Supporting Information

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SI Materials and Methods

Cells. Gaucher disease (GD) human fibroblasts were derived from skin biopsy of patients with types 1, 2, and 3 GD, harboring N370S/N370S, L444P/Rec*NciI*, and L444P/L444P mutations in glucocerebrosidase (GC), respectively. Skin biopsies were obtained under Institutional Review Board-approved and informed consent by E.S.'s laboratory (National Institutes of Health, Bethesda, MD). GD fibroblasts were maintained in DMEM supplemented with 15% (vol/vol) FBS (HyClone), Glutamax (Invitrogen), and penicillin/streptomycin. HEK 293T cells were obtained from J. Silvio Gutkind (National Institutes of Health, Bethesda, MD) and were maintained in DMEM/10% (vol/vol) FBS/penicillin/streptomycin/glutamine. Control iPS-DF4-7T.A cells and H9/WA09 human embryonic stem cells (hESC) were purchased from the WiCell Repository. hESC and human induced pluripotent stems cells (hiPSC) were grown in hESC/hiPSC medium: DMEM-F12 (Invitrogen), 20% (vol/vol) Knockout Serum Replacement (Invitrogen), L-glutamine, penicillin/streptomycin, β-mercaptoethanol (β-ME), nonessential amino acids (NEAA), and 10 ng/mL basic fibroblast growth factor (bFGF) (Stemgent). DR4 mouse embryonic fibroblasts (MEF) were obtained from embryonic day 13.5 (13.5E) embryos of DR4 male (1) and CF1 female mice and were maintained in fibroblast culture medium.

Plasmid DNAs. The hSTEMCCA vector encoding OCT4, SOX2, KLF4, and c-MYC and the packaging plasmids HDM-Hgpm2, RC-CMV-Rev1b, HDM-tat1b, and HDM-VSV-G were obtained from G. Mostovslavsky (Boston University, Boston, MA) (2).

Antibodies. Mouse antibodies to OCT4, SSEA-4, TRA-1–60, TRA-1–81, SSEA-1, and rat anti–SSEA-3 were from Millipore (ES Cell Marker Kit; catalog no. SCR002); mouse anti-MAP2, rabbit anti-SOX2, and anti-GFAP were from Millipore (Neural Stem Cell Characterization Kit, catalog no. SCR019); mouse anti-dopamine β hydroxylase (DβH) antibody was from Millipore (catalog no. MAB308); rabbit anti-NANOG was from Abcam (catalog no. ab21624). Rabbit anti-GATA4 (catalog no. sc-25310) and anti-brachyury (catalog no.sc-20109) were from Santa Cruz Biotechnology. Mouse anti-tyrosine hydroxylase (TH) (catalog no. T2928) and rabbit anti-GABA (catalog no. SAB4501067) were from Sigma-Aldrich. Mouse anti-Tuj1 (catalog no. MO15013) and anti-O4 (catalog no. MO15002) were from Neuromics. Rabbit anti-GlcCer IgG was from Glycobiotech GmBH (catalog no. RAS-0011). Rabbit polyclonal anti-GC has been described (3). PE-conjugated mouse anti-NANOG (catalog no. 560483), anti–TRA-1-60 (catalog no. 560193), anti–TRA-1-81 (catalog no. 560161), anti-CD68 (catalog no. 556078), anti-CD163 (catalog no. 556018), anti-CD34 (catalog no. 555822), allophycocyanin (APC)-conjugated anti-CD14 (catalog no. 555399), and anti-CD11b (catalog no. 550019) were from BD Bioscience. Phycoerythrin (PE)-conjugated mouse anti-SOX2 (catalog no. IC2018P), anti-OCT4 (catalog no. IC1759P), and APC–SSEA-4 (catalog no. FAB1435A) were from R&D Systems. Secondary antibodies DyLight 488- or 549-conjugated mouse, rabbit or rat Ig-specific antibodies were from Jackson ImmunoResearch Laboratories.

Virus Preparation. HEK 293T cells were cotransfected with hSTEMCCA, HDM-Hgpm2, RC-CMV-Rev1b, HDM-tat1b, HDM-VSV-G, using Trans-IT (Mirus) as described (2). After 48 h, the viral supernatant was harvested and concentrated by ultracentrifugation as previously described (4); then the virus particles were resuspended in 1/100 of the original volume and stored at −80 °C until use.

hiPSC Generation. hiPSC generation was carried out as described (2). Human fibroblasts were seeded in gelatin-coated 6-cm plates at cell densities of 5×10^4 and 1×10^5 cells per plate in fibroblast growth medium. The next day, the cultures were infected with concentrated hSTEMCCA virus in the presence of 5 μg/mL of Polybrene for 24 h. Then the culture medium was replaced with hESC/hiPSC culture medium. Five days postinfection cells were split and plated at a density of 2×10^4 infected cells per 6-cm plate on irradiated DR4 MEFs (1) and were cultured in hiPSC medium for 15–30 d. Colonies with typical hESC-like morphology began emerging 10–15 d postinfection. These colonies were manually picked on days 18 and 31 postinfection and were plated onto irradiated DR4 MEFs. Upon growth and development into typical hiPSCs, the colonies were passaged into six-well plates and further expanded to form stable cell lines for characterization. Of 50 hiPSCs manually picked from the plates, five hiPSC lines of each genotype were chosen and expanded for further characterization.

Karyotype Analysis. On the day of karyotyping, 20 randomly selected metaphases from GD hiPSC clones were fully analyzed, and three cells were karyotyped at the Cytogenetic Core Facility at the Johns Hopkins Cancer Center.

In Vitro Differentiation of hiPSC to Three Germ Layers. Human embryoid bodies (EBs) were maintained in six-well ultra-low-attachment plates in EB culture medium for 15–20 d with half of the medium changed every other day. EB medium consisted of DMEM-F12, 20% (vol/vol) Knockout Serum Replacement, L-glutamine, β-ME, and NEAA.

Teratoma Formation from GD hiPSC in NOG/SCID Mice. Six- to eight-week-old male NOG/SCID mice (NOD.Cg-Prkdc <scid> Il2rg <tm1Sug>/JicTac; Taconic Farms) were injected s.c. with the indicated hiPSC lines. One six-well plate of cells was prepared per mouse. The cells were grown to 90% confluence before harvesting using a cell scraper without enzymatic treatment. The cell pellet was resuspended carefully in 200 μL of DMEM-F12. An equal volume of Growth Factor-Reduced Matrigel (BD Biosciences) was added to the cells, mixed, and then placed immediately on ice. The cell/Matrigel mixture was injected s.c. into the hind legs of the mice, and the mice were monitored weekly for teratoma formation. Once the teratomas reached 1.5 cm in diameter (about 8–10 wk postinjection), they were harvested and fixed in 4% (vol/vol) paraformaldehyde. Tumors were subsequently embedded in paraffin and sectioned, followed by staining with H&E.

Differentiation of GD hiPSC into Monocytes. Directed differentiation of GD hiPSC to monocyte/macrophages was carried out essentially as described (5). For EB formation, hiPSC were detached from plate and feeder cells by treatment with 0.2% dispase for 4 min and were collected by scraping. The hiPSC were transferred into six-well, ultra-low-attachment plates (Costar) in EB culture medium and were cultured for 4 d at 37 °C. For monocyte differentiation, 4–10 large EBs were transferred into gelatin-coated six-well plates containing monocyte differentiation medium (MDM) [DMEM (Sigma), 10% (vol/vol) FBS, 50 ng/mL human macrophage colony-stimulating factor (hM-CSF), 25 ng/mL hIL-

3, 1 mM L-glutamine (GIBCO), 1× NEAA, and 0.1 mM β-ME]. Medium was replaced every 4 d. The hM-CSF concentration was increased to 100 ng/mL after the first medium change. Continuous monocyte production started within 15–20 d, and monocytes were harvested every 4–5 d.

Differentiation of Monocytes into Macrophages. Monocytes harvested from EB factories were resuspended in macrophage differentiation medium [RPMI/10% (vol/vol) FBS, supplemented with 100 ng/mL hM-CSF, glutamine, and penicillin/streptomycin] and plated in four-well chamber slides at a density of $2–3 \times 10^4$ cells per well or at equivalent densities in plates. Medium was changed every 2–3 d.

Differentiation of GD hiPSC to Neuronal Cells. EBs were maintained in EB culture medium for 10 d with half the medium changed on alternate days and then for another 4 d in EB medium supplemented with 5 μM dorsomorphin and 10 μM of the TGF-β receptor inhibitor SB431542 (Sigma-Aldrich) (6). Then EBs were transferred to Matrigel (BD Biosciences)-coated four-well chamber slides (one EB per well) or 60-mm plates (four EBs per plate) containing DMEM-F12 medium with N2 supplement (GIBCO) and 20 ng/mL bFGF, with a medium change every other day. Within ∼5 d, the hiPSC aggregates attached and formed individual colonies. Neuroepithelial cells, organizing into neural tube-like rosettes, developed within 10 d. The neural rosettes were picked manually and replated on Matrigel-coated chamber slides in the same medium. Spreading of neuronal cells started within 2 d. To generate mature neurons, the rosettes were triturated and plated on Matrigel-coated plates and were maintained for another 10–15 d in medium without bFGF, during which time the neurons matured and established interconnections. Neural progenitors capable of differentiating into neurons can be maintained on Matrigel-coated plates with a change of medium every 2 d and a plate change every 12–15 d.

May–Grünwald–Giemsa Staining for Macrophages. Macrophages cultured in four-well chamber slides were stained with May–Grünwald (MG 500)–Giemsa (G-9641; Sigma-Aldrich) according to the manufacturer's instructions.

Immunofluorescence Analysis. ***Staining of hiPSC for pluripotency surface markers.*** Chamber slides (Lab-Tek) containing hiPSC plated on MEFs were fixed using 4% (vol/vol) paraformaldehyde in PBS for 15 min, and slides were processed for immunostaining as previously described (7). The following primary antibodies were used at the dilution specified in parentheses: mouse anti-OCT4 (1:50), mouse anti–SSEA-4 (1:50), mouse anti–TRA-1–60 (1:50), rat anti–SSEA-3 (1:50), mouse anti–SSEA-1 (1:50), mouse anti–TRA-1–81 (1:50), rabbit anti-SOX2 (1:50), and rabbit anti-NANOG (1:30). hiPSC also were analyzed for pluripotency marker expression by flow cytometry as described below.

Staining of EBs for trilineage marker analysis. EBs were fixed in 4% (vol/vol) paraformaldehyde for 15 min and then were processed for immunostaining. Two or three EBs were used for each staining. The antibodies used were rabbit anti–GATA-4 (1:50), rabbit anti-brachyury (1:50), mouse anti-Tuj1 (1:50), mouse anti-MAP2 (1:50), and rabbit anti-GFAP (1:50). Stained EBs were mounted on glass slides.

Staining of monocytes and macrophages. Monocytes from EB factory supernatants were collected by centrifugation at $500 \times g$ for 5 min and were fixed in 4% (vol/vol) paraformaldehyde. The cells were then stained using APC-conjugated mouse anti-CD14 antibody. Stained cells were seeded onto glass slides by cytospin centrifugation (750 rpm for 5 min). Monocytes also were analyzed by flow cytometry using APC-conjugated anti-CD14 to determine monocyte yields. Macrophages grown on chamber slides were fixed and stained with the indicated antibodies.

Staining of neuronal cells. Neurons that were differentiated and grown on Matrigel-coated chamber slides for more than 2 wk were stained as described above. Antibodies used were mouse anti-Tuj1 (1:50), mouse anti-MAP2 (1:50), rabbit anti-GFAP, mouse anti-O4 (1:50), mouse anti-tyrosine hydroxylase (TH) (1:50), rabbit anti-GABA (1:50), mouse anti-DβH (1:50), and rabbit anti-Sox2 (1:50). Antibody-antigen reaction was developed by species- and Ig-specific DyLight 488- or 549-conjugated secondary antibody (1:100). Cell nuclei were stained with DAPI-containing mounting medium (Vectashield; Vector Laboratories). As negative controls, the primary or secondary antibodies were omitted in the immunostaining procedures. Staining was visualized with a Zeiss Axioscope II fluorescence microscope.

Gel Electrophoresis and Immunoblotting. Cells were lysed directly in Tricine sample buffer. Twenty microliters of protein lysate was loaded onto 16.5% (wt/vol) Tricine SDS/PAGE gel (Bio-Rad) to separate the proteins, followed by transfer of the proteins onto 0.2-μm nitrocellulose membrane. Membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% (wt/vol) nonfat dry milk (blocking buffer) overnight and were incubated with primary antibodies for 2 h. Then membranes were washed, followed by a 1-h incubation in blocking buffer containing HRP-conjugated secondary antibodies. Films were developed using ECL Plus Western Blot detection reagent (Amersham).

Flow Cytometry. ***hiPSC analysis.*** Seventy to eighty percent confluent GD hiPSC were treated with 0.05% trypsin-EDTA (Life Technologies) for 5 min at 37 °C. Cell suspensions were stained with mouse monoclonal anti-human antibodies directed against APC-SSEA-4, PE-TRA-1-60, PE-TRA-1-81, PE-SOX2, PE-OCT4, or PE-NANOG. For staining of SOX2, OCT4, and NANOG, cells were fixed and permeabilized using Fix & Perm Cell Fixation and Permeabilization kit (Life Technologies catalog no. GAS-003) before antibodies were added. Isotype-matched antibodies were used as controls. Data were acquired by flow cytometry using a BD LSRII Flow Cytometer and were analyzed using FlowJo software (Tree Star Inc.).

Monocytes and macrophages. Induced monocytes and macrophages were fixed in 4% (vol/vol) paraformaldehyde, washed with PBS, and incubated in blocking buffer consisting of PBS, human IgG (1 mg/mL; Sigma), 8% (vol/vol) FBS, and 0.01% sodium azide. Cells were incubated with the indicated antibodies for 1 h in buffer containing PBS, 0.2% saponin, 8% (vol/vol) FBS, and sodium azide. Then cells were washed three times in blocking buffer without human IgG, were washed twice with PBS, and were kept at 4 °C until FACS analysis.

Analysis of Cytokine mRNA Induction by LPS. GD and control hiPSC-macrophages (3×10^5 cells per well) were cultured in 12-well plates for 5 d. Then cells were incubated with 100 ng/mL LPS for the indicated times. After incubation, mRNA was isolated using an RNA isolation kit (Qiagen). cDNA was synthesized using the iScript kit (Bio-Rad). Transcriptional regulation of different cytokines in the macrophages before and after treatment was analyzed by quantitative PCR (7900 HT; Applied Biosystems) using the SYBR Green method. The relative mRNA expression of the corresponding cytokine was normalized to the values of GAPDH mRNA for each reaction. The following primers were used: TNF-α, 5′-CACTTTGGAGTGATCGGCC (forward) and 5′-CTCAGCTTGAGGGTTTGCTACAAC (reverse); IL-10, 5′-GCTGTCATCGATTTCTTCCC (forward) and 5′-CTCATGG-CTTTGTAGATGCCT (reverse); IL12-p35, 5′-TCAGCAACAT-GCTCCAGAAG (forward) and 5′-CCTCCACTGTGCTGGTT-TTAT (reverse); and IL12-p40, 5′-GTTTCAGGGCCATTGGACT (forward) and 5′-GAGATGCCAGAAAAACCAGG (reverse).

GC Assay. The assay for GC enzymatic activity in cells was carried out as described (8). Briefly, the intact cell assay reaction was started with the addition of 2.5 mM 4-methylumbelliferyl β-D-glucopyranoside (MUG) in 0.2 M acetate buffer (pH 4.0). The cells were incubated at 37 °C for 6 h, and the reaction was stopped with the addition of 0.2 M glycine buffer (pH 10.8). Released 4-methylumbelliferone was measured using a fluorescence plate reader (excitation 365 nm, emission 445 nm). To control for non-GC enzymatic activity, 1 mM conduritol B epoxide was added to replicate wells for the duration of the assay. For GC enzymatic activity in cell lysates, cells were lysed as described (9). Cell lysates were assayed in 0.1 M acetate buffer (pH 5.0) containing 2.5 mM MUG in the presence of 0.15% Triton X-100 and 0.15% taurodeoxycholate. After incubation at 37 °C the reaction was terminated, and fluorescence intensity was measured as described above.

Phagocytosis Assay for Macrophage Function. Sheep RBC (Lampire Biologicals) were washed several times with saline and opsonized by incubation with anti-sheep RBC IgG (10 μL for 1×10^9 cells in 1 mL) (Cordis Laboratories) at 37 °C for 1 h. Then RBC were washed with saline to remove the unbound antibody. Macrophages cultured in chamber slides ($2–5 \times 10^4$ cells per well) for 5 d were incubated with opsonized RBC ($2–5 \times 10^6$ cells per well) at 37 °C for 1–4 h. After incubation, the cultures were washed to remove excess RBC and were treated with ammonium-chloride-potassium lysing buffer (Lonza), to remove RBC still attached on the surface of the macrophages (10). The macrophages were incubated further for several days, as indicated in the text, and clearance of RBC was monitored by direct microscopic observation. A minimum of 300 macrophages per field was scored for the presence of ingested RBC. Assays were carried out in triplicate. Non-opsonized RBC were used as negative controls.

Treatment with Recombinant GC. GD hiPSC-macrophages were treated with the indicated concentrations of recombinant human GC (Cerezyme; Genzyme) for 30 min. Treated macrophages were incubated with opsonized RBC for 2 h and washed as described above. Then the macrophages were incubated in Cerezyme-supplemented medium for 3 d and were monitored for RBC clearance. Cerezyme was obtained from remnants in patient-infusion bags.

Treatment with Isofagomine. GD hiPSC-monocytes were plated in macrophage differentiation medium supplemented with the indicated concentrations of isofagomine (Toronto Research Chemicals). After 5 d, the treated macrophages were incubated with opsonized RBC for 2 h, and then washed. Then the cells were cultured in the presence of isofagomine and were monitored for RBC clearance for 3 d.

Analysis of Glucosylsphingosine by HPLC-MS/MS. To measure glucosylsphingosine, 500 μL of 2:3 (vol/vol) $CHCl_3$:MeOH solution was added to cell lysates in 2-mL siliconized Eppendorf tubes. The samples were sonicated for 5 min followed by vortexing for 30 s. The cell extracts were cleared by centrifugation at 13,000 × *g* for 5 min. The supernatant was collected and evaporated to dryness using a rotatory evaporator (Heto Vacuum Centrifuge; ATR, Inc.). The residue was dissolved in 500 μL of water, and 500 μL each of $CHCl_3$ and 2-BuOH were added to the samples. The samples were vortexed for 30 s followed by centrifugation at 3,000 × *g* for 10 min. The lower organic phase was collected, and the sample was re-extracted using 1 mL of 1:1 (vol/vol) $CHCl_3$:2-BuOH. The combined extracts were evaporated to dryness under a nitrogen stream at 50 °C (reacti-Vap III; Pierce). The extracted glucosylsphingosine was resuspended in 75 μL of 1:1 (vol/vol) buffer A and B solutions (see below) and was analyzed by injecting 10 μL of this solution onto a column as described below. Glucosylsphingosine was quantitated by external calibration using a curve prepared from six standard samples of glucosylsphingosine (Avanti Polar Lipids) present in a concentration range spanning 0.02–25 pmol prepared in charcoal-stripped serum. Calibration curves were found to be linear, with correlation coefficients obtained from $1/x^2$ weighted linear regressions of 0.9998. The quantitative results were considered to be the amount of glucosylsphingosine present in the total sample extracted from a single well of a six-well plate.

LC-MS/MS analysis was conducted using a TSQ Discovery triple quadrupole mass spectrometer (Thermo Scientific) coupled to a Shimadzu UFLC XR HPLC (Shimadzu Corp.). Reversed-phase chromatography was conducted using a 5 cm × 1.0 mm × 5 μm Discovery C18 column (Supelco) operating at a flow rate of 100 μL/min and maintained at 37 °C. Mobile phase A consisted of 5 mM $HCOONH_4$ in 2:1 (vol/vol) H_2O/CH_3OH containing 0.5% HCOOH; mobile phase B consisted of 5 mM $HCOONH_4$ in 99.5:0.5 (vol/vol) CH_3OH:HCOOH. For each LC-MS/MS experiment, the extracted samples were diluted in 75 μL of 1:1 (vol/vol) buffer A and B solutions, and 10 μL of this sample solution was injected onto the column. Fractionation was achieved using the following gradient conditions: after sample injection, the initial 2% B gradient was increased to 97% B in 20.80 min and then was increased to 100% B in 0.2 min, where it was held for next 3 min. The gradient was brought to initial conditions in 0.5 min and was held there for next 5.5 min, for a total run time of 30 min.

Electrospray ionization (ESI) source conditions were optimized using a standard containing 1 pmol/μL glucosylsphingosine. The source parameters were as follows: ionization mode, positive; spray voltage, 4,500 V; capillary temperature, 300 °C; skimmer offset, −7 V. Collision-induced dissociation was performed using nitrogen gas within Q2, which was offset from Q1 by 10 V. To measure glucosylsphingosine, single-reaction monitoring measuring the transition *m/z* 462.33 (corresponding to $[M+H]^+$ ion of glucosylsphingosine) to *m/z* 264.18 and 282.23 (corresponding to the double -and single-dehydrated carbocation of the sphingoid-base backbone of the glucosylsphingosine molecule) was performed. The acquisition parameters were scan width (*m/z*) 0.010; scan time, 100 ms for each transition; peak width (FWHM) 0.70 for both Q1 and Q3; collision pressure 1.5 mTorr, and skimmer offset of −7 V. Data acquisition and analysis were accomplished using Xcalibur software v.2.0.5 (Thermo Scientific).

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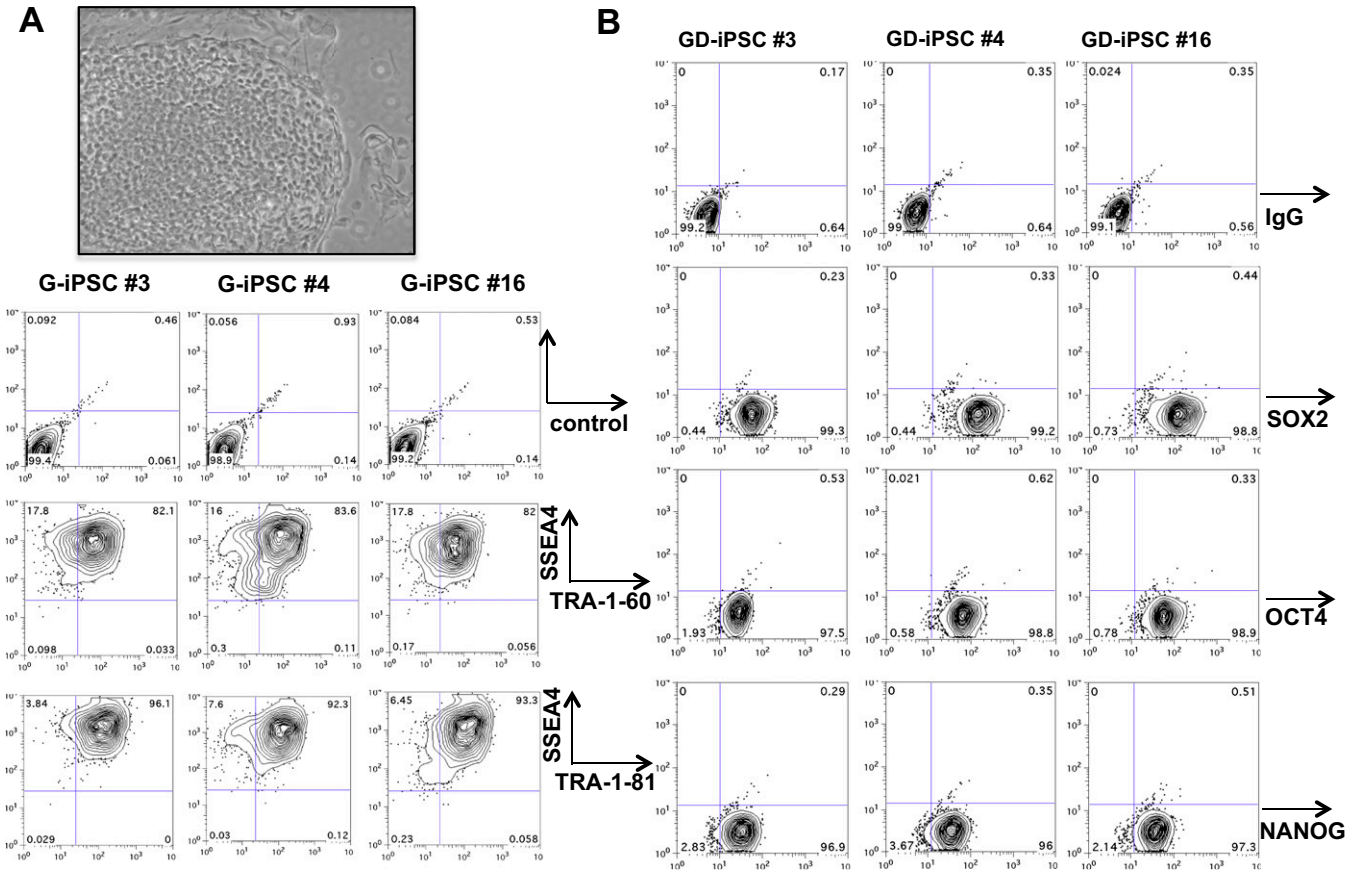

Fig. S1. Characterization of human GD-specific hiPSC by FACS analysis. (*A*) Phase-contrast image of an L444P/Rec*NciI* hiPSC colony 3 d after seeding. (*B*) Three different lines of L444P/Rec*NciI* hiPSC (#3, #4, and #16) were stained with the following antibodies: APC-SSEA-4, PE-TRA-1–60, PE-TRA-1–81, PE-SOX2, PE-OCT4, PE-NANOG, or with the corresponding IgG isotype controls. The stained cells were analyzed by flow cytometry. Contour plots represent the expression of each marker.

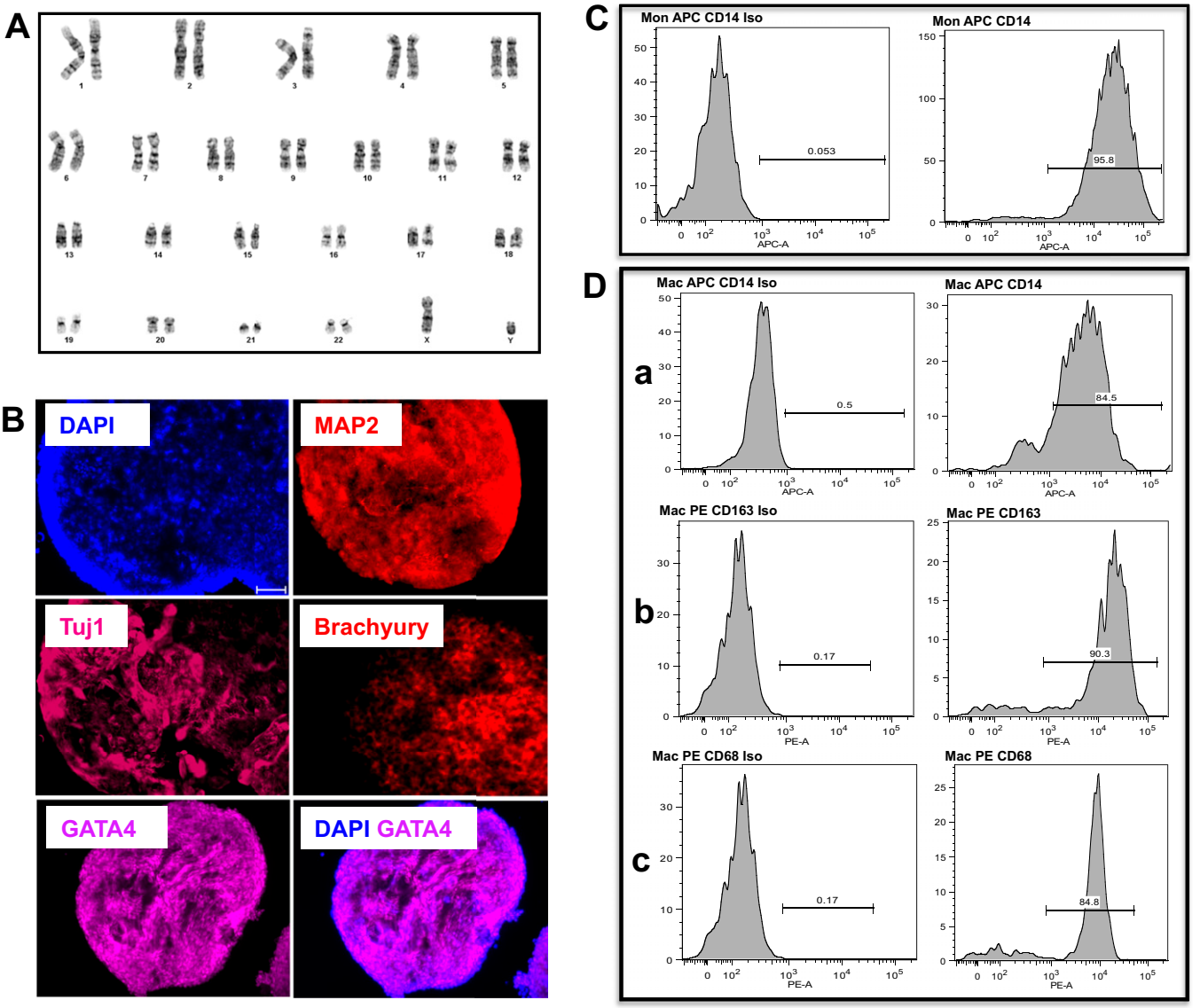

Fig. S2. Karyotype, trilineage differentiation of L444P/Rec*NciI* hiPSC, and characterization of control hiPSC-monocyte/macrophages. (*A*) Normal karyotype of L444P/Rec*NciI* hiPSC. (*B*) EBs differentiated into three germ layers were stained with antibodies to the lineage-specific markers MAP2, Tuj1, Brachyury, and GATA4. Where indicated, nuclei were stained with DAPI. (Scale bar, 100 μm.) (*C* and *D*) FACS analysis of control hiPSC-monocyte/macrophages. Histograms show the percentage of cells stained with antibodies to specific markers (*Right*) and isotype controls (*Left*). (*C*) CD14 expression in monocytes. (*D*) Expression of CD14 (*a*), CD163 (*b*), and CD68 (*c*) in macrophages.

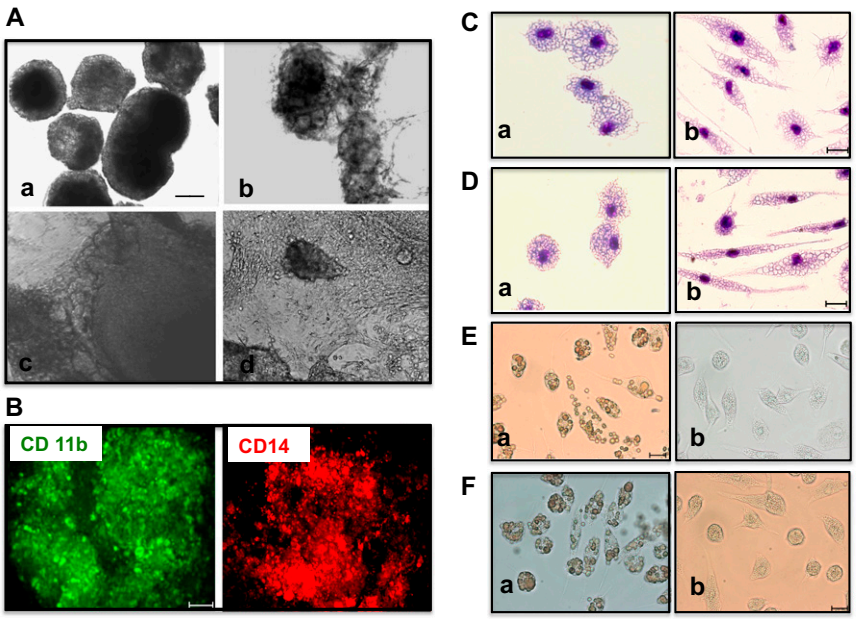

Fig. S3. Setting up L444P/Rec*NciI* monocyte factories and characterization of macrophages. (*A*) (*a*) Four-day-old EBs in suspension culture. (*b*) Initial attachment of EBs to the plate in monocyte differentiation medium. (*c*) Well-established flattened EBs after 12 d are ready to produce monocytes. (*d*) EB factories producing monocytes. (*B*) Staining of flattened EBs showing the expression of CD11b (green) and CD14 (red). (*C*) May–Grünwald–Giemsa staining of control hiPSC-macrophages. (*D*) May–Grünwald–Giemsa staining of H9/hESC-macrophages. Panels *a* and *b* in *C* and *D* show the appearance of macrophages with different morphologies. (*E*) (*a*) Live-cell images of control hiPSC-macrophages with ingested opsonized RBC. (*b*) Non-opsonized RBC control. (*F*) (*a*) Live-cell images of H9/hESC-macrophages with ingested opsonized RBC. (*b*) Non-opsonized RBC control. (Scale bars, 100 μm in *A* and *B*, 20 μm in *C*–*F*.)

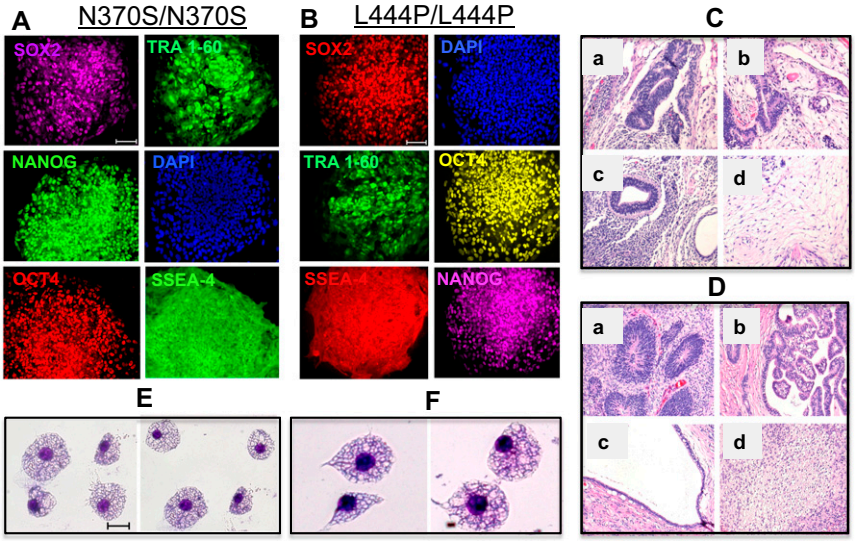

Fig. S4. Characterization of N370S/N370S and L444P/L444P hiPSC. (*A* and *B*) N370S/N370S (*A*) and L444P/L444P (*B*) hiPSC were stained with antibodies to SOX2, OCT4, NANOG, SSEA4, and TRA-1–60. Nuclei were stained with DAPI. (*C*) H&E staining of teratomas induced by injection of N370S/N370S hiPSC. (*a*) Neuronal rosettes. (*b*) Neuronal rosettes and keratinocytes. (*c*) Glandular epithelium. (*d*) Connective tissue. (*D*) H&E staining of teratomas induced by injection of L444P/L444P hiPSC. (*a*) Neuronal rosettes. (*b*) Intestinal epithelium. (*c*) Lung epithelium. (*d*) Connective tissue. (Magnification: 20×.) (*E* and *F*) May–Grünwald–Giemsa staining of N370S/N370S (*E*) and L444P/L4444P (*F*) hiPSC-macrophages. (Scale bars, 100 μm in *A* and *B*, 20 μm in *E* and *F*.)

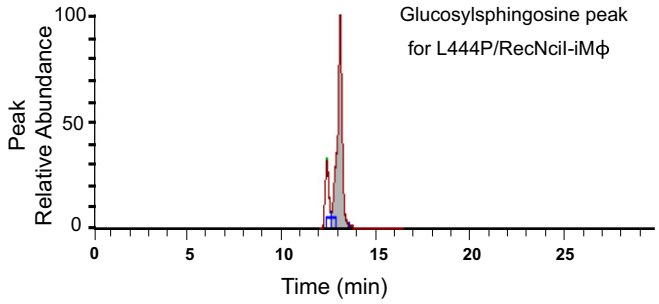

Fig. S5. HPLC-MS/MS analysis of glucosylsphingosine in GD hiPSC-macrophages. Representative chromatogram showing the peak of glucosylsphingosine obtained from HPLC-MS/MS analysis of L444P/Rec*NciI* hiPSC-macrophage samples, processed as described in *SI Materials and Methods*. Glucosylsphingosine in each sample was quantitated by external calibration, using a curve prepared from six standard samples of glucosylsphingosine present in a concentration range of 0.02–25 pmol.

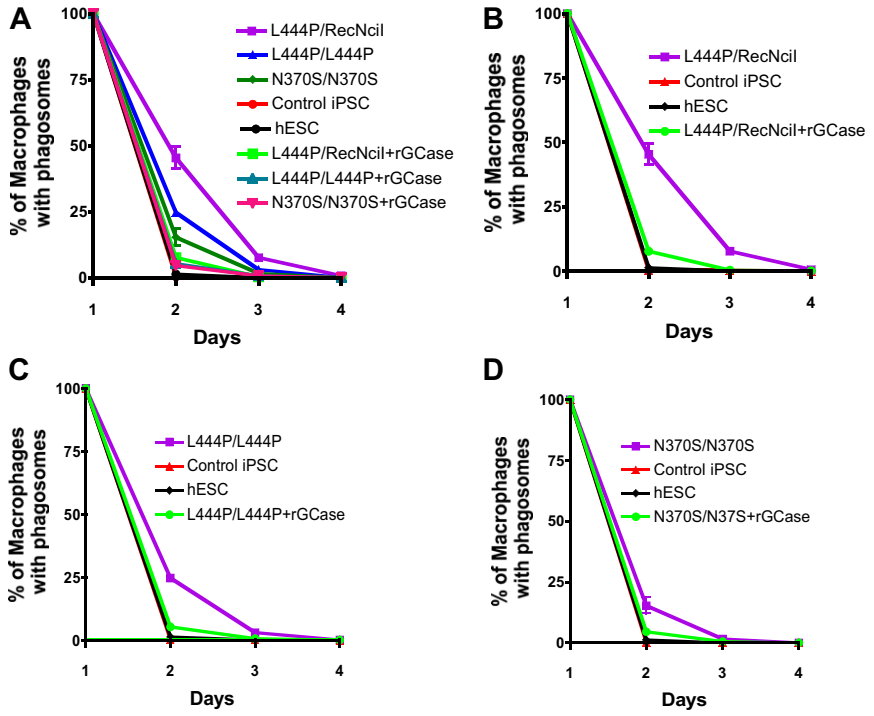

Fig. S6. Recombinant GC reverses the phagocytosed RBC clearance defect of types 1, 2, and 3 hiPSC-macrophages. (*A*) Composite figure with all GD genotypes. The indicated untreated induced macrophages and induced macrophages treated with 0.08 U/mL recombinant GC were assayed for RBC clearance as described in *SI Materials and Methods*. Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/Rec*NciI* hiPSC-macrophages (purple); untreated L444P/L444P-hiPSC-macrophages (dark blue); untreated N370S/N370S-hiPSC-macrophages (dark green); GC-treated L444P/Rec*NciI* hiPSC-macrophages (light green); GC-treated L444P/L444P-hiPSC-macrophages (light blue); GC-treated N370S/N370S-hiPSC-macrophages (pink). (*B–D*) Individual GD genotypes are shown separately for clarity. The indicated untreated induced macrophages and induced macrophages treated with 0.08 U/mL recombinant GC were assayed for RBC clearance as described above. (*B*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/Rec*NciI* hiPSC-macrophages (purple); GC-treated L444P/Rec*NciI* hiPSC-macrophages (light green). (*C*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/L444P hiPSC-macrophages (purple); GC-treated L444P/L444P hiPSC-macrophages (light green). (*D*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated N370S/N370S hiPSC-macrophages (purple); GC-treated N370S/N370S hiPSC-macrophages (light green). On day 2, $P < 0.0001$ (*B*), $P < 0.0006$ (*C*), $P < 0.0334$ (*D*).

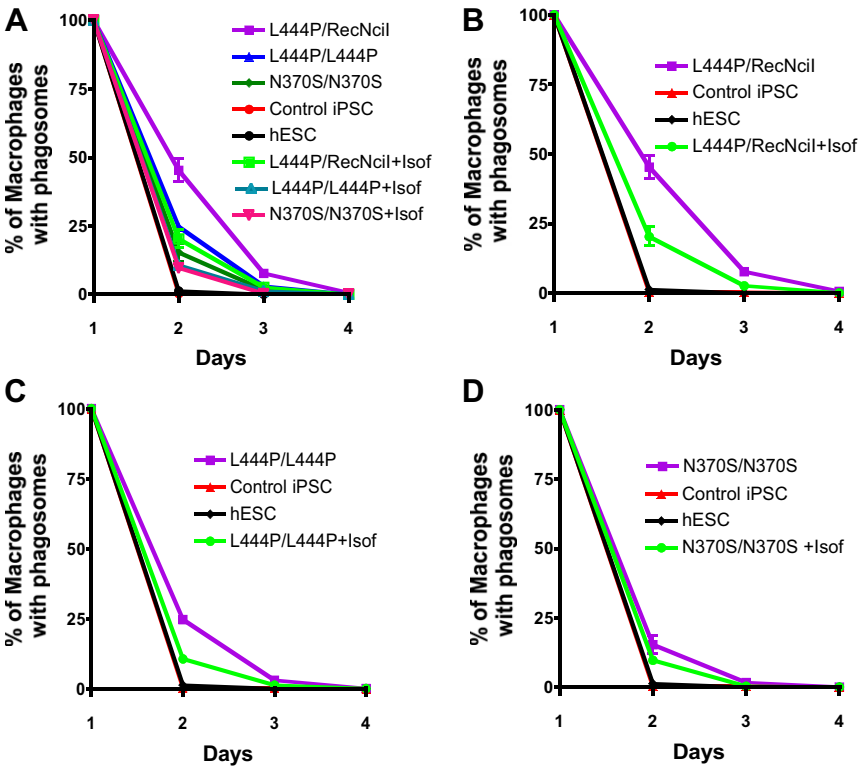

Fig. S7. Response of types 1, 2, and 3 GD hiPSC-macrophages to isofagomine. (*A*) Composite figure. The indicated untreated induced macrophages and induced macrophages treated with 60 μM isofagomine for 5 d were assayed for RBC clearance as described in *SI Materials and Methods*. Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/Rec*NciI* hiPSC-macrophages (purple); untreated L444P/L444P hiPSC-macrophages (dark blue); untreated N370S/N370S hiPSC-macrophages (dark green); isofagomine-treated L444P/Rec*NciI* hiPSC-macrophages (light green); isofagomine-treated L444P/L444P hiPSC-macrophages (light blue); isofagomine-treated N370S/N370S hiPSC-macrophages (pink). (*B–D*) Individual GD genotypes are shown separately for clarity. The indicated untreated induced macrophages and induced macrophages treated with 60 μM isofagomine for 5 d were assayed for RBC clearance as described above. (*B*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/Rec*NciI* hiPSC-macrophages (purple); isofagomine-treated L444P/Rec*NciI* hiPSC-macrophages (light green). (*C*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated L444P/L444P hiPSC-macrophages (purple); isofagomine-treated L444P/L444P hiPSC-macrophages (light green). (*D*) Untreated control hiPSC-macrophages (red); untreated H9/hESC-macrophages (black); untreated N370S/N370S hiPSC-macrophages (purple); isofagomine-treated N370S/N370S hiPSC-macrophages (light green). On day 2, $P < 0.0097$ (*B*), $P < 0.0015$ (*C*), $P < 0.161$ (*D*).

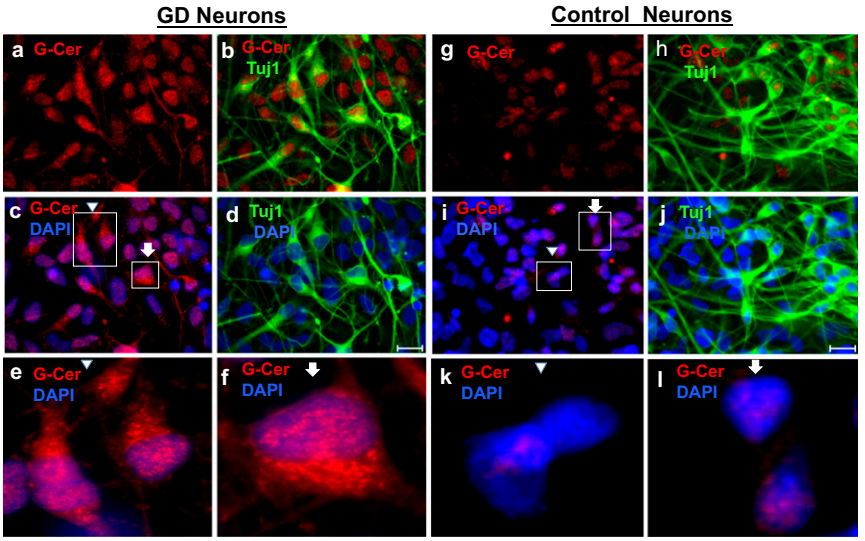

Fig. S8. GD hiPSC-neuronal cells have elevated levels of glucosylceramide. L444P/Rec*NciI* and control hiPSC-derived neurons were stained with antibodies to glucosylceramide (G-Cer, red) and Tuj1 (green) with counterstain of DAPI (blue) as indicated in the figure. Fluorescence images of the stained cells were taken using a fixed exposure for both GD and control hiPSC-macrophages. (*A–F*) L444P/Rec*NciI* hiPSC-neurons. The boxed area denoted by an arrowhead in *C* is shown at higher magnification in *E*; the boxed area denoted by an arrow in *C* is shown at higher magnification in *F*. (*G–L*) Control hiPSC-neurons. The boxed area denoted by an arrowhead in *I* is shown at higher magnification in *K*; the boxed area denoted by an arrow in *I* is showed at higher magnification in *L*. (Scale bars, 20 μm in *A–D* and *G–J*.)