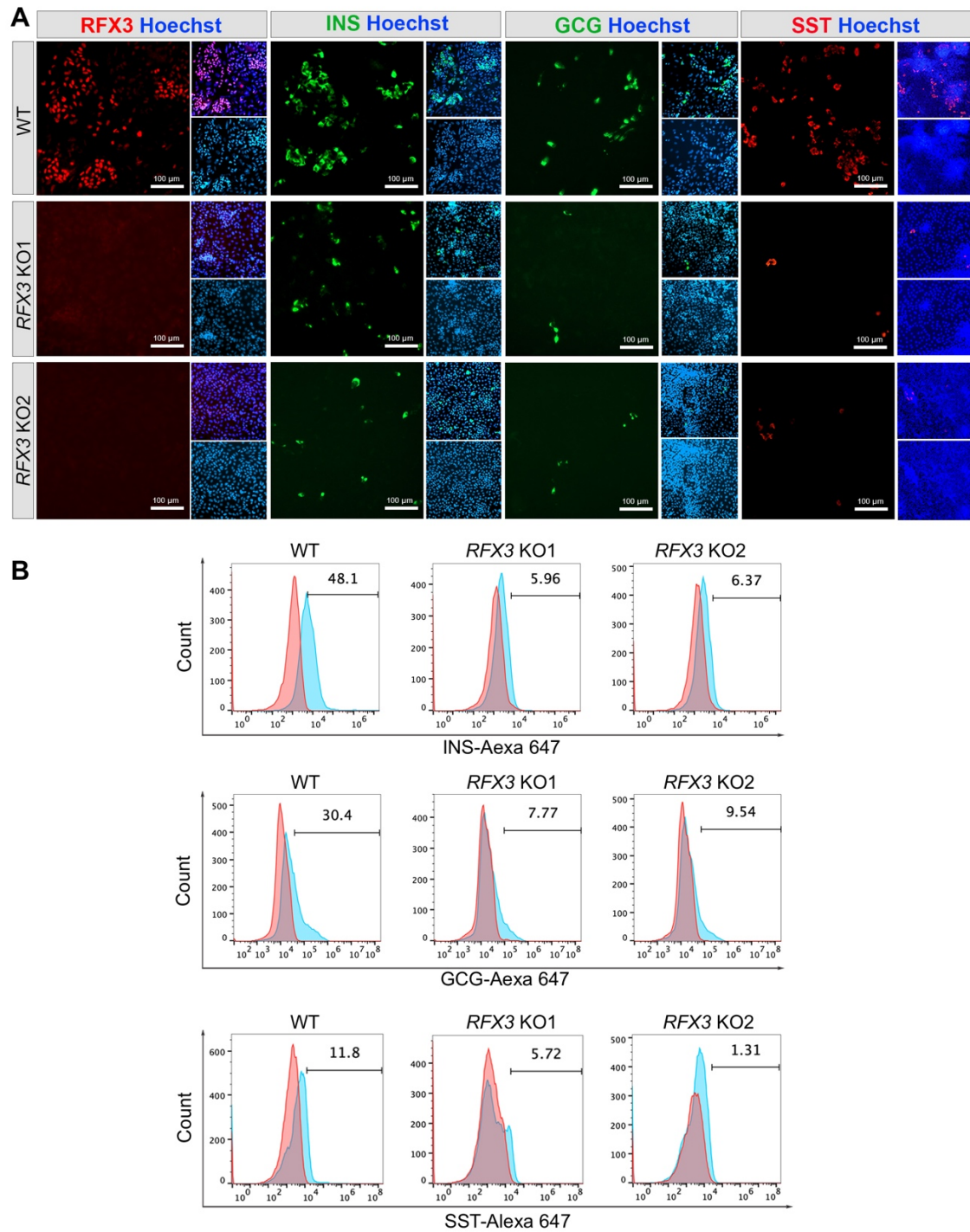


## Supplementary Figures

Figure S1

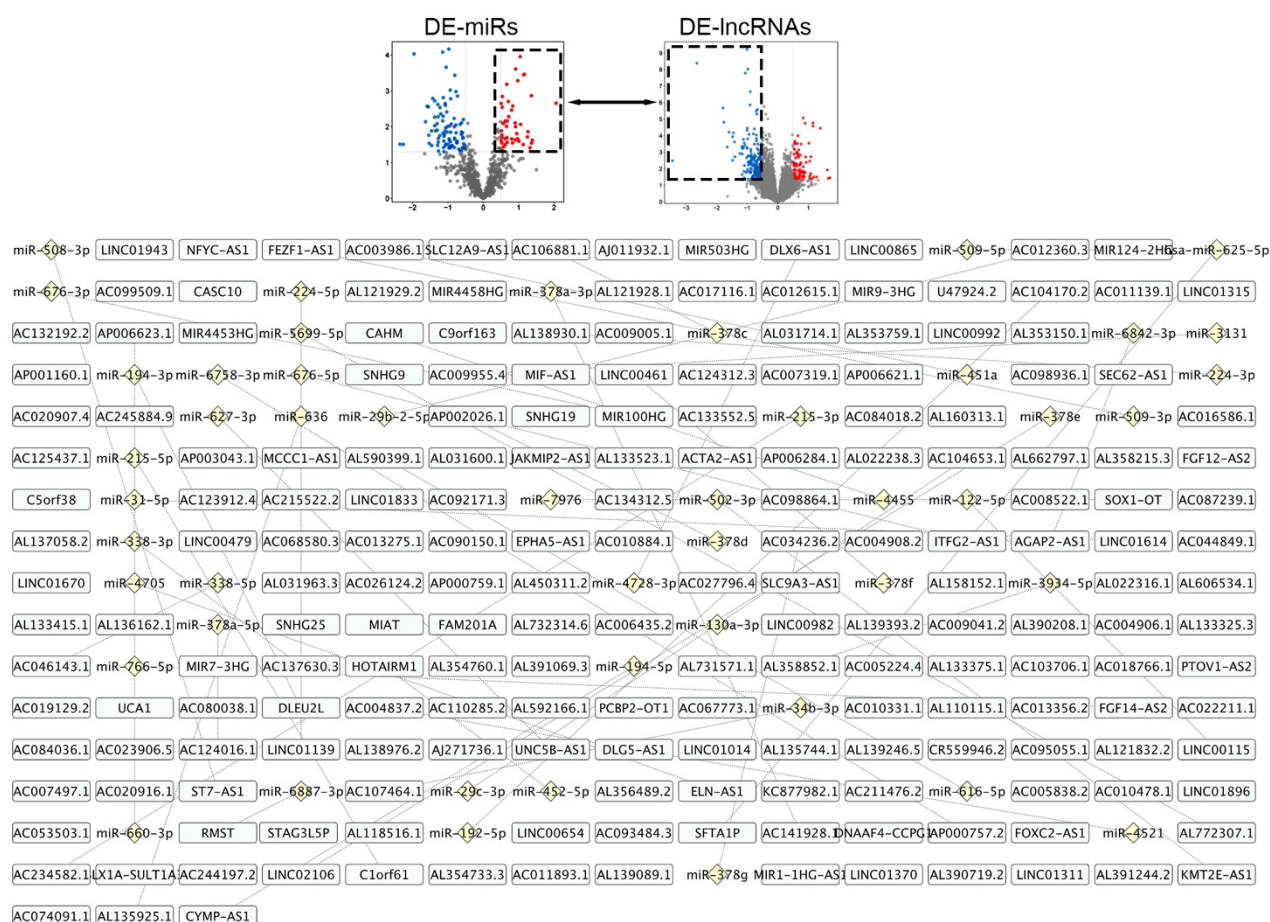


**Figure S1. Effect of *RFX3* loss on pancreatic islet markers.** (A) Immunofluorescence staining confirming the loss of *RFX3* expression in islets lacking *RFX3* and the effect of *RFX3* deficiency on key islet hormone markers, *INS*, *GCG*, and *SST*. Scale bar = 100  $\mu$ m. (B) Flow cytometry analysis showing the expression of *INS*, *GCG*, and *SST* in islets differentiated from *RFX3* KO iPSCs compared with WT controls.

**Figure S2. Lack of RFX3 results in dysregulation of coding and non-coding RNAs related to lipid metabolism, enterochromaffin cells, and apoptosis in pancreatic islets.** (A) Heatmap of upregulated DEGs related to lipid metabolism, enterochromaffin cells, and apoptosis. (B) Integration analysis of selected upregulated DEGs linked to lipid metabolism and enterochromaffin cells targeted by downregulated DEmiRs. (C) Integration analysis of selected upregulated DEGs linked to apoptosis targeted by downregulated DEmiRs. The interaction networks between the downregulated DEmiRs and the upregulated DEGs were constructed using the Ingenuity Pathway Analysis (IPA) platform. Green lines indicate experimentally validated targets, red lines denote interactions with high predicted confidence, whereas grey lines indicate moderate prediction confidence.

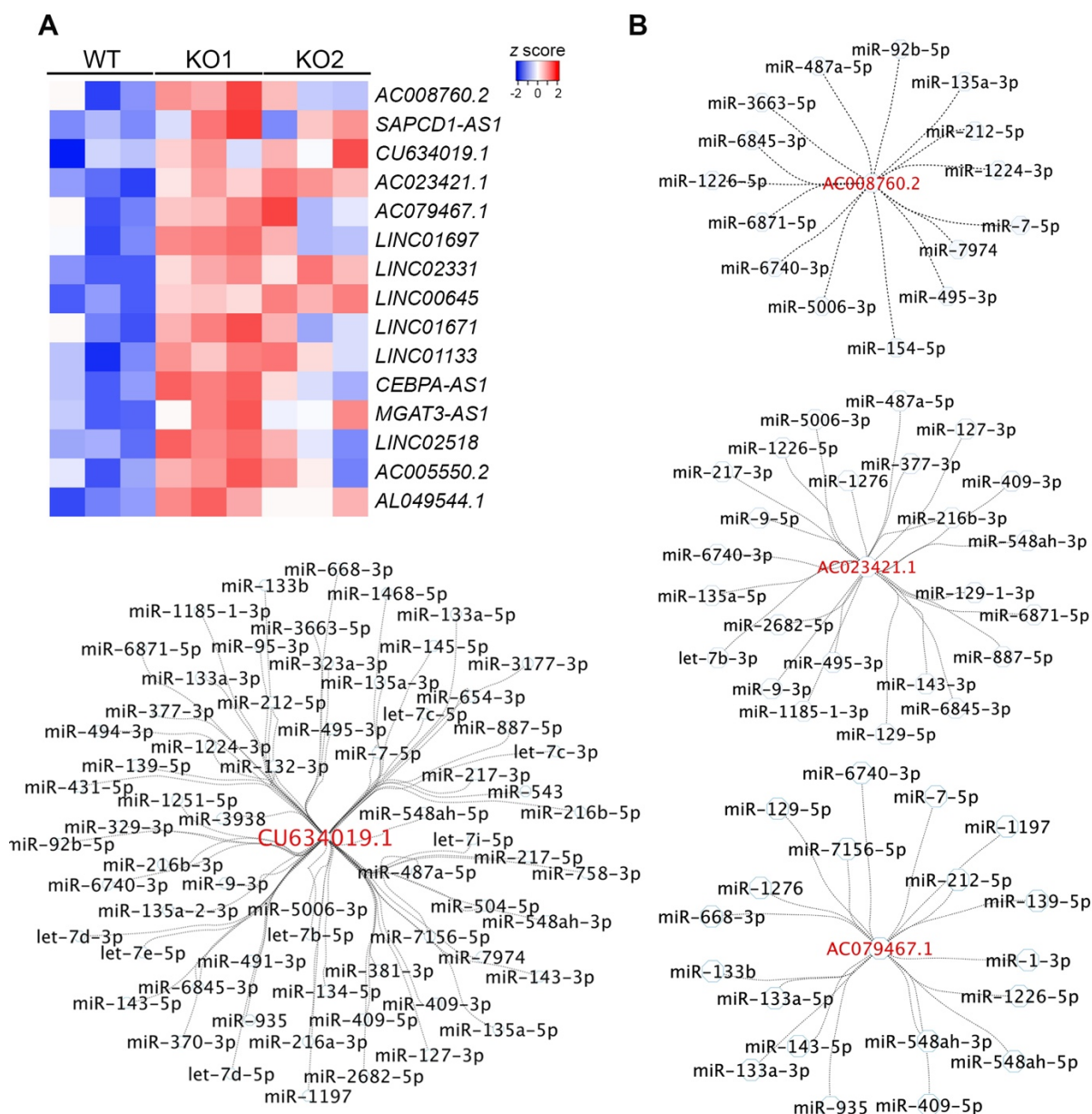


**Figure S3**



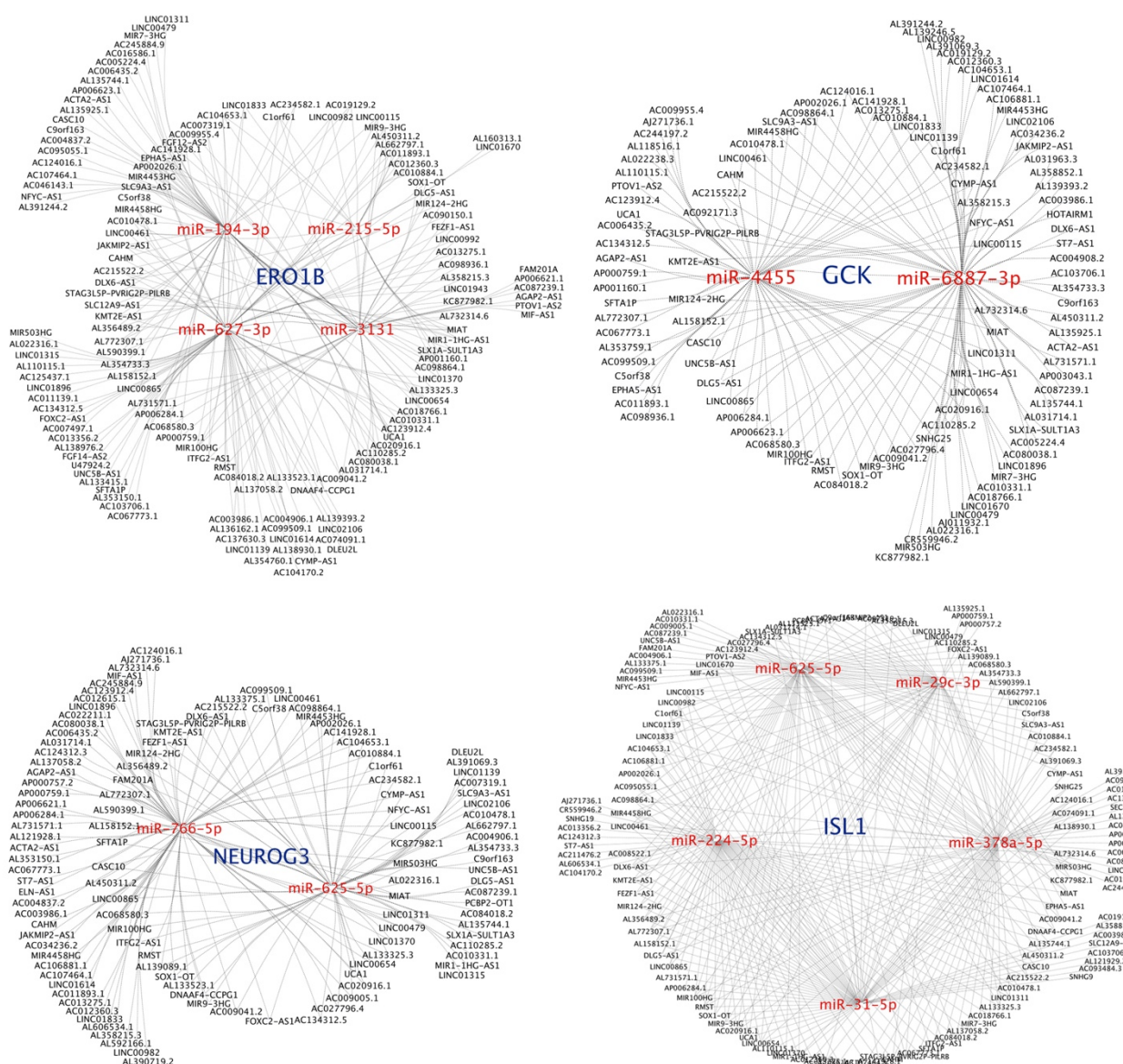
**Figure S3.** Correlation analysis between upregulated DEMiRs aligned against the sequences of 196 significantly downregulated DElncRNAs using the default miRanda algorithm.

### Figure S4



**Figure S4. lncRNA-miRNA network analysis in RFX3-deficient islets.** (A) Heatmap of the significantly upregulated DElncRNAs ( $n=3$ ) in *RFX3* KO islets ( $n=3$ ). (B) Networks of the top upregulated DElncRNAs and their interacting downregulated miRNAs in *RFX3* KO islets.

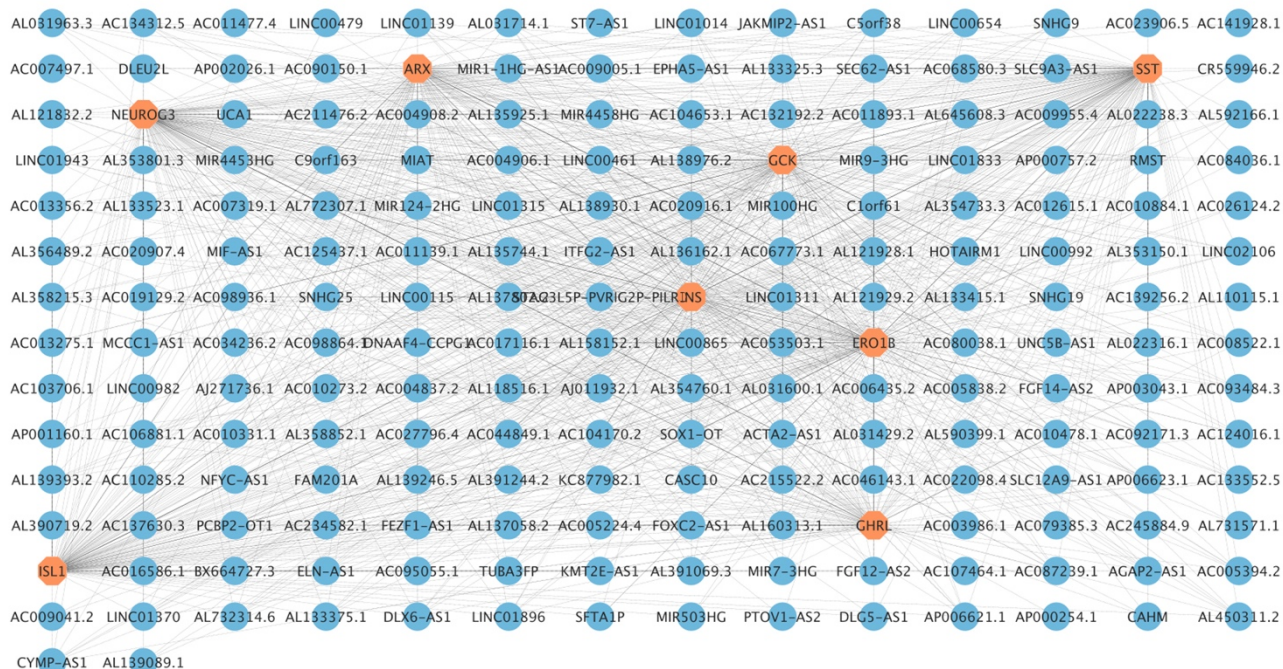
**Figure S5**



**Figure S5.** Representative miRNA-mRNA-lncRNA correlation networks in RFX3-deficient pancreatic islets. The networks illustrate predicted interactions between DE miRNAs, DE lncRNAs, and DEGs. miRNA target prediction was performed using Ingenuity Pathway Analysis (IPA) and miRanda for DEGs and DE lncRNAs, respectively. Selected pancreatic islet markers were used to construct ceRNA networks. Networks were generated using Cytoscape software.

**Figure S6**

**A**

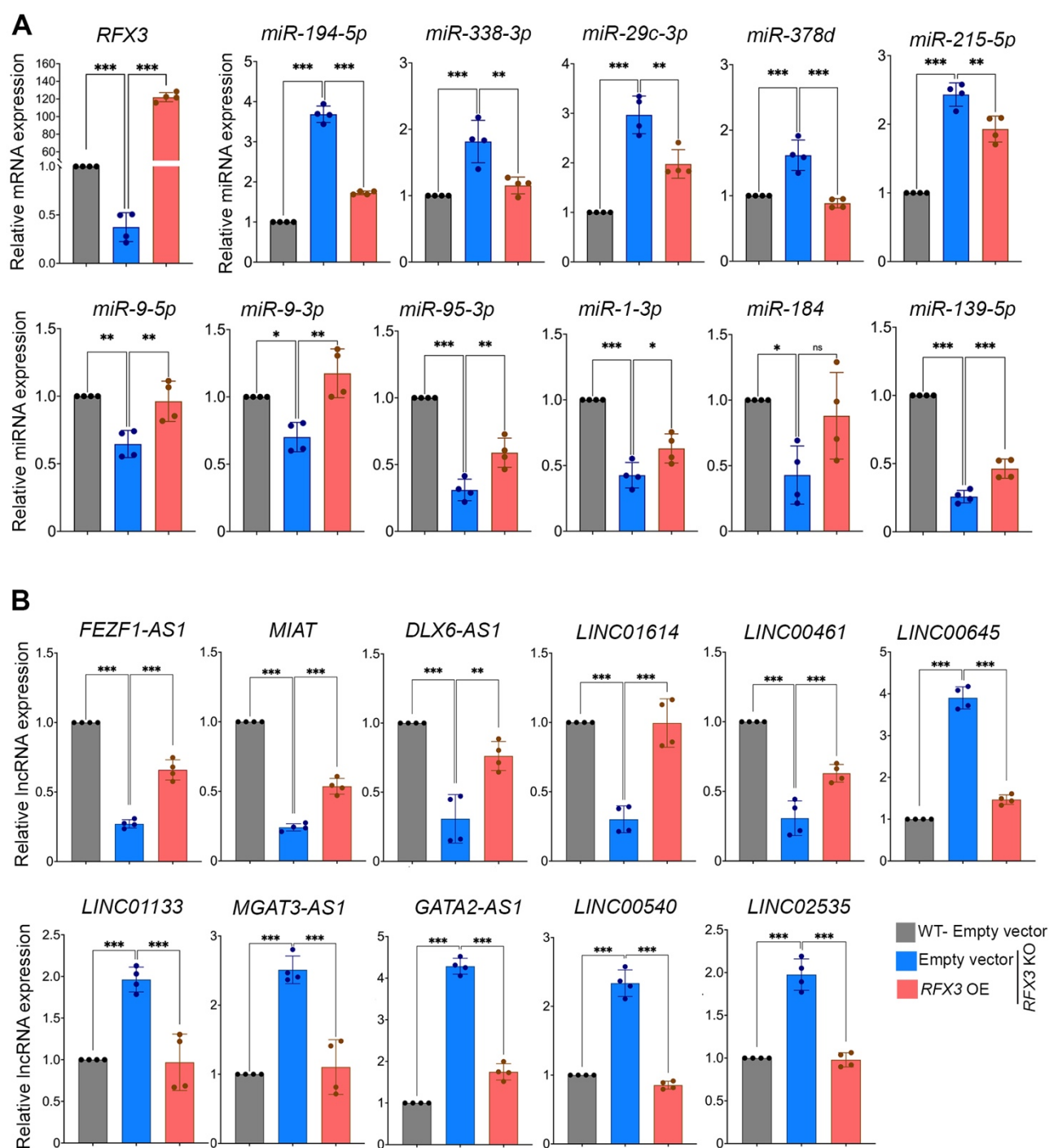


**B**



**Figure S6. Direct correlation analysis between DElncRNAs and DEGs.** (A) Co-expression network analysis of the positive correlation analysis between downregulated DElncRNAs and selected downregulated DEGs in pancreatic islets derived from *RFX3* KO iPSCs. (B) Co-expression network analysis of the positive correlation analysis between upregulated DElncRNAs and selected upregulated DEGs in pancreatic islets lacking *RFX3*.

**Figure S7**



**Figure S7: RFX3 overexpression rescues the expression of dysregulated ncRNAs in iPSC-derived pancreatic islets lacking RFX3.** (A) RT-qPCR validation of the effect of RFX3 OE on the selected dysregulated DemiRs in *RFX3* KO islets ( $n=4$ ). (B) RT-qPCR validation of the effect of RFX3 OE on the selected dysregulated lncRNAs in *RFX3* KO islets ( $n=4$ ). Data are represented as mean  $\pm$  SD. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , ns; non-significant.