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Supplemental Information

Inappropriate Intrusion of an Axonal Mitochondrial Anchor into Dendrites Causes Neurodegeneration

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Figure S1

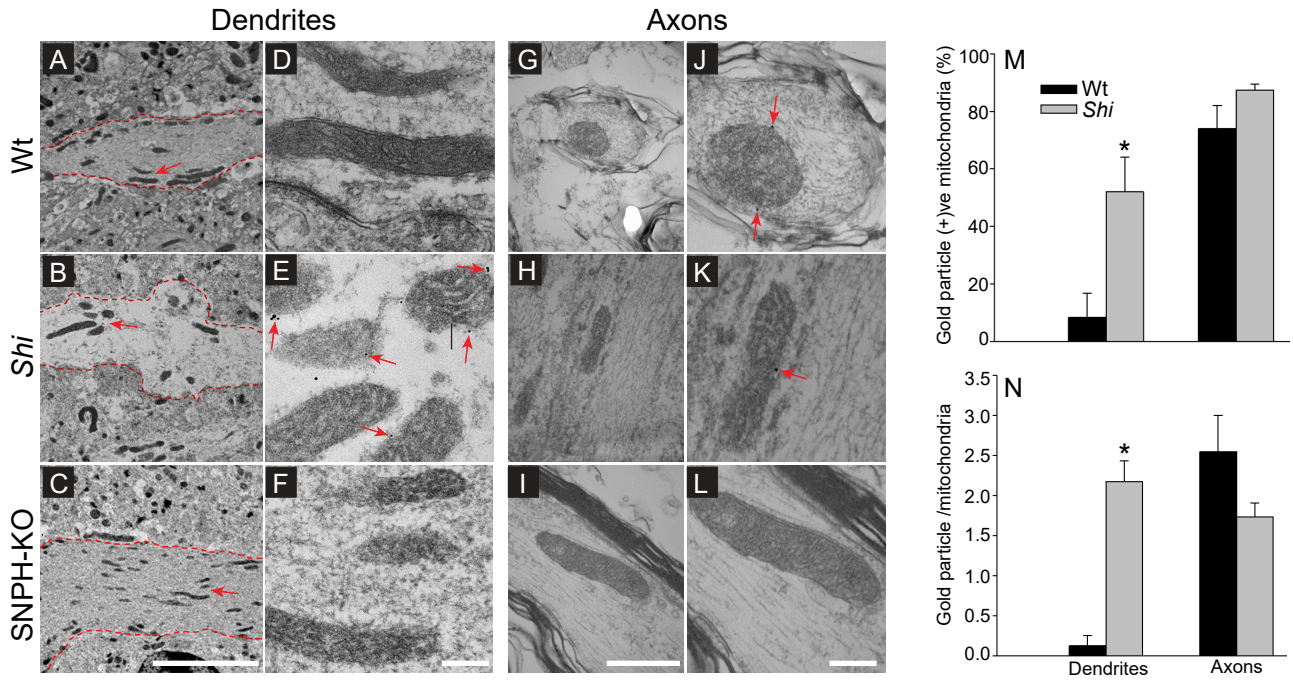

Figure S1. SNPH immuno-EM gold particle labeling in cerebellar tissue sections. Related to Figure 1. (A-C) low magnification EM imaging of SNPH labeled ultrathin sections from cerebellar molecular layers identifying dendrites (marked with dashed Red line) in age matched 3.5 month Wt, *Shi* and SNPH-KO mice. Scale bar 5 μm. (D-F) Enlarged images of dendritic ultrastructure showing mitochondria in Wt (D), *Shi* (E) and SNPH-KO (F) cerebellum. Scale bar 200 nm. Arrows in E indicates gold particle on the mitochondrial surface in *Shi* dendrites. (G-I) EM imaging of SNPH labeled cerebellar white matter axons in Wt (G), *Shi* (H) and SNPH-KO (I) mice. Scale bar 1 μm. Enlarged images of axonal region containing mitochondria are shown in J (Wt), K (*Shi*) and L (SNPH-KO) respectively. Scale bar 200 nm. Arrows in J and K indicate EM gold particles on mitochondria of Wt and *Shi* mice respectively. (M-N) Quantification of EM immuno gold positive mitochondria (M) and gold particle per mitochondria (N) in dendrites and axons from 8-10 different fields in 2 mice each of Wt and *Shi* mice. Data shown as mean ± SEM. *p<0.05.

Figure S2

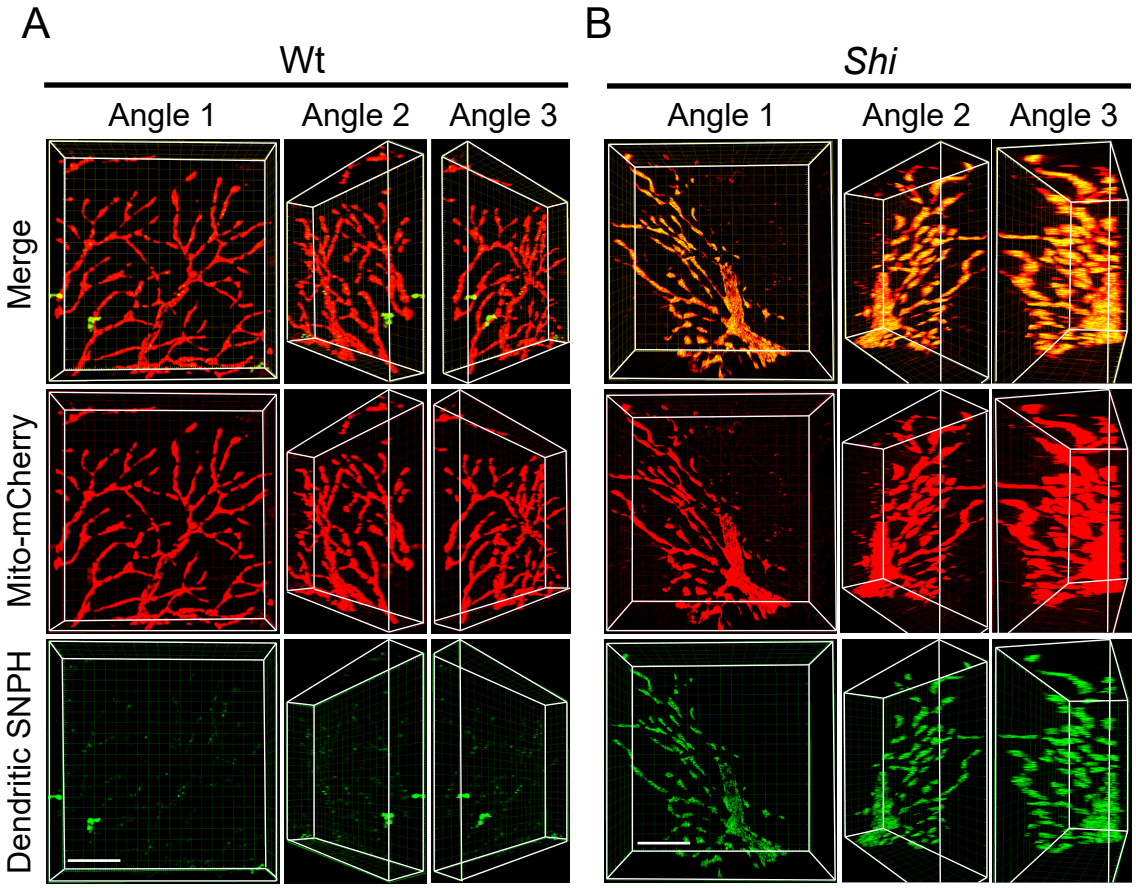

Figure S2. Adenoviral pre-tagging of mitochondria in PC capturing SNPH intrusion. Related to Figure 1. AAV-Mito-mCherry viral particles were injected in DCN of Wt and *Shi* mice by stereotactic injection (see method for detail) and cerebellar sections were labeled with SNPH antibody after 3 weeks of viral injection. Static images from 3D rotation of Mito-mCherry and SNPH at 3 different angles in Wt and *Shi* sections are shown in panels A and B respectively. Merge of SNPH and Calbindin is shown in top panels while Mito-mCherry and SNPH are shown separately in middle and bottom panels respectively. Scale bar 5μm. Note that all SNPH labeling co-localizes (yellow) with Mito-mCherry in *Shi* (panel B) and co-rotates with Mito-mCherry (remains yellow) in all 3 angles. On the other hand the green puncta in Wt (panel A), appears to be random nonspecific dots, which does not colocalize with Mito-mCherry. Massive dendritic SNPH intrusion is captured by this method in *Shi* (bottom).

Figure S3

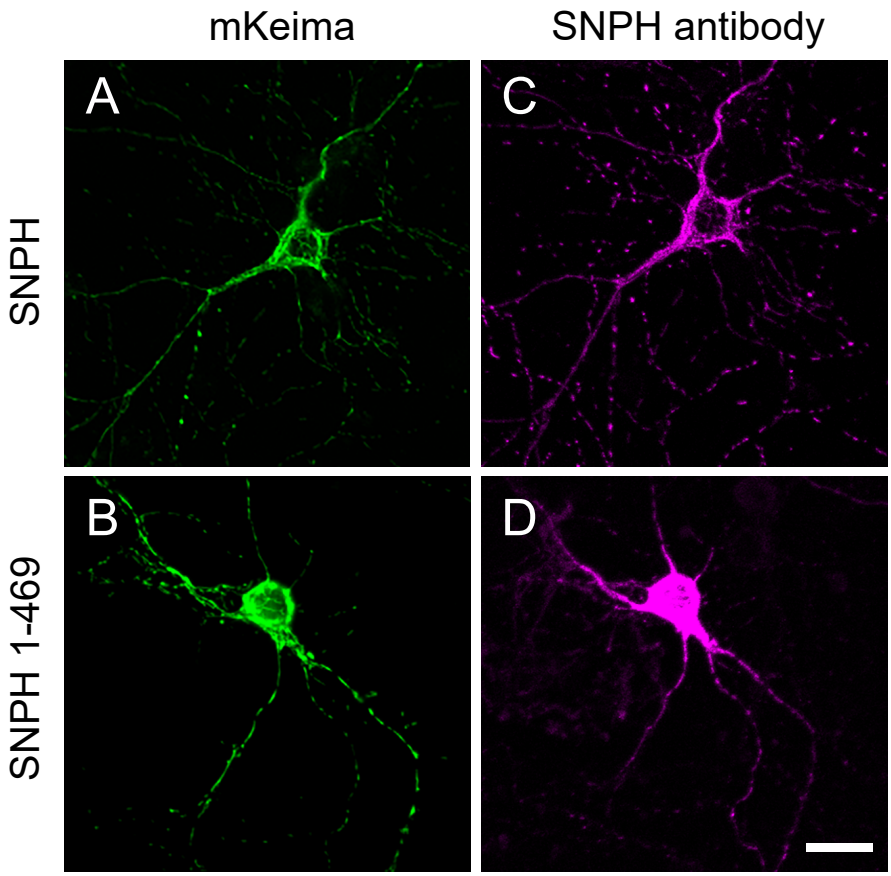

Figure S3. Verification of non-GFP variant of SNPH and SNPH1-469 co-transfection in neurons. Related to Figure 5. After mKeima imaging, neurons were fixed with paraformaldehyde and processed for immunofluorescence of SNPH antibody. Immunolabeling of SNPH in mKeima–SNPH (A; Ac in figure 5) and mKeima SNPH1-469 (B; Ad in figure 5) transfected neurons shown in panels C and D respectively. Scale bar 20 μm.

Figure S4

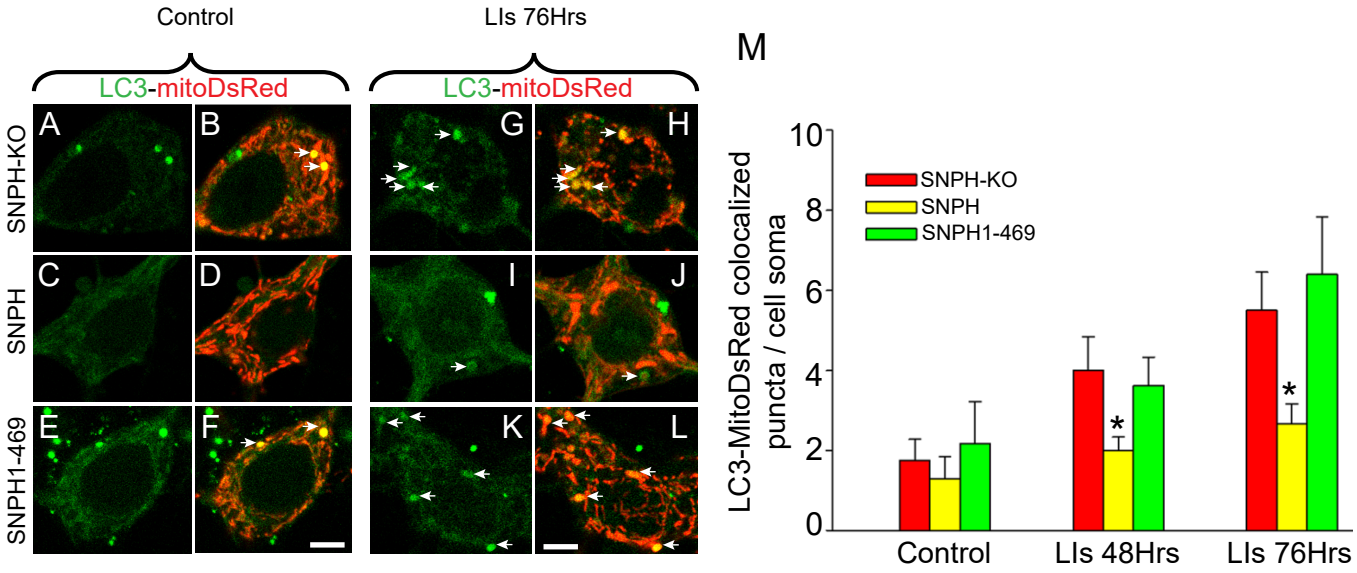

Figure S4. SNPH overexpression blocks somal mitophagy revealed by LC3 *in vitro*. Related to Figure 5. Hippocampal neurons cultured from SNPH-KO mice were co-transfected with SNPH, SNPH1-469, LC3 and mitoDsRed plasmids and flux of somal mitophagy was monitored as co-localization of LC3-MitoDsRed puncta in neuronal soma. (A-F) Representative images of mitophagy in control (vehicle treated) culture from SNPH-KO (A-B), SNPH overexpressed (C-D) and SNPH1-469 overexpressed (E-F) neurons. Scale bar 10 µm. Transfected neurons were treated with lysosomal inhibitors (LIs) for 48 and 76 hrs in culture medium and mitophagy flux was measured. Representative images from SNPH-KO (G-H), SNPH overexpressed (I-J) and SNPH1-469 overexpressed (K-L) groups shown at 76 hrs of LIs treatment. Scale bar 10 µm. (M) Quantification of mitophagy in SNPH-KO, SNPH and SNPH1-469 groups in presence or absence of LIs inhibitors. Data shown as mean ±SEM from 10 neurons from 3 different experiments in each group. *P<0.05

Figure S5

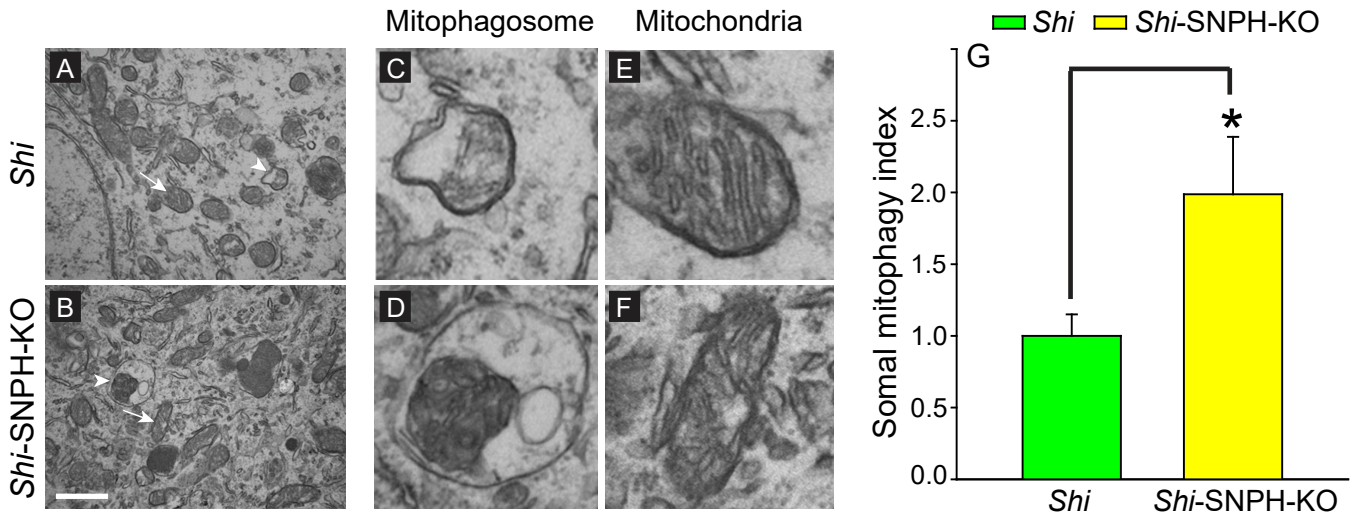

Figure S5. SNPH-KO Increases Mitophagosomes in Soma of PC in *Shiverer* in EM analysis. Related to Figure 5. (A – B) Low magnification EM images from cerebellar PC of *Shi* (A) and *Shi*-SNPH-KO (B) mice at 4.5 months. Arrow in A and B indicate healthy mitochondria while arrowhead in these panels indicates mitophagosomes. Scale bar 1 µm. An enlarged image of mitophagy structures (C-D) and healthy mitochondria (E-F) shown from A and B respectively. (G) Quantification of somal mitophagy index, normalized with respect to *Shi* in total 250 cerebellar sections from 5 mice of each genotype at 4.5 months. Data shown as mean±SEM. * $p<0.05$.