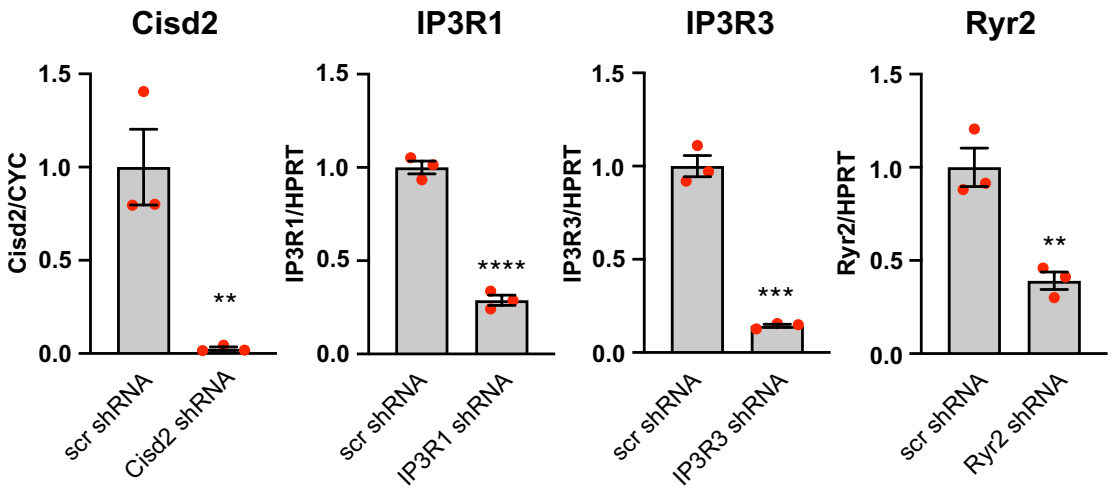

Figure S1. shRNAs against Cisd2, IP3R1, IP3R3, and RYR2 efficiently suppress the expression of respective mRNAs. PC6 cells were transfected with either scrambled shRNA or specific shRNAs and selected for 7 days with 200 µg/ml G418. Total RNA was extracted using RNAeasy mini kit (Qiagen), and first-strand synthesis was performed using 5 µg of total RNA with Maxima First Strand cDNA Synthesis Kit (Thermo). cDNAs were subjected to qPCR using specific primers for genes of interest and CYC or HPRT (housekeeping gene), and using a QuantStudio 12K Flex from Applied Biosystems by Life technologies. Acquired data were analyzed using the delta Ct method and normalized to transfection efficiency, which was estimated separately. **P < 0.01, ***P < 0.001, ****P < 0.0001 compared with scrambled shRNA group (n = 3, unpaired t-test).

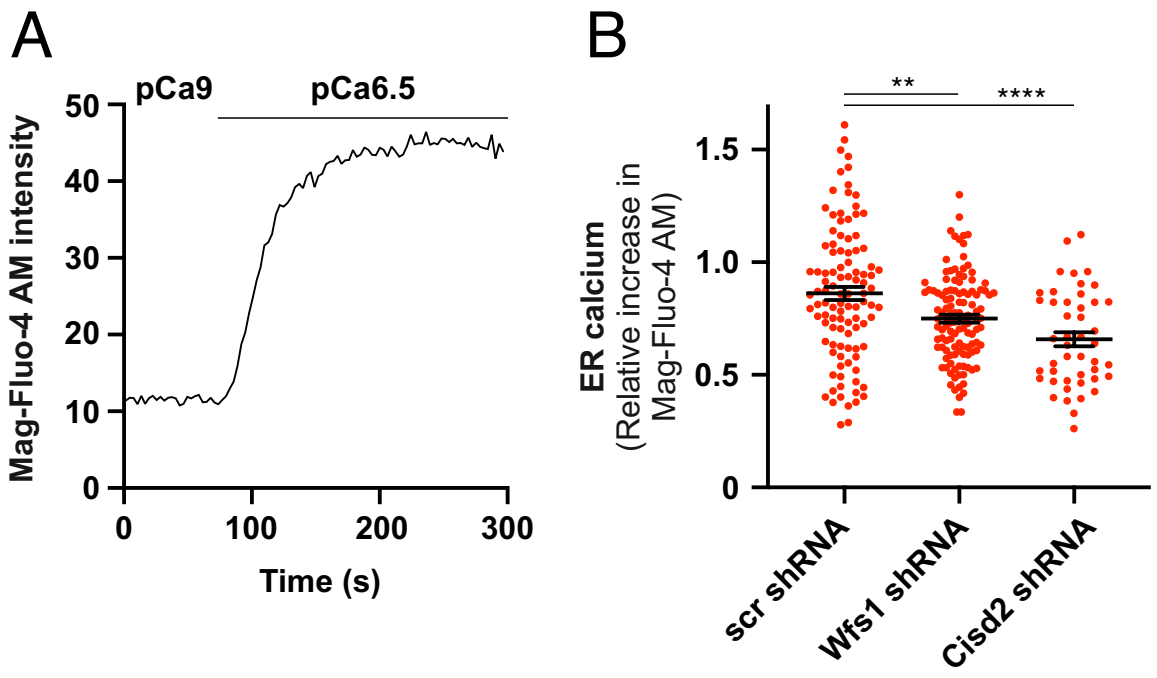

Figure S2. Decreased ER Ca2+ uptake in WFS1- and CISD2 deficient neurons. Neuronal cells growing on glass bottom dishes (Mattek) coated with poly-L-lysine were transfected at DIV2-3 with scrambled, *Wfs1* or *Cisd2* shRNA and mitochondrial marker Mito2Kate. At DIV 9-10 growth media was removed, and cells were further incubated in Krebs-Ringer solution containing 1 mM Ca^{2+} and 5 μM Mag-Fluo-4 AM for 1 hour at 37°C. Then cells were permeabilized in a basic solution containing (in mM) ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetra-acetic acid 10 (EGTA); N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid 30 (BES, pH 7.1), free Mg^{2+} 1; taurine 20; glutamic acid 5.56; malic acid 1.5; K_2HPO_4 3.9; dithiothreitol 0.5; NaATP 3.16; ionic strength was adjusted to 160 mM with potassium methanesulfonate (pH 7.1) with saponine (50 μg/ml) for 16 minutes at 4°C and washed with the same solution containing sodium azide (3 mM). Images were collected using an LSM 780 confocal microscope (Plan Apochromat 20X/0,5 objective) at room temperature. From each dish, one random field with size 424 X 424 μm was selected and imaged at 3-second intervals over 10 minutes. After 20 frames, the $CaCl_2$ was added to achieve pCa 6.5. (A). Data are presented as relative Ca^{2+} uptake of the transfected cell to Ca^{2+} uptake in nontransfected neurons in the same field (B). ** $P < 0.01$ and **** $P < 0.00011$, n = 104, 119 or 46 neurons in respective groups from 22, 23 or 11 dishes, Welch's ANOVA and Dunnett's T3 multiple comparisons test.

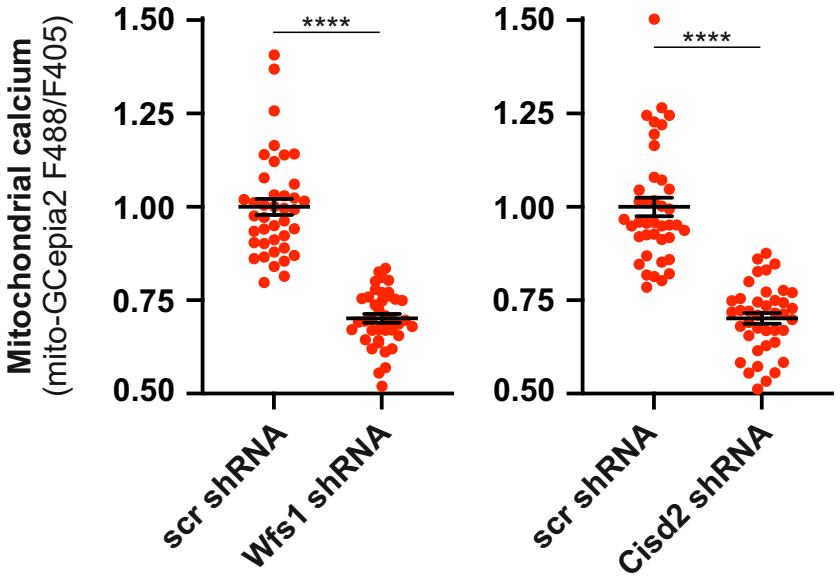

Figure S3. Decreased resting mitochondrial Ca^{2+} levels in the axons of WFS1 and CISD2 deficient neurons as measured by higher affinity mitochondrial Ca^{2+} indicator Cepia 2mt. ****P < 0.0001, n = 40 neurons from 4 dishes per group (average from 3-8 individual mitochondria per axon), Mann-Whitney test.

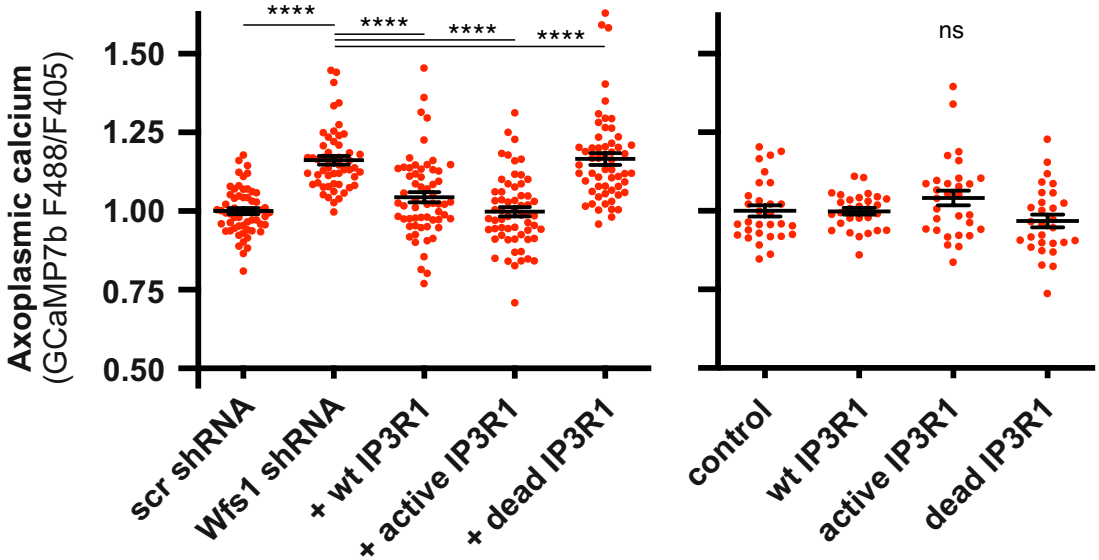

Figure S4. Overexpression of IP_3R1 but not its pore-dead mutant restores cytosolic Ca^{2+} levels in the axons of WFS1 deficient neurons (left panel). ****P < 0.0001, 53-60 axons from 6 dishes per group for left panel, Kruskal-Wallis test and Dunn's multiple comparisons test; right panel: 29-30 axons from 3 dishes per group, Brown-Forsythe ANOVA and Dunnett's T3 multiple comparisons test.

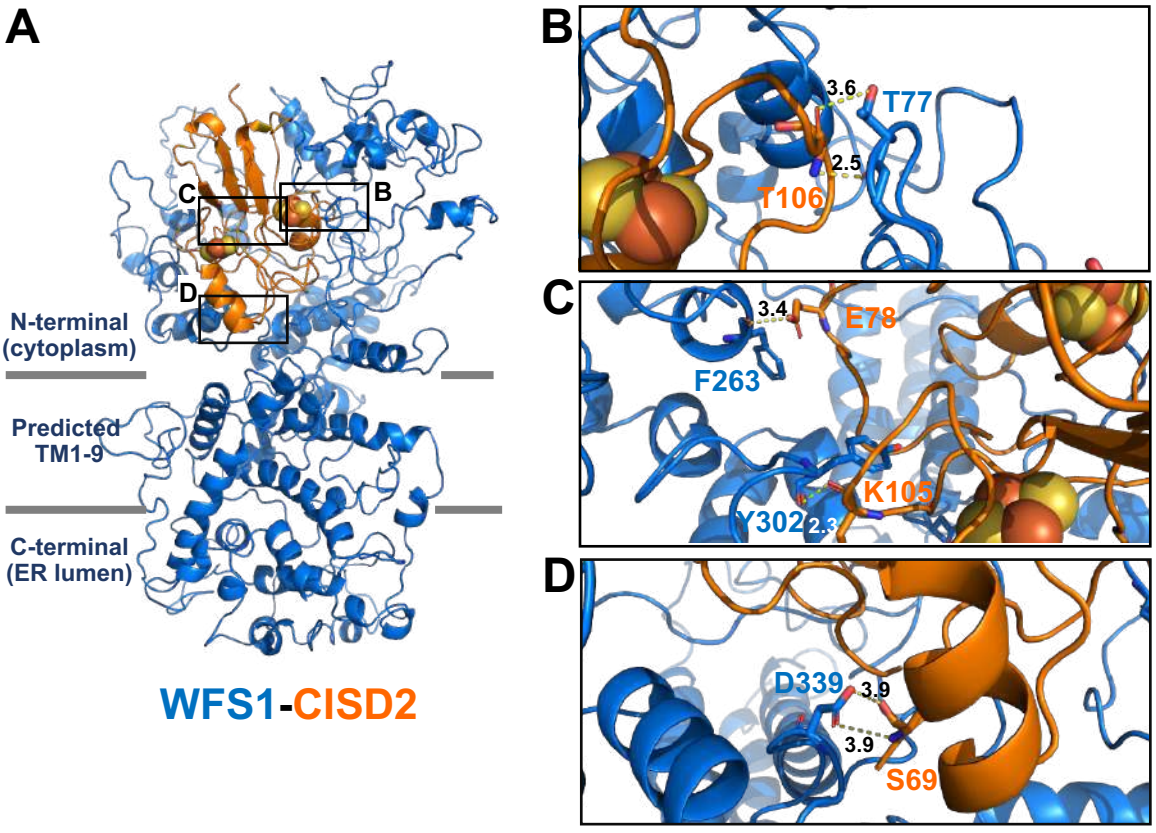

Figure S5. Computational docking model for human CISD2 and WFS1. (A) ZDOCK web server (http://zdock.umassmed.edu/) was used to perform protein-protein molecular docking. The ZDOCK score for the CISD2/WFS1 docking model was 1430.867. The crystal structure of CISD2 (orange) was available in Protein Data Bank (PDB ID: 3FNV). The predicted structure and transmembrane domains of WFS1 (blue) were obtained from[81]. Magnified views of WFS1-CISD2 interactions were shown in (B)-(D) to demonstrate the potential hydrogen bonds formed between WFS1 and CISD2 (yellow lines).

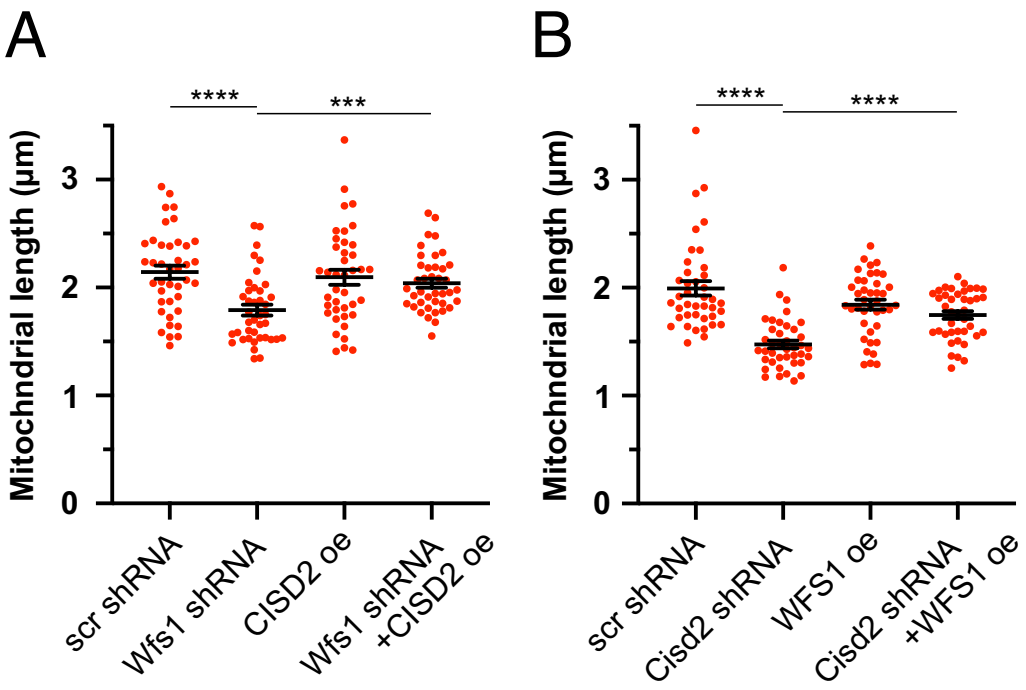

Figure S6. Mitochondrial length is restored in WFS1 deficient neurons when overexpressing CISD2 (A) and in CISD2 deficient neurons when overexpressing WFS1 (B). (A) ***$P < 0.001$ and ****$P < 0.0001$ compared with indicated group (n = 40 axons from 4 dishes per group, Brown-Forsythe ANOVA & Dunn's multiple comparisons test. (B) ****$P < 0.0001$ compared with indicated group, n = 40 axons from 4 dishes per group, Kruskal-Wallis test and Dunn's multiple comparisons test.

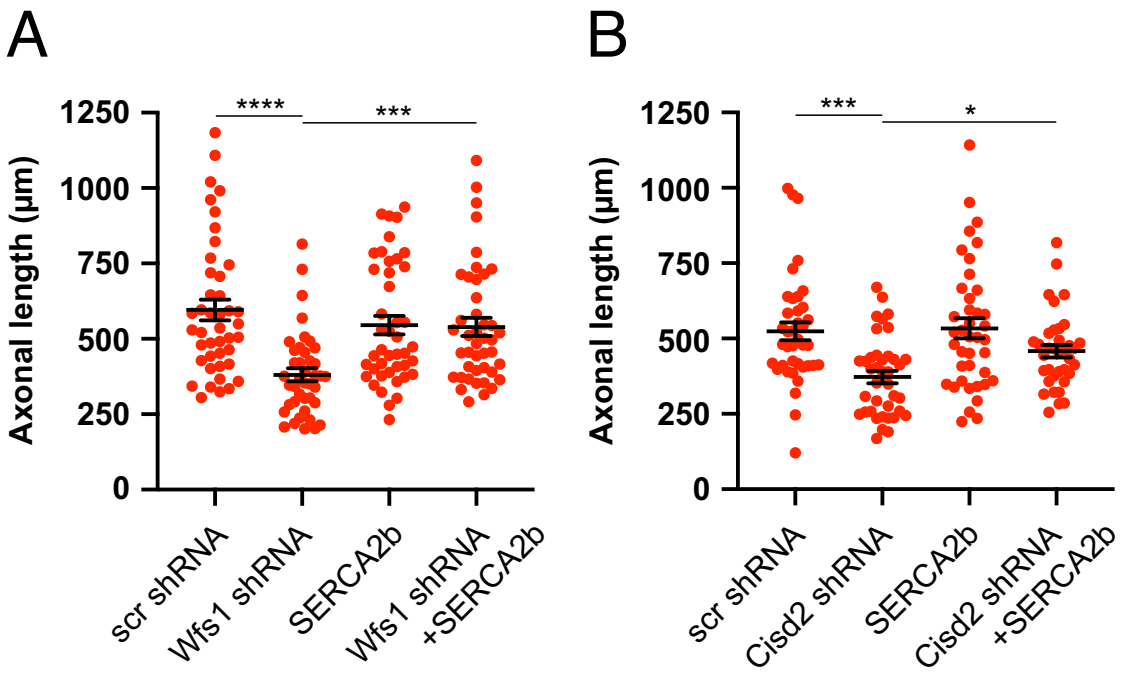

Figure S7. Suppressed axonal growth in WFS1- (A) or CISD2 deficient (B) neurons is restored when expressing SERCA2b. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$ compared with an indicated group (n = 36-43 axons from 4 dishes per group, Kruskal-Wallis test and Dunn's multiple comparisons test).

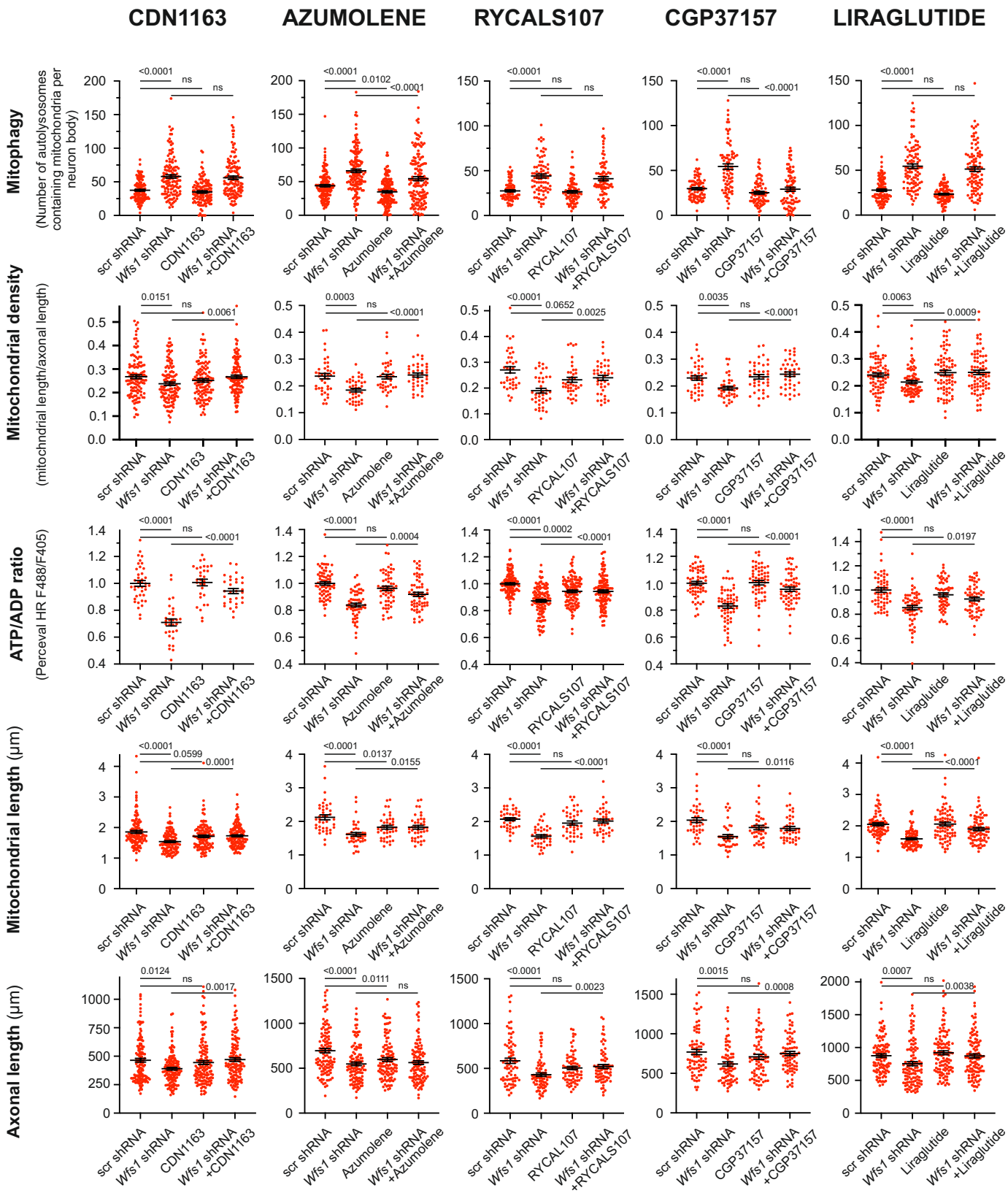

Figure S8. Effects of different compounds on mitophagy, mitochondrial density, ATP/ADP ratio, mitochondrial length, and axonal development.