A) Control vs MAPK8IP3 KO

B) Western blot analysis for MAPK8IP3 and Tubulin.

C) Immunofluorescence images for DQ-Red BSA and Lamp1-GFP.

D) Bar graph showing % Cells with Active Lysosomes (DQ-Red BSA) with No Treatment vs Bafilomycin 100nM.

Supplemental Figure 1
**Supplemental Figure 1: Validation of axonal phenotypes of MAPK8IP3 KO i3Neurons**

A, B) Representative images of cells labeled with lysotracker showing axonal buildup of acidic organelles in the MAPK8IP3 KO i3Neurons while Control i3Neurons have relatively fewer organelles in their axons. C) Western blot showing loss of MAPK8IP3 protein in MAPK8IP3 KO i3Neurons. MAPK8IP3 (arrow) and tubulin (loading control) are probed in Control, MAPK8IP3 KO, and MAPK8IP3 KO LAMP1-GFP DIV21 i3Neurons. Asterisk indicates non-specific band. (D) Quantification of DQ-Red BSA positive lysosomes in MAPK8IP3 KO DIV10 i3Neurons expressing LAMP1-GFP following 5-hour incubation with DQ-Red BSA with or without addition of Bafilomycin A (mean ± SEM from three independent experiments; Control n = 47 cells; Bafilomycin n = 46 cells; ****P < 0.0001, unpaired t test). E) Representative images showing DQ-Red BSA positive vesicles and lack of DQ-Red BSA vesicles with Bafilomycin treatment. Scale bar, 10µm.
A) Lysosomal Degradation
DQ-Red BSA / BSA-488

B) DQ-Red BSA

C) BSA-488

D) E) F)

G) Alexa-647 Dextran

Supplemental Figure 2
Supplemental Figure 2: Depiction of cell-to-cell variability in lysosomal proteolytic and endocytic assays in i3Neurons

Graphs (A, D) depict the lysosomal degradation (DQ-Red BSA/BSA-488 ratio) in each cell across three experiments, where ratio from each cell was in turn normalized to population mean of Control i3Neurons from same experiment. Graphs show DQ-Red BSA (B,E), BSA-488 (C,F) and Dextran (G) uptake in individual cells across experiments normalized to population mean of Control i3Neurons from their respective experiments.