

## Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: Analysis of differentially expressed genes between *Neurod1ST* mutant and control endocrine cells at E15.5 identified by bulk RNA-seq.

File Name: Supplementary Data 2

Description: Differentially expressed genes between control and *Neurod1CKO* mutant beta cells and alpha cells identified by scRNA-seq.

File Name: Supplementary Movie 1

Description: **E18.5 Control tdTomato GLP1 INS.** Microdissected pancreas of tdTomato reporter *Control-Ai14* mice was cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas at E18.5; tdTomato<sup>+</sup> endocrine cell population (magenta),  $\beta$  cells with expression of insulin (white), and  $\alpha$  cells expressing glucagon-like peptide-1 (GLP1, green).

File Name: Supplementary Movie 2

Description: **E18.5 Neurod1ST tdTomato GLP1 INS.** Microdissected pancreas of tdTomato reporter *Neurod1ST-Ai14* mice were cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas; tdTomato<sup>+</sup> endocrine cell population (magenta),  $\beta$  cells with expression of insulin (white), and  $\alpha$  cells expressing glucagon-like peptide-1 (GLP1, green).