Suppl. figure 1

Exogenous wtMfn2 transgene is efficiently transfected and expressed in primary hippocampal neurons.

A) Hippocampal primary neurons were transfected by Nucleofector kit (AMAXA) with plasmid expressing Flag-tag alone, as internal control, or Flag-tagged wtMfn2 for 24h and then total protein extracts were analyzed by Western blotting with anti-Flag (M2 clone) antibody to test the successful expression/delivery of exogenous Mfn2 transgene. β-III tubulin was used as loading control.

B) Hippocampal primary neurons were transfected by Nucleofector kit (AMAXA) with plasmid expressing Flag-tag alone (left), as internal control, or Flag-tagged wtMfn2 (right) for 24h and then stained by immunofluorescence analysis with anti-Mfn2 antibody to check the increased intracellular levels of total protein. Scale bar:15µm

Suppl. Figure 2

The physiological full-length human myc-tau 1-441 (htau40) does not alter the distribution of Parkin and UCHL-1 in transduced neuronal cultures.

Confocal microscopy analysis of double immunofluorescence for myc-tag (green channel) and for Parkin (red channel, A) or UCHL-1 (red channel, A') carried out on primary hippocampal cultures at16 h post-infection with myc-tau 1-441 (htau40) (MOI 50). Nuclei were stained with DAPI (blue channel). Notice that the physiological full-length human myc-tau1-441 (htau40) -when overexpressed in neuronal at similar low levels (MOI 50) of toxic NH_2htau fragment, as checked by Western blotting analysis with antibody against myc-tag (clone 9E10, B)- was unable to alter the uniform and mainly cytoplasmatic distribution of both Parkin and UCHL-1. Scale bar:25µm

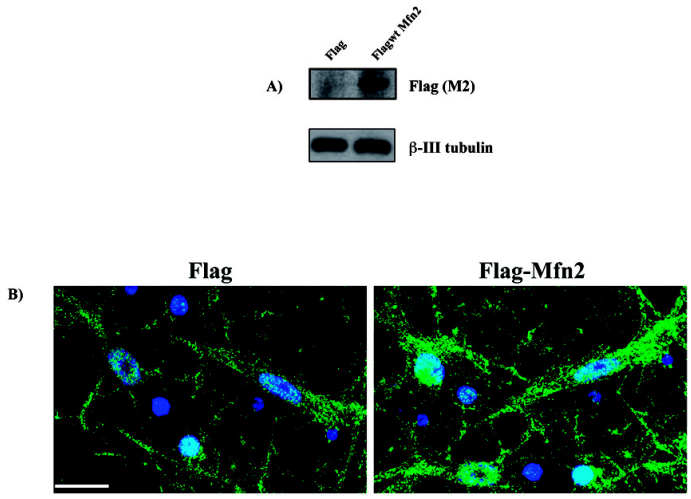
Suppl. figure 3

A reproducible and significant proportion of UCHL-1 and Parkin actually relocalizes in human AD mitochondrial fractions.

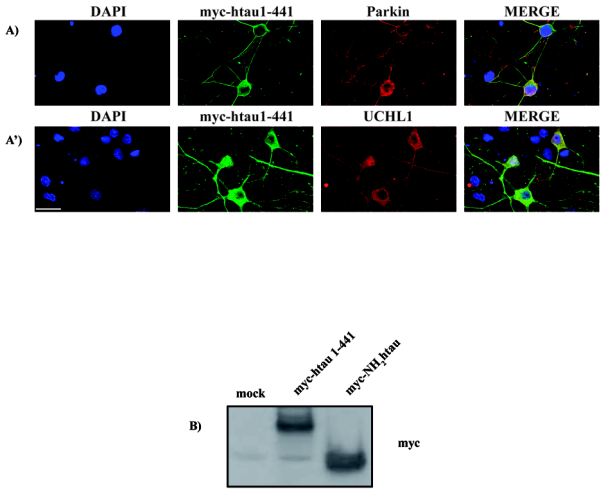
Equal proteins amount of cytosolic and mitochondrial fractions (25µg) from purified synaptosomes of AD (n=3) and age-matched, cognitively intact, not-demented (ND; n=1) human subjects were analyzed by Western blotting to check the *in vivo* mitochondrial co-distribution of

endogenous NH_2-derived 20-22 kDa tau fragment, Parkin and UCHL-1. Immunoblots were probed with CCP-NH_2 tau (NH_2 aa26-36 of human tau) antiserum recognizing the endogenous 20-22kDa NH_2hfragment without any cross-reaction with intact human full-length tau (17, 73), or with Parkin (clone Park8) or with UCHL-1 (PGP9.5 clone 31A3) antibodies. The efficiency and the purity of mitochondrial preparation was checked by probing fractionated protein fractions with several specific antibodies reacting with cytoplasmatic (copper/zinc-Cu/Zn-SODI) (D) and mitochondrial markers (VDAC).

Suppl. Figure 1



Suppl. Figure 2



Suppl. Figure 3