SUPPLEMENTAL DATA

FIGURE LEGENDS

Supplemental Fig. 1. Enzyme activities of normal, ML-II, and enzyme-treated ML-II skin fibroblasts. (A) These data are supplementary to Fig. 1B. (B) These data are supplementary to Fig. 1C. Units are nmol/h/mg protein.

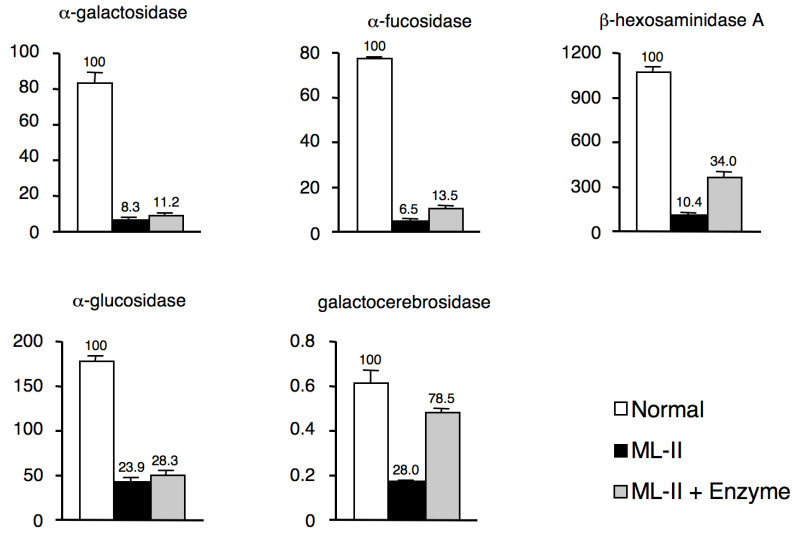
Supplemental Fig. 2. M6P-receptor-independent targeting of β-glucosidase. (A) Activity of β-glucosidase was measured in normal, ML-II, and enzyme-treated ML-II skin fibroblasts. Measurements were carried out in triplicate for each sample and means ± SD are indicated. Units are nmol/h/mg protein. Relative activities (%) compared to normal cells are shown above each bar. (B) Cultured ML-II skin fibroblasts with various concentration of M6P (0, 5, and 10 mM) were collected before enzyme treatment (pre), and at 48 and 72 h after treatment, and β-glucosidase activity was measured. Means are indicated and units are nmol/h/mg protein. (C) β-glucosidase was co-stained with LysoTracker. Merging of β-glucosidase and LysoTracker signals in untreated ML-II skin fibroblasts indicates that β-glucosidase was targeted to lysosomes in an M6P-receptor-independent manner. Scale bars, 20 μm.

Supplemental Fig. 3. Lyso-endosomal entrapment of BODIPY-Cer in ML-II skin fibroblasts. BODIPY-Cer and LysoTracker fluorescence merged in untreated ML-II cells. This indicates that extrinsic BODIPY-Cer was trapped in endosomes and lysosomes, where undigested substrates are accumulated, and could not reach Golgi apparatus smoothly. Scale bars, 20 μm.

Supplemental Fig. 4. Protein analysis for V-ATPase and mucolipin-1. (A) Western blotting of V-ATPase and mucolipin-1. Intensities of V1B2/V0d1 and mucolipin-1/β-actin were acquired by ImageJ software. In each group, three samples were measured and means ± SD are shown. (B) Co-localization of V1B2 subunit and LysoTracker. Immunocytochemistry of V-ATPase V1B2 antibody were performed with LysoTracker staining. In both normal and ML-II, V1B2 subunit and LysoTracker were merged. Scale bars, 20 μm.

Supplemental Figure 1

A



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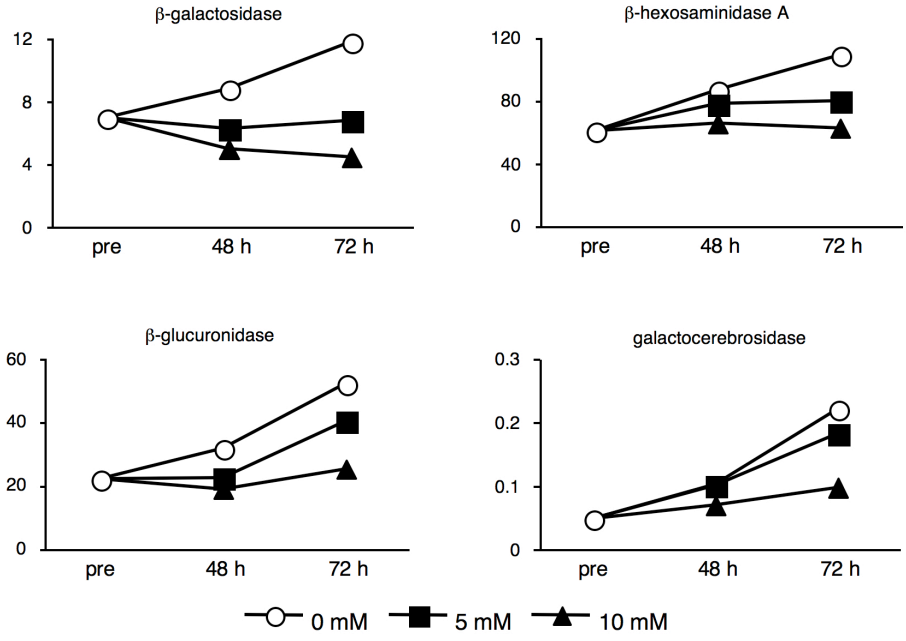
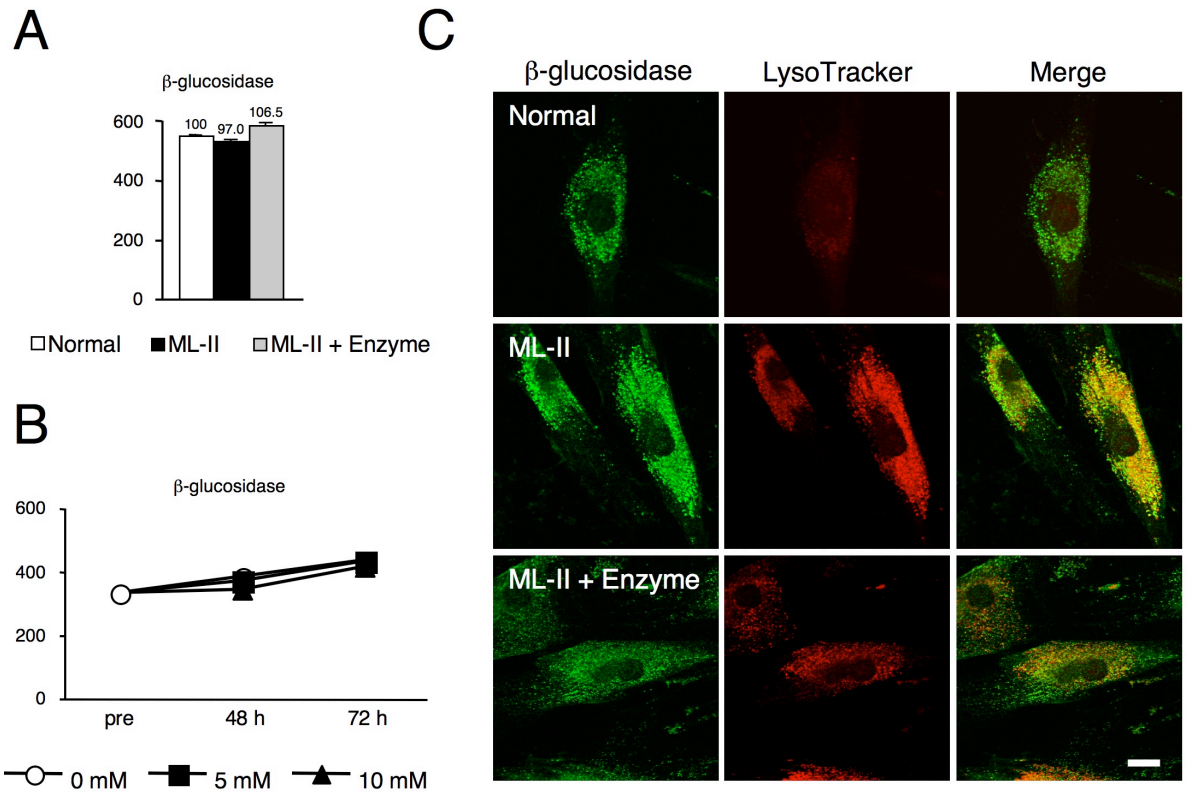
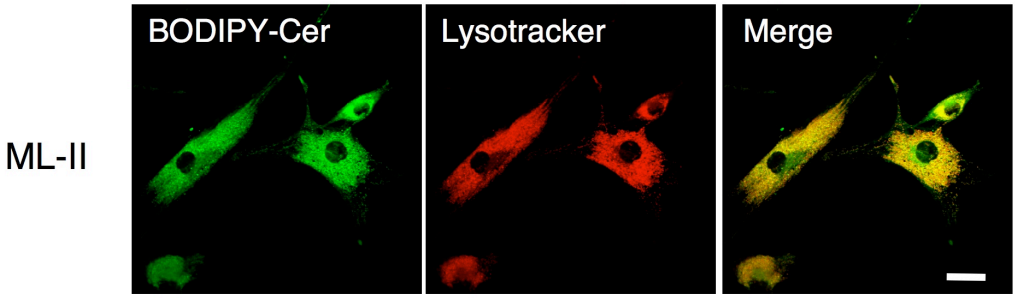


Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4