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SUPPLEMENTARY INFORMATION

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Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease

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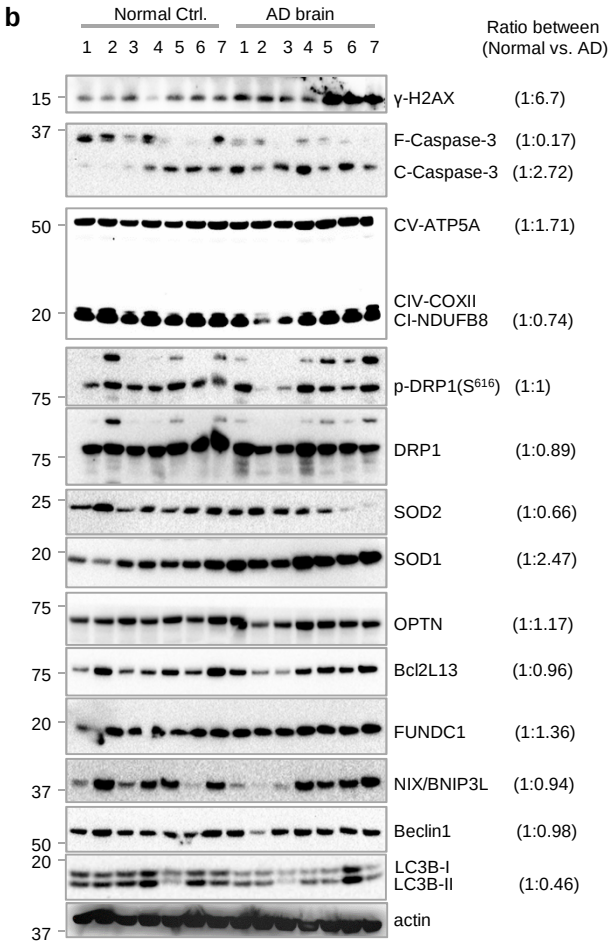
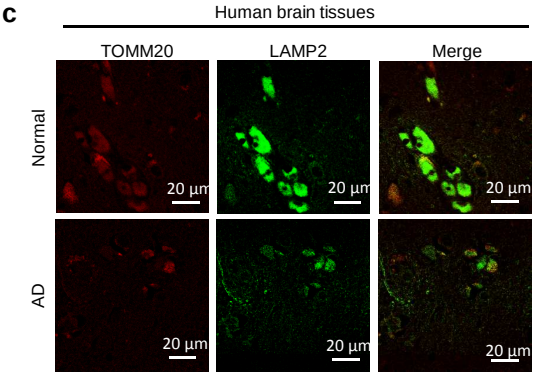
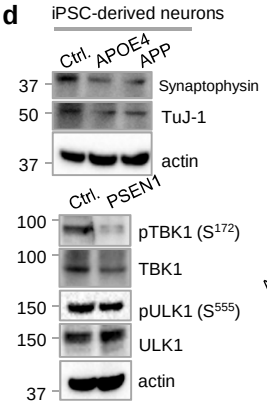
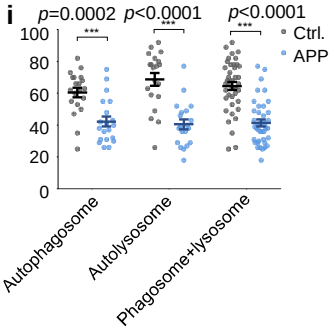
a

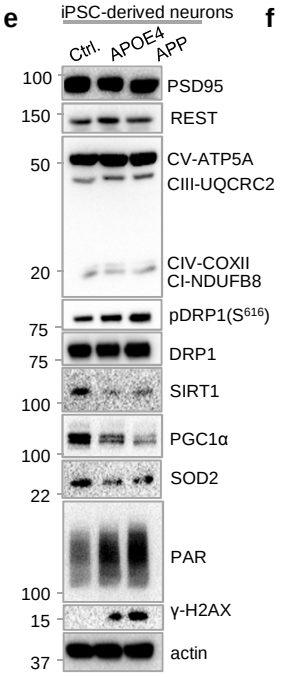
Human brain tissues (vs. Ctrl.)		
Proteins	**Ctrl.**	**AD**
pAMPK/AMPK	1.0 ± 0.40	4.4 ± 1.13* (p=0.014)
pMFF/MFF	1.0 ± 0.14	11.4 ± 4.45* (p=0.032)
pTBK1/TBK1	1.0 ± 0.29	0.19 ± 0.10* (p=0.014)
pULK1/ULK1	1.0 ± 0.22	0.35 ± 0.16* (p=0.027)

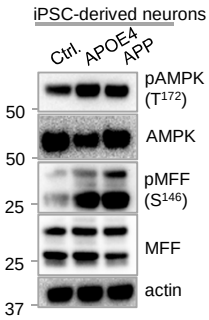












g

iPSC-derived neurons (vs. Ctrl.)			
Proteins	**Ctrl.**	**APOE4**	**APP**
PINK1 /actin	1.0 ± 0.13	0.36 ± 0.22* (p=0.035)	0.20 ± 0.15* (p=0.022)
pTBK1 /TBK1	1.0 ± 0.23	0.06 ± 0.12*** (p=0.0004)	0.52 ± 0.35
pULK1 /ULK1	1.0 ± 0.31	0.20 ± 0.13* (p=0.018)	0.24 ± 0.09* (p=0.023)
LC3-II /actin	1. 0 ± 0.29	0.12 ± 0.08** (p=0.007)	0.06 ± 0.13** (p=0.035)
Beclin1	1.0 ±	0.34 ± 0.14*	0.22 ± 0.13*

h

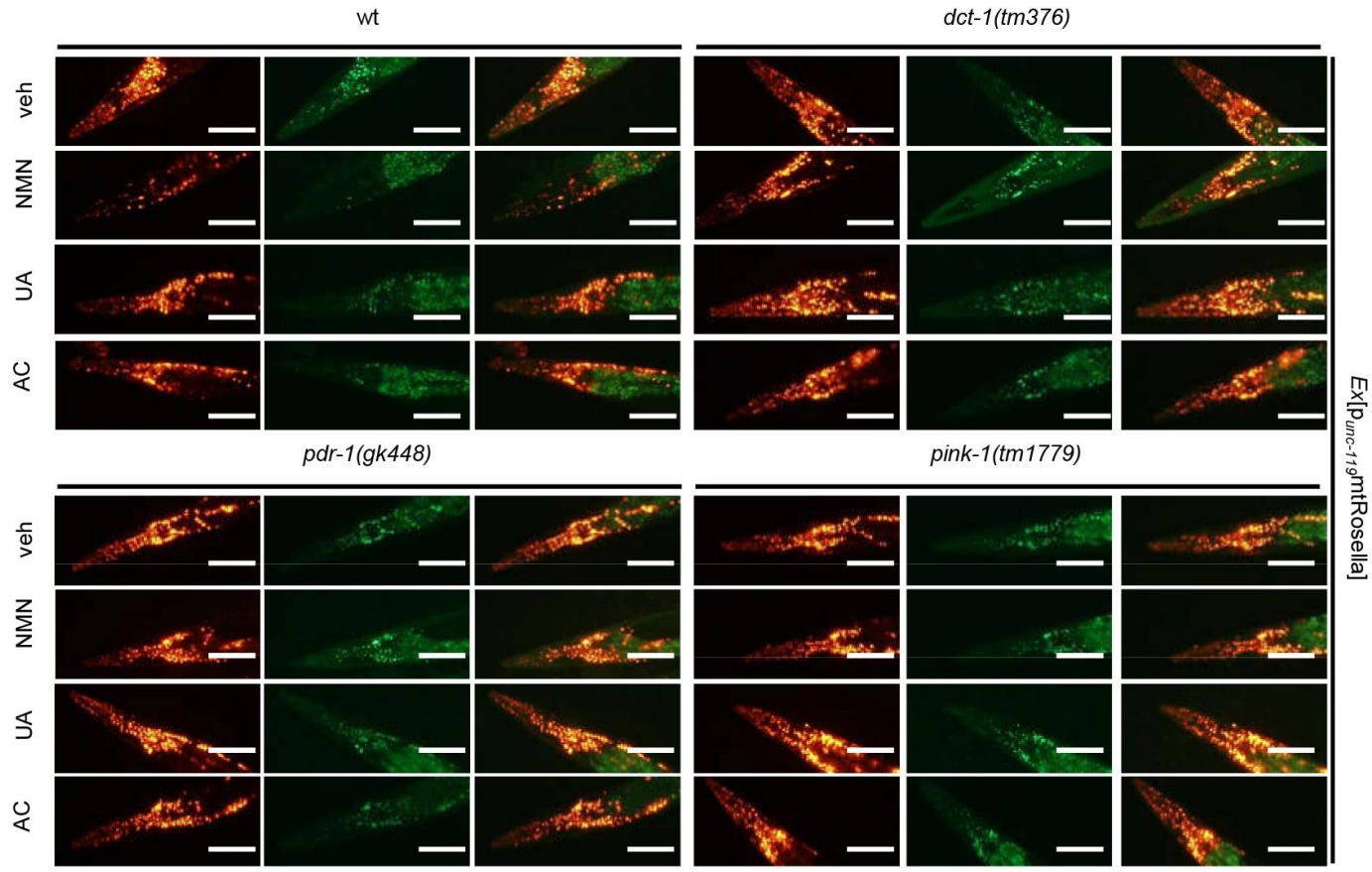
ATP (nmol/mg protein)
Human brain tissues (vs. Ctrl) Ctrl: 4.3 ± 0.13 AD: 1.9 ± 0.17*** (p=0.0009)
iPSC-derived neurons (vs. Ctrl) Ctrl: 3.2 ± 0.07 APOE4: 1.4 ± 0.06*** (p=0.0003) APP: 1.5 ± 0.12*** (p=0.0008)

Supplementary Fig. 1

Supplementary Figure 1

Characterization of postmortem hippocampal samples and iPSC-derived neurons from AD patients and neurologically normal controls.

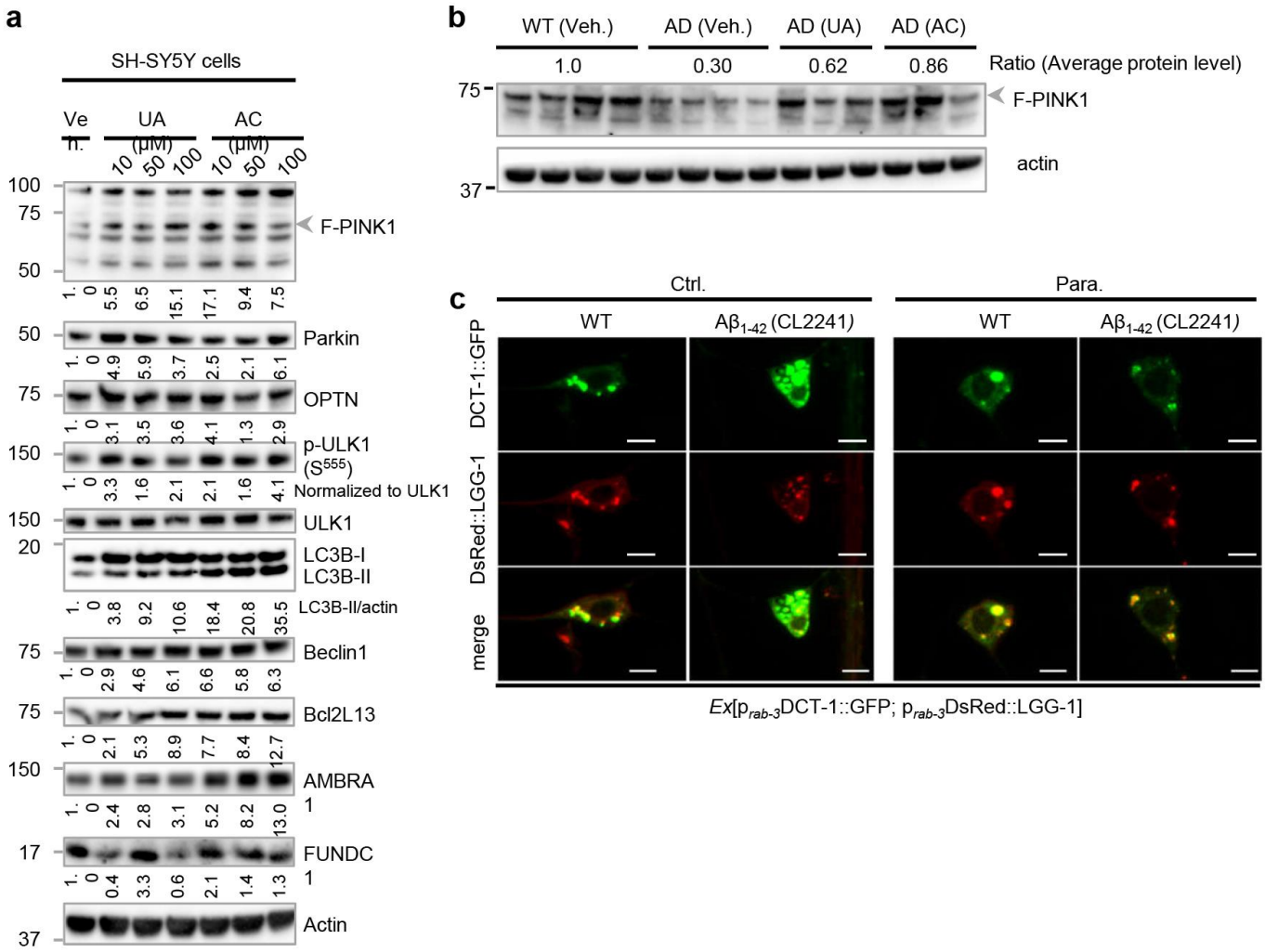
a, Quantification of Western blot data of the indicated proteins. Data were shown in mean ± s.e.m. (n = 7 biologically independent samples from 7 AD patients or 7 healthy controls; **p*<0.05; Two-sided Student's *t*-test). **b**, Levels of proteins related to mitochondrial homeostasis in postmortem hippocampal samples from AD patients and age-matched controls. The right panel shows quantified data between normal and AD groups. Experiments were repeated independently twice with similar results. **c**, Representative IHC images showing colocalization of TOMM20 (mitochondrial outer membrane protein) and LAMP2 (lysosomal protein) in postmortem hippocampal regions of AD patients and age-matched healthy controls (n = 3 samples/group). Experiments were repeated independently twice with similar results. **d**, (upper) Levels of neuronal markers in two AD patient iPSC-derived neuronal lines and a sex- and age-matched control. (lower) Western blotting showing levels of mitophagy-related proteins in a PSEN1 AD patient iPSC-derived neuronal lines and matched control. Experiments were performed once. **e** and **f**, Levels of proteins involved in mitochondrial function and dynamics, metabolism, DNA repair, and mitophagy in two AD patient iPSC-derived neuronal lines and a shared sex- and age-matched control. **g**, Quantification of Western blot data of the indicated proteins. Data are shown in mean ± s.e.m. (n = 3 independent experiments; n.s., *p*>0.05 and **p*<0.05, ***p*<0.01, ****p*<0.001; One-way ANOVA). Experiments were performed three times. **h**, ATP levels in postmortem hippocampal samples from AD patients and age-matched controls. Data are shown in mean ± s.e.m. (n = 7 biologically independent samples; ****p*<0.001; Two-sided Student's *t*-test). **i**, Quantification of autophagosomes and autolysosomes in the APP and control cells with the ptfLC3 plasmid, with data shown in mean ± s.e.m. (n = 20 neurons from 3 independent experiments; ****p*<0.001; Two-sided Student's *t*-test). Full scans of all the blots are in Supplementary Note.



Supplementary Figure 2

Monitoring of neuronal mitophagy in vivo.

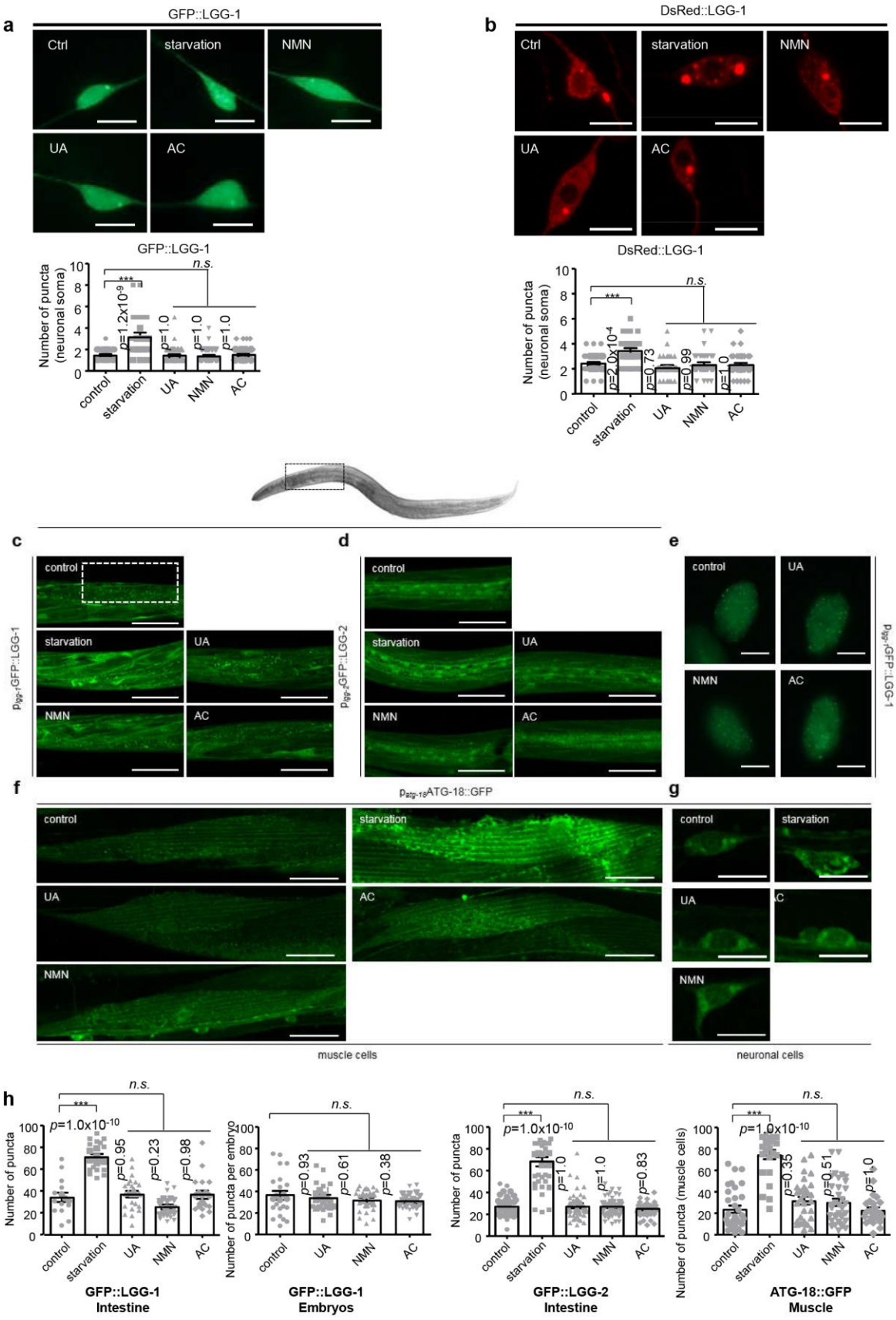
Transgenic nematodes expressing mtRosella biosensor in neuronal cells treated with NMN, UA, and AC. Decreased GFP/DsRed ratio of mtRosella indicates neuronal mitophagy stimulation. DCT-1, PDR-1 and PINK1 were required for neuronal mitophagy induction in response to UA, NMN and AC treatment. Scale bars, 20μm. Quantitative data shown in Fig. 2d. Experiments were repeated independently three times with similar results.



Supplementary Figure 3

Molecular mechanisms of mitophagy induction by UA and AC and defective mitophagy in the $A\beta_{1-42}$ (CL2241) nematodes.

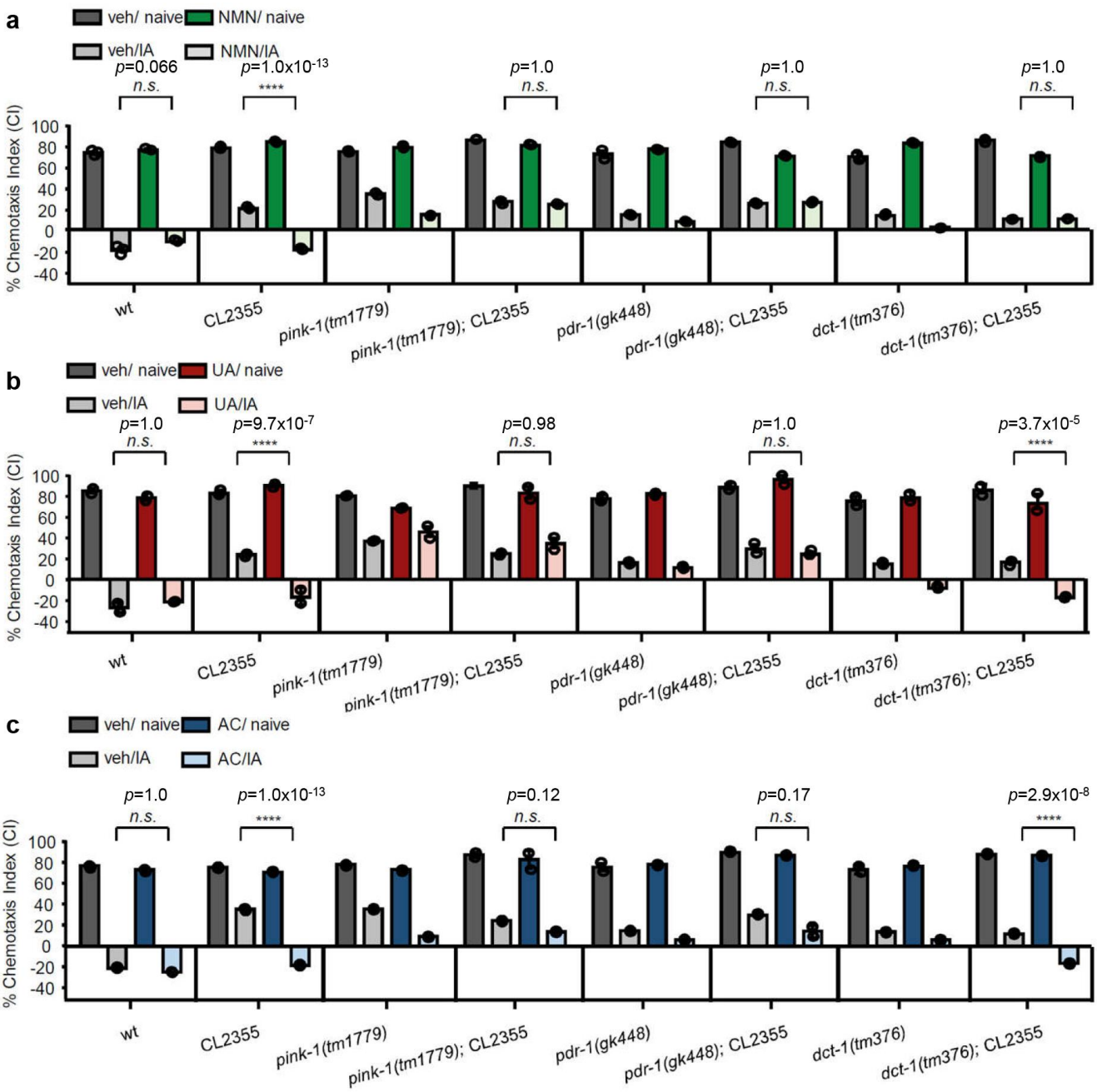
a, Effects of UA and AC on the protein levels of a list of mitophagy-related proteins. The human SH-SY5Y cells were treated with UA (10-100 μM) and AC (10-100 μM) for 24 h, followed by detection of protein expression through western blotting. **b**, UA induces PINK1 expression in the APP/PS1 mice. The APP/PS1 mice were treated with UA (200 mg/kg/day) by daily gavage for 2 months starting from 6 months of age, and then the hippocampal tissues were subjected to western blotting analysis. **c**, Representative images showing changes of neuronal mitophagy in $A\beta_{1-42}$ (CL2241) nematodes under normal and oxidative stress conditions (paraquat/para. 8 mM). Co-localization between the mitophagy receptor DCT-1 fused with GFP and the autophagosomal protein LGG-1 fused with DsRed depicts mitophagy events. Scale bars, 2 μm. For (a), experiments were repeated independently twice with similar results. For (b), one repeat. For (c), experiments were repeated independently twice with similar results. Full scans of all the blots are in Supplementary Note.


a
GFP::LGG-1
Ctrl
starvation
NMN
UA
AC
GFP::LGG-1
Number of puncta (neuronal soma)
***
n.s.
p=1.2x10-9
p=1.0
p=1.0
p=1.0
control
starvation
UA
NMN
AC
b
DsRed::LGG-1
Ctrl
starvation
NMN
UA
AC
DsRed::LGG-1
Number of puncta (neuronal soma)
***
n.s.
p=2.0x10-4
p=0.73
p=0.99
p=1.0
control
starvation
UA
NMN
AC
c
control
starvation
UA
NMN
AC
pgp-4GFP::LGG-1
d
control
starvation
UA
NMN
AC
pgp-2GFP::LGG-2
e
control
UA
NMN
AC
pgp-4GFP::LGG-1
f
control
starvation
UA
AC
NMN
patg-18ATG-18::GFP
muscle cells
g
control
starvation
UA
C
NMN
neuronal cells
h
Number of puncta
***
n.s.
p=1.0x10-10
p=0.95
p=0.23
p=0.98
control
starvation
UA
NMN
AC
GFP::LGG-1
Intestine
Number of puncta per embryo
n.s.
p=0.93
p=0.61
p=0.38
control
UA
NMN
AC
GFP::LGG-1
Embryos
Number of puncta
***
n.s.
p=1.0x10-10
p=1.0
p=1.0
p=0.83
control
starvation
UA
NMN
AC
GFP::LGG-2
Intestine
Number of puncta (muscle cells)
***
n.s.
p=1.0x10-10
p=0.35
p=0.51
p=1.0
control
starvation
UA
NMN
AC
ATG-18::GFP
Muscle

Supplementary Figure 4

The optimized doses of NMN, UA, and AC do not induce general macro-autophagy in *C. elegans*.

a-b, Supplementation with NMN (5 mM), UA (0.1 mM), and AC (1 mM) does not alter general autophagy levels in *C. elegans* neurons. Representative images showing transgenic nematodes expressing the autophagosomal marker LGG-1 fused with GFP (a) or DsRed (b) (n = 30 neuronal cells/group; ***p<0.001; one-way ANOVA followed by *Sidak*'s multiple comparisons test). Scale bars, 5 µm. **c-h**, *C. elegans* transgenic animals expressing full-length p_{lgg-1}GFP::LGG-1, p_{lgg-2}GFP::LGG-2 and p_{atg-18}ATG-18::GFP fusion proteins indicative of autophagic activity treated with NMN (5 mM), UA (0.1 mM), and AC (1 mM) (n = 30 nematodes/group; ***p<0.001; one-way ANOVA followed by *Sidak*'s multiple comparisons test). Several tissues were evaluated, including intestines (c-d; Scale bars, 40 µm), embryos (e; Scale bars, 10 µm), muscles (f; Scale bars, 20 µm), and neurons (g; Scale bars, 5 µm). Starvation-induced autophagy used as an internal positive control. Error bars, ± s.e.m.



d

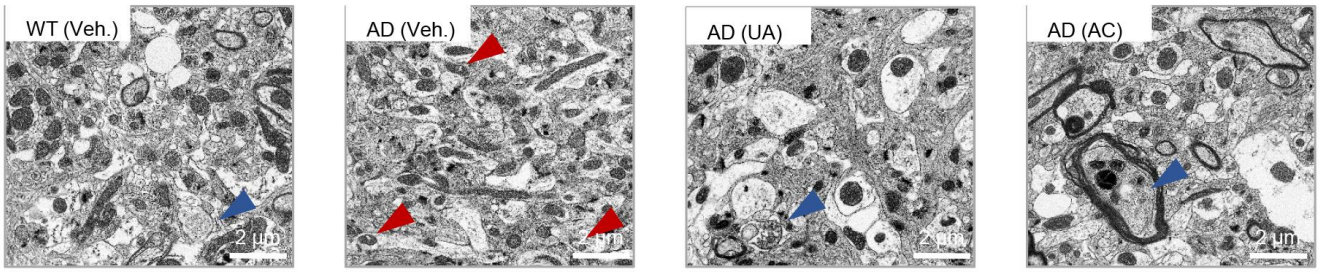
Aβ1-42 (CL2355)			
Mitophagy inducers	Mitophagy gene (memory dependent)		
	pink-1	*pdr-1*	*dct-1*
NMN	yes	yes	yes
UA	yes	yes	no
AC	yes	yes	no

Supplementary Figure 5

Mitophagy induction protects against memory impairment in *C. elegans* AD models.

We evaluated memory defects in the AD nematodes using an aversive olfactory learning paradigm. To determine whether memory retention is dependent on mitophagy in the $A\beta_{1-42}$ (CL2355) nematodes, we generated three mitophagy mutant strains, *pink1(tm1779)*;CL2355, *pdr-1(gk488)*;CL2355, and *dct-1(tm376)*;CL2355. **a-c**, NMN-, UA-, and AC-induced memory improvement depends differentially on PINK1, PDR-1 and DCT-1 activity (n = 400 nematodes/group; n.s., $p>0.05$, ****$p<0.0001$; Two-way ANOVA followed by Tukey's multiple comparisons test). **d**, Beneficial effects of NMN, UA, and AC on memory of $A\beta_{1-42}$ expressing nematodes (CL2355) differentially depend on mitophagy genes. For all experiments, nematodes were treated with NMN (5 mM) and AC (1 mM) for 2 generations, and with UA (0.1 mM) from eggs to the day of experiment for one generation. Adult Day 1 nematodes were used for the memory assay. For all worm experiments, 2 to 4 independent experiments were performed. For all 'dot-plot' figures, center value represents mean and error bars represent s.e.m.

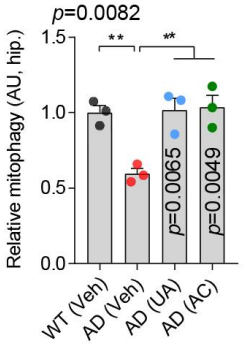
a



b

Parameters	Hippocampus			
	WT (Veh.) n=1377	APP/PS1 (Veh.) n=1228	APP/PS1 (UA) n=2733	APP/PS1 (AC) n=1271
Mito. length (nm)	503±8	432±11	620±6	574±8
Mito. diameter (nm)	371±3	197±4	339±3	328±4
Area (μm²) / mitochondrion	0.156±0.003	0.071±0.004	0.187±0.003	0.160±0.004
Damaged mito. (%)	11.3±2.2	35.6±2.6*** (p=0.39x10^{-4})	14.9±2.0### (p=0.08x10^{-4})	18.2±2.9### (p=0.92x10^{-4})
Mitophagy (%)	28.4±2.6	9.3±1.1*** (p=0.57x10^{-4})	24.9±2.5### (p=0.82x10^{-4})	26.0±2.1### (p=0.46x10^{-5})

c



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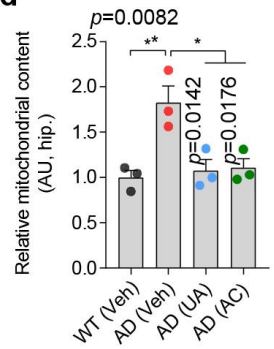

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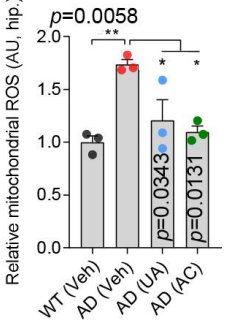

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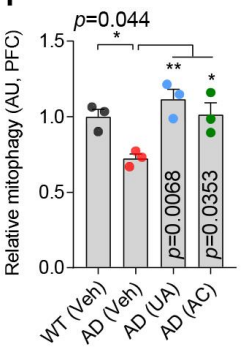

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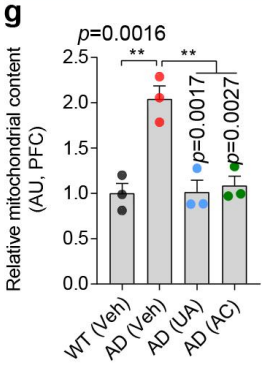

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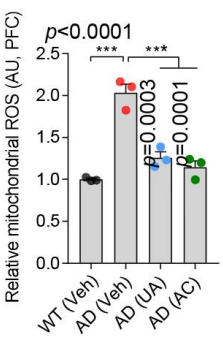

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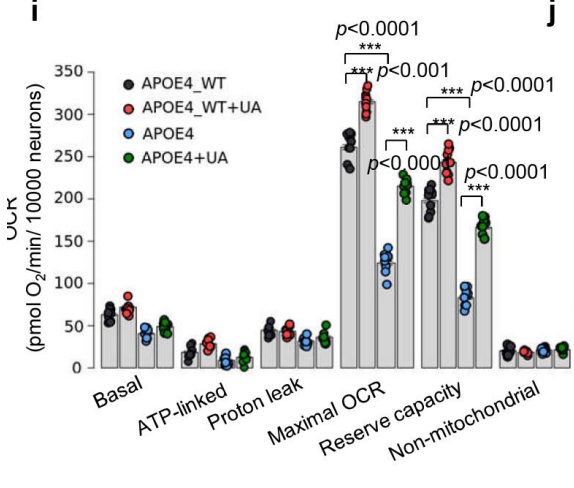

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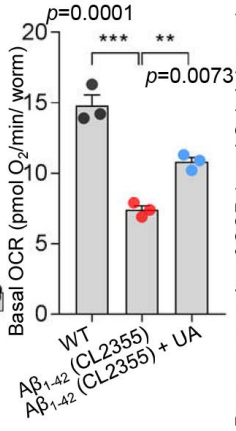

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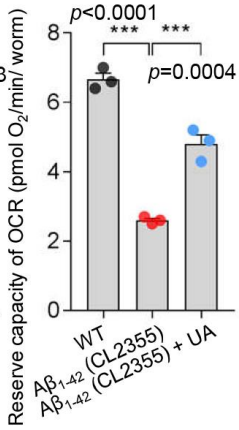


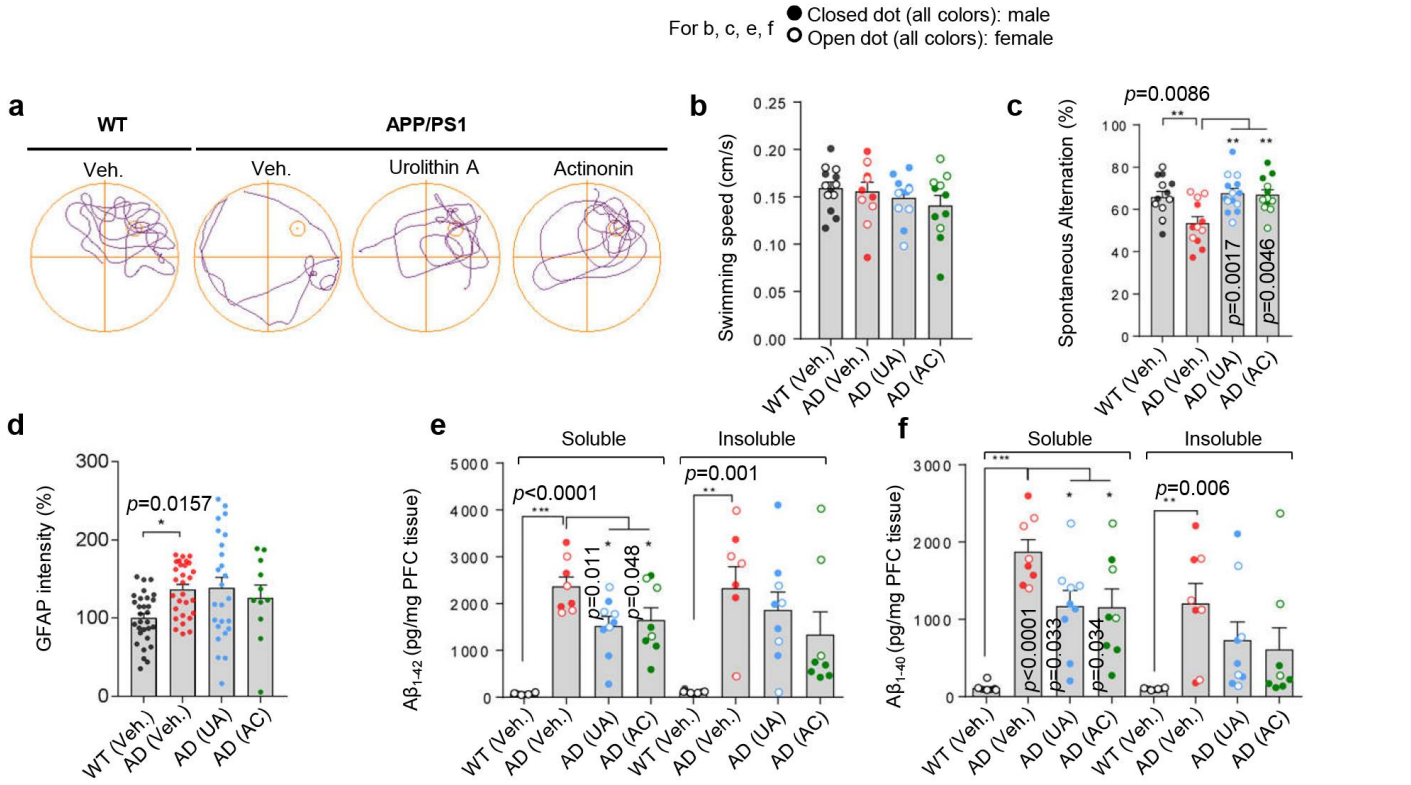
l

Mitochondrial ROS	Relative values
WT (veh):	1.0 ± 0.05
Aβ$_{1-42}$(CL2355) (veh)	1.8 ± 0.06*** (p=0.0005)
Tau(BR5270) (veh):	1.6 ± 0.05*** (p=0.0007)
WT (UA):	0.7 ± 0.04## (p=0.006)
Aβ$_{1-42}$(CL2355) (UA)	1.4 ± 0.05## (p=0.009)
Tau(BR5270) (UA):	1.2 ± 0.04### (p=0.0006)

Supplementary Figure 6

Mitophagy induction improves mitochondrial homeostasis and mitochondrial function in APP/PS1 AD mice and APOE4/E4 iPSC-derived neurons.

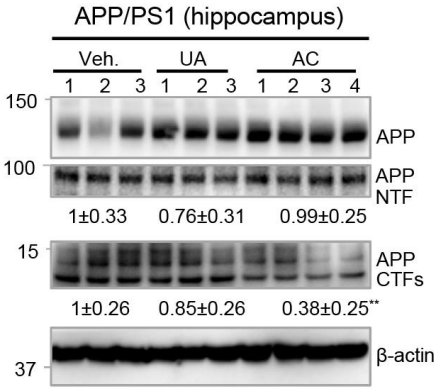
APP/PS1 AD mice were treated with UA (200 mg/kg/day) or AC (30 mg/kg/day) by daily gavage for 2 months starting at 6 months of age, and subsequently tested in behavioral studies. Thereafter, mice were sacrificed for tissue collection. **a**, Representative EM images of neuronal mitochondria (n = 3 mice/group). **b**, Summary of mitochondrial parameters and mitophagy events in hippocampus tissues upon EM analysis. Data were shown in mean ± s.e.m. (n = 3 mice/group; ***p<0.001 compared with WT (veh), $^{###}p$<0.001 compared with APP/PS1 (veh); One-way ANOVA). **c-e**, Measurement of mitophagy levels (**c**), mitochondrial content (**d**) and mitochondrial ROS (**e**) in freshly isolated neurons from hippocampal tissues of treated and untreated animals. Center value represents mean and error bars represent s.e.m. (n = 3 mice/group; *p<0.05, **p<0.01, and ***p<0.001; One-way ANOVA). **f-h**, Measurement of mitophagy (**f**), mitochondrial content (**g**), and mitochondrial ROS (**h**) in isolated neurons from prefrontal cortex (PFC) tissues of treated and untreated animals. Center value represents mean and error bars represent s.e.m. (n = 3 mice/group; *p<0.05, **p<0.01, and ***p<0.001; One-way ANOVA). **i**, UA improves mitochondrial function in APOE/E4 iPSC-derived neurons. The APOE/E4 iPSC-derived neurons and WT controls were treated with UA (50 μM) for 24 h, followed by the evaluation of OCR using a seahorse machine. Data were shown in mean ± s.e.m. (n = 6 technical repeats; One-way ANOVA with ***p<0.001). **J-k**, UA increases OCR in the $A\beta_{1-42}$ (CL2355) nematodes. Experiments for (j-k) were performed together with Fig. 2 e-f and, thus share the same data for the vehicle groups (n = 3 independent experiments; **p<0.01, and ***p<0.001; One-way ANOVA). l, UA treatment reduced mitochondrial ROS levels in WT, $A\beta_{1-42}$ (CL2355), and Tau (BR5270) worms. UA (0.1 mM) was fed from L4 to adult day 3, followed by isolation of fresh mitochondria, stained with DFCA, and the ROS signals were quantified through FACS. Data were shown in mean ± s.e.m. (n = 3 independent experiments; ***p<0.001 compared with WT veh.; $^{##}p$<0.01 or $^{###}p$<0.001 compared with each own respective vehicle control; One-way ANOVA).



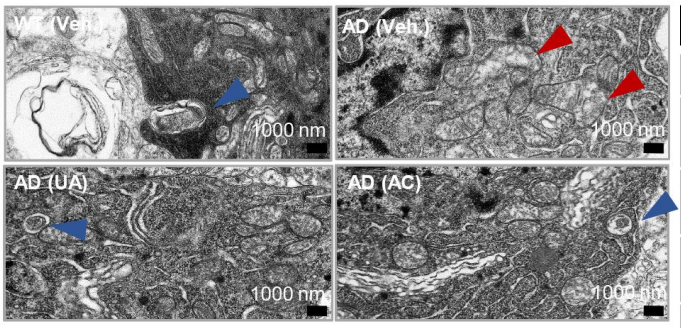
g

APP/PS1 mice			
Proteins	**Veh.**	**UA**	**AC**
Synap./actin	1.0 ± 0.14	3.62 ± 0.67* (*p*=0.035)	3.60 ± 0.76* (*p*=0.037)
TREM2/actin	1.0 ± 0.31	2.17 ± 0.25	7.67 ± 1.55* (*p*=0.023)
CD68/actin	1.0 ± 0.50	5.29 ± 0.91* (*p*=0.024)	6.75 ± 1.89 (*p*=0.09)
IRF7/actin	1.0 ± 0.38	4.26 ± 0.11* (*p*=0.017)	3.56 ± 0.99 (*p*=0.06)
CSF2RA/actin	1.0 ± 0.19	1.98 ± 0.21* (*p*=0.041)	2.05 ± 0.27
Osteopo./actin	1.0 ± 0.68	0.58 ± 0.17	2.68 ± 1.20

h



i



j

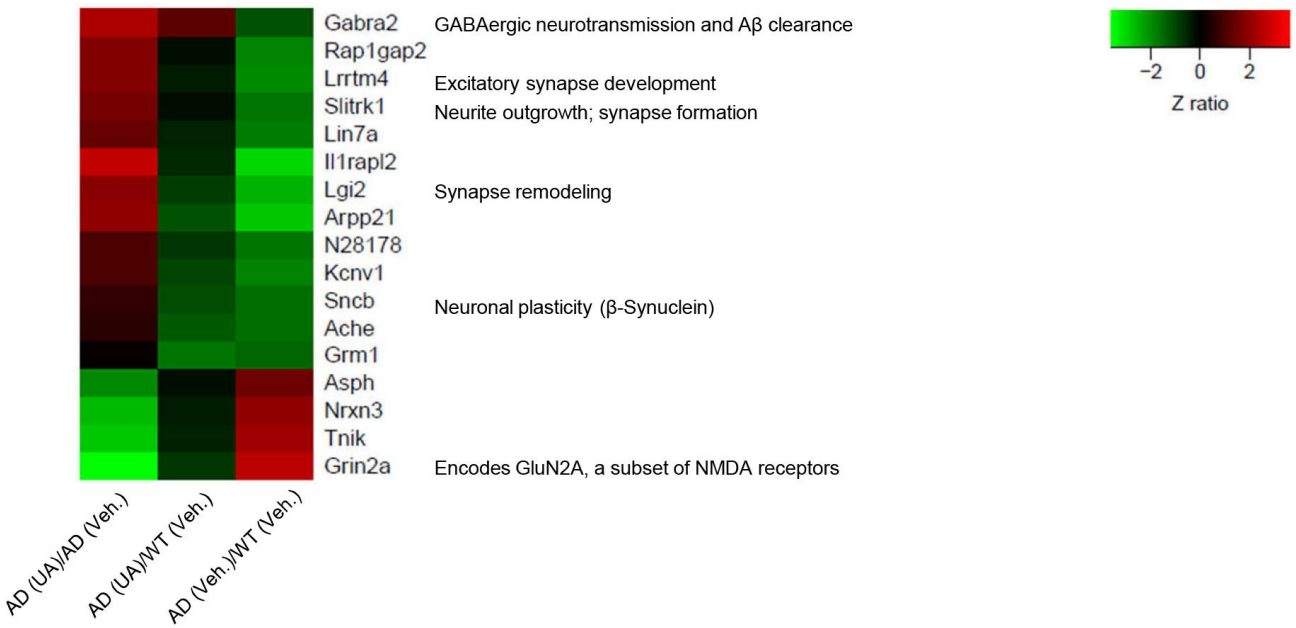
APP/PS1 mice (compared with Veh.)			
Parameters	**Veh.**	**UA**	**AC**
Microglia with Aβ/Total microglia	1.0 ± 0.1	2.5 ± 0.3***	2.9 ± 0.2***
Number of microglia/ROI	8.11 ± 0.45	13.0 ± 0.91***	7.29 ± 1.07
Processes/microglial cell	3.15 ± 0.16	2.66 ± 0.15*	2.56 ± 0.15*
Process length (μm)	5.99 ± 0.15	5.12 ± 0.21**	5.32 ± 0.19*

Supplementary Figure 7

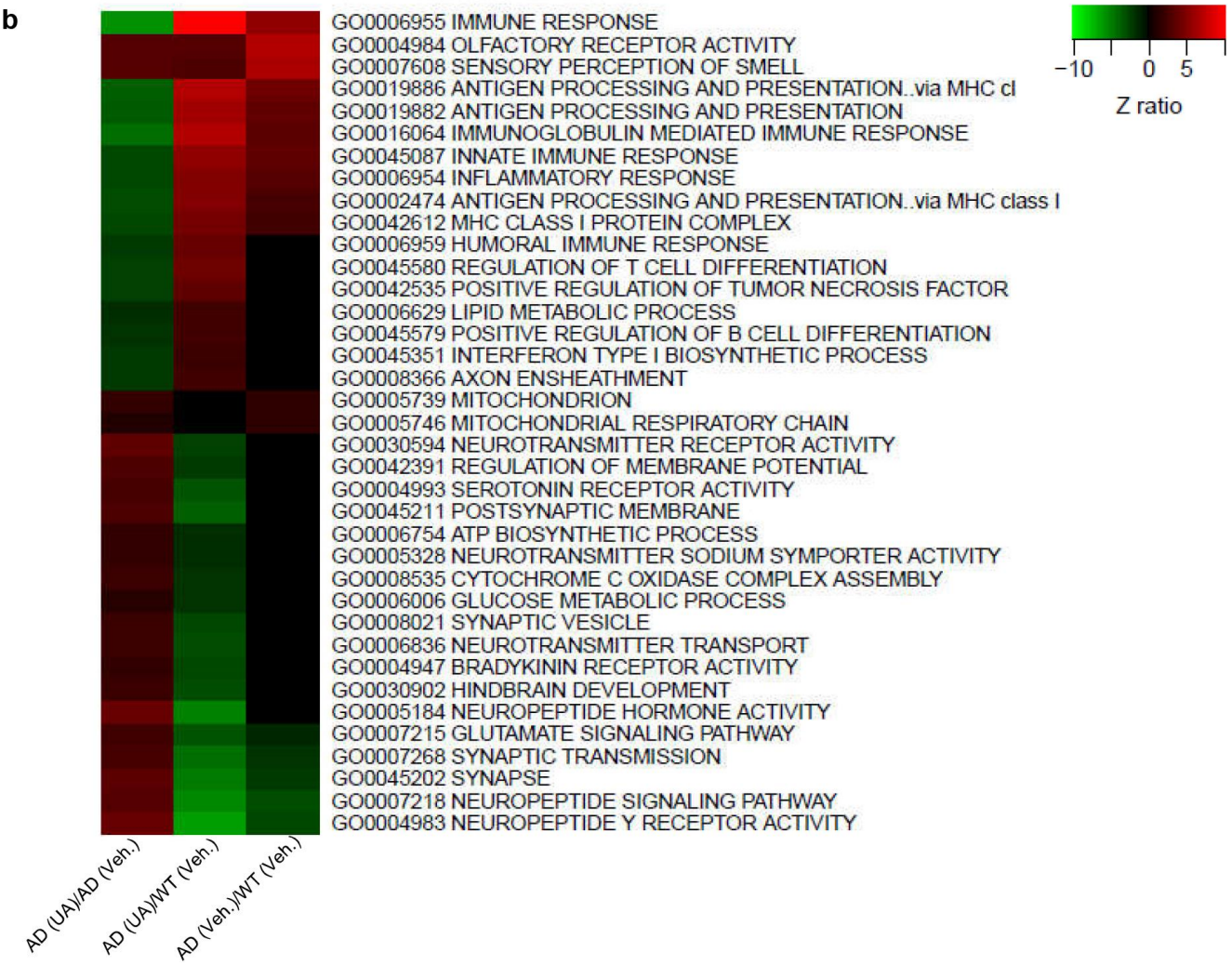
Mitophagy induction increases memory, diminishes soluble Aβ in PFC, and improves the quality of microglial mitochondria in the APP/PS1 AD mice.

APP/PS1 AD mice were treated with UA (200 mg/kg/day) or AC (30 mg/kg/day) by daily gavage for 2 months starting from 6 months to 8 months, tested in behavioral assays, and then sacrificed for tissue collection. **a**, Representative images of the latency to a hidden platform in the Morris water maze test (n = 13 mice in the WT veh. group, or 11 mice in all the other groups). **b**, There was no difference in swimming speed between groups in Morris water maze test. Center value represents mean and error bars represent s.e.m. (n = 13 mice in the WT veh. group, or 11 mice in all the other groups). **c**, Mitophagy stimulation improves spatial memory in the Y-Maze. Center value represents mean and error bars represent s.e.m. (n = 13, 12, 14, 12 mice for each group). **d**, Quantification of GFAP using histological tissues. Center value represents mean and error bars represent s.e.m. (n = 30, 30, 25, 11 sections, from 3 mice, for each group). **e-f**, Analysis of soluble and insoluble $A\beta_{1-42}$ and $A\beta_{1-40}$ levels in prefrontal cortex (PFC) tissues using an ELISA method. Center value represents mean and error bars represent s.e.m. (n = 9 mice in the AD UA. group, or 8 mice in all the other groups; **p*<0.05, ***p*<0.01; One-way ANOVA). **g**, Quantification of Western blot data of the indicated proteins from 3 mice/group (associated with Fig. 4b). Data are shown in mean ± s.e.m. (n.s., *p*>0.05 and **p*<0.05, ***p*<0.01, ****p*<0.001; One-way ANOVA). **h**, Protein levels of APP intermediates in hippocampal samples in response to UA and AC supplementation (n = 3 in the veh. group and the UA group, n = 4 in the AC group). Quantification values are shown in mean ± s.e.m. (***p*<0.01 with *p* =0.029; One-way ANOVA). **i**, Representative EM images of mitochondria in microglial cells (n = 3 mice/group). Error bars, ± s.e.m. **j**, Quantification of a list of parameters related to microglial phagocytosis, numbers and morphology (activation). Supplementation of UA and AC resulted in enhanced Aβ plaque sequestration by microglia in hippocampus (n = 5 mice/group; ****p*<0.001; One-way ANOVA). UA and AC treatment influences the microglia population, the number of processes and the process length of microglia in hippocampus. Data are shown in mean ± s.e.m. (n=5 mice/group; ****p*<0.001; One-way ANOVA). Full scans of all the blots are in Supplementary Note.

a


Gabra2
Rap1gap2
Lrrtm4
Slitrk1
Lin7a
Il1rapl2
Lgi2
Arpp21
N28178
Kcnv1
Sncb
Ache
Grm1
Asph
Nrxn3
Tnik
Grin2a
GABAergic neurotransmission and Aβ clearance
Excitatory synapse development
Neurite outgrowth; synapse formation
Synapse remodeling
Neuronal plasticity (β-Synuclein)
Encodes GluN2A, a subset of NMDA receptors
−2
0
2
Z ratio
AD (UA)/AD (Veh.)
AD (UA)/WT (Veh.)
AD (Veh.)/WT (Veh.)

b


GO0006955 IMMUNE RESPONSE
GO0004984 OLFACTORY RECEPTOR ACTIVITY
GO0007608 SENSORY PERCEPTION OF SMELL
GO0019886 ANTIGEN PROCESSING AND PRESENTATION..via MHC cl
GO0019882 ANTIGEN PROCESSING AND PRESENTATION
GO0016064 IMMUNOGLOBULIN MEDIATED IMMUNE RESPONSE
GO0045087 INNATE IMMUNE RESPONSE
GO0006954 INFLAMMATORY RESPONSE
GO0002474 ANTIGEN PROCESSING AND PRESENTATION..via MHC class I
GO0042612 MHC CLASS I PROTEIN COMPLEX
GO0006959 HUMORAL IMMUNE RESPONSE
GO0045580 REGULATION OF T CELL DIFFERENTIATION
GO0042535 POSITIVE REGULATION OF TUMOR NECROSIS FACTOR
GO0006629 LIPID METABOLIC PROCESS
GO0045579 POSITIVE REGULATION OF B CELL DIFFERENTIATION
GO0045351 INTERFERON TYPE I BIOSYNTHETIC PROCESS
GO0008366 AXON ENSHEATHMENT
GO0005739 MITOCHONDRION
GO0005746 MITOCHONDRIAL RESPIRATORY CHAIN
GO0030594 NEUROTRANSMITTER RECEPTOR ACTIVITY
GO0042391 REGULATION OF MEMBRANE POTENTIAL
GO0004993 SEROTONIN RECEPTOR ACTIVITY
GO0045211 POSTSYNAPTIC MEMBRANE
GO0006754 ATP BIOSYNTHETIC PROCESS
GO0005328 NEUROTRANSMITTER SODIUM SYMPORTER ACTIVITY
GO0008535 CYTOCHROME C OXIDASE COMPLEX ASSEMBLY
GO0006006 GLUCOSE METABOLIC PROCESS
GO0008021 SYNAPTIC VESICLE
GO0006836 NEUROTRANSMITTER TRANSPORT
GO0004947 BRADYKININ RECEPTOR ACTIVITY
GO0030902 HINDBRAIN DEVELOPMENT
GO0005184 NEUROPEPTIDE HORMONE ACTIVITY
GO0007215 GLUTAMATE SIGNALING PATHWAY
GO0007268 SYNAPTIC TRANSMISSION
GO0045202 SYNAPSE
GO0007218 NEUROPEPTIDE SIGNALING PATHWAY
GO0004983 NEUROPEPTIDE Y RECEPTOR ACTIVITY
−10
0
5
Z ratio
AD (UA)/AD (Veh.)
AD (UA)/WT (Veh.)
AD (Veh.)/WT (Veh.)

Supplementary Figure 8

Bioinformatic analysis indicates that mitophagy induction improves neuronal function in APP/PS1 AD mice.

APP/PS1 AD mice were treated with UA (200 mg/kg/day) by daily gavage for 2 months starting from 6 months of age. Hippocampal tissue was collected for microarray analysis. **a**, Effects of UA treatment on mRNA levels of designated neuron-specific genes. Right side, neuronal functions of designated proteins. **b**, Changes of GO pathways among designated groups. n = 5, 6, 4 mice for AD (veh.), AD (UA), and WT (veh.), respectively.

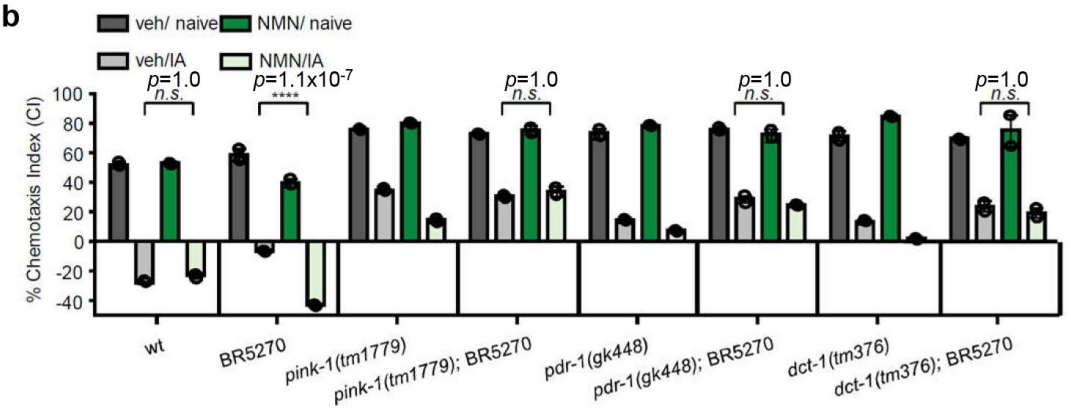
a

Inhibition of pTau site				
Human Tau isoforms	**pTau sites**			
	Thr^{181}	Ser^{202}+ Thr^{205}	Thr^{23}_{1}	Ser^{262}
2N4R	yes	yes	yes	yes
1N4R	no	yes	no	yes
2N3R	yes	yes	no	yes

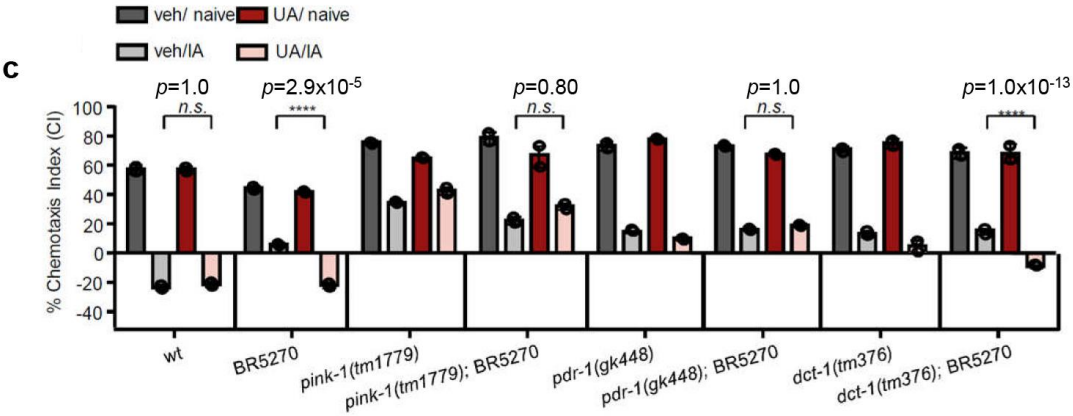
e

Tau (BR5270)			
Mitophagy inducer	**Mitophagy gene (memory dependency)**		
	pink-1	*pdr-1*	*dct-1*
NMN	yes	yes	yes
UA	yes	yes	no
AC	yes	no	no

b



c



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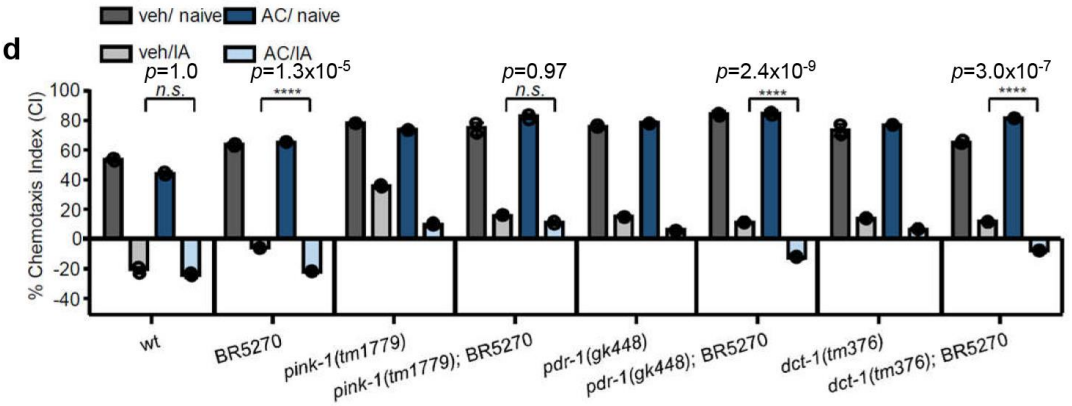
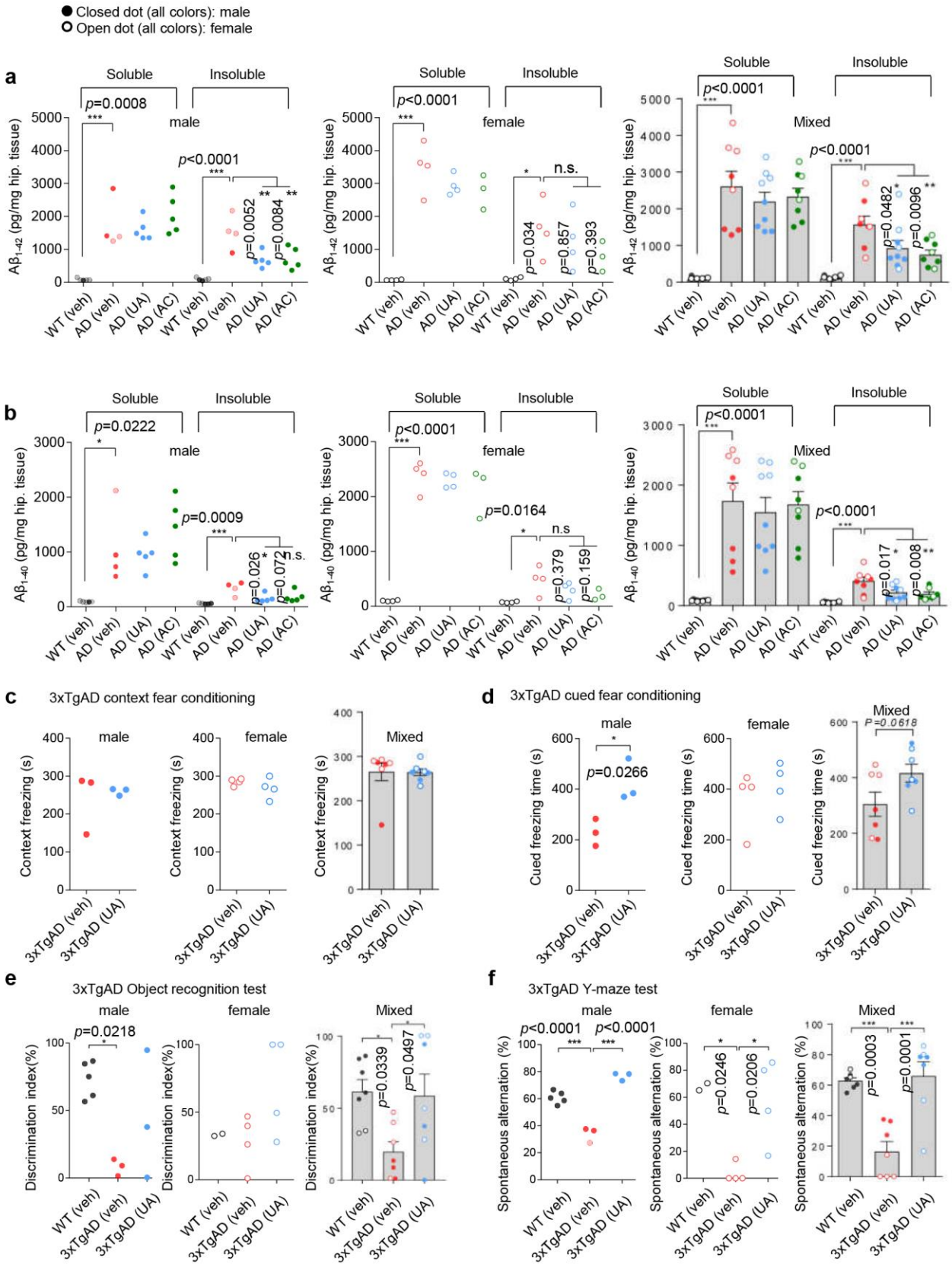


Supplementary Figure 9

Rescue of learning and memory deficits by NMN, UA, and AC is dependent on mitophagy.

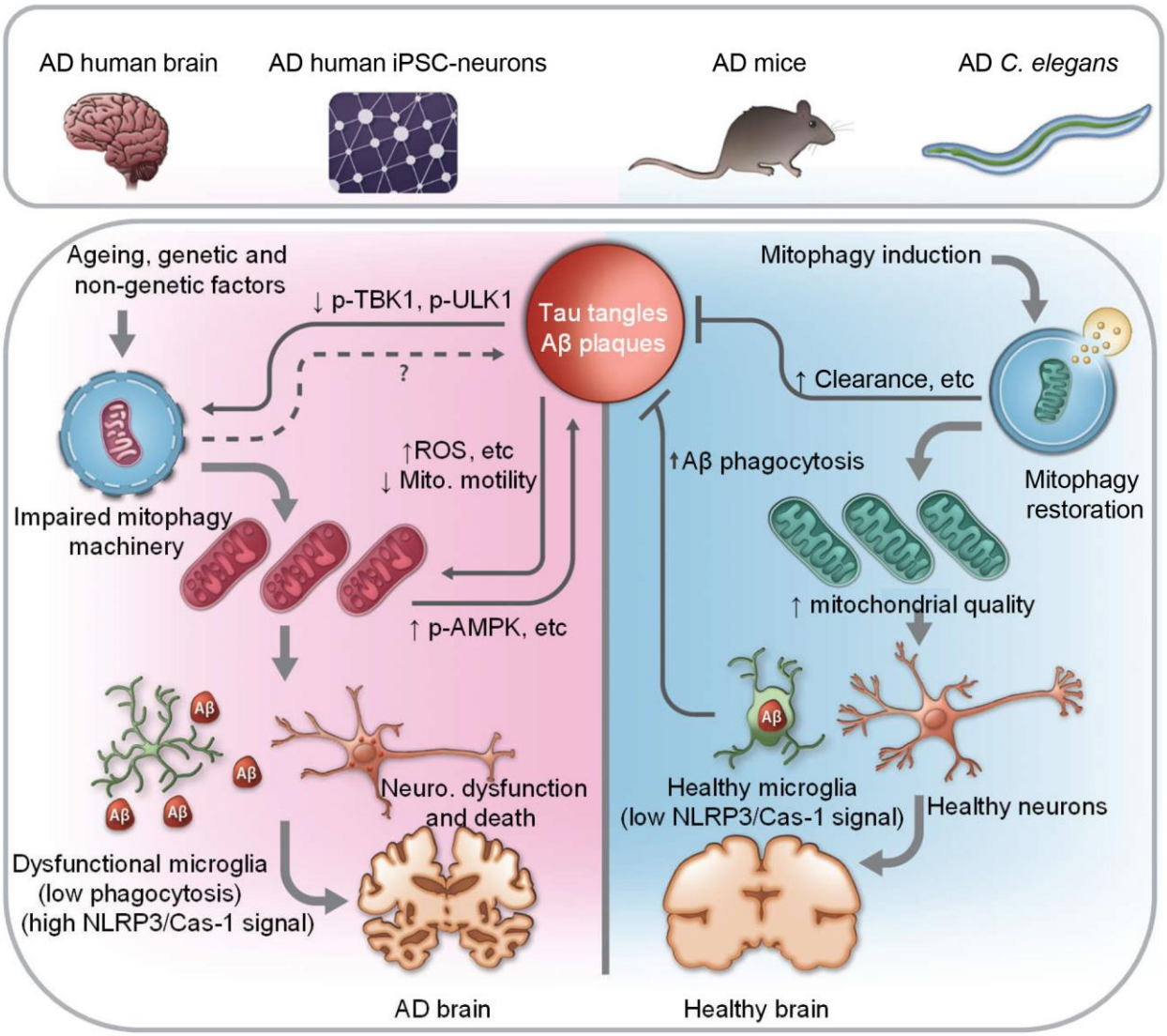
a, A summary of the effects of UA on pTau levels (associated to Fig. 5a). Conclusions were based on two independent experimental repeats and with similar results. **b-e**, Using an aversive olfactory learning paradigm, we investigated whether memory retention is dependent on mitophagy in the Tau (BR5270) nematodes. We generated three mitophagy mutant Tau strains, *pink-1(tm1779)*;BR5270, *pdr-1(gk488)*;BR5270, and *dct-1(tm376)*;BR5270. **b-d**, PINK1, PDR-1 and DCT-1 were differentially required to mediate memory improvement in transgenic animals expressing Tau (BR5270) upon NMN, UA and AC supplementation. Center value represents mean and error bars represent s.e.m. (n = 400 nematodes/group; n.s., $p>0.05$, ****$p<0.0001$; Two-way ANOVA followed by Tukey's multiple comparisons test). For all experiments, nematodes were treated with NMN (5 mM) and AC (1 mM) for 2 generations, or with UA (0.1 mM) from eggs to the day of experiment. Adult Day 1 nematodes were used for the memory assay. For all worm experiments, 2 to 4 independent experiments were performed. **e,** Beneficial effects of NMN, UA, and AC on memory of Tau (BR5270) nematodes depend differentially on mitophagy genes. Conclusions were based on two independent experimental repeats and with similar results.


Closed dot (all colors): male
Open dot (all colors): female
a
Soluble
Insoluble
male
female
Mixed
Aβ1-42 (pg/mg hip. tissue)
p=0.0008
p<0.0001
p=0.0052
p=0.0084
p=0.034
n.s.
p=0.857
p=0.393
p=0.0482
p=0.0096
WT (veh)
AD (veh)
AD (UA)
AD (AC)
b
Aβ1-40 (pg/mg hip. tissue)
p=0.0222
p=0.0009
p=0.026
p=0.072
p=0.0164
p=0.379
p=0.159
p=0.017
p=0.008
c
3xTgAD context fear conditioning
Context freezing (s)
3xTgAD (veh)
3xTgAD (UA)
d
3xTgAD cued fear conditioning
Cued freezing time (s)
p=0.0266
P=0.0618
e
3xTgAD Object recognition test
Discrimination index(%)
p=0.0218
p=0.0339
p=0.0497
f
3xTgAD Y-maze test
Spontaneous alternation (%)
p<0.0001
p<0.0001
p=0.0246
p=0.0206
p=0.0003
p=0.0001

Supplementary Figure 10

Effects of sex difference on mitophagy-induction-induced benefits in two AD mouse models.

a-b, The APP/PS1 mice were treated with UA (200 mg/kg/day) or AC (30 mg/kg/day) by daily gavage for 2 months starting from 6 months of age, and then the $A\beta_{1-42}$ and $A\beta_{1-40}$ levels in the hippocampal region were detected using standard ELISA techniques. Data shown in changes of hippocampal $A\beta_{1-42}$ (a) or $A\beta_{1-40}$ (b) levels in male and female mice. For the mouse numbers in (a) and (b), n = 8 (4 males + 4 females) in WT (veh.), n = 8 (4 males + 4 females) in AD (veh.), n = 9 (5 males + 4 females) in AD (UA), and n = 8 (5 males + 3 females) in AD (AC). Center value represents mean and error bars represent s.e.m. (**p*<0.05, ***p*<0.01, ****p*<0.001; One-way ANOVA). Sex difference of the data shown in Figure 3f, g were reanalyzed here. **c-f**, effects of one-month UA treatment on memory performance in 3xTgAD mice. Thirteen-month old 3xTgAD mice were treated with UA (200 mg/kg/day) by daily gavage for 1 month. To investigate any sex difference, the data show in Figure 5i-l were further analyzed here. Contextual and cued fear conditioning test (**c, d**), object recognition test (**e**), and Y-maze test (**f**) were performed. For the mouse numbers using in c-f, n = 7 (5 males + 2 females) in WT (veh.), n = 7 (3 males + 4 females) in 3xTgAD (veh.), n = 7 (3 males + 4 females) in 3xTgAD (UA). Center value represents mean and error bars represent s.e.m. (**p*<0.05, ***p*<0.01, ****p*<0.001; Two-sided Student's *t*-test was used for the comparison between 2 groups, while One-way ANOVA was used to compare three groups.).



Supplementary Figure 11

Working model.

Upper panel: The roles of mitophagy were evaluated in AD pathology utilizing postmortem human AD brain samples, AD iPSC-derived neurons, and transgenic animal models of AD, including *C. elegans* and mice. Lower panel: We propose the synergistic roles and relationships between Aβ, p-Tau, and defective mitophagy in AD progression. In the red panel: The underlying cause in most AD cases is complex, likely reflecting risk associated with aging, multiple genetic factors as well as non-genetic (e.g., environmental, lifestyle/behavioural and metabolic) factors. These factors can directly/indirectly cause mitophagy defects, leading to accumulation of damaged mitochondria, a major feature in both familial and sporadic AD patients. Defective mitophagy, damaged mitochondria, and Tau tangles/Aβ plaques, exacerbate one another (dashed arrow, further work necessary), causing neurodegeneration and impaired phagocytosis by microglia, and the gradual development of AD pathology in brain. Evidence of Tau/Aβ-induced reduction of mitochondrial motility were from (Ram D. et al., Science 2008; Tammineni P et a., Autophagy 2017). In the blue panel: Mitophagy induction maintains a healthy mitochondrial pool through efficient clearance of dysfunctional organelles. Healthy mitochondria augment neuronal function and survival as well as promote the clearance of extracellular Aβ plaques by microglia. Proficient mitophagy maintains a healthy brain.

Western blots full scans

Figure 1c

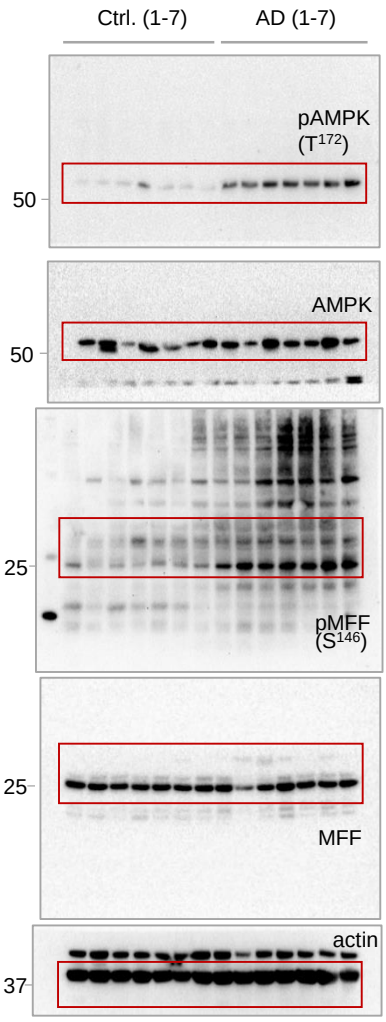


Figure 1f

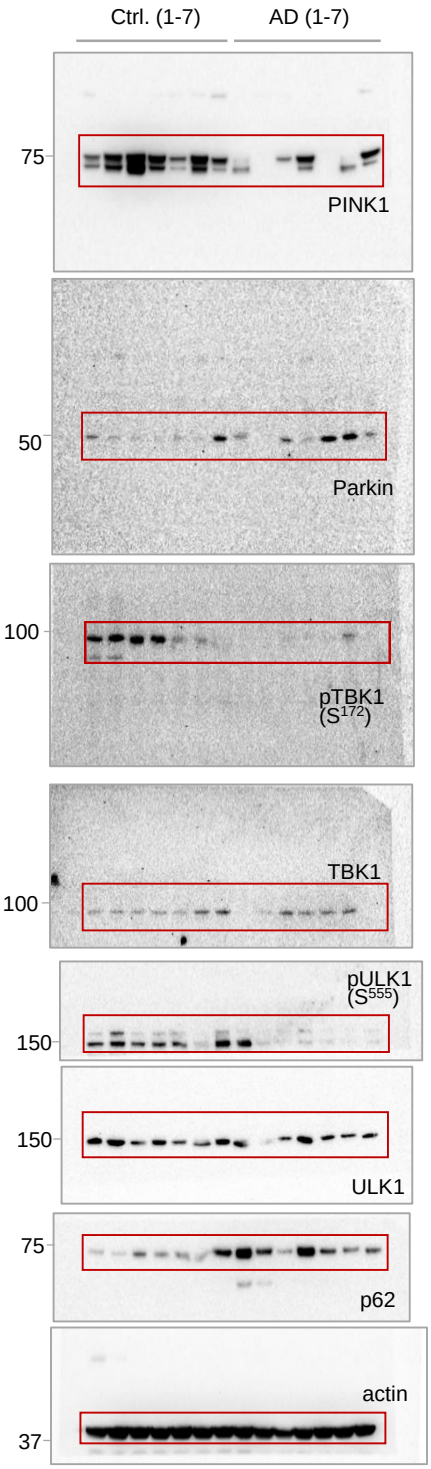
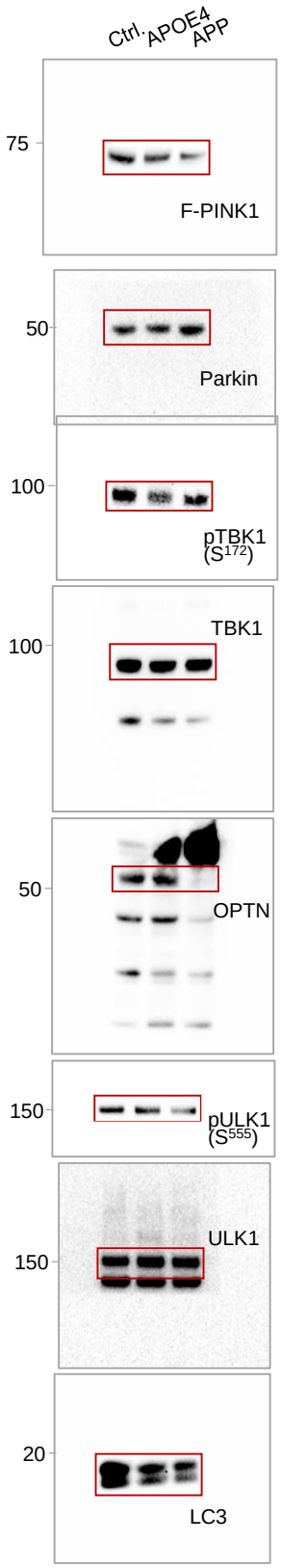


Figure 1j



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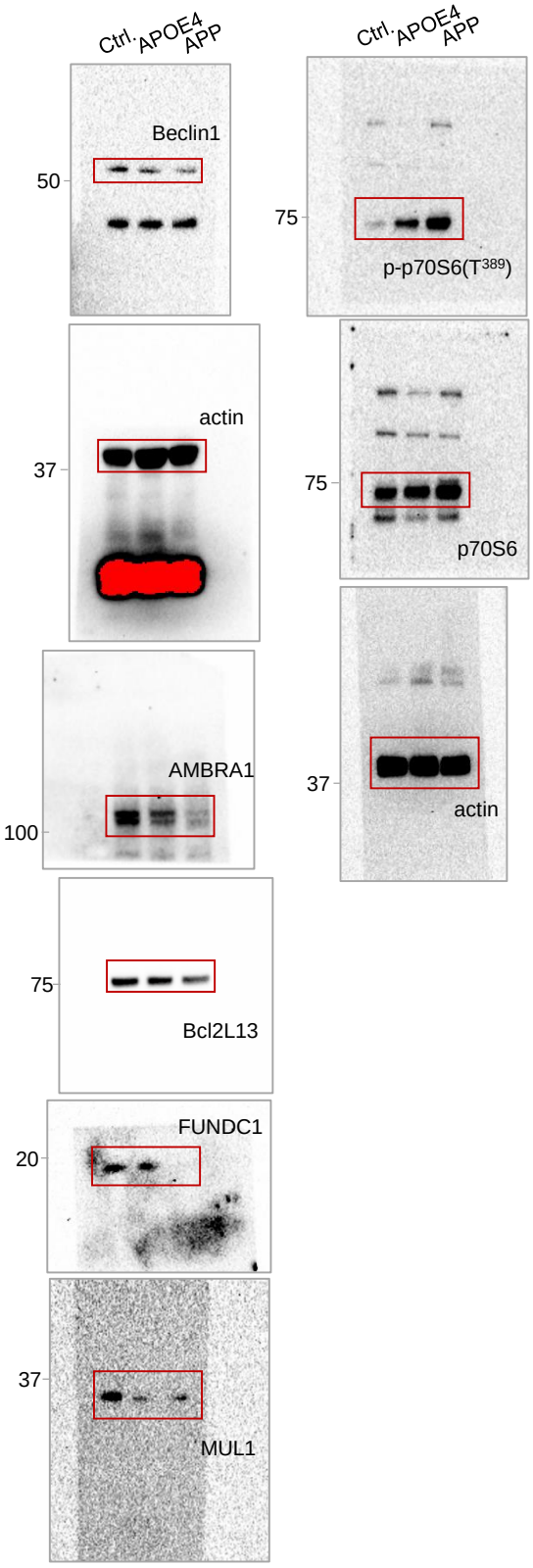


Figure 4b

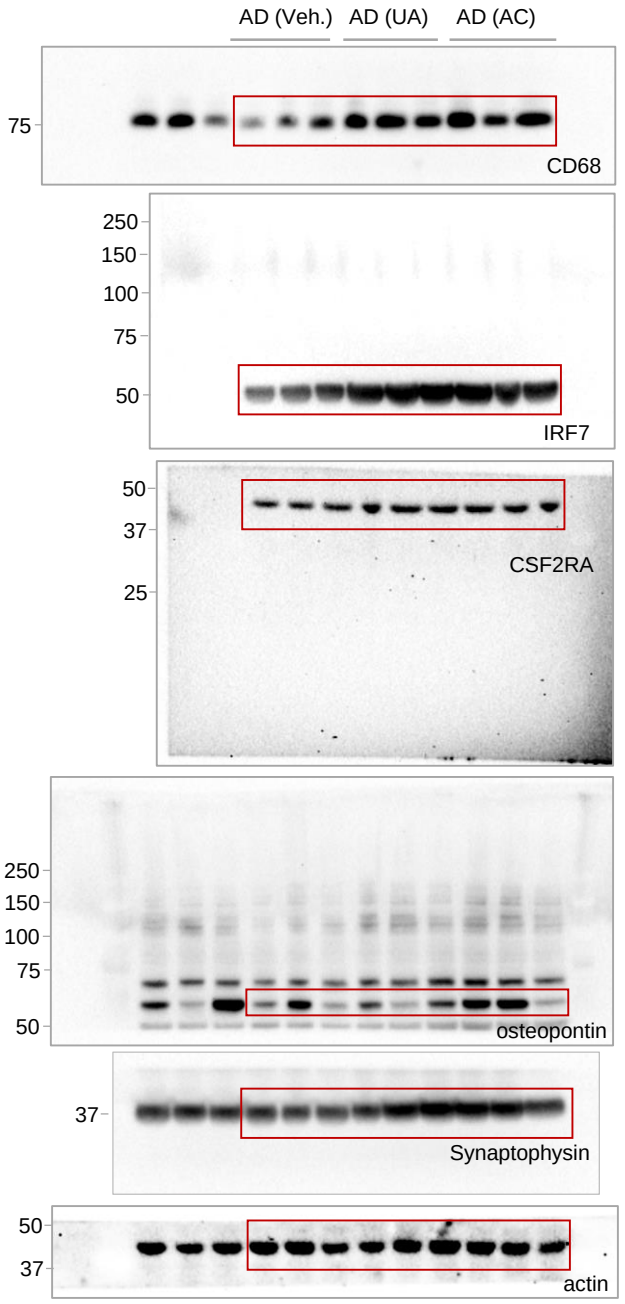


Figure 4j

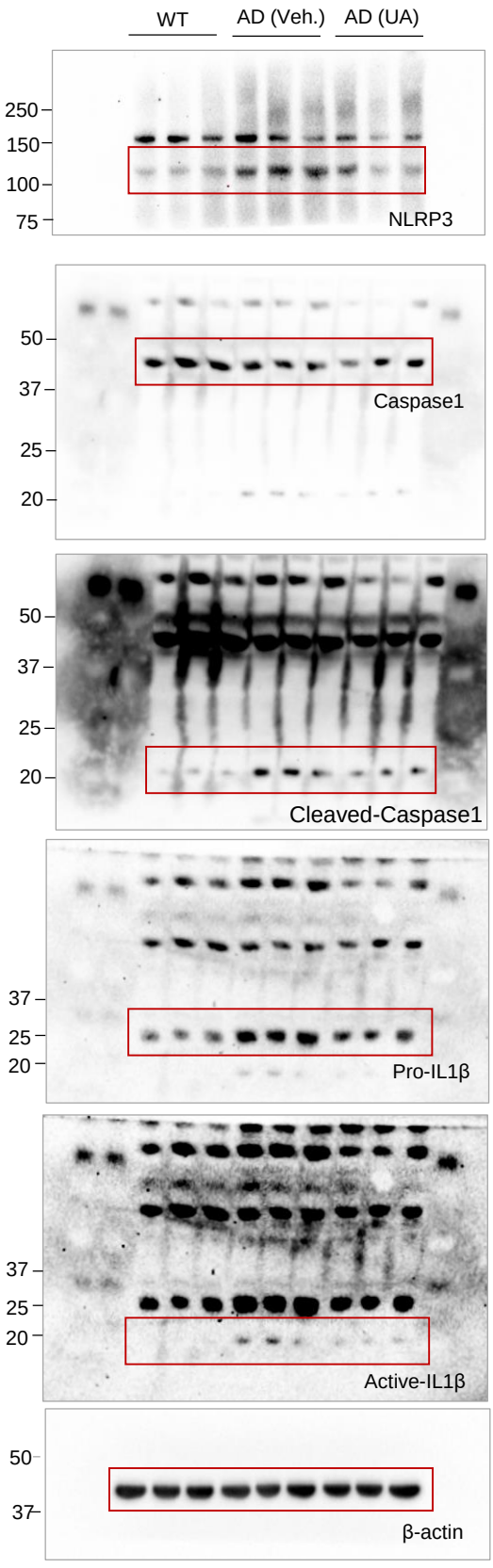


Figure 5a

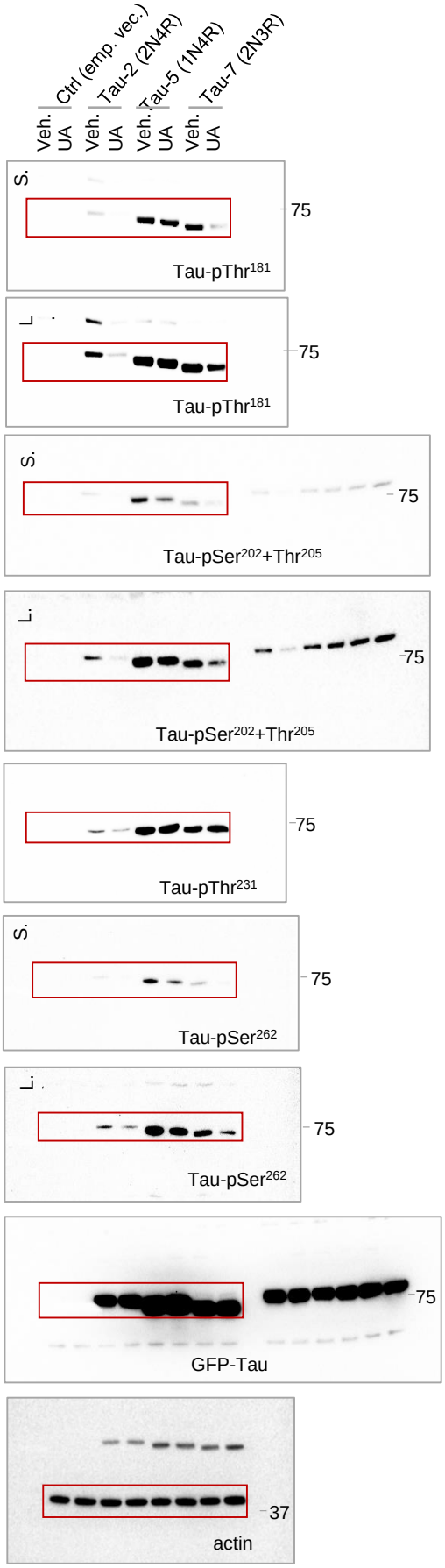


Figure 5b

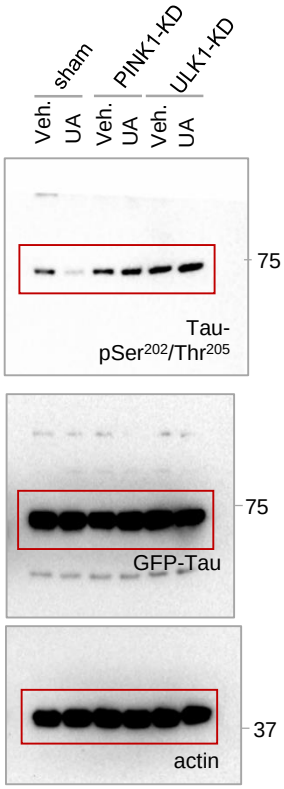


Figure 5m

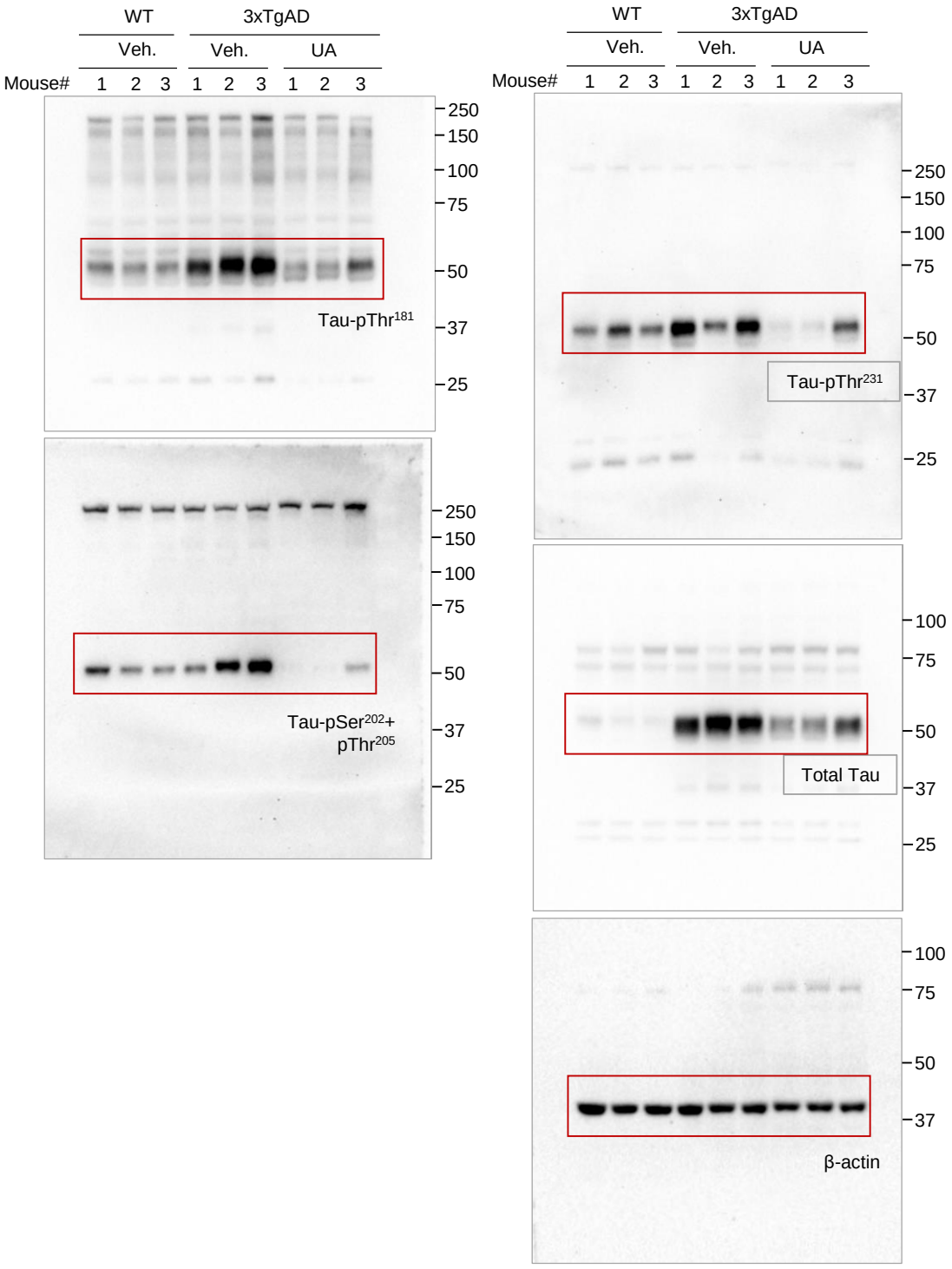
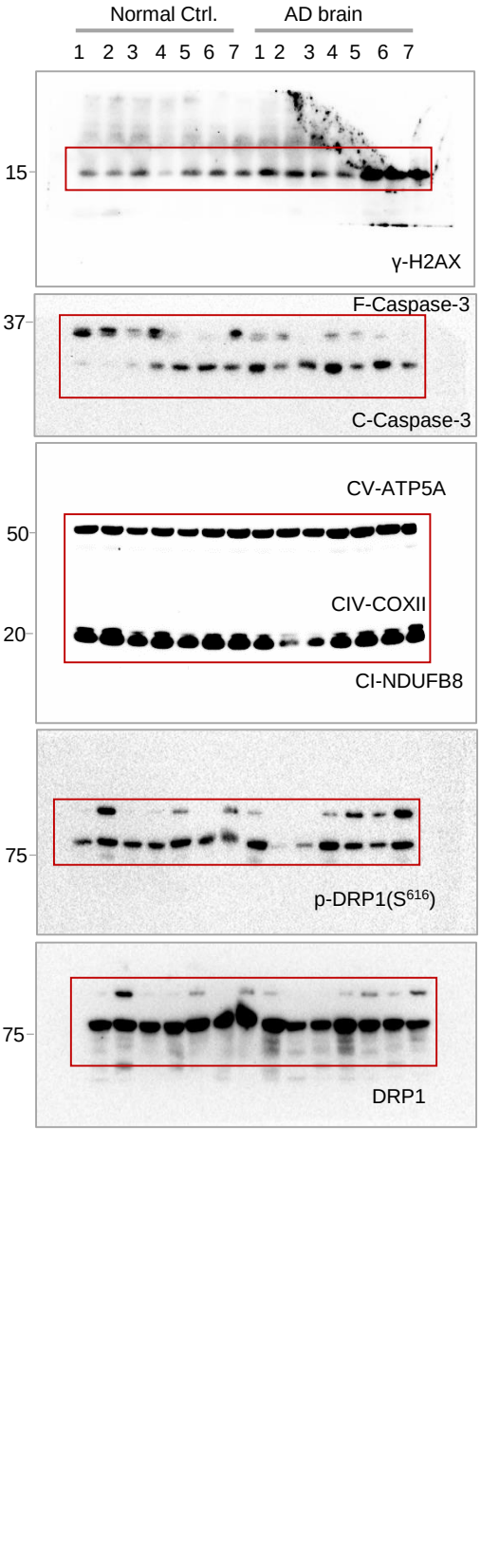

WT
3xTgAD
Veh.
Veh.
UA
Mouse#
1 2 3 1 2 3 1 2 3
250
150
100
75
50
37
25
Tau-pThr181
Tau-pSer202+
pThr205
Tau-pThr231
Total Tau
β-actin

Figure S1b



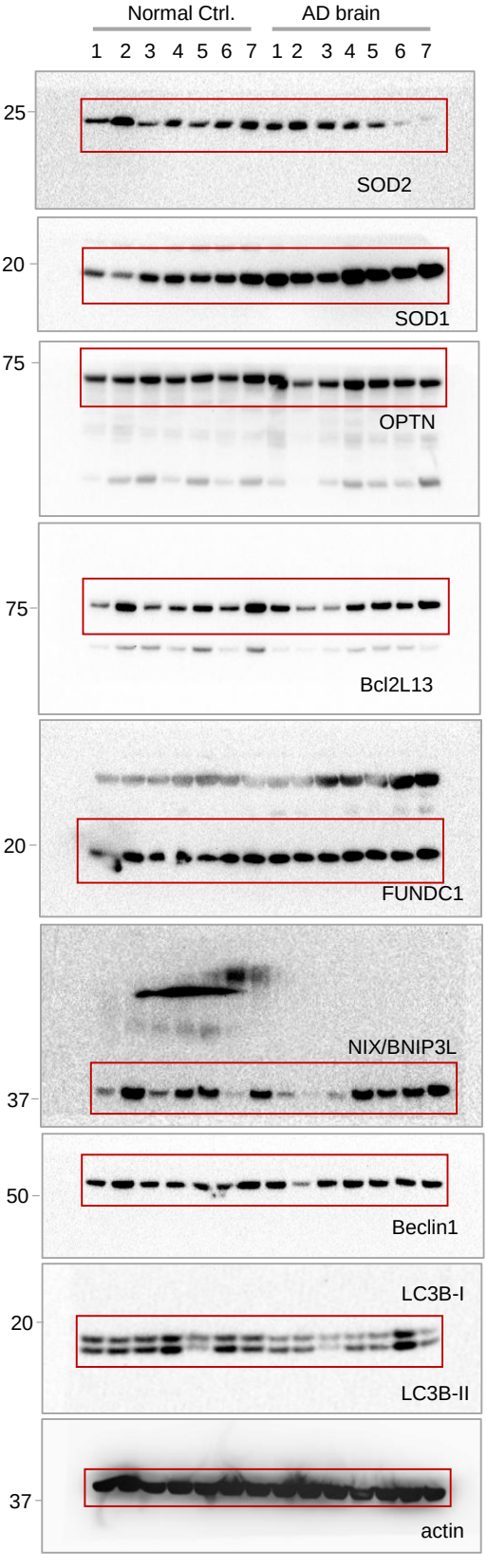
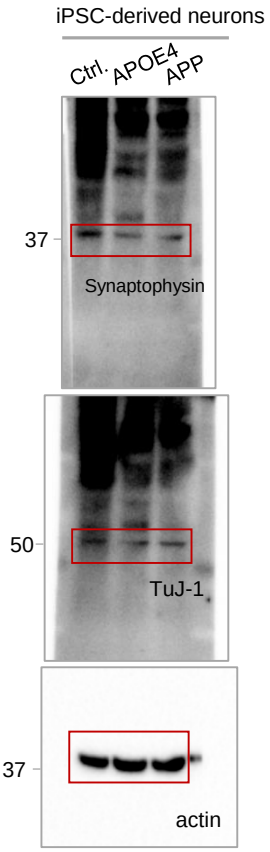


Figure S1d



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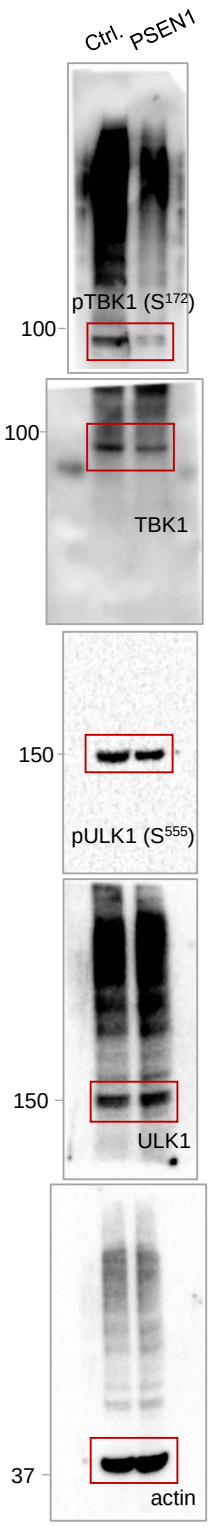
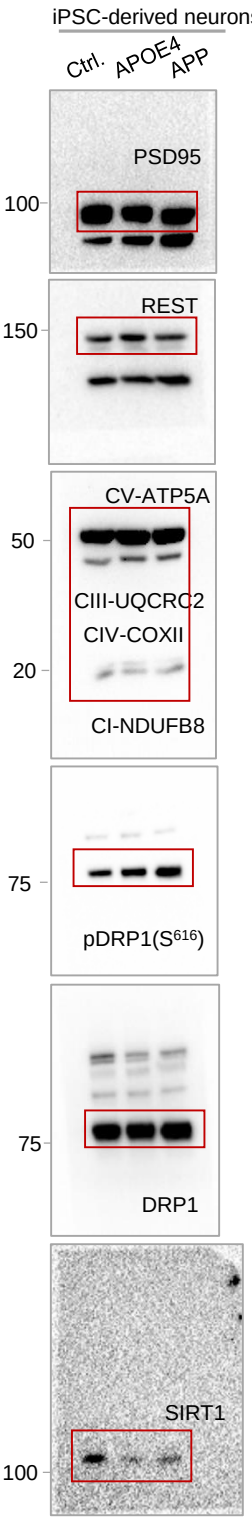


Figure S1e



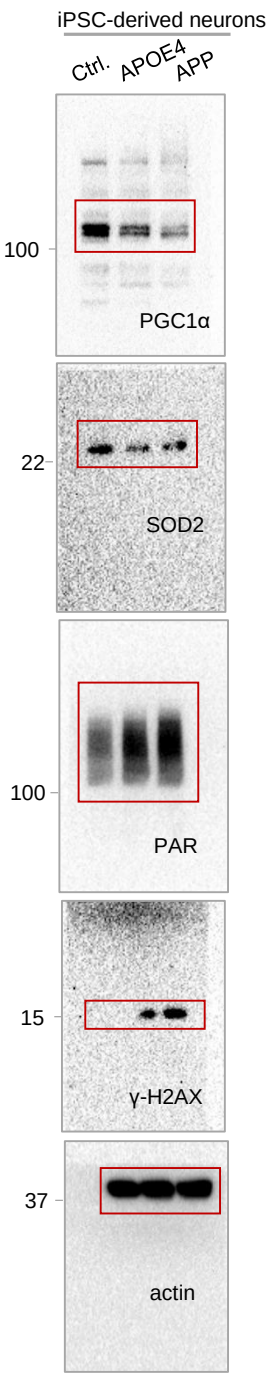


Figure S1f

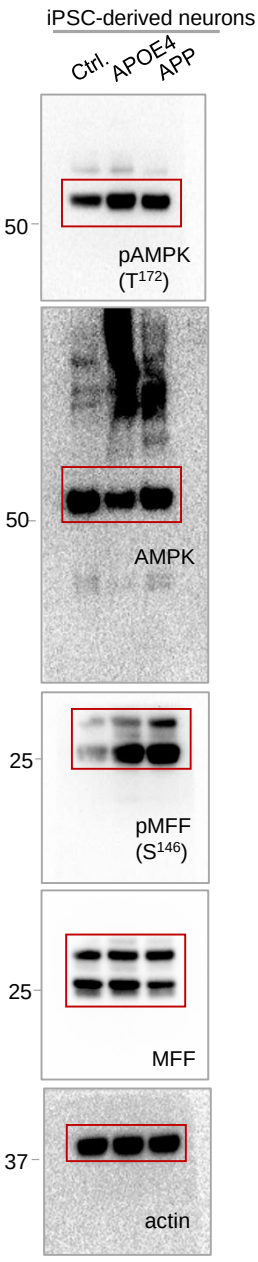
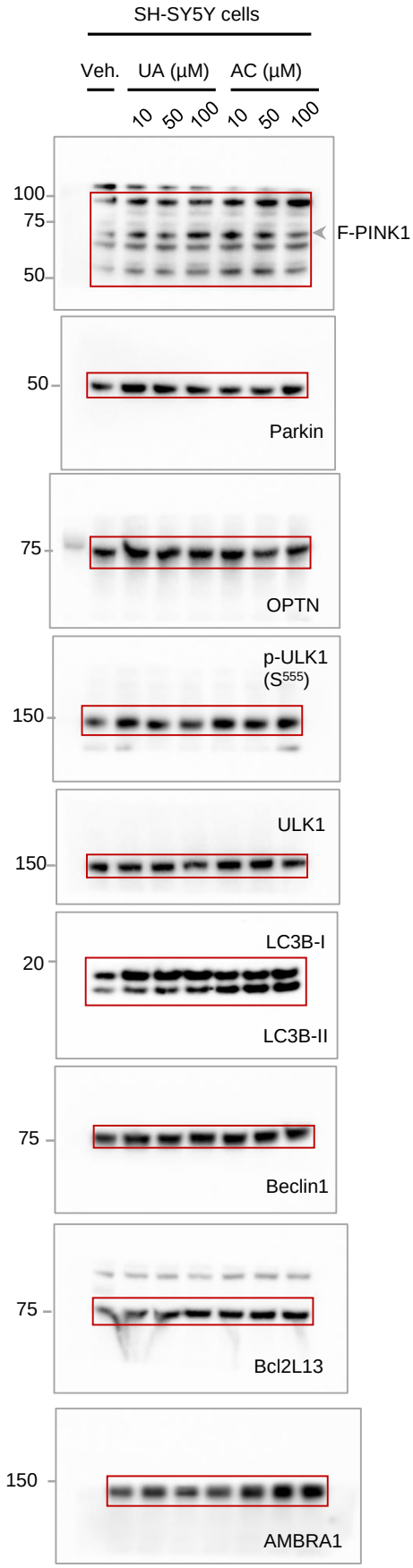


Figure S3a



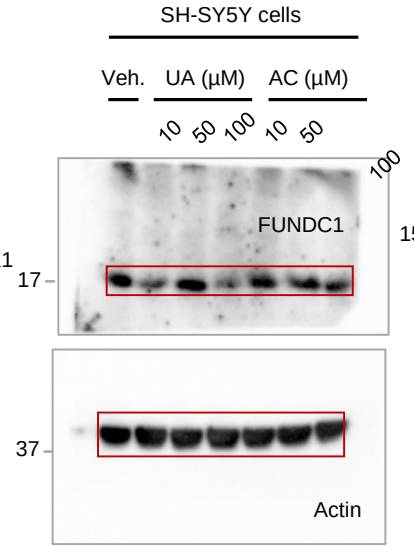


Figure S7h

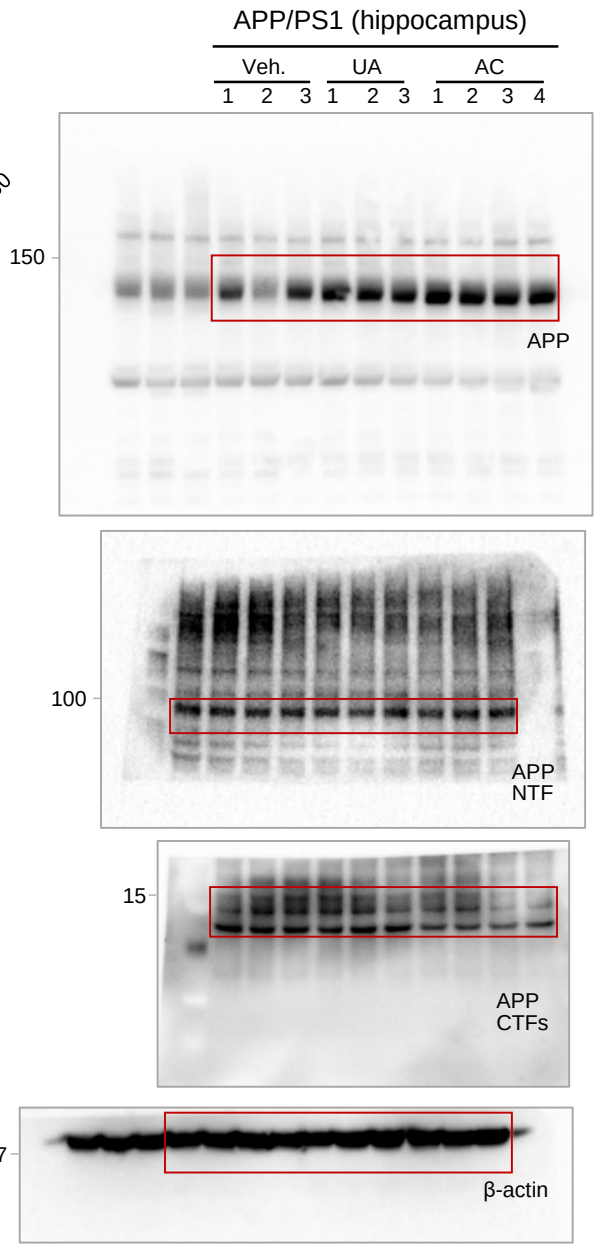


Figure S3b

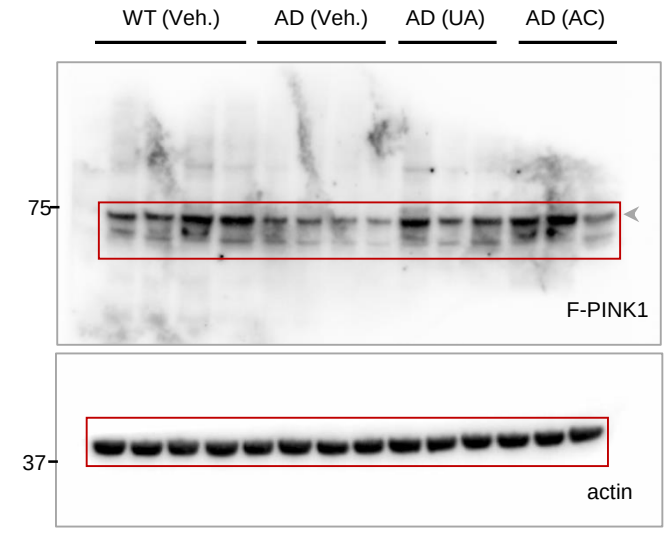


Table S1. Detailed information of human brain samples

Disease	Gender	Age	Years in storage	UMB#	Post mortem interval
Alzheimer Disease	Female	70	11	UMB4556	7
Control	Female	70	13	UMB1490	23
Alzheimer Disease	Male	78	15	UMB1252	9
Control	Male	78	2	UMB5671	19
Alzheimer Disease	Female	82	9	UMB4979	9
Control	Female	83	10	UMB4743	16
Alzheimer Disease	Female	59	9	UMB4833	19
Control	Female	59	17	UMBM2884M	24
Alzheimer Disease	Female	82	NA	AN14331	17
Control	Female	82	NA	AN18592	24
Alzheimer Disease	Male	78	NA	AN14184	15
Control	Male	77	NA	AN12916	25
Alzheimer Disease	Male	74	NA	AN08341	20.5
Control	Male	74	NA	AN10212	20.5

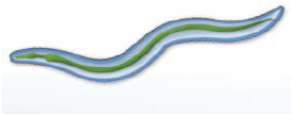
Samples were provided by the University of Maryland Brain Bank (UMB) or Harvard Brain Bank (AN) with MTA agreement.

Table S3. A summary of the cross-species model systems used in this study

 Human brain	AD human brain samples (N = 7)
	Control human brain samples (N = 7)

 Cell line and iPSC-derived neuron	Sporadic AD (APOE4/E4) human iPSC-derived neuron
	Familial AD (APP/V717L) human iPSC-derived neuron
	Age- and sex-matched healthy control (SBAD03-01) human iPSC-derived neuron
	Familial AD (PSEN1) human iPSC-derived neuron
	2N4R, 1N4R, and 2N3R-overexpressed SH-SY5Y cells

 mouse	APP/PS1 with their control mice
	3xTgAD with their control mice

 C. elegans	$A\beta_{1-42}$ worm model (CL2355)
	Tau worm model (BR5270)
	pink-1(tm1779) , *pdr-1(gk488)* , *dct-1(tm376)*
	pink-1(tm1779) ;CL2355, *pdr-1(gk488)* ;CL2355, *dct-1(tm376)* ;CL2355
	pink-1(tm1779) ;BR5270, *pdr-1(gk488)* ;BR5270, *dct-1(tm376)* ;BR5270
	and more information in Methods.