DefiniGEN
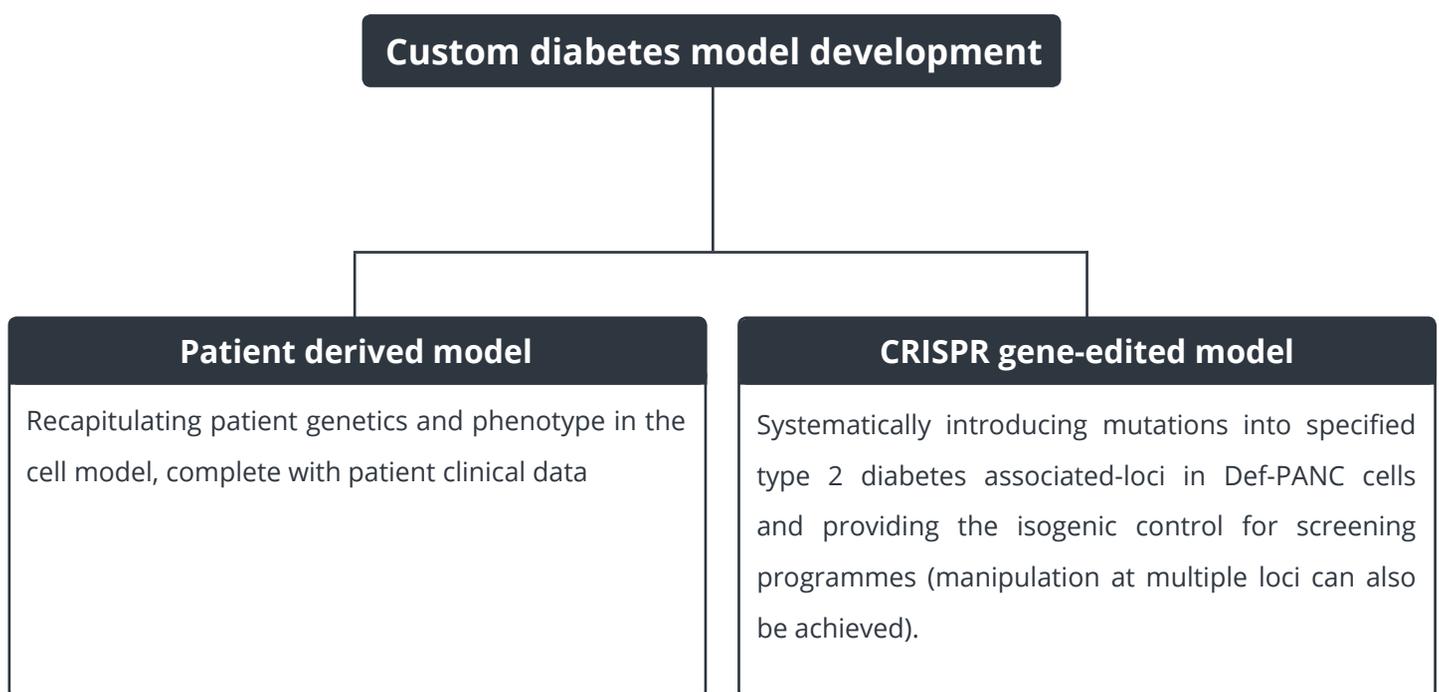

DefiniGEN custom type 2 diabetes disease model development

Currently although over 120 type 2 diabetes-associated loci identified, it has proven challenging to identify causal genes. iPS-derived pancreatic T2D cell models can help to elucidate cellular mechanisms and identify/validate causal mutations. DefiniGEN's mature and functional beta cell phenotype and marker profile provides a human primary-like cell and a more predictive preclinical model for the study of diabetes.

Custom diabetes model development

Patient derived model

Recapitulating patient genetics and phenotype in the cell model, complete with patient clinical data

CRISPR gene-edited model

Systematically introducing mutations into specified type 2 diabetes associated-loci in Def-PANC cells and providing the isogenic control for screening programmes (manipulation at multiple loci can also be achieved).

T2 diabetes gene variants

Melatonin receptor 1B gene (MTNR1B)

Zinc transporter (ZnT8) SLC30A8

Zinc finger MIZ domain-containing protein 1 (ZMIZ1) ADP-ribosylation factor-like 15 (ARL15)

Thyroid adenoma (THADA)

Sprouty homolog 2 (Spry2)

StAR-related lipid transfer protein 10 (STARD10)

Advance your Diabetes research and drug discovery

The Def-PANC cell products are highly functional iPS-derived pancreatic cells. Yamanaka iPS technology in combination with fully defined differentiation conditions enable the generation of standardized populations of pancreatic cell products. Through a 25 day differentiation process the cells proceed through key developmental stages ultimately producing functional pancreatic cells.

Monolayer culture
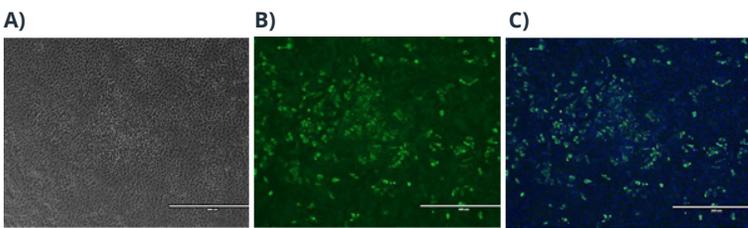
Figure 1. Pancreatic cell type distribution in monolayer. When grown in standard laboratory 96 well plates Def-PANC cells show typical tightly-packed pancreatic cell morphology (A) and a high proportion of C-peptide secretion from beta cells (green) (B). Panel C depicts DAPI staining of nuclear DNA (blue) as well as c-peptide (green).

Microislet formation
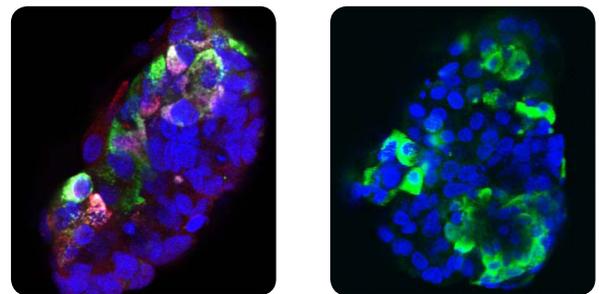
Figure 2. Immunostaining analysis of Def-PANC cells. Microislet formation of Def-PANC in low adherent plates. Insulin secreting beta cells (green) are the dominant cell population observed alongside lower populations of glucagon and somatostatin expressing cells (size 100 - 150 µm)

## Glucose stimulated insulin secretion assay (GSIS)
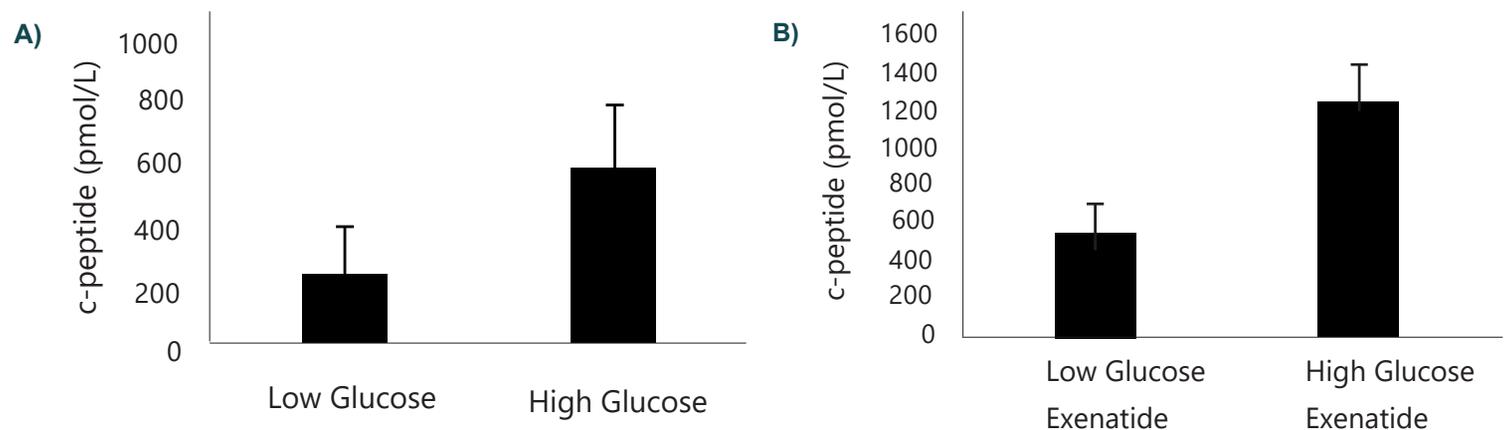
Figure 3. Def-PANC WT cells display a dose dependent GSIS response to key diabetes reference drugs. (A) shows GSIS assay results for Def-PANC cells stimulated with low and high concentrations of glucose only. Panel **(B)** overviews the GSIS response of Def-PANC cells stimulated with low and high concentrations of glucose and exenatide. Low glucose concentration 1.6mM, high glucose concentration 16.7mM, exenatide concentration 25nM.

Modelling MODY3 Diabetes

MODY3 diabetes is caused by mutations in the HNF1-alpha gene which is a key regulatory transcription factor controlling the downstream regulation of multiple genes involved in the differentiation of beta cells. These cell products can offer disease modelling and drug discovery researchers unique tools for elucidating the mechanistic basis of MODY. Our custom services can also engineer bespoke mutations for additional forms of the eleven known types of MODY.

Cell morphology

In contrast to Def-PANC WT cells which display normal tightly packed pancreatic cell morphology, Def-PANC MODY3 cells display aberrant morphology expected from developmental retardation caused by the HNF1a mutation.
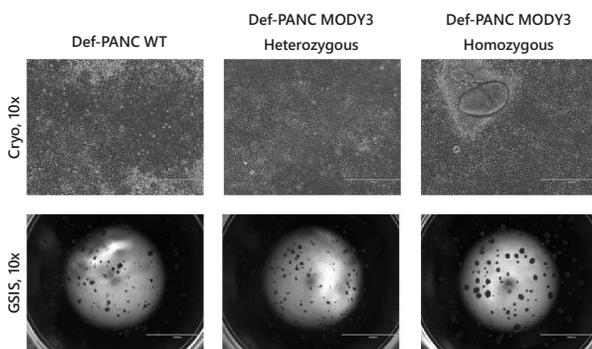
Figure 1. Morphological analysis of Def-PANC MODY cells. Def-PANC MODY3 cells display aberrant morphology in both monolayer and microislet culture.
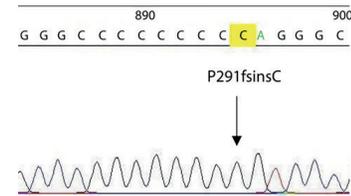
Pancreatic key marker analysis

The expression of crucial pancreatic genes is sequentially reduced in disease modelled Def-PANC cells.
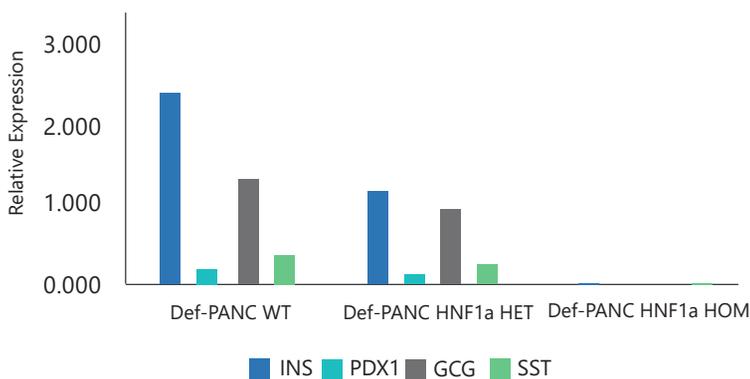

Figure 2. Analysis of pancreatic-specific markers in Def-PANC MODY3 cells. Key marker analysis by qPCR shows the expected reduction in key pancreatic cell markers in the Def-PANC disease models.

Disease circuit verification

Figure 3. Confirmation of disease circuit. Sanger sequencing shows heterozygous HNF1a mutation with single base insertion of a C in a polyC tract around codon 291 for Proline.

GLUT-2 + PKLR expression analysis
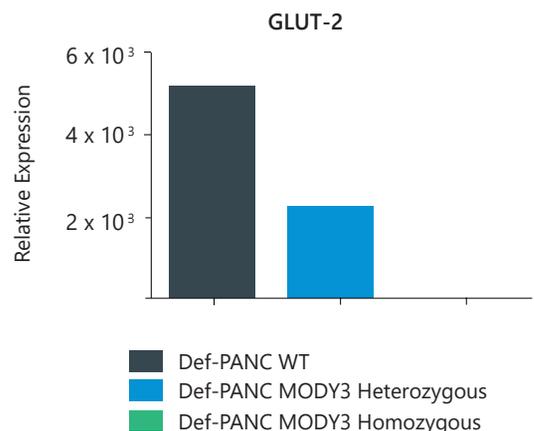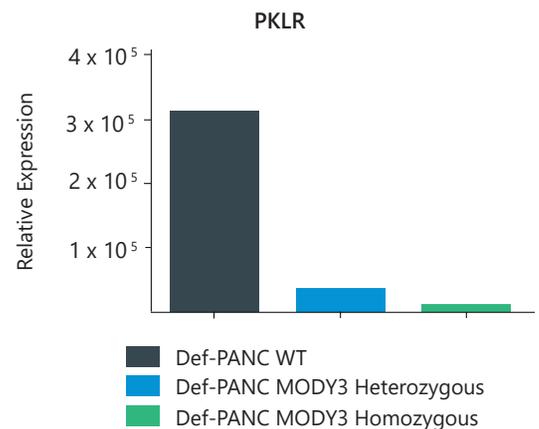
Figure 4. Down regulation of pancreatic function in MODY cells. Heterozygous and homozygous Def-PANC MODY3 cells show progressive gene change effects in key HNF1a gene regulated genes including PKLR and GLUT-2.

Modelling Neonatal Diabetes

DefiniGEN's neonatal diabetes human pancreatic cells are an effective model of this form of monogenic diabetes. Neonatal diabetes mellitus (NDM) is a rare but potentially devastating metabolic disorder characterized by hyperglycemia combined with low levels of insulin. The neonatal diabetes cell products display the disease phenotype in combination with general function comparable to human primary pancreatic islets.
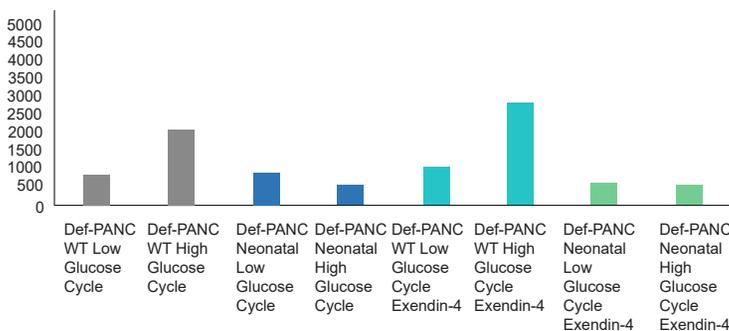
Cell Morphology

Def-PANC WT cells and Neonatal disease model variants display typical pancreatic cell morphology.
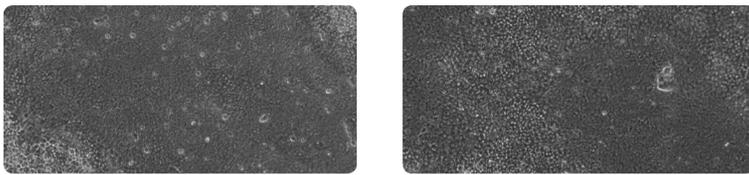
Figure 1. Morphological analysis of Def-PANC KCNJ11 disease modelled pancreatic cells. Morphology of pancreatic cells grown as a monolayer.

Glucose stimulated insulin secretion assay

Figure 3. Def-PANC WT isogenic control shows expected glucose cycling GSIS response at low and high concentrations. The Def-PANC Neonatal disease model shows dysfunction in its glucose responsive insulin production in contrast to the isogenic WT control. Genetic information and phenotypic GSIS response confirms that Def-PANC Neonatal cells are an effective model of Neonatal Diabetes.

Disease circuit verification
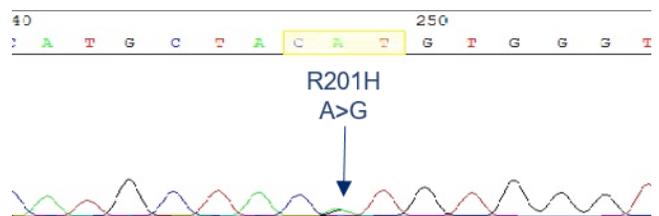
Figure 2. Confirmation of disease circuit. Sanger sequencing showing heterozygous KCNJ11 mutation with single base change (CAT>CGT) at codon 201 in the gene encoding for the ATP-sensitive potassium channel subunit Kir6.2.

References:

Modelling Neonatal and MODY Diabetes in Vitro Using IPS Cell-derived human Pancreatic Beta Cells. Soares F, Santos R, Schofield C, Lowe C, Vallier L. **ISSCR 2016** June 22-25.

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