Multi-modal Proteomic Characterization of Lysosomal Function and Proteostasis in Progranulin-Deficient Neurons

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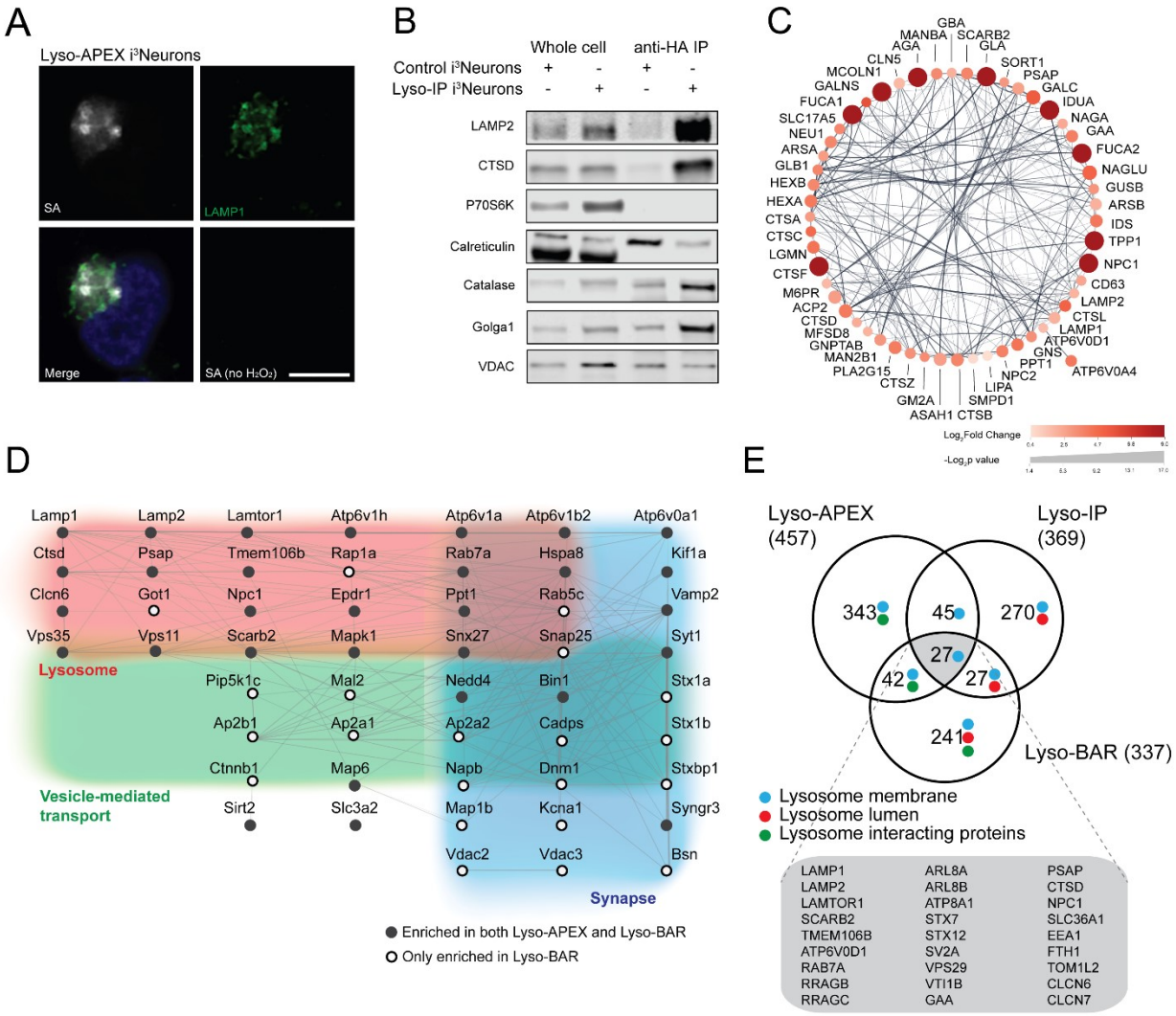
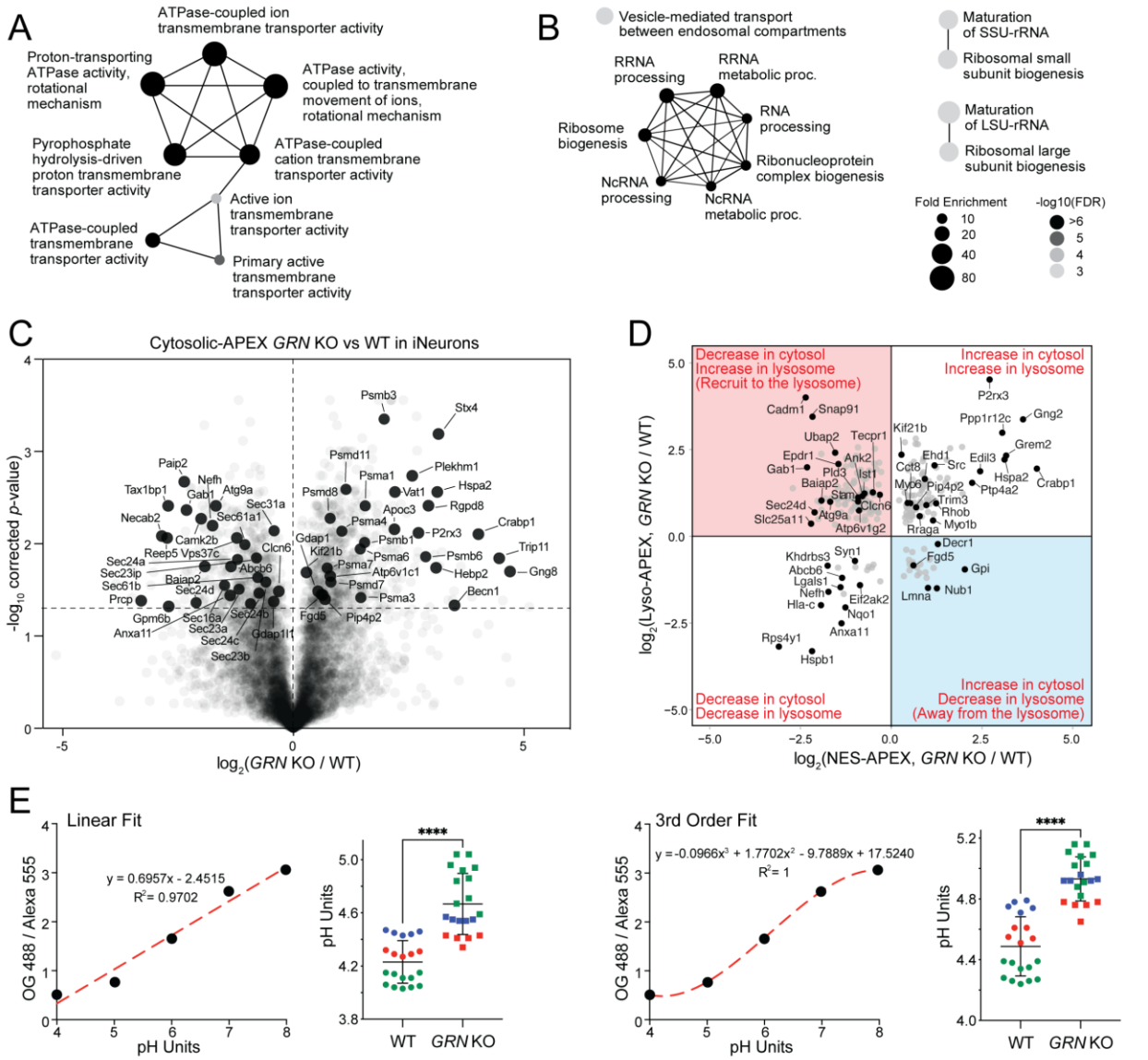
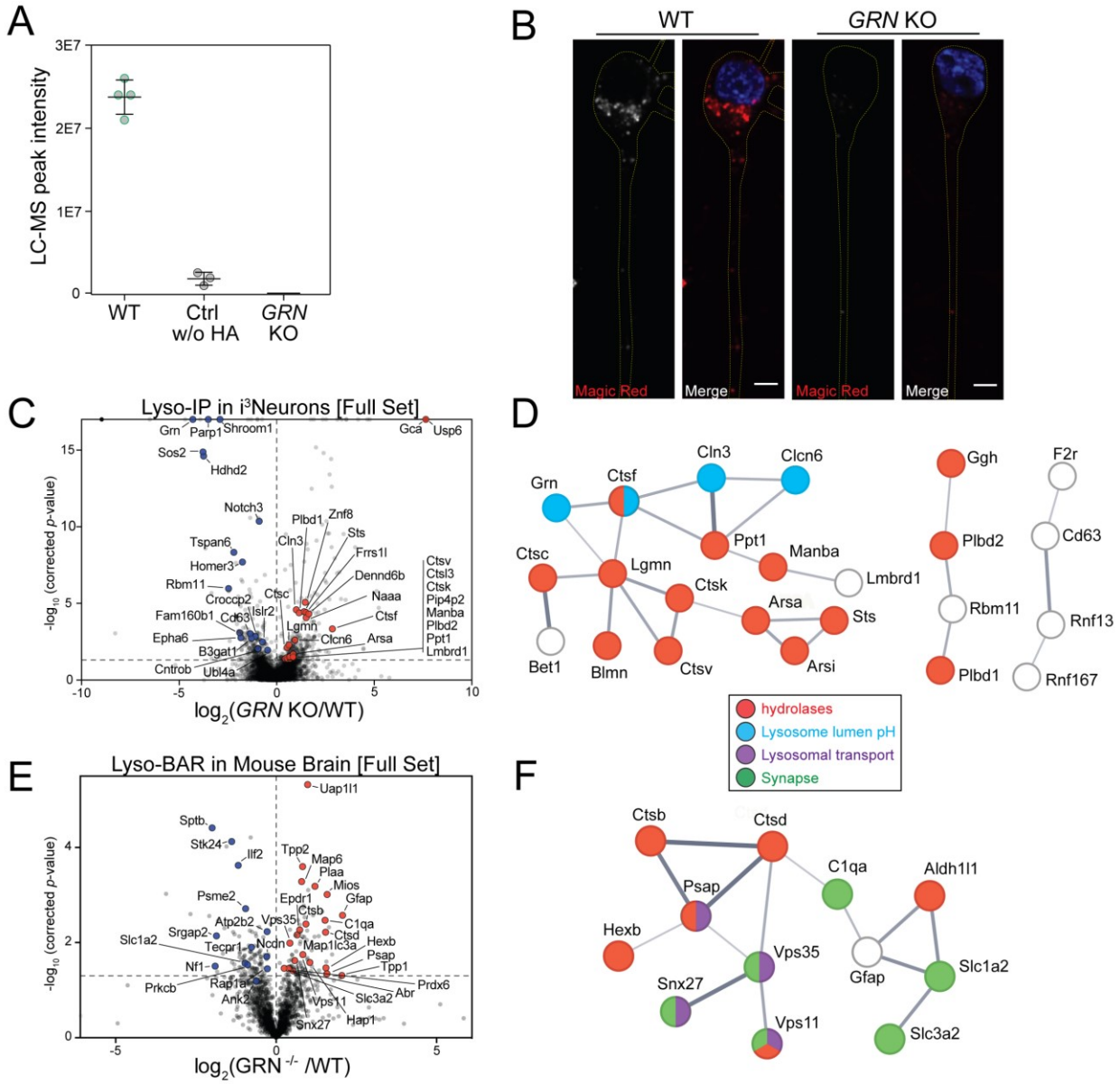
Supplementary Figures



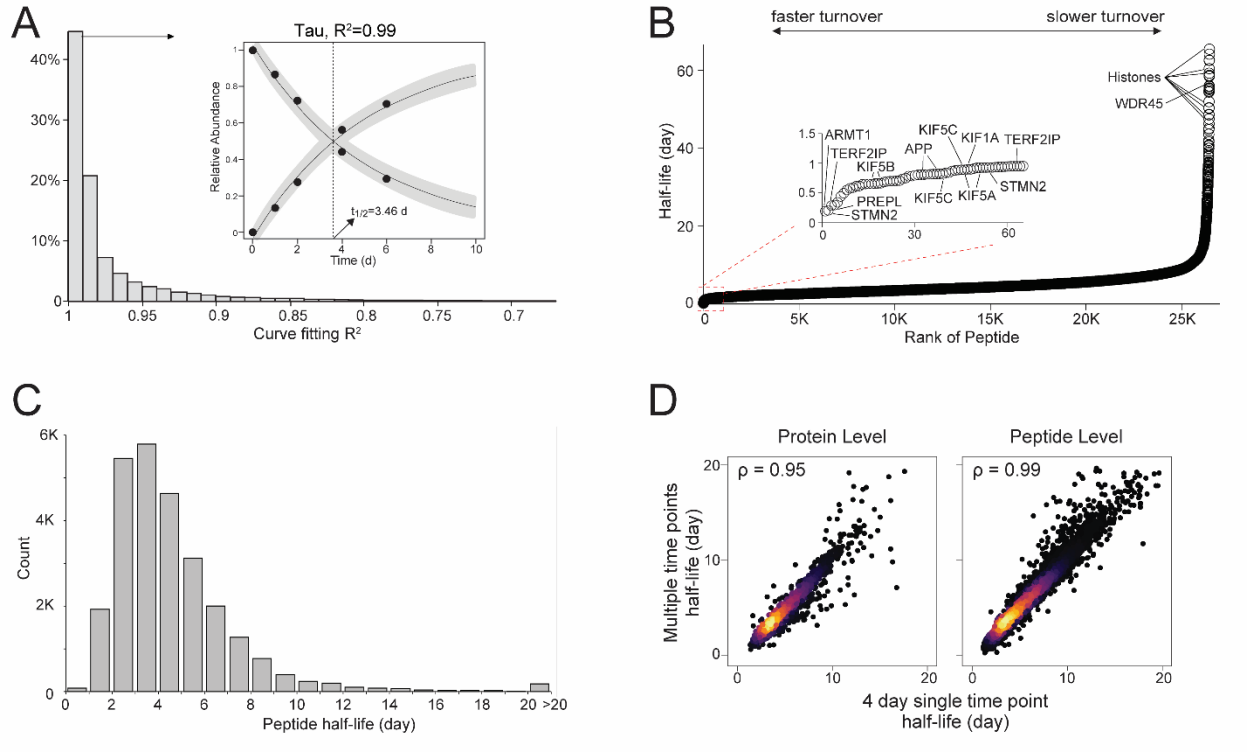
Supplementary Figure S1. **Confirmation of the Lyso-IP, Lyso-BAR, and Lyso-APEX probe locations at the lysosomes, related to Figure 1.** (**A**) Fluorescence imaging of Lyso-APEX i^{3}Neurons and negative control neurons without H_2O_2 treatment. Biotinylation cloud stained with streptavidin (SA-680), colocalizes with the bait protein LAMP1. Negative control group does not have SA signal. Nuclei were stained with Hoechst. Scale bar is 10 μm. (B) Western blot analysis of isolated lysosomes from Lyso-IP i^{3}Neurons compared to whole cell lysate and negative control without HA expression. (**C**) Protein network analysis of Lyso-IP enriched known lysosome proteins from i^{3}Neurons. (**D**) Protein network analysis showing enriched lysosome, vesicle-mediated transport, and synapse processes from Lyso-Bar enriched proteins in mouse brains compared to Lyso-APEX enriched proteins in i^{3}Neurons. (**E**) Venn diagram comparison of significantly enriched proteins in Lyso-APEX, Lyso-IP, and Lyso-BAR proteomics.



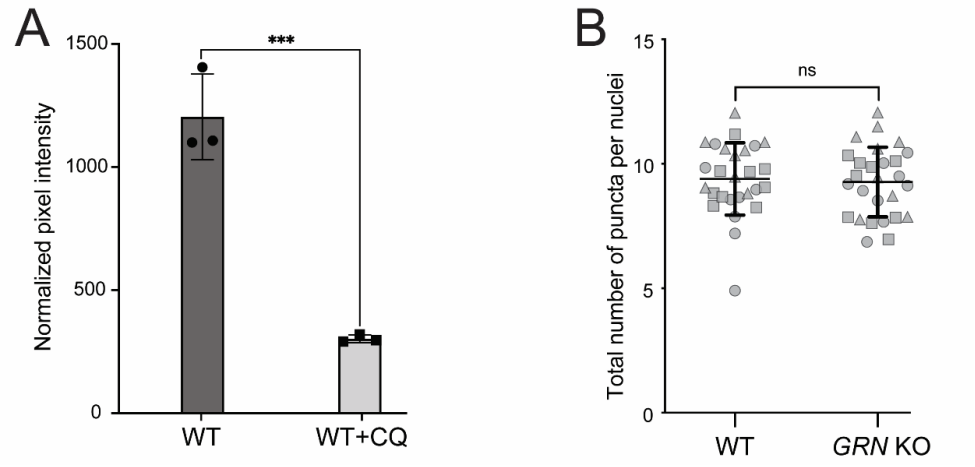
Supplementary Figure S2. Proximity labeling proteomics and lysosome pH measurement in progranulin-null i^{3}Neurons, related to Figure 2. (A) GO enrichment analysis of significantly upregulated molecular functions in *GRN* KO vs. WT Lyso-APEX proteomics. **(B)** GO enrichment analysis of significantly downregulated molecular functions in *GRN* KO vs. WT Lyso-APEX proteomics. **(C)** Volcano plot of cytosolic-APEX proteomics in *GRN* KO vs. WT i^{3}Neurons. **(D)** Scatter plot of significantly changed proteins (corrected *p*-value <0.05) in both Lyso-APEX and cytosolic-APEX proteomics showing potential protein translocation in neurons. (**E**) Lysosomal pH measurements in WT vs. *GRN* KO i^{3}Neurons with linear (left) and 3rd order (right) calibration curve fitting.



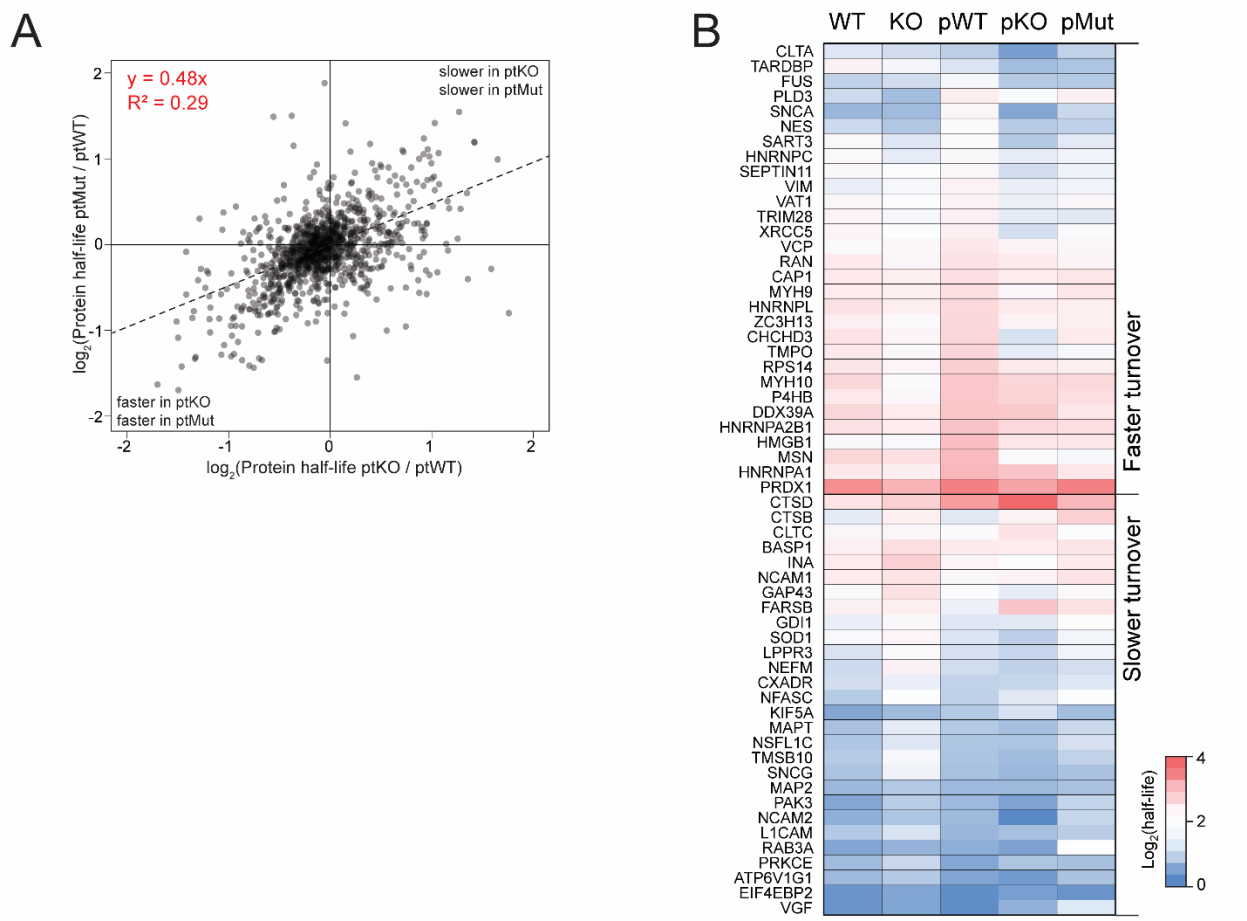
Supplementary Figure S3. Loss of progranulin results in elevated levels of lysosomal catabolic enzymes and decreased cathepsin B activity in human i^{3}Neurons and mouse brains, related to Figure 3. (**A**) PGRN is enrichment in isolated WT lysosomes and is absent in *GRN* KO lysosomes from Lyso-IP proteomics data. (**B**) Additional replicate of Magic Red assay showing reduced cathepsin B activity in *GRN* KO i^{3}Neurons compared to WT. Scale bar is 10 µm. (**C**) Volcano plot of Lyso-IP proteomics in KO vs. WT i^{3}Neurons without filtering, related to Figure 3B. (**D**) Protein network analysis of selected lysosome and synaptic proteins that are significantly changed in *GRN* KO vs. WT Lyso-IP proteomic. (**E**) Volcano plot of Lyso-BAR proteomics in GRN$^{-/-}$ vs. WT mouse brains without filtering, related to Figure 3E. (**F**) Protein network analysis of selected lysosome and synaptic proteins that are significantly changed in *GRN*$^{-/-}$ vs. WT Lyso-BAR proteomics.



Supplementary Figure S4. Developing dynamic SILAC proteomics in i^{3}Neurons to measure global neuron protein half-lives, related to Figure 4. (**A**) Histogram distribution of peptide level curve fitting R^2 to first-order exponential decay. An example of Tau peptide degradation and synthesis curves is shown in the inset. (**B**) Scatter plot of ranked peptide level half-lives in WT i^{3}Neurons. (**C**) Histogram distribution of peptide half-lives, consistent with protein level results in Figure 4D. (**D**) Scatter plot showing strong correlation of protein/peptide half-lives measured by multiple-time-point method (1, 2, 4, 6 days) and single-time-point method (4 day).



Supplementary Figure S5. DQ-BSA Red Assay to measure lysosomal degradative function, related to Figure 5. (**A**) Chloroquine treatment (30 μM for 12 hours) impairs the lysosomal degradative function with significantly reduced DQ-BSA signals compared to the untreated WT i^{3}Neurons (*** denotes *p*-value < 0.001). (**B**) WT and *GRN* KO i^{3}Neurons have similar total number of DQ-BSA fluorescent puncta, indicating similar overall levels of endocytosis and lysosomal biogenesis, related to Figure 5I.



Supplementary Figure S6. **Protein half-life changes caused by progranulin deficiency in GRN-KO, WT, ptKO, ptMut, and ptWT i^{3}Neurons, related to Figure 6.** (**A**) Scatter plot showing potential gene dosage effect of protein half-life changes in ptKO vs. ptWT and ptMutant vs. ptWT i^{3}Neurons. (**B**) Heatmap showing overlapping protein half-lives in WT, *GRN*-KO, ptWT, ptKO, and ptMut i^{3}Neurons. Heatmap colors represent the absolute half-life measurements in days.