Supplemental material

Supp. Figure 1. Size distribution and motility of vesicles accumulating in MPSIIIB neurons.

Quantification of vesicles labelled with anti-LAMP1 antibodies on fixed embryonic cortical neuron cultures at day 12 (A and B) or with IDUA-GFP on living embryonic cortical neuron cultures transduced at day 7 and examined at day 12 (C).

(A) The number of LAMP1-positive vesicles per 1500 µm2 of neurite surface (the mean neuritic area for a single neuron) was scored in wild type (153±14, 570 vesicles, 22 neurons) and MPSIIIB neurons (175±14, 833 vesicles, 28 neurons, p<0.01). Differences between numbers of vesicles scored in MPSIIIB and wild type neurons are indicated for each size category in the left diagram, and expressed relatively to the number of scored wild type vesicles in the right diagram, showing that increased LAMP1 vesicle number in MPSIIIB neurons affects all size categories, including small vesicles, though it is more obvious in large-size categories (values are means ± SEM).

(B) LAMP1-positive vesicles present in neurites of wild type (n=22), MPSIIIB (n=28) or genetically corrected MPSIIIB (+ HIV-NaGlu, n=8) neurons were scored and their size was measured (wild type: 0.48±0.02 µm2, MPSIIIB: 0.97±0.04 µm2, MPSIIIB+HIV-NaGlu : 0.40±0.02 µm2). Numbers of vesicles scored in each size category are expressed as percents of the total number of scored vesicles, showing a switch of the size distribution diagram to the right in MPSIIIB compared to wild type neurons, and the correction of this phenotype in cells expressing the missing enzyme NaGlu following lentivirus-mediated gene transfer.

(C) The size distribution of IDUA-GFP-positive mobile and static vesicles is shown in the diagram. Numbers of vesicles scored in each size category are expressed as percents of the total number of scored vesicles.

Supp. Figure 2. Single labelling for markers of the endocytic pathway.

Confocal or apotome images of cells stained with each individual antibody are shown as complements to Fig. 3A. Scale bars, 10 µm.

Supp. Figure 3. Secondary storage products are not associated with LAMP1 positive vesicles in neurites.
Parasagittal sections of the rostral cortex of 8-month-old MPSIIIB mice, or day 10 MPSIIIB neuron cultures were immuno-labeled with anti-LAMP1 antibodies (in purple) in combination (in green) with anti-HS (left panel), anti-ScMAS (middle panel), anti-ubiquitin (middle panel), or anti-GM3 (right panel) antibodies. Nuclei were counterstained in blue. Merged confocal images (cortex sections), or apotome views (neuron culture) are shown. Rare co-localization of LAMP1 with ScMAS is visible in peri-nuclear aggregates, whereas co-localization was not observed in other areas, or for HS and ubiquitin. GM3 colocalizes with few LAMP1 positive vesicles in soma only. Scale bars, 10 µm.

**Supp. Figure 4.** Transport kinetics in the secretory pathway.

Plasmid DNA coding for ts045VSV-G-YFP was nucleofected in wild type or MPSIIIB neurospheres from E17 mouse embryonic ganglionic eminences. These cells can more efficiently transfected with exogenous DNA than primary neurons. After 24 hours of adhesion on coated coverslips at non-permissive temperature (40°C), cultures were switched to 32°C, fixed at different time points and examined by fluorescence microscopy.

(A) The protein is blocked in the ER at 0 min, concentrated at ERES at 15 min, transited through Golgi complex at 17 min and reaches cell surface at 60 min. Similar kinetics were observed in wild type and MPSIIIB cells. Scale bars 10 µm.

(B) Cells were fixed 15 minutes after temperature switch and examined for YFP fluorescence signal and simultaneously stained for LAMP1. Signal co-localisation was quantified (see text). Scale bars 10 µm.

**Supp. Figure 5.** Western blot analysis of glycoproteins contained in storage vesicles.

(A, B) Total proteins were extracted from cortical neurons cultures at day 10 and processed for western blot analysis without treatment (control: C), or after treatment with endoglycanase H (E, 49 units per µg of proteins, or 10-fold more concentrated, E_{x10}), or with PNGase (P, 0.17 nanomolar units per µg of proteins). Membranes were revealed with anti-LAMP1 or the anti-ID1A antibody to reveal IDUA.

(C) Total or membrane bound (Mb) proteins were processed for western blot analysis to reveal
GM130. Actin and CD56 signals were revealed to assess protein load on each lane.

**Supp. Video 1.** Trafficking of IDUA-GFP-positive vesicles along wild type neuronal processes.

Wild type embryonic cortical neuron cultures were transduced at day 7 with a lentivirus vector coding for the fluorescent lysosomal enzyme IDUA-GFP and were examined by time-lapse fluorescent microscopy at day 12. The neuronal soma is visible on the left of the field with one prolongation oriented to the bottom right. Small IDUA-GFP-positive vesicles circulate along the neurite in both directions. Scale bars, 10 µm.

**Supp. Video 2.** Clustering of IDUA-GFP-positive vesicles along MPSIIIB neuronal processes.

MPSIIIB embryonic cortical neuron cultures transduced at day 7 with a lentivirus vector coding for IDUAGFP were examined at day 12. The neuronal soma is visible on the left of the field with a short prolongation oriented to the top left and another one oriented to the bottom right. Enlarged and immobile IDUA-GFP-positive vesicles are clustered in neurites. Scale bars, 10 µm.

**Supp. Video 3.** Altered trafficking of IDUA-GFP-positive vesicles along MPSIIIB neuronal processes.

MPSIIIB embryonic cortical neuron cultures transduced at day 7 with a lentivirus vector coding for IDUAGFP were examined at day 12. The neuronal soma is on the left (outside the field) with its prolongation extending from the top left to the bottom right. A proximal cluster of six IDUA-GFP-positive enlarged vesicles blocks the trafficking of two small IDUA-GFP-positive vesicles appearing on the bottom right at 65 seconds. Scale bars, 10 µm.