Supplemental Figures

Figure S1 (Lin et al)

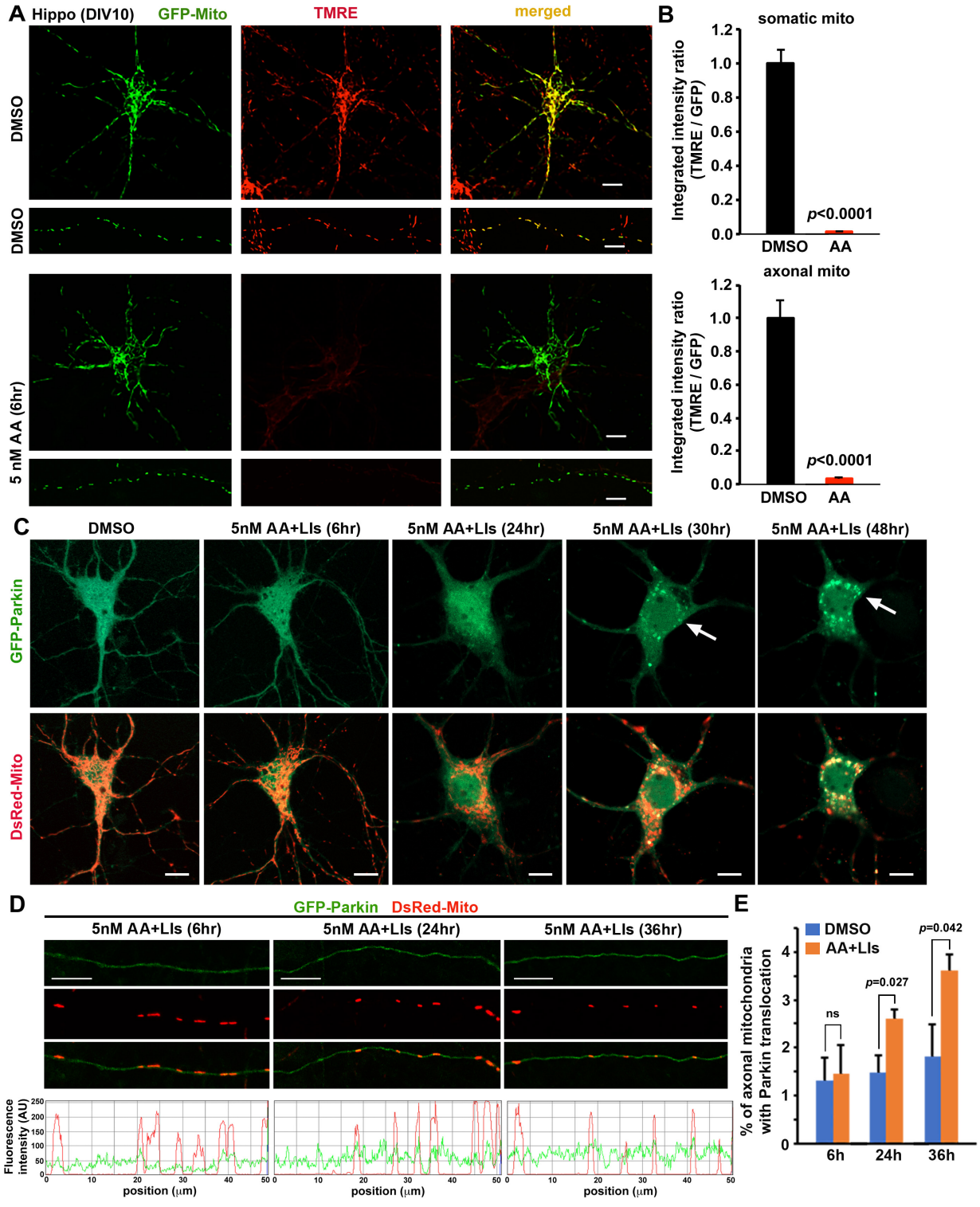

Figure S1 (related to Figure 1A). **Nanomolar AA Induces Delayed Parkin-Mediated Mitophagy**

(A, B) Representative images (A) and quantitative analysis (B) showing depolarized mitochondria after treating hippocampal neurons with 5 nM AA. Neurons at DIV10 were treated with 5 nM AA for 6 hours or DMSO as control, followed by the loading of the mitochondrial $\Delta\psi_m$-dependent dye TMRE for 20 minutes before imaging. Note that the majority of mitochondria in both somatodendritic regions (upper panels) and distal axons (lower panels) lose TMRE staining after 5-nM AA treatment for 6 hours. The integrated intensity of TMRE was normalized by the relative fluorescence intensity within individual GFP-Mito masked areas and compared to DMSO control.

(C) Representative images showing delayed Parkin-mediated mitophagy after prolonged treatment with 5 nM AA. Hippocampal neurons expressing GFP-Parkin and DsRed-Mito at DIV10 were treated with 5 nM AA plus lysosomal inhibitors (LIs, 10 μM E64D and 10 μM pepstatin A) for different time periods, as indicated. Note that the translocation of cytosolic Parkin to depolarized mitochondria was not observed until 30-hour AA treatment. The arrows point to GFP-Parkin-labeled mitochondria.

(D, E) Line scan and quantitative analysis showing no detectable co-localization of Parkin and mitochondria along axons following mild AA treatment (5 nM) for 6 hours. Line scan analysis was performed on raw images in which the brightness and contrast were adjusted identically before and after image sets. A Parkin-targeted mitochondrion was considered when the relative intensity of GFP-Parkin peak was at least twice that of the neighboring peaks (Ashrafi et al., 2014). Note that after prolonged treatment for 36 hours, only a small population (3.62 ± 0.45%) of axonal mitochondria displays Parkin translocation.

Data were analyzed from a total of number of n=10 (B) and 242-335 mitochondria in n=20 axons (E) for each condition in three independent experiments and were expressed as mean ± SEM. The Mann-Whitney test was used to determine the difference between two groups. Scale bars: 10 μm.

Figure S2 (Lin et al)

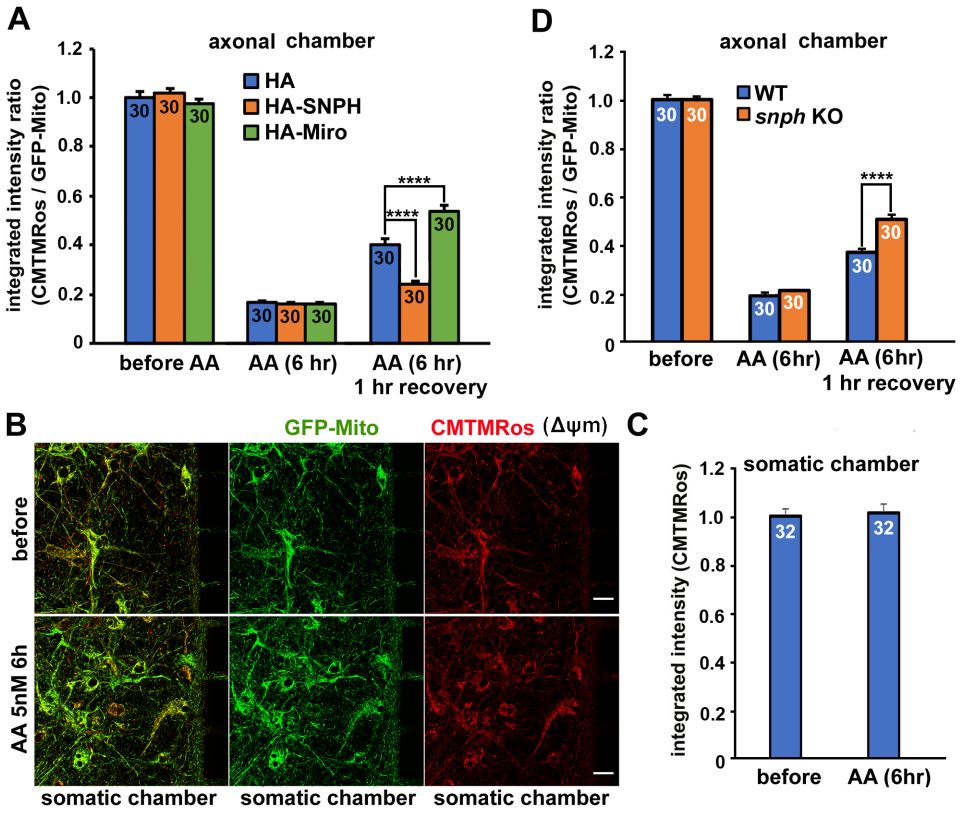

Figure S2 (related to Figures 1C, 1D). **Mitochondrial Transport Is Critical to Maintaining Axonal Mitochondrial $\Delta\psi_m$ Following Mild AA Treatment**

(A) Quantitative analysis of axonal chambers showing the impaired maintenance of axonal mitochondria $\Delta\psi_m$ by arresting mitochondrial transport following 5 nM AA treatment for 6 hours. Cortical neurons infected with lentivirus encoding GFP-Mito and HA, HA-SNPH, or HA-Miro1 at DIV0 were grown on the microfluidic chamber. At DIV8, 5 nM AA was applied to the axonal chamber for 6 hours and then washed out with fresh medium. After a 1-hour recovery, 100 nM MitoTracker CMTMRos (CMTMRos) was loaded in the axonal chamber for 20 minutes before fixation and subsequent imaging of mitochondrial $\Delta\psi_m$. Note that while arresting mitochondrial transport by expressing SNPH reduces the maintenance of axonal mitochondria $\Delta\psi_m$ when compared to the control neurons (p<0.0001), expressing Miro1 enhances the maintenance (p<0.0001).

(B, C) Representative somatic chamber imaging (B) and quantitative analysis (C) showing the fluidic isolation of AA in the microfluidic chambers. Cortical neurons expressing GFP-Mito were

grown on the microfluidic chamber. At DIV8, 5 nM AA was applied to the axonal chamber for 6 hours. CMTMRos was applied to the somatic chamber for imaging. Note that the restricted AA application within the axonal chamber does not induce the global loss of somatic mitochondrial $\Delta\psi_m$.

(D) The quantitative data showing the enhanced maintenance of axonal mitochondria $\Delta\psi_m$ in *snph* KO neurons following the axon-restricted application of 5 nM AA treatment. WT and *snph* KO cortical neurons were infected with lentivirus encoding GFP-Mito at DIV0 and grown on the microfluidic chamber. At DIV8, 5 nM AA was applied to the axonal chamber for 6 hours. After 1-hour recovery, 100 nM CMTMRos was loaded in the axonal chamber for 20 minutes followed by fixation and subsequent imaging of mitochondrial $\Delta\psi_m$. Note that the enhanced mitochondrial transport in the *snph* KO neurons improves the maintenance of axonal mitochondria $\Delta\psi_m$.

The integrated intensity ratio of CMTMRos against GFP-Mito was measured within individual mitochondria and normalized by the control group without AA treatment. Data were quantified from n=30 axonal chamber images (A, D) or n=32 somatic chamber images (C) from six microfluidic chambers per condition in three independent experiments. Data are expressed as mean ± standard error with the Ordinary one-way ANOVA test (A, D) or the unpaired *t*-test (C) (****, $p<0.0001$). Scale bars: 20 μm.

Figure S3 (Lin et al)

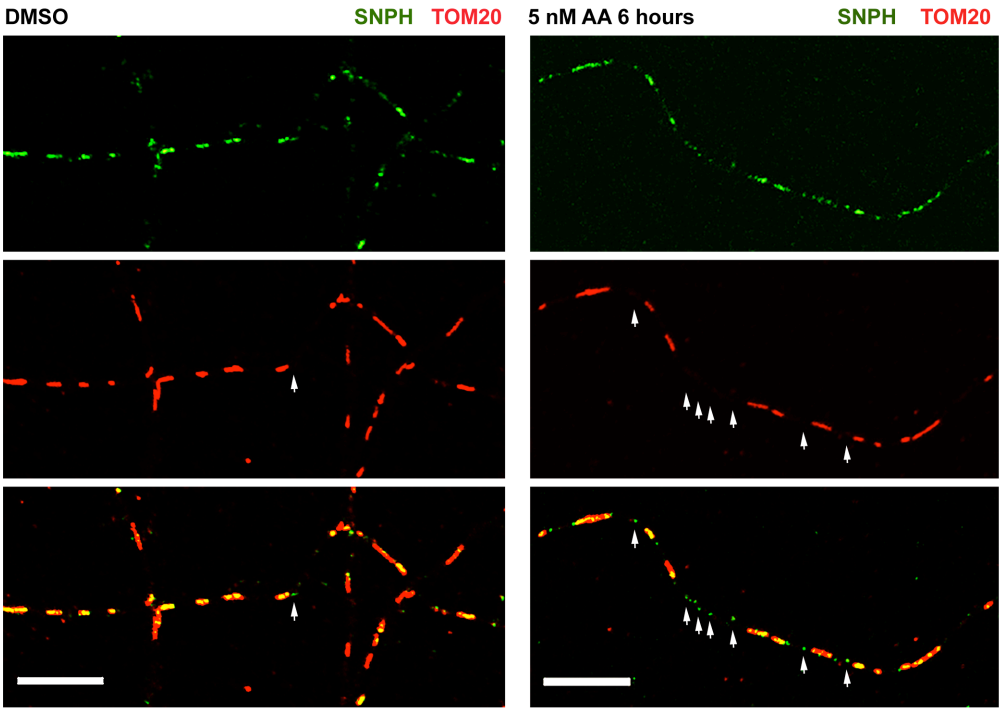

Figure S3 (related to Figure 3E). **TOM20 Is Not Present in the SNPH Cargos**

Representative images show no detectable TOM20 in SNPH cargo vesicles. Hippocampal neurons at DIV10 were treated with DMSO or 5 nM AA for 6 hours, followed by co-immunostaining with antibodies against SNPH and DyLight 550-conjugated-TOM20. Scale bars: 10 μm.

Figure S4 (Lin et al)

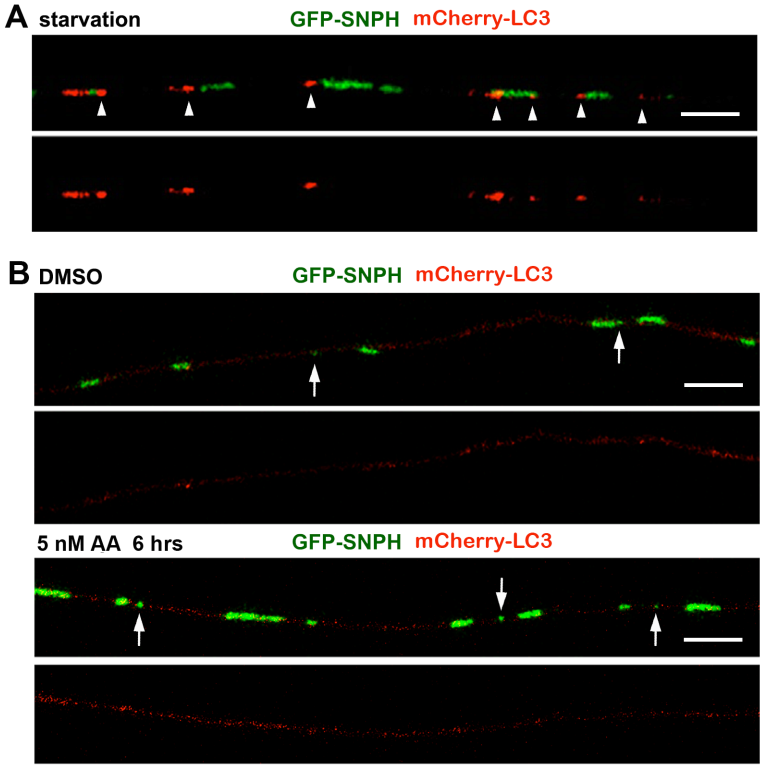

Figure S4 (related to Figure 4). **The generation of SNPH Cargo Vesicles is Independent of Autophagy Induction**

Representative images showing starvation-induced autophagic vacuoles (AVs) labeled by LC3 (A) and AA-induced SNPH vesicles (B). DRG neurons were co-transfected with GFP-SNPH* and mCherry-LC3 at DIV0 and incubated with serum-free medium (starvation) (A) or treated with DMSO or AA (5 nM for 6 hours) (B) at DIV3, followed by live imaging. Arrows indicate SNPH cargo vesicles while arrowheads point to AVs. Note that while a 3-hour starvation efficiently induces AVs (A), SNPH vesicles are generated in the absence of autophagy induction along the same axons under mild mitochondrial stress condition (B). Scale bars: 5 μm.

Figure S5 (Lin et al)

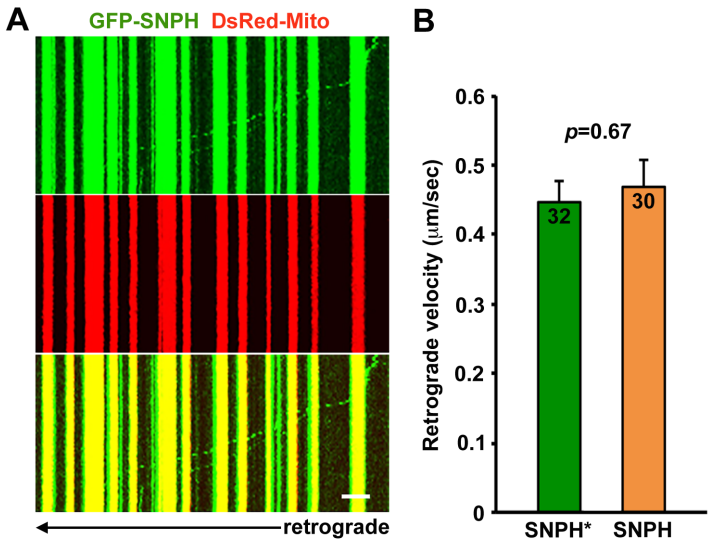

Figure S5 (relative to Figure 6A). **SNPH Cargo Vesicles Undergo Retrograde Transport with a Similar Velocity to SNPH* Vesicles**

(A, B) Representative kymograph (A) and velocity analysis (B) showing retrograde transport of SNPH cargos after 5 nM AA treatment. DRG neurons were co-transfected with DsRed-Mito and GFP-SNPH* or GFP-SNPH at DIV0 and treated with AA (5 nM for 6 hours) at DIV3, followed by two-channel time-lapse imaging at 2-second intervals for a total of 3 minutes. Note that SNPH cargo vesicles share a similar retrograde velocity to SNPH* vesicles following AA treatment (0.45 ± 0.03 μm/sec for GFP-SNPH*; 0.47 ± 0.04 μm/sec for GFP-SNPH, p=0.67). Data were collected from the total number of vesicles for velocity analysis indicated in the bars from three P8-P10 rats and were expressed as mean ± SEM with the unpaired t-test with Welch's correction, Scale bar: 5 μm.

Figure S6 (Lin et al)

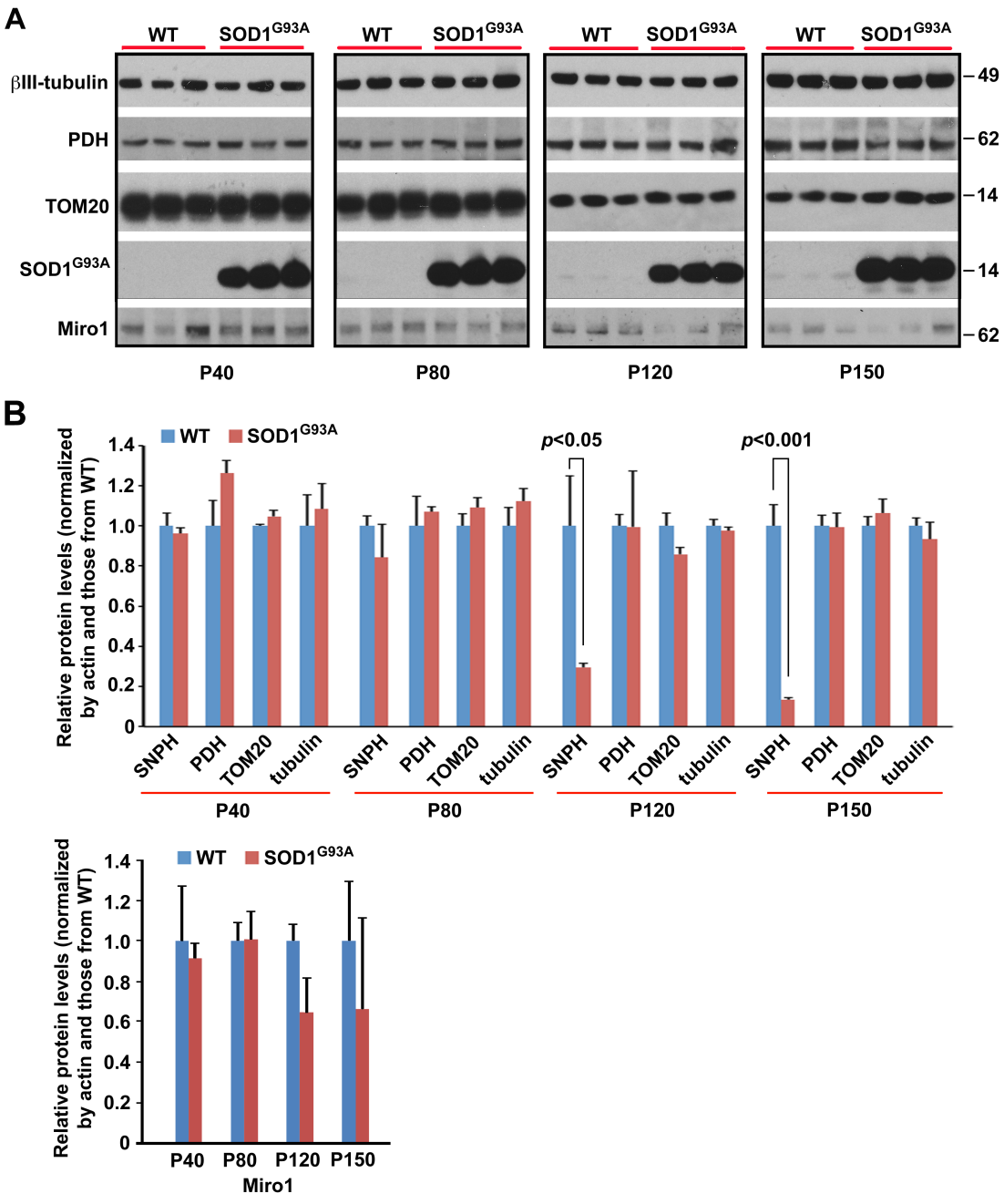

Figure S6 (related to Figures 7D, 7E). **Selective SNPH Depletion During Disease Progression of the hSOD1^{G93A} Mice**

Representative western blots (A) and quantitative analysis (B) showing SNPH depletion in the hSOD1^{G93A} mice after disease onset at age P120. A total of 10 μg of homogenates from the sciatic nerve of age-matched controls and hSOD1^{G93A} mice was sequentially detected on the

same membrane. Note that SNPH, but not the outer mitochondrial membrane proteins TOM20 and the mitochondrial matric protein PDH, is selectively diminished after disease onset at P120, while in WT mice SNPH shows no decline with age (Also see Figures 7D, 7E). Miro1 is reduced in one of three hSOD1^{G93A} mice examined starting at P120. Protein intensities were normalized by actin from the same mice and compared with those in an age-matched control group. For each time point, data were collected from (n=3) pairs of WT and *hSOD1*^{G93A} mice for each time point and were expressed as mean ± SEM with the unpaired Student's *t*-test.

II. Supplementary Movies

Movie S1 (Related to Figure 2A) **Axonal Mitochondrial Motility before AA Treatment**

Movie S2 (Related to Figure 2A) **Axonal Mitochondrial Motility After 5 nM AA Treatment for 2 Hours**

Movie S3 (Related to Figure 2A) **Axonal Mitochondrial Motility After 5 nM AA Treatment for 4 Hours**

Movie S4 (Related to Figure 2A) **Axonal Mitochondrial Motility After 5 nM AA Treatment for 6 Hours**

Movie S5 (Related to Figure 6C) **3D Surface Reconstitution Showing a SNPH Cargo Vesicle (Green) Wrapped by a Rab7-labeled Late Endosome (Red).**