Supporting Information

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SI Materials and Methods

Immunostaining and Alkaline Phosphatase Staining. Immunofluorescence staining was performed as described previously (1). Primary antibodies were mouse monoclonal antibodies to β-tubulin III (TUJ1; Babco), AFP (Sigma-Aldrich), Gb3 (Seikagaku), α-actinin (Sigma-Aldrich), cardiac troponin T (Thermo Fisher Scientific), SSEA1 (Santa Cruz Biotechnology), and rabbit polyclonal antibodies to GFAP (Dako), desmin (Sigma-Aldrich), GST-π (MBL), GATA-4 (Santa Cruz Biotechnology), nestin (Abcam), and E-cadherin (Cell Signaling Technology). Cytochemical staining of alkaline phosphatase activity was performed using AP staining kit (Sigma-Aldrich) according to manufacturer's recommendation.

Cardiomyocyte Differentiation and Treatment with Diltiazem. EB formation in suspension culture was initiated as described above. After 4 days, EBs were collected and plated onto gelatin-coated 24-well plates (one EB per well) in differentiation medium. The medium was replaced every 2 days. The presence of spontaneous beating areas was monitored. Beating was observed 6 days after attachment. The beating areas were dissected under a microscope and seeded on gelatin-coated dishes or coverslips for further analysis. Some EBs with a beating frequency of ~30 beats/min were treated with diltiazem (Sigma-Aldrich) at 10^{-9} to 10^{-4} M. Three independent EBs were analyzed for each concentration of diltiazem, and the beating frequency was monitored for 1 min after adding the drug.

Neural Stem Cell Differentiation. EB formation in suspension culture was induced and maintained in DMEM with 15% FBS, 2 mM L-glutamine, 0.1 mM nonessential amino acid, and 0.1 mM 2-mercaptoethanol for 4 days. Then medium was switched to neural basal medium (Invitrogen) supplemented with G-5 supplement (Invitrogen), 10 ng/mL of recombinant human basic fibroblast growth factor (bFGF; Invitrogen), and 2 mM of L-glutamine for an additional 4 days. EBs were plated on gelatin-coated dishes and maintained in the foregoing medium for 8 days. The neural epithelium–like cells that migrated from EBs were harvested by mild trypsinization. The EBs were removed by settlement. Cells in the supernatant were seeded on collagen-coated dishes in neural basal medium supplemented with N2-supplement (Invitrogen), 200 μM ascorbic acid (Sigma-Aldrich), and 10 ng/mL bFGF.

GALC Enzyme Assay. Homogenates (10 μL) made in water were added to 20 μL of substrate mixture consisting of 0.4 mM substrate, 0.3% sodium taurocholate, and 0.09% oleic acid in citric acid/phosphate buffer (pH 5.2). The enzymatic reaction was performed at 37 °C for 16 h in 200-μL PCR tubes, and then was terminated by the addition of 0.2 M glycine-NaOH buffer (pH 10.7) with 0.2% SDS and Triton X-100. Fluorescence was measured at excitation wavelength at 360 nm and at emission wavelength at 448 nm. Activity was expressed as nmol/mg/16 h. Protein concentration was determined using BCA protein assay reagent (Pierce).

Western Blot Analysis. EBs in suspension cultures were collected on day 4, washed with PBS, and then lysed in RIPA buffer with protease inhibitor mixture (Santa Cruz Biotechnology). Clarified lysates (15 μg protein) were denatured in sample buffer with 2.5% 2-mercaptoethanol at 98 °C for 5 min. Electrophoresis and Western blot analyses were performed as described previously (1). Primary antibodies used were rabbit polyclonal antibodies to CD44 (Santa Cruz Biotechnology) and E-cadherin (Cell Signaling Technology), mouse monoclonal antibodies to PCNA (Dako) and β-actin (Sigma-Aldrich), and rabbit monoclonal antibody to activated caspase-3 (Epitomics).

1. Shen JS, et al. (2008) Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule expression in Fabry disease endothelial cells. *Mol Genet Metab* 95:163–168.

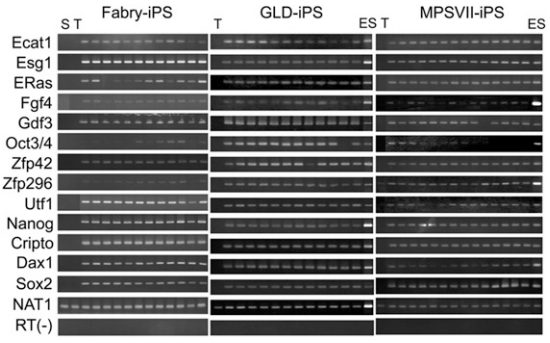


Fig. S1. Expression of ES marker genes in iPS cells. The expression of ES marker genes in these iPS cells was analyzed by RT-PCR. More than 10 iPS clones from each mouse model were analyzed. S, SNL feeder cells; T, tail-tip fibroblasts; ES, embryonic stem cells; RT(-), NAT1 PCR products amplified from cDNA samples without reverse transcriptase.

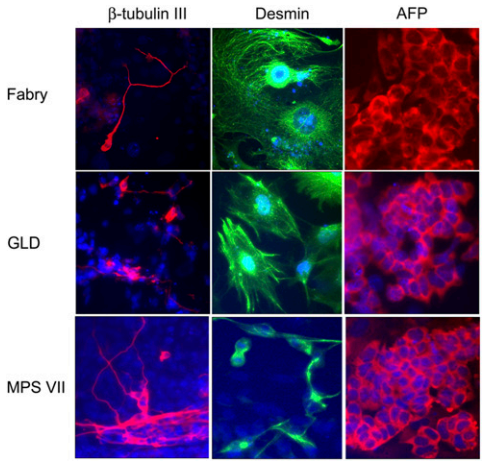
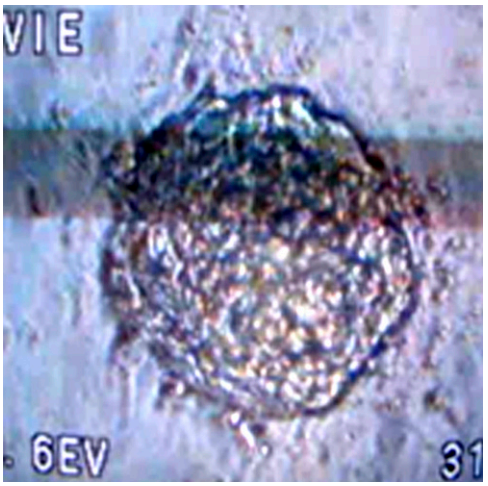


Fig. S2. In vitro analysis of pluripotency of disease-specific iPS cells. Fabry-, GLD-, and MPSVII-iPS cells were allowed to be differentiated in vitro. Cells positive for β-tubulin III, desmin, and AFP were detected in all three disease-derived iPS cells.



Movie S1. Lineage-directed differentiation of Fabry-iPS cells into cardiomyocytes. The differentiated cells demonstrated spontaneous contraction suggesting they are cardiomyocytes.

Movie S1