

Figure S1. Increased Recruitment of Parkin and LC3-II to Mitochondria in Cortical Neurons of Mutant hAPP Tg Mice

Cultured WT (red box) or mutant hAPP Tg (green box) neurons at DIV16 were subjected to fractionation into post-nuclear supernatant (P), mitochondrial-enriched membrane fraction (M), and cytosol supernatant (S). An equal amount of fractions (10 mg) was sequentially immunoblotted with antibodies against Parkin and LC3 (mitophagy/autophagy), SOD2 (mitochondria), and APP on the same membranes after stripping between each antibody application. Note that mitochondria in mutant hAPP Tg neurons recruited more Parkin and LC3-II (green box) relative to WT mitochondria (red box). The blots are representative from three independent experiments.

Figure S2. Expressing Mutant hAPP^{swe} or Applying A β Oligomers Induces Parkin-Mediated Mitophagy in Non-Tg Neurons

(A and B) Representative images (A) and quantitative analysis (B) showing Parkin distribution pattern in mature WT cortical neurons expressing vector, WT hAPP, or hAPP^{swe}. Neurons were transfected at DIV7 and imaged at DIV17-19. Note that expressing mutant hAPP^{swe}, but not WT hAPP, is sufficient to induce Parkin-mediated mitophagy.

(C and D) Representative images (C) and quantitative analysis (D) showing enhanced recruitment of LC3 to fragmented mitochondria in the soma of neurons expressing mutant hAPP^{swe}. Arrows indicate mitochondria engulfed in LC3-labeled autophagosomes. Mitochondria marked with autophagic marker LC3 are referred as mitophagosomes.

(E) Quantitative analysis of Parkin translocation in non-Tg neurons at DIV17 following treatment with a relative low concentration (500 nM) of A β 1-42 oligomers or vehicle as control for 72-96 h. Note that A β 1-42 treatment resulted in increased percentage of neurons with Parkin translocation to mitochondria.

(F and G) Representative images (F) and quantitative analysis (G) showing translocation of YFP-Parkin to depolarized mitochondria in non-Tg neurons treated with a low concentration of A β 1-42 oligomers. Non-Tg

cortical neurons were co-transfected with YFP-Parkin and CFP-Mito and treated with a low concentration (500 nM) of A β 1-42 oligomers for 72-96 h, followed by incubating with TMRE for 30 min prior to imaging at DIV17. Note that TMRE mean intensity is reduced in the soma of neurons with Parkin translocation following treatment with A β 1-42 oligomers. Arrows indicate Parkin targeted depolarized mitochondria labeled by CFP-Mito but unlabeled by TMRE; arrowheads represent remaining polarized “healthy” mitochondria marked by both CFP-Mito and TMRE, but not labeled by Parkin.

(H) Representative images showing increased LC3 recruitment to fragmented mitochondria in the soma of non-Tg neurons treated with A β 1-42 oligomers (500 nM) for 72 h. Arrows indicate mitochondria engulfed by LC3-marked autophagosomes.

(I-K) Autophagic marker p62/SQSTM1 were recruited to mitochondria labeled by HSP60 (arrows) in the soma of non-Tg neurons treated with 500 nM of A β 1-42 oligomers for 72 h (I). Co-localization was analyzed as the percentage of neurons showing mitophagosomes (J) and the averaged number of mitophagosomes per neuron (K).

(L) Representative images showing co-localization of Parkin-targeted mitochondria with p62 in oligomeric A β 1-42 treated non-Tg neurons. Right panels are close-up view of the boxed areas. Arrows point to structures containing Parkin, p62, and SOD2.

Scale bars: 10 μ m. Data were quantified from a total number of neurons (n) as indicated in parentheses (B, D, E, J, K) or on the top of bars (G). Data were collected from > 3 independent experiments. Error bars: SEM. Student's t test: *** P < 0.001; ** P < 0.01; * P < 0.05.

Figure S3. Compartmental Distribution of Parkin-Targeted Fragmented Mitochondria in Mutant hAPP Neurons

Representative images showing that Parkin-tagged mitochondria were relatively enriched in somatodendritic regions, but not readily detected in distal axons, of mutant hAPP Tg neurons. Lower and right panels are

enlarged views of the axon and the soma, respectively. Arrows indicate Parkin rings surrounding fragmented mitochondria in the somatodendritic region. Scale bars: 10 mm.

Figure S4. Mitophagosome Accumulation in Mutant hAPP Tg Neurons and Mouse Brains

(A) Autophagic marker p62/SQSTM1 co-localizes with mitochondrial marker cytochrome *c* (Cyto *c*) (arrows) in the soma of mutant hAPP neurons at DIV18. Representative image (right) showing accumulation of p62-ring-like structures in the soma of MAP2-labeled mutant hAPP Tg neurons at DIV18.

(B) Increased targeting of p62 to clustered mitochondria (arrows) marked by cytochrome *c* (Cyto *c*) in the soma of hippocampal regions from mutant hAPP Tg mice.

(C) Increased association of p62 with lysosomes (arrows) labeled by LAMP-1 in the soma of hippocampal regions in mutant hAPP Tg mouse brain.

(D and E) Representative images (D) and quantitative analysis (E) showing increased lysosomal retention of fragmented mitochondria in the soma of mutant hAPP Tg neurons. Arrows indicate abnormal accumulation of mitochondria within clustered and enlarged LAMP-1-marked lysosomes.

Scale bars: 10 mm. Data were quantified from a total number of neurons (*n*) as indicated in parentheses (E).

Data were collected from > 3 independent experiments. Error bars: SEM. Student's *t* test: ****P* < 0.001; ***P* < 0.01; **P* < 0.05.

Figure S5. PARK2 mRNA Levels in Control and AD Patient Brains

(A and B) Representative RT-PCR (A) and quantitative analysis (B) showing no significant alteration in PARK2 mRNA levels in AD patient brains relative to control subjects. Data were quantified from brain specimens of 4 control cases and 8 AD patients (4 in Braak I & II; 2 in Braak III & IV; and 2 in Braak V & VI), and expressed as mean ± SEM with Student's *t* test.

Figure S1 (Cai)

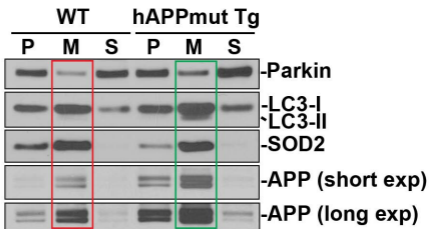


Figure S2 (Cai)

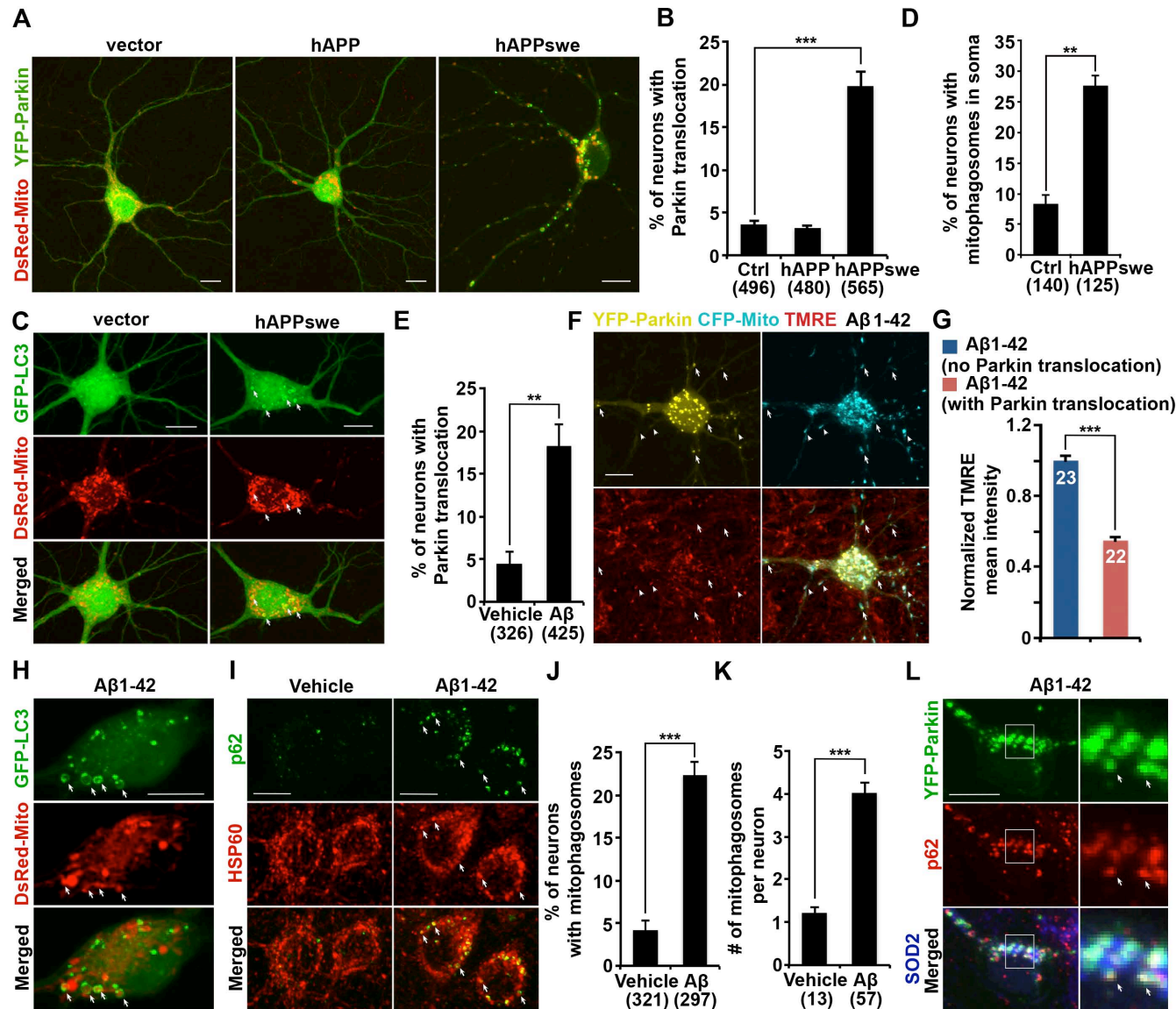


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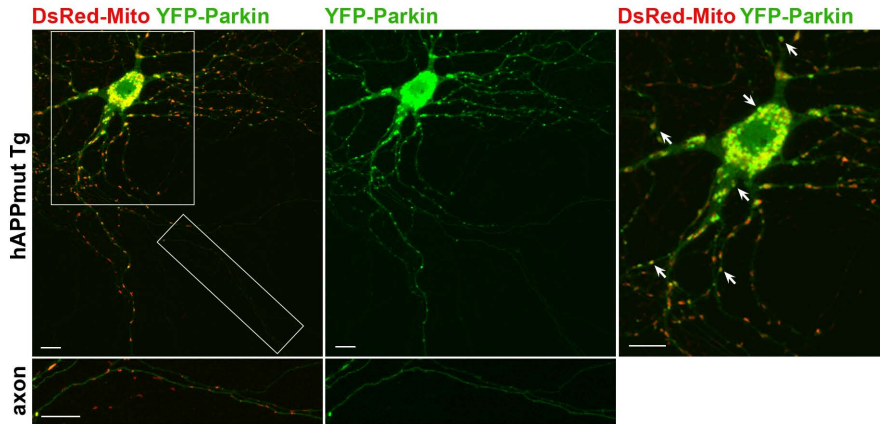


Figure S4

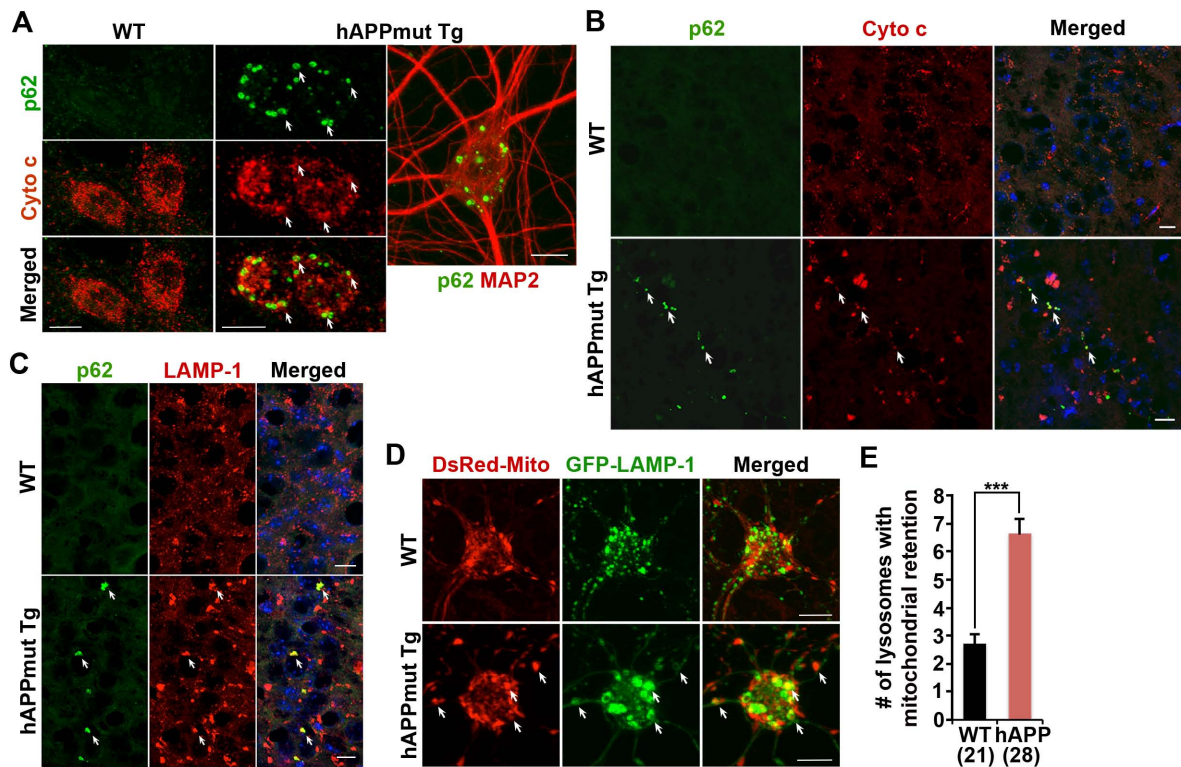
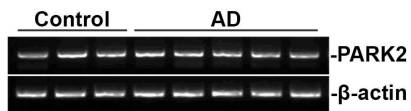


Figure S5 (Cai)

A



B

