Supplementary Figure Legends

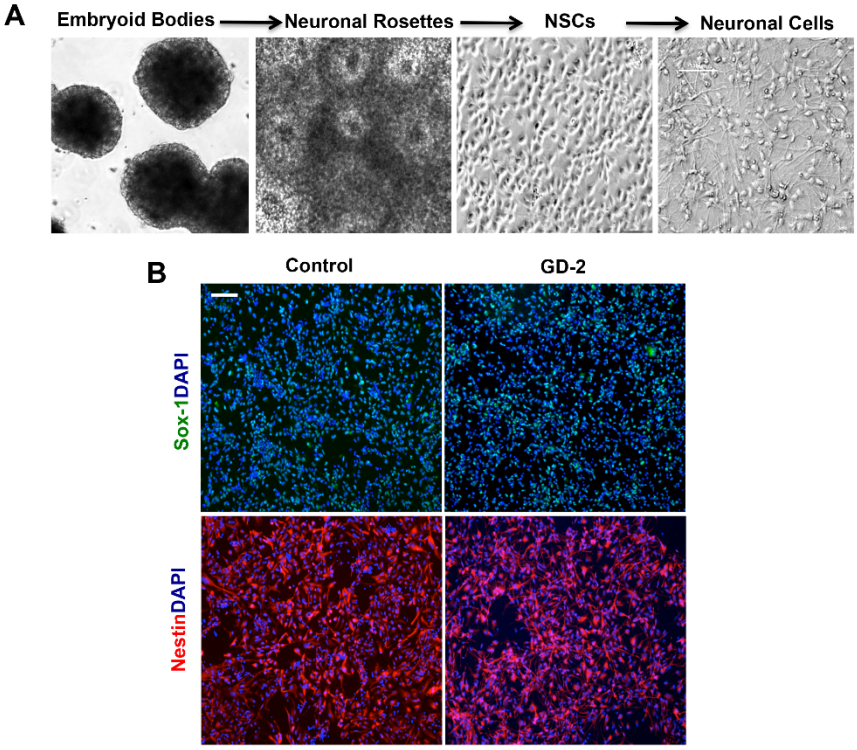

Figure S1. Generation of GD iPSC-NCs. (A) Outline of neuronal differentiation steps showing phase contrast images for iPSCs-derived embryoid bodies, neuronal rosettes and NSCs after expansion in culture. After 3 weeks in neuronal differentiation media, NSCs were differentiated into neuronal cells as shown by the characteristic morphology in last panel. Magnification 10x; scale bar, 100 μm. (B) Representative immunofluorescence images of control and GD-2 NSCs stained with anti-SOX1 (green) or anti-Nestin (red) antibodies, and co-labeled with DAPI (blue). Magnification 10x; Scale bar, 100 μm.

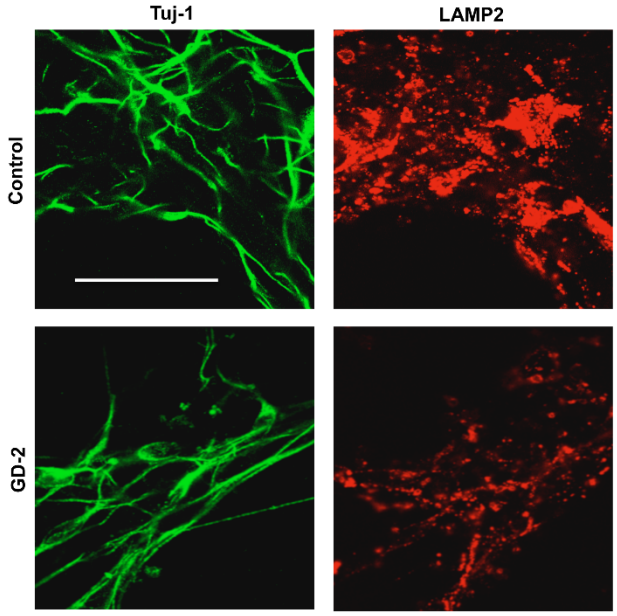

Figure S2. Lysosomal depletion in GD-2 iPSC-NCs. Representative confocal images of control and GD-2 iPSC-NCs stained with anti-Tuj1 (green) and anti-LAMP2 (red) antibodies. Magnification, 63x; Scale bar, 50 μm.

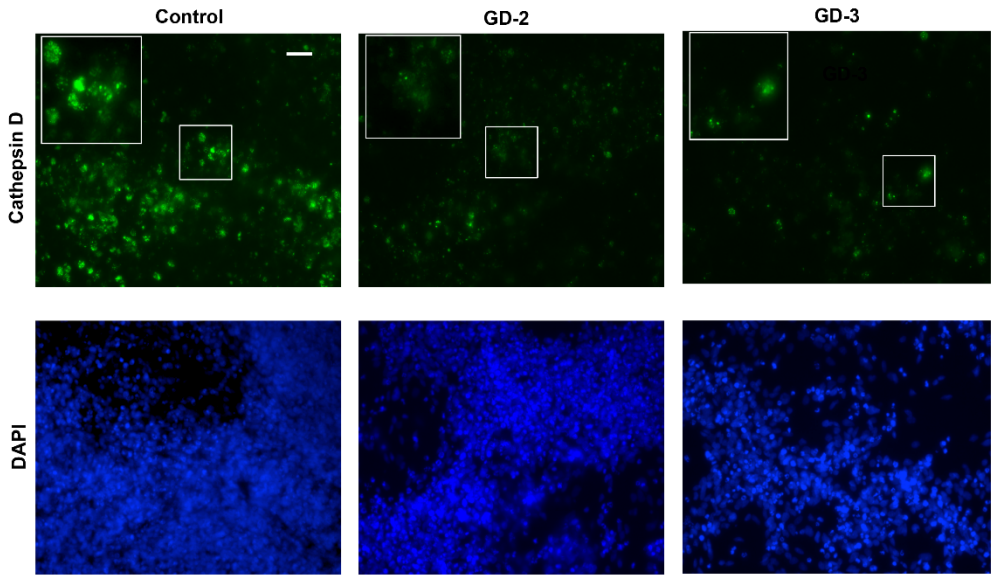

Figure S3. Decreased Cathepsin D level in neuropathic GD-iPSC-NCs. Representative fluorescence images for control, GD-2 and GD-3 iPSC-NCs stained with anti-Cathepsin D (green) antibody and with DAPI (blue); insets are enlarged image of the enclosed area. Magnification 20x; Scale bar, 100 μm.

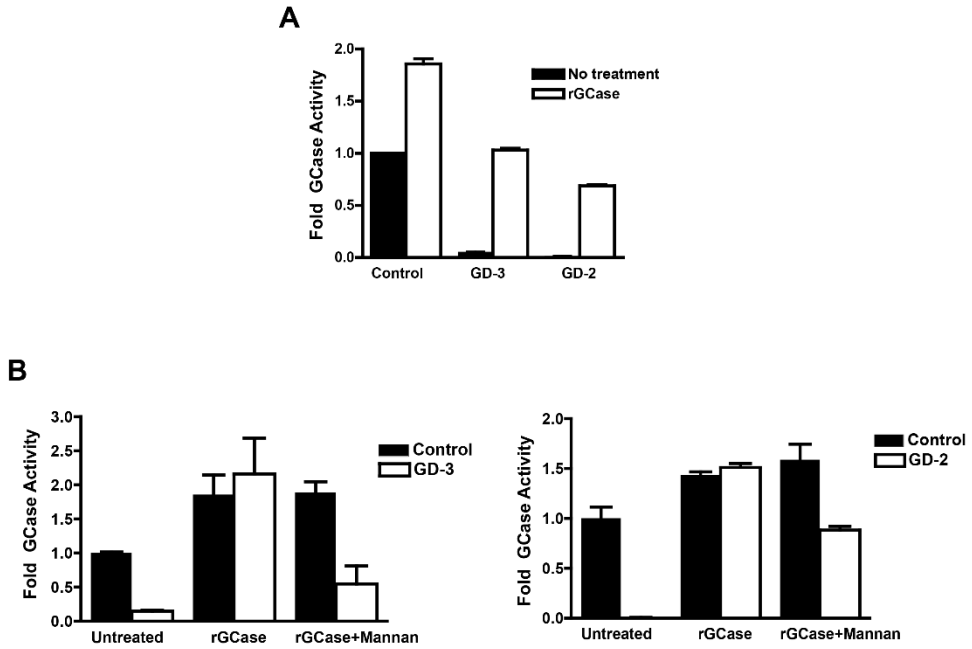

Figure S4. Assay of GCase enzyme activity in iPSC- NCs treated with rGCase in the presence or absence of mannan. (A) GCase enzyme activity assay in cell lysates from control and GD iPSC-NCs that were either left untreated or were incubated with rGCase (0.24 U/mL) for five days. Data represent fold activity relative to untreated control, as measured by fluorescence plate reader in duplicate wells ±SEM. (B) GD iPSC-NCs were incubated in the presence or absence of 2 mg/ml mannan for 1 hour. After 1 hour, cells were treated with 0.24 U/mL rGCase and after another hour, an additional dose of mannan was added to the medium. 16 hours later, the cells were washed and GCase enzyme activity was assayed as described in the Materials and Methods. Data represent fold activity relative to untreated control, as measured by fluorescence plate reader in duplicate wells ±SEM.

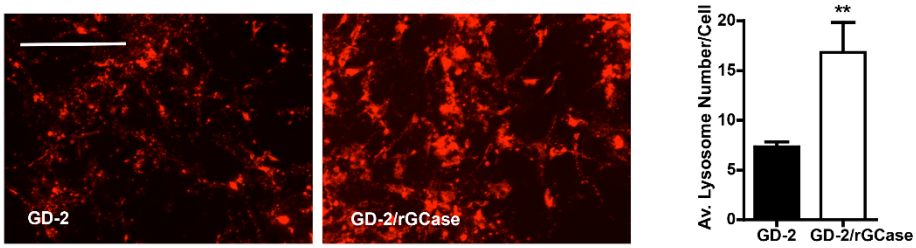

Figure S5. Reversal of GD iPSC-NCs lysosomal phenotype by rGCase treatment.

Representative fluorescence images for GD-2 iPSC-NCs that were either left untreated or were incubated with 0.24 U/mL rGCase for five days, followed by staining with anti-LAMP1 antibody. Magnification 20x; scale bar, 100 μm. Bar graph to the right shows quantitative analysis of number of LAMP1-labeled lysosomes in untreated and rGCase-treated GD-2 iPSC-NCs. Data represent automated compiled measurements from >1000 cells assayed in least 4 independent fields from 2 independent experiments. Error bars = SEM, $p<0.005$ assessed by Student's *t*-test.

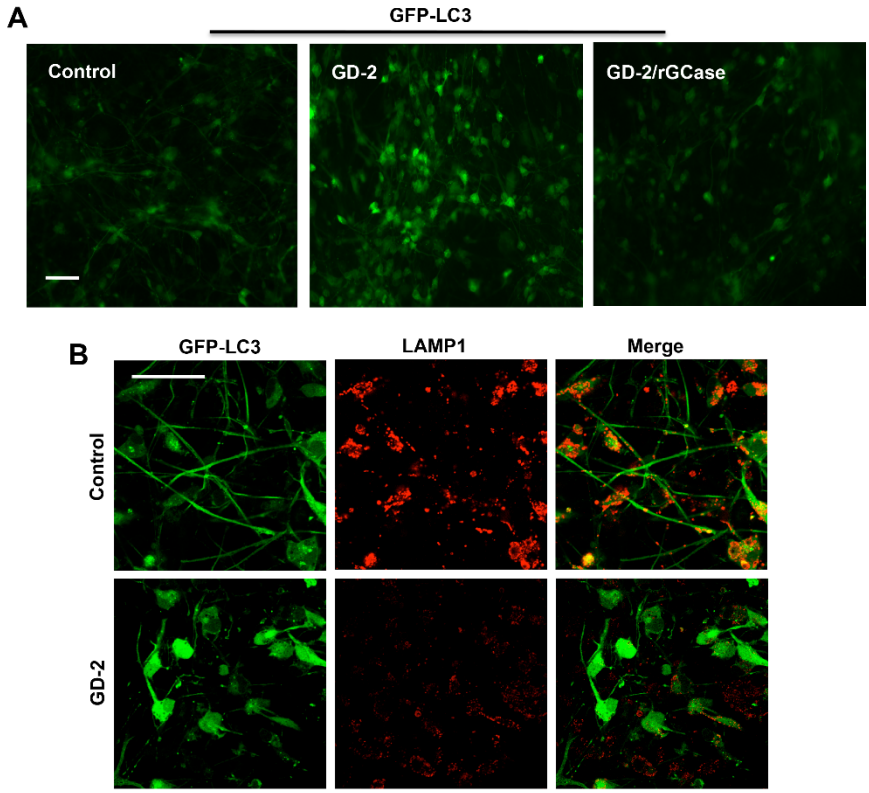

Figure S6. Autophagy phenotype in GD-2 iPSC-NCs. (A) Representative fluorescence images for control and GD-2 iPSC-NCs infected with GFP-LC3 virus and treated with 100 nM Rapamycin for 24 hours. GD-2 iPSC-NCs were either left untreated or were incubated for 5 days in the presence of 0.24 U/ml rGCase before Rapamycin treatment. Magnification 20x; scale bar, 100 μm. (B) Representative confocal images for control and GD-2 iPSC-NCs that were infected with GFP-LC3 virus and then treated with 100 nM Rapamycin for 24 hours, followed by staining with anti-LAMP1 antibody. Green: GFP-LC3; red: LAMP1. Overlay of the two markers is shown in right panel. Magnification 63x; scale bar, 50 μm.

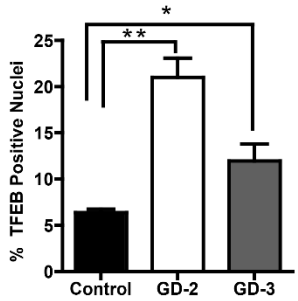

Figure S7. TFEB nuclear localization in GD iPSC-NCs. Quantitation of the number of cells with TFEB nuclear localization in control, GD-2 and GD-3 iPSC-NCs. Bar graph represents the percentage of TFEB-positive nuclei as assessed by TFEB immunoflurescence signal co-localization with DAPI, relative to total cell number. Data represent cell numbers counted in five different high power fields in a representative experiment ± SEM. *$p<0.05$ and **$p<0.005$ between the indicated groups as assessed by One-way ANOVA.

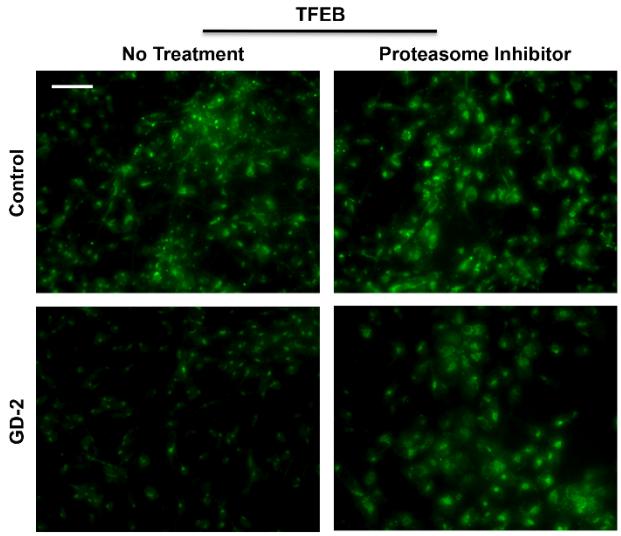

Figure S8. TFEB degradation in GD-2 iPSC-NCs. Representative fluorescence images for control and GD-2 iPSC-NCs that were either left untreated or were treated with proteasome inhibitor as described in the Materials and Methods. Cells were stained with anti-TFEB antibody (green). Magnification 20x; scale bar, 100 μm.

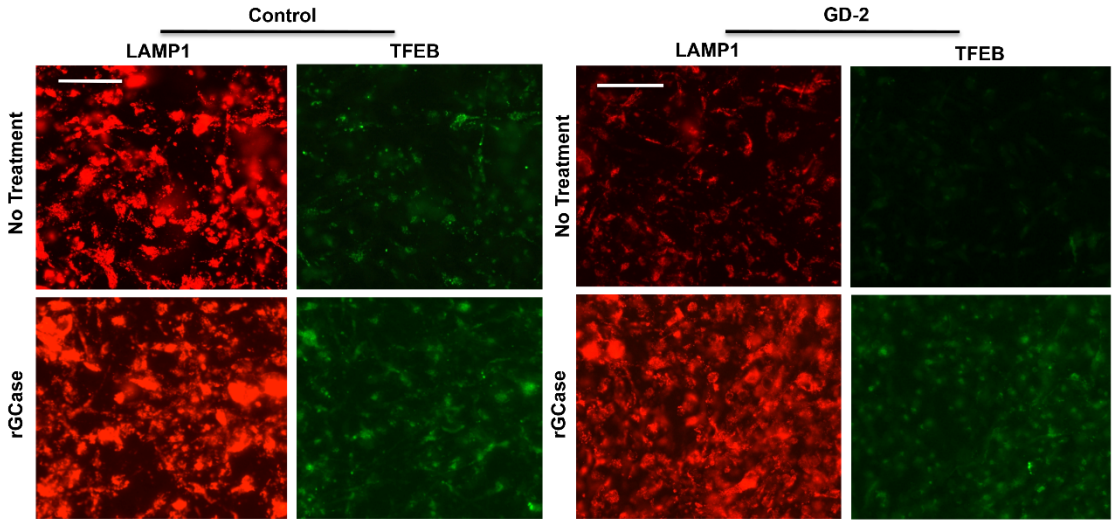

Figure S9. Decreased levels of LAMP1 and endogenous TFEB in GD-2 neuronal stem cells (NSC). Representative fluorescence images for control and GD-2 NSCs that were either left untreated or were treated with rGCase for 5 days as described in the Materials and Methods. Cells were stained with antibodies to LAMP1 (red) or TFEB (green). Magnification 20x; scale bar, 100 μm.

Supplementary Table 1. Genotypes of control and GD-iPSCs lines.

Cell type	iPSC line	Sub-clones	Genotype
Control	MJ DF4-7T.A	#4	Wild type Wild type
GD-1	MNG-09-232	#1	N370S/N370S
GD-2	MNG-10-257 MNG-09-246	#4,3 #13,16	L444P/Rec*Nci*I W184R/D409H
GD-3	MNG-98-12-9	#4,1	L444P/L444P

Supplementary Table 2. RT-PCR primer sets and their corresponding sequences.

TFEB-F	ACCTGTCCGAGACCTATGGG
TFEB-R	CGTCCAGACGCATAATGTTGTC
GBA -F	TGGGTACCCGGATGATGTTA
GBA-R	AGATGCTGCTGCTCTCAACA
HEXA-F	CAACCAACACATTCTTCTCCA
HEXA-R	CGCTATCGTGACCTGCTTTT
LAMP1-F	ACGTTACAGCGTCCAGCTCAT
LAMP1-R	TCTTTGGAGCTCGCATTGG
CTSD-F	AACTGCTGGACATCGCTTGCT
CTSD-R	CATTCTTCACGTAGGTGCTGGA
CTSB-F	AGTGGAGAATGGCACACCCTA
CTSB-R	AAGAAGCCATTGTCACCCCA
GNS-F	CCCATTTTGAGAGGTGCCAGT
GNS-R	TGACGTTACGGCCTTCTCCTT