

## Supplementary Materials

### Methods

#### Immunofluorescence staining

Cell clusters were obtained from 1.5-mL tubes after suspension culture for insulin staining. The cell clusters were then incubated with 4% paraformaldehyde for 15 minutes at 4 °C and washed thrice in phosphate-buffered saline. Subsequently, they were incubated with 0.1% Triton X-100 for 10 minutes and with 10% normal donkey serum for 1 hour at room temperature. The primary antibodies, that is, anti-KLF4 (222235; Abcam), anti-TRA-1-60 (MAB4360; Millipore Sigma), and anti-TRA-1-81 (MAB4381; Millipore Sigma), were incubated at 4 °C overnight. On the next day, they were incubated with a secondary Cyanine3 (Jackson ImmunoResearch)-conjugated antibody for 2 hours at room temperature. Subsequently, they were stained with 4'6-diamidino-2-phenylindole (DAPI; Vector Laboratories) for nucleic acid staining. Images were obtained using a Zeiss LSM700 confocal microscope (Carl Zeiss MicroImaging GmbH).

#### Karyotype analysis

Pluripotent cells were cultured in culture plates coated with Matrigel in conditioned media for 3 to 5 days. The cells were transported GenDix Research Center (GenDix Inc.) where cell harvest and karyotype analysis of metaphase chromosomes was performed using G-banding.

#### Cell Counting Kit-8 assay

Induced pluripotent stem cells (iPSCs) were differentiated in 96-well microplates for the Cell Counting Kit-8 (CCK-8) assay. After differentiation, the cells were subjected to various tacrolimus (Tac) treatments for specified durations. CCK-8 solution (CK04-01; Dojindo Molecular Technologies) was added to each well for 2 hours. Subsequently, absorbance was measured at 450 nm using a VersaMax ELISA Reader (Molecular Devices).

### Results

#### Induced pluripotent stem cell characterization

Using the patient iPSCs generated, we confirmed the expression of the pluripotency markers KLF4, TRA-1-61, and TRA-1-81 at the protein level using immunofluorescence (Supplementary Fig. 1A, available online). To confirm that the iPSCs generated were genomically normal, we analyzed their karyotypes using the GTG banding method. The iPSCs showed a normal karyotype of 44 + XX or 44 + XY, except in the case of DM-C (trisomy 20) (Supplementary Fig. 1B, available online).

#### Effect of tacrolimus on insulin expression in patient-specific induced pluripotent stem cells

We confirmed the pluripotency of patient-specific iPSCs from the diabetes mellitus (DM) and non-DM groups. To examine Tac-induced toxicity, we differentiated the patient iPSCs (non-DM individuals, n = 4; DM patients, n = 4) in a 96-well plate. On the next day, Tac was administered for 24 hours at serial doses of 0, 30, 40, 50, and 60 µg/mL, and toxicity was confirmed via a cell viability assay involving CCK-8 (Supplementary Fig. 2A, available online). We calculated the area under the curve, indicating the individual cell viability rates at various Tac levels and exposure times (Supplementary Fig. 2B, available online); the findings did not significantly differ between the non-DM and DM groups. Consistent with these results, the insulin messenger RNA levels at the end of the experiment did not differ between the groups (Supplementary Fig. 2C, available online).