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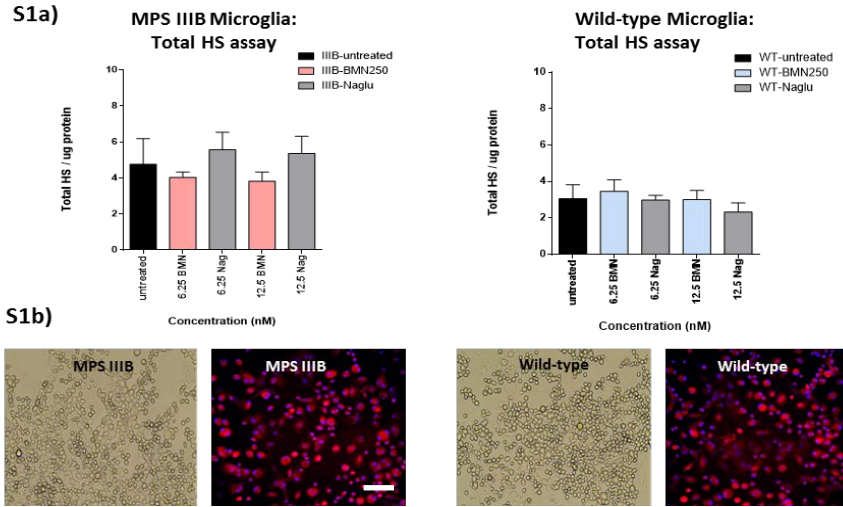
Supplemental Information

**Differential Uptake of NAGLU-IGF2
and Unmodified NAGLU in Cellular Models
of Sanfilippo Syndrome Type B**

Heather Prill, Amanda Luu, Bryan Yip, John Holtzinger, Melanie J. Lo, Terri M. Christianson, Gouri Yogalingam, Mika Aoyagi-Scharber, Jonathan H. LeBowitz, Brett E. Crawford, and Roger Lawrence

Differential uptake of NAGLU-IGF2 and unmodified NAGLU in cellular models of Sanfilippo syndrome Type B - Supplemental Figures:

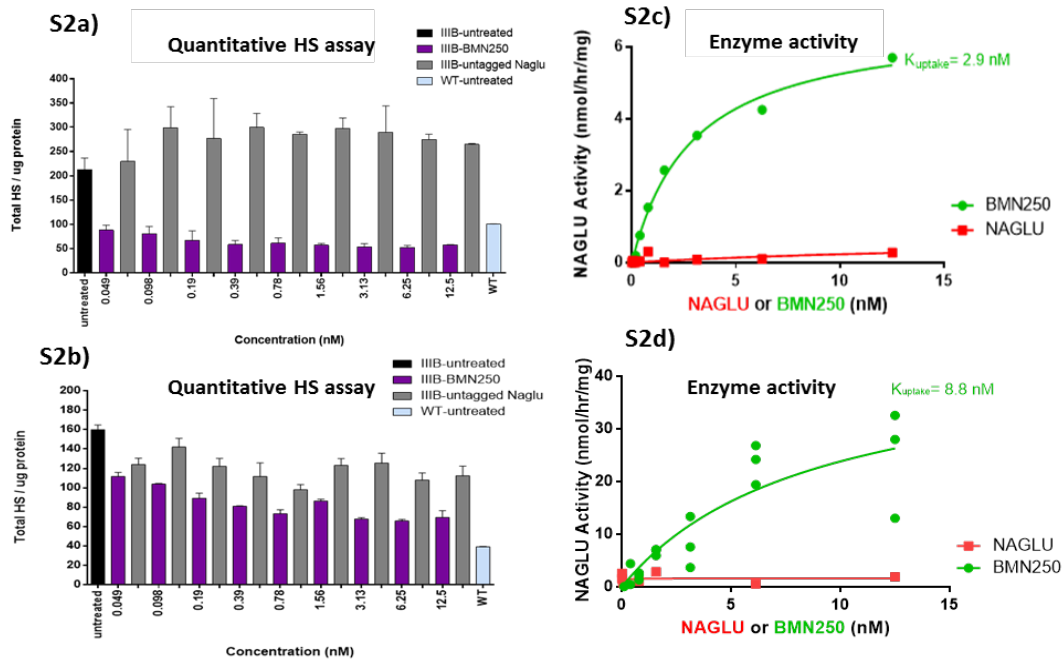
Supplemental Figure 1: MPS IIIB & wild-type microglia



Supplemental Figure 1: HS levels in MPS IIIB & wild-type mouse microglia.

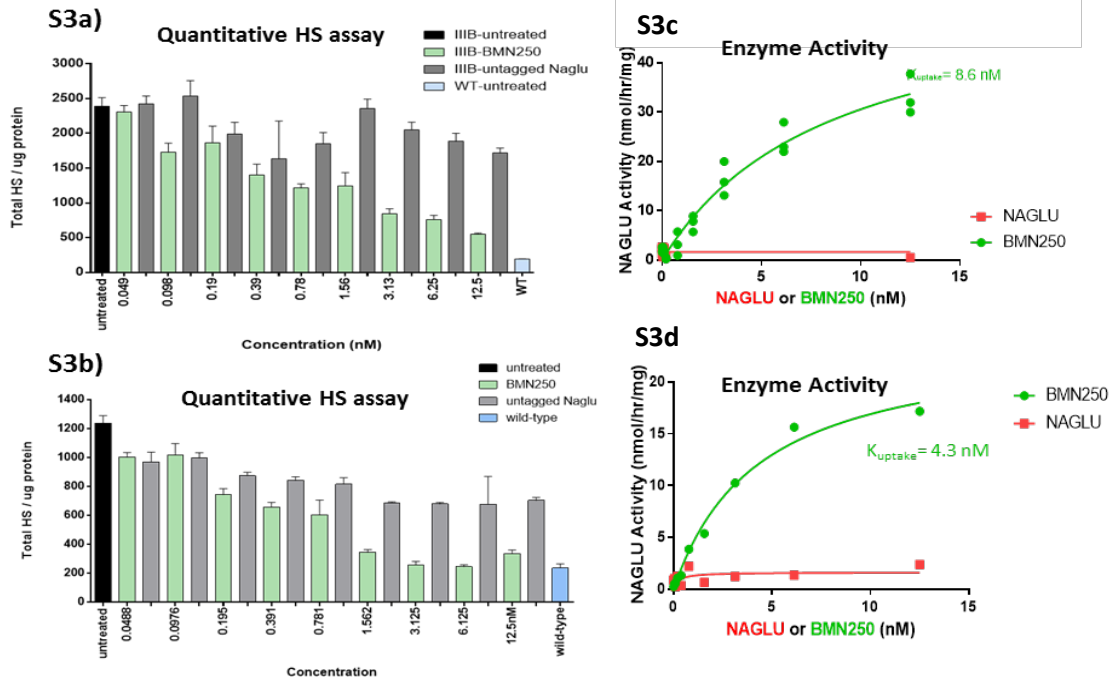
S1a) Quantitative total HS assay results indicated MPS IIIB microglia accumulated little to no HS storage in culture compared to wild-type microglia cultures using our culturing methods. Not surprisingly, no significant differences in HS levels were observed between untreated, NAGLU-IGF2 (BMN250) treated, and unmodified NAGLU (Naglu, Nag) treated MPS IIIB microglial cells after 30 minutes exposure (either 6.25 or 12.5 nM concentrations). Data shown as pmoles total HS per μg protein per well mean \pm SD ($n=3$). **S1b)** MPS IIIB & wild-type microglia showed similar general morphology by bright field microscopy and immunocytochemistry; Iba1 antibody (1:1000; red); Hoechst dye-nuclei ($1\mu\text{g}/\text{mL}$; blue). Scale bar is shown in the MPS IIIB fluorescence image ($10\mu\text{m}$).

Supplemental figure 2: MPS IIIB primary neurons



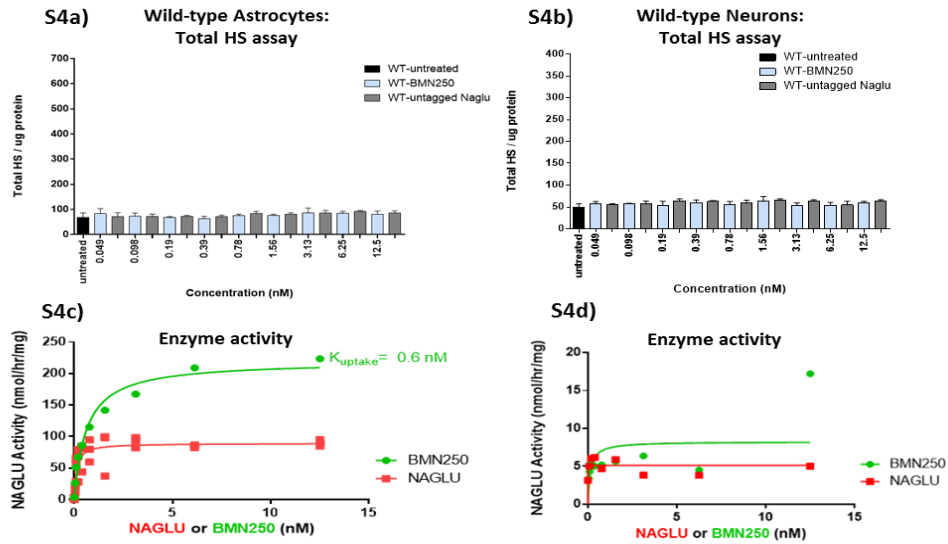
Supplemental Figure 2: Replicate experimental results for quantitative total HS and enzyme activity assay in MPS IIIB primary neurons. S2a-b) 30-minute exposure with 0 to 12.5 nM NAGLU-IGF2 (BMN250) resulted in a dose-dependent decrease in total HS in MPS IIIB primary mouse cortical neurons (purple bars), whereas there was little to no decrease in total HS storage with unmodified enzyme (untagged Naglu) at any concentration tested (gray bars). Data shown as pmoles total HS per μ g protein per well mean \pm SD (n=3). **S2c-d)** Enzyme activity from neuronal cell lysates confirms NAGLU-IGF2 (BMN250, green traces) was rapidly taken up in neurons with 30-minute enzyme exposure ($K_{\text{uptake}} = 2.9 \text{ nM}$ and 8.8 nM), but cell lysates showed no activity for unmodified NAGLU after 30 minute exposure (NAGLU, red traces).

Supplemental Figure 3: MPS IIIB Astrocytes



Supplemental Figure 3: Replicate experimental results for quantitative total HS & enzyme activity assay in MPS IIIB Astrocytes. S3a-b) 30-minute exposure with 0 to 12.5 nM NAGLU-IGF2 (BMN250) resulted in a dose-dependent decrease in total HS in MPS IIIB primary astrocytes (green bars), whereas there was little to no decrease in total HS storage with unmodified enzyme (untagged Naglu) at any concentration tested (gray bars). Data shown as pmoles total HS per μg protein per well mean \pm SD (n=3). **S3c-d)** Enzyme activity from astrocyte cell lysates confirms NAGLU-IGF2 (BMN250, green traces) was rapidly taken up in astrocytes with 30-minute enzyme exposure ($K_{\text{uptake}} = 8.6 \text{ nM}$ and 4.3 nM). However, cell lysates showed no activity for unmodified NAGLU after 30 minute exposure (NAGLU, red traces).

Supplemental Figure 4: Wild-type Astrocytes & Neurons



Supplemental Figure 4: HS measurements and enzyme uptake analysis in wild-type primary mouse astrocytes and neurons. Age-matched wild-type mouse primary astrocytes and neurons were cultured, treated, and analyzed in parallel plates for each experiment with representative data from one experiment shown. **S4a)** As expected, wild-type astrocytes and neurons have much lower levels of total HS than the corresponding cells cultured from MPS IIIB mice. NAGLU-IGF2 (BMN250) and unmodified NAGLU (untagged Naglu) showed little to no effect on total HS compared to untreated (black bars). Data shown as pmoles total HS per μg protein per well mean \pm SD (n=3). **S4b)** Limited, possibly preferential uptake and activity of NAGLU-IGF2 (BMN250, green traces; $K_{\text{uptake}} = 0.6 \text{ nM}$) observed in wild-type astrocytes.