SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Representative decay curves of individual proteins from the SILAC proteomics in iNs. (a) Percent light protein remaining values are shown with exponential decay curves fit. The percentage of light protein remaining was calculated for each individual protein and summarized for each line and differentiation by the median value of all proteins. Mean and SD are shown (n = 3 differentiations). (**b**) Representative western blot of CAPZA1 and CFL1 protein following 48 hours of 20 μM CHX incubation with or without co-incubation with 40 μM chloroquine (Chlor) or 10 nM bortezomib (Bort).

Supplemental Figure 2. Generation of BAG3 isogenic lines using CRISPR/Cas9 and correlations of BAG3 protein abundance to protein degradation enrichment scores. (**a**) Bortezomib gene set variation analysis (GSVA) was used to create an enrichment score from the top 100 downregulated differentially expressed proteins in iNs treated with 5 nM bortezomib for 72 hours (cutoff of q = .05) [33]. This enrichment score was then correlated to BAG3 protein abundance across 53 ROSMAP iNs (Spearman correlation) [33]. (**b**) Chloroquine GSVA was used to create an enrichment score from the top 100 downregulated differentially expressed proteins in iNs treated with 40 μM chloroquine for 24 hours (cutoff of q = .05) [4]. This enrichment score was then correlated to BAG3 protein abundance across 53 ROSMAP iNs (Spearman correlation) [33]. (**c**) Table listing the BAG3 isogenic lines generated in this study. *BAG3* WT, HET, and KO iPSCs were generated in two isogenic sets of lines: BR33 (male) and BR24 (female), derived from non-cognitively impaired participants in the ROSMAP cohort. CRISPR/Cas9 editing was performed at the iPSC stage with a gRNA targeted to exon 2 of BAG3, followed by monoclonal selection and sequencing. For analysis, three clones for each iPSC line were selected: two targeted (biallelic frameshift mutations, KO or HET), and an unaltered wildtype clone (WT).

Supplemental Figure 3. Ubiquitinated protein and global protein turnover is unchanged following loss of BAG3 in neurons. (**a**) Representative western blot (WB) of total ubiquitin in BAG3 WT and KO iNs treated with or without 5 nM bortezomib for 72 hours. (**b**) Quantification of total ubiquitin normalized to GAPDH in BAG3 WT and KO iNs treated with or without 5 nM bortezomib for 72 hours. n = 4 differentiations across 2 genetic backgrounds with 3-6 technical replicates per differentiation. Statistics were performed using a paired one-way ANOVA with Tukey's multiple comparisons test. Data is displayed using SEM. BR33 is represented with red dots and BR24 is represented with blue dots. Solid dots represent a differentiation while lighter dots in the background represent all technical replicates. (**c**) Global protein turnover as measured by % Biotin Remaining after a 7 day chase period in BAG3 WT and KO iNs. BR33 is represented with red dots. Statistics were performed with a paired t-test (n=4 differentiations). For all comparisons: ns = not significant.

Supplemental Figure 4. Assaying aggregation prone proteins in BAG3 WT and KO iNs. (**a**) Representative western blot is shown and the ratio of aggregated phosphorylated tau to total phosphorylated tau (HMW/(Total Major + HMW pTau)) or total phosphorylated tau to total tau (Major pTau/Total Tau) was calculated after probing for tau phospho-epitopes, (**b-c**) $pTau^{181}$ and (**d-e**) $pTau^{217}$ or (**f**) total tau (n = 6 differentiations across 2 genetic backgrounds with 3-6 technical replicates per differentiation). Statistical tests were performed using a paired t-test. All data is displayed using SEM. BR33 is represented with red dots and BR24 is represented with blue dots. Solid dots represent a differentiation while lighter dots in the background represent all technical replicates. (**g-h**) Amyloid-ß 40 and 42 were assayed in the condition media of *BAG3* WT and KO neurons and the 42:40 ratio was calculated (n = 2 differentiations across 2 genetic backgrounds with 4 technical replicates per differentiation). (**i**) Representative western blot showing α-synuclein protein abundance across *BAG3* WT, HET, and KO iNs with and without

40 μM chloroquine. For all comparisons: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns = not significant.

Supplemental Figure 5. BAG3 upregulation following AD brain extract exposure. (**a**) iNs treated with AD brain extract for 72 hours from Figure 4c were separated into LPNCI and AD diagnoses. The fold change in BAG3/REVERT following brain extract exposure was then calculated (n = 10 differentiations across 5 genetic backgrounds and 3-4 technical replicates per differentiation for each diagnosis category). (**b-c**) Autophagic flux was assayed via western blot in iNs treated with AD brain extract for 72 hours. Chloroquine (40 μM for 24 hours) was used to measure autophagic flux as the change in LC3B-II between vehicle control and chloroquine (normalized to GAPDH). n = 6 differentiations across 2 genetic backgrounds with 3-6 technical replicates per differentiation. BR33 is represented with red dots and BR24 is represented with blue dots. For all graphs, statistical testing was performed using a paired t-test. All data is displayed using SEM. For all comparisons: ns = not significant.