Supplementary material

Enhancing autophagy maturation with CCZ1-MON1A complex alleviates neuropathology and memory defects in Alzheimer disease models

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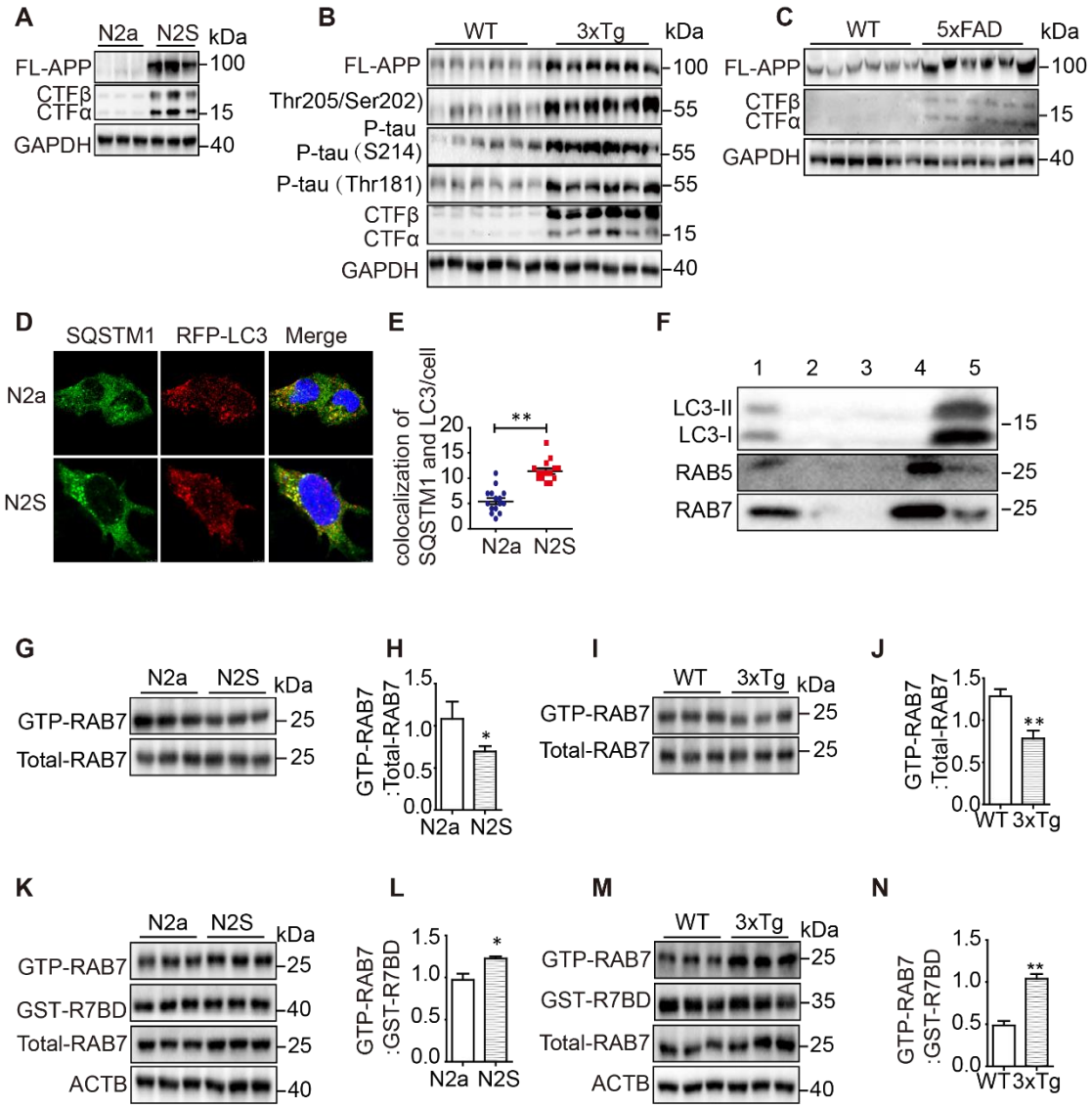

Figure S1. (**A**) Immunoblotting was used to detect the expression of FL-APP, and APP-CTFs levels in N2a and N2S, GAPDH was used as an internal control. (**B**) Immunoblotting was used to detect the expression of FL-APP, and APP-CTFs levels in the hippocampus region of 12-month-old C57BL/6 and 3xTg AD mice; GAPDH was used as an internal control. (**C**) Immunoblotting was used to detect the expression of FL-APP, and APP-CTFs levels in the hippocampus region of 12-month-old WT and 5xFAD AD mice; GAPDH was used as an internal control. (**D-E**) N2a and N2S cells were transiently transfected with RFP-LC3, and the

colocalization of RFP-LC3 with endogenous SQSTM1 was visualized under confocal microscopy. (**F**) Enrichment of LC3-II in purified autophagosomes. (**G-H**) GTP-RAB7 in autophagosomes isolation from N2a and N2S were determined by GTP-beads affinity-isolation assay. Data are quantified as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, *vs*. the relative control. (**I-J**) GTP-RAB7 in autophagosomes isolation from WT and 3xTg were determined by GTP-beads affinity-isolation assay. Data are quantified as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, vs. the relative control. (**K-L**) The total amount of GTP-RAB7 in whole cell from N2a and N2S were determined by GST-R7BD affinity-isolation assay. Data are quantified as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, *vs*. the relative control. (**M-N**) The total amount of GTP-RAB7 in whole cell from WT and 3xTg AD mouse were determined by GST-R7BD affinity-isolation assay. Data are quantified as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, *vs*. the relative control.

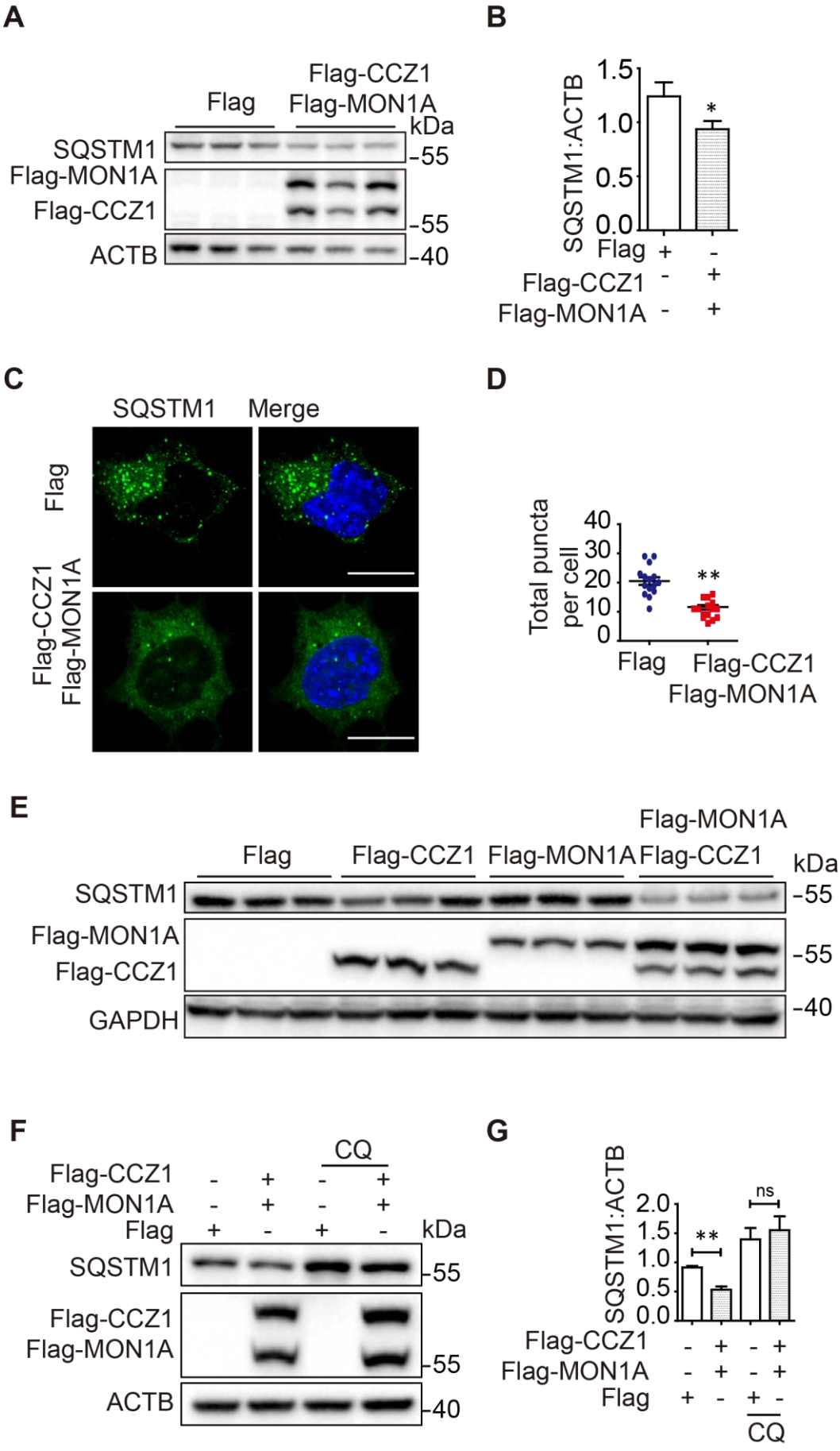
A
Flag
Flag-CCZ1
Flag-MON1A
kDa
SQSTM1
Flag-MON1A
Flag-CCZ1
ACTB
-55
-55
-40
B
SQSTM1:ACTB
1.5
1.0
0.5
0.0
*
Flag + -
Flag-CCZ1 - +
Flag-MON1A - +
C
SQSTM1
Merge
Flag
Flag-CCZ1
Flag-MON1A
D
Total puncta per cell
40
30
20
10
0
**
Flag
Flag-CCZ1
Flag-MON1A
E
Flag
Flag-CCZ1
Flag-MON1A
Flag-MON1A
Flag-CCZ1
kDa
SQSTM1
Flag-MON1A
Flag-CCZ1
GAPDH
-55
-55
-40
F
CQ
Flag-CCZ1 - + - +
Flag-MON1A - + - +
Flag + - + -
kDa
SQSTM1
Flag-CCZ1
Flag-MON1A
ACTB
-55
-55
-40
G
SQSTM1:ACTB
2.0
1.5
1.0
0.5
0.0
**
ns
Flag-CCZ1 - + - +
Flag-MON1A - + - +
Flag + - + -
CQ

Figure S2. (**A-B**) SQSTM1 levels were determined by immunoblotting after transfection of FLAG-CCZ1/FLAG-MON1A or FLAG plasmid in N2S cells. (**C-D**) SQSTM1 levels were determined by immunofluorescence after transfection of FLAG-CCZ1/FLAG-MON1A or FLAG plasmid in N2S cells. Quantification data were presented as the mean ± SEM, n = 20-25 cells from 3 independent experiments. *P < 0.05, **P < 0.01, *vs*. the relative control. Scale bar: 5 μm. (**E**) SQSTM1 levels were determined by immunoblotting after transfection of FLAG-CCZ1 and FLAG-MON1A plasmids alone or in combination. (**F-G**) N2a cells were transfected with FLAG-CCZ1/FLAG-MON1A plasmids and co-treated with vehicle or 30 μM of CQ for 12 h. Immunoblotting was used to detect the SQSTM1 levels. *P < 0.05, **P < 0.01, ***P < 0.001 *vs*. the relative control.

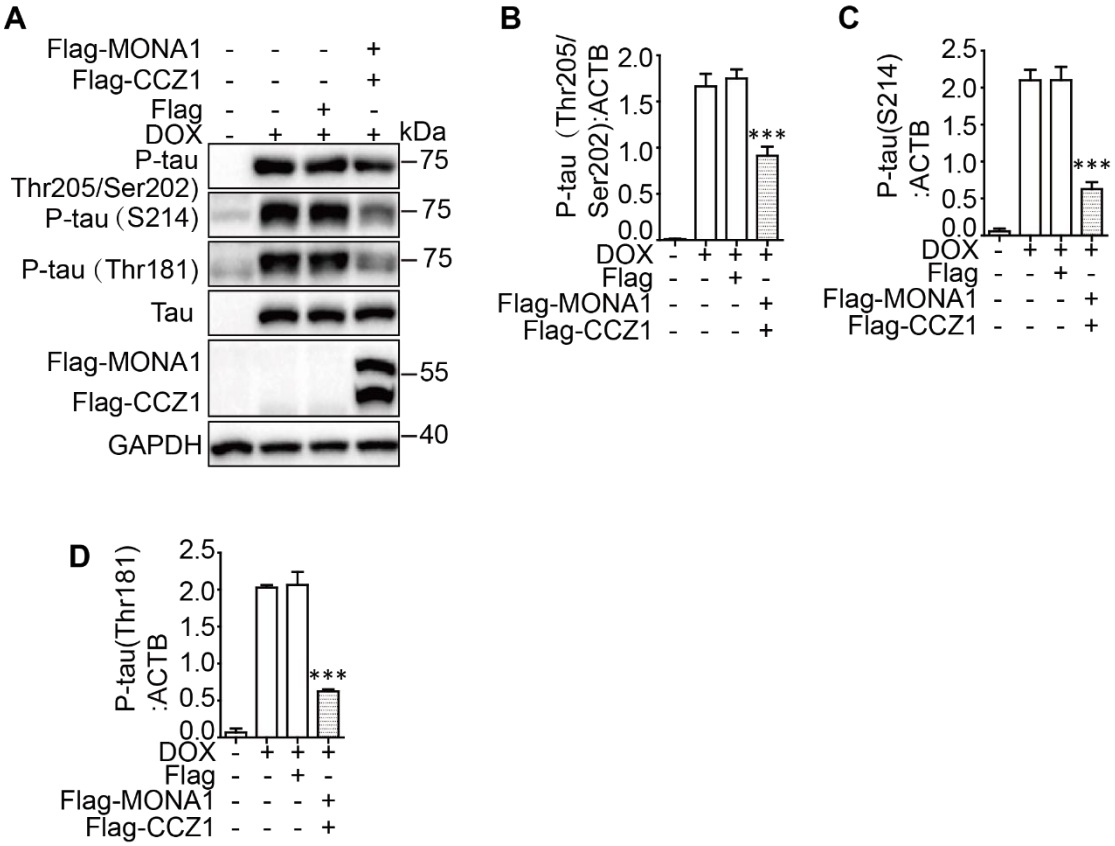

Figure S3. (**A**-**D**) HEK 293 cells expressing pTRE3G-mcherry-BI promoter-EGFP Tau P301L (HEK 293 3G-EGFP-Tau P301L/mCherry) were treated with doxycycline (DOX) for 24 h to induce the expression of Tau P301L. Then the cell was transfected with Flag-CCZ1, Flag-MON1A and Flag plasmids, and the levels of P-tau, Flag-CCZ1 and Flag-MON1A were examined by immunoblotting. Data are quantified as mean ± SEM (n = 3). *P < 0.05, **P < 0.01, *vs*. the relative control.

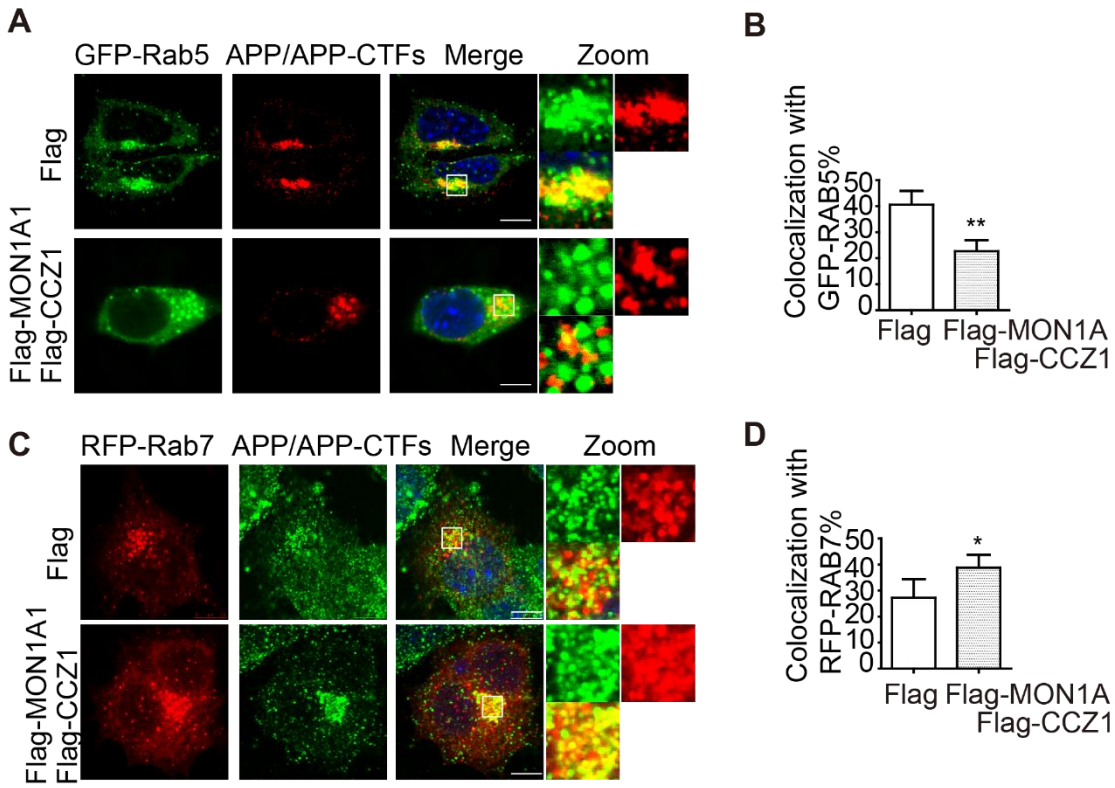

Figure S4. (**A-B**) N2S cells were transiently transfected with GFP-RAB5 and stained with APP antibody. The colocalization of GFP-RAB5 and APP/APP-CTFs was visualized under confocal microscope. Quantification data were presented as the mean ± SEM, n = 20-25 cells from 3 independent experiments. Scale bar: 7.5 μm. *P < 0.05, **P < 0.01, *vs*. the relative control. (**C-D**) N2S cells were transiently transfected with RFP-RAB7 and stained with APP antibody. The colocalization of RFP-RAB7 and APP, APP-CTFs was visualized under confocal microscope. Quantification data were presented as the mean ± SEM, n = 20-25 cells from 3 independent experiments. *P < 0.05, **P < 0.01, *vs*. the relative control. Scale bar: 7.5 μm.

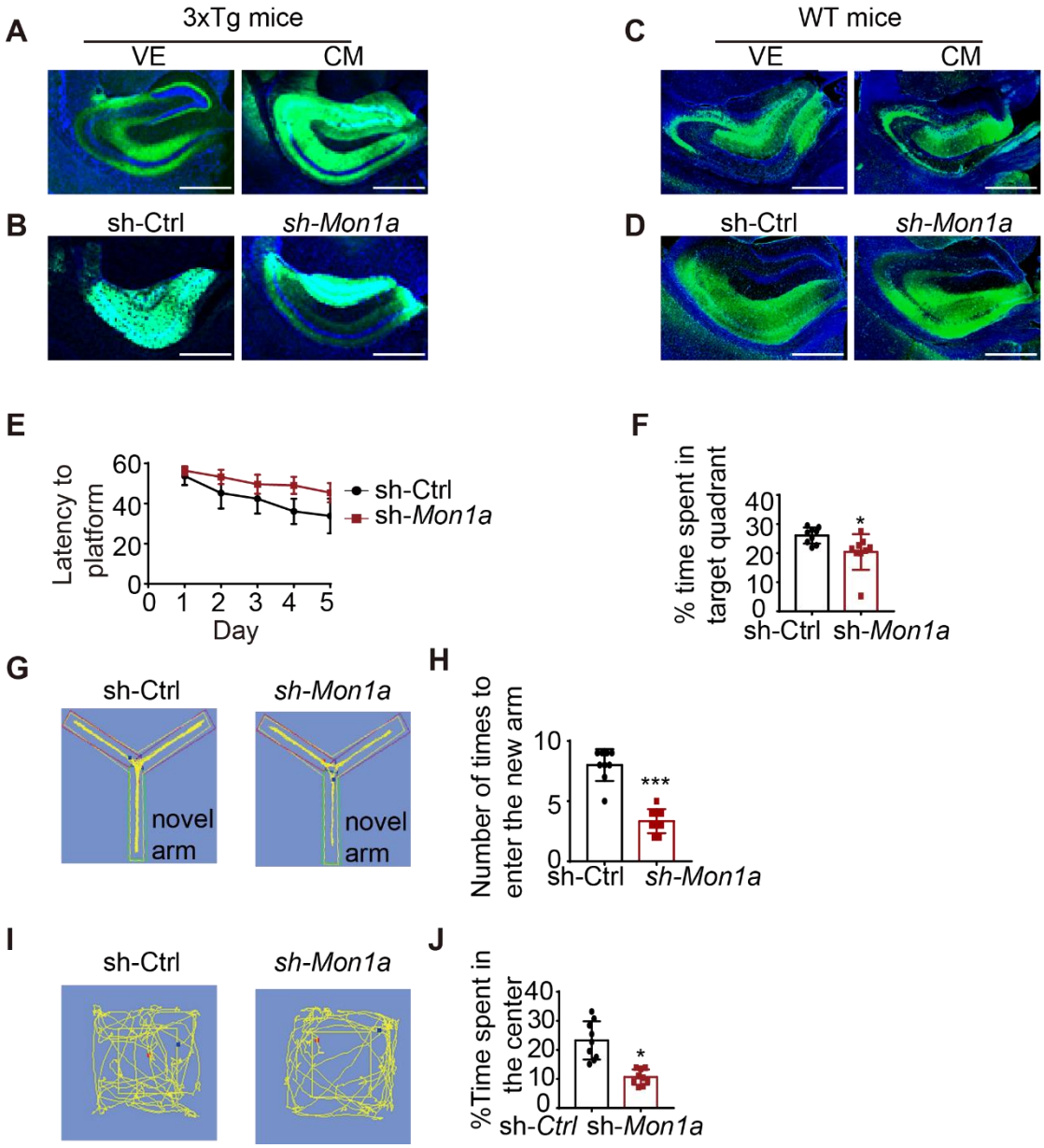

Figure S5. (**A**) Representative image of AAV-Flag-ccz1and AAV-Flag-mon1ainjection of hippocampus. AAV-Flag-Ccz1, AAV-Flag-Mon1 efficiently transduces all types of neurons of the hippocampus in 3xTg AD mouse. bar: 800 μm. (**B**) Representative image of AAV-GFP-sh-mon1ainjection of hippocampus. AAV-GFP-sh-mon1a, AAV-GFP-sh-Ctrl efficiently transduces all types of neurons of the hippocampus in 3xTg AD mouse. bar: 8000 μm. (**C**) Representative image of AAV-Flag-ccz1and AAV-Flag-mon1ainjection of hippocampus. AAV-Flag-Ccz1, AAV-Flag-Mon1 efficiently transduces all types of neurons of the

hippocampus in 12-month-old C57BL/6 WT mouse. bar: 800 μm. (**D**) Representative image of AAV-GFP-sh-mon1ainjection of hippocampus. AAV-GFP-sh-mon1a, AAV-GFP-sh-Ctrl efficiently transduces all types of neurons of the hippocampus in 12-month-old C57BL/6 WT mouse. bar: 8000 μm. (**E-F**) The spatial memory of 3xTg or 3xTg KD MON1A mice receiving AAV injection was evaluated by morris water maze (MWM). Training trials (60 sec each) were performed 4 times a day for 5 days and the time for mice to find the platform was recorded. After 5 days training, the platform was removed and the time that mice stay in the platform quadrant was recorded. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. (**G-H**) Short-term memory was test by Y-maze. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. (**I-J**) Open field test, representative exploratory patterns of mice in each group. Quantification of time spent in the center for each treatment. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control.

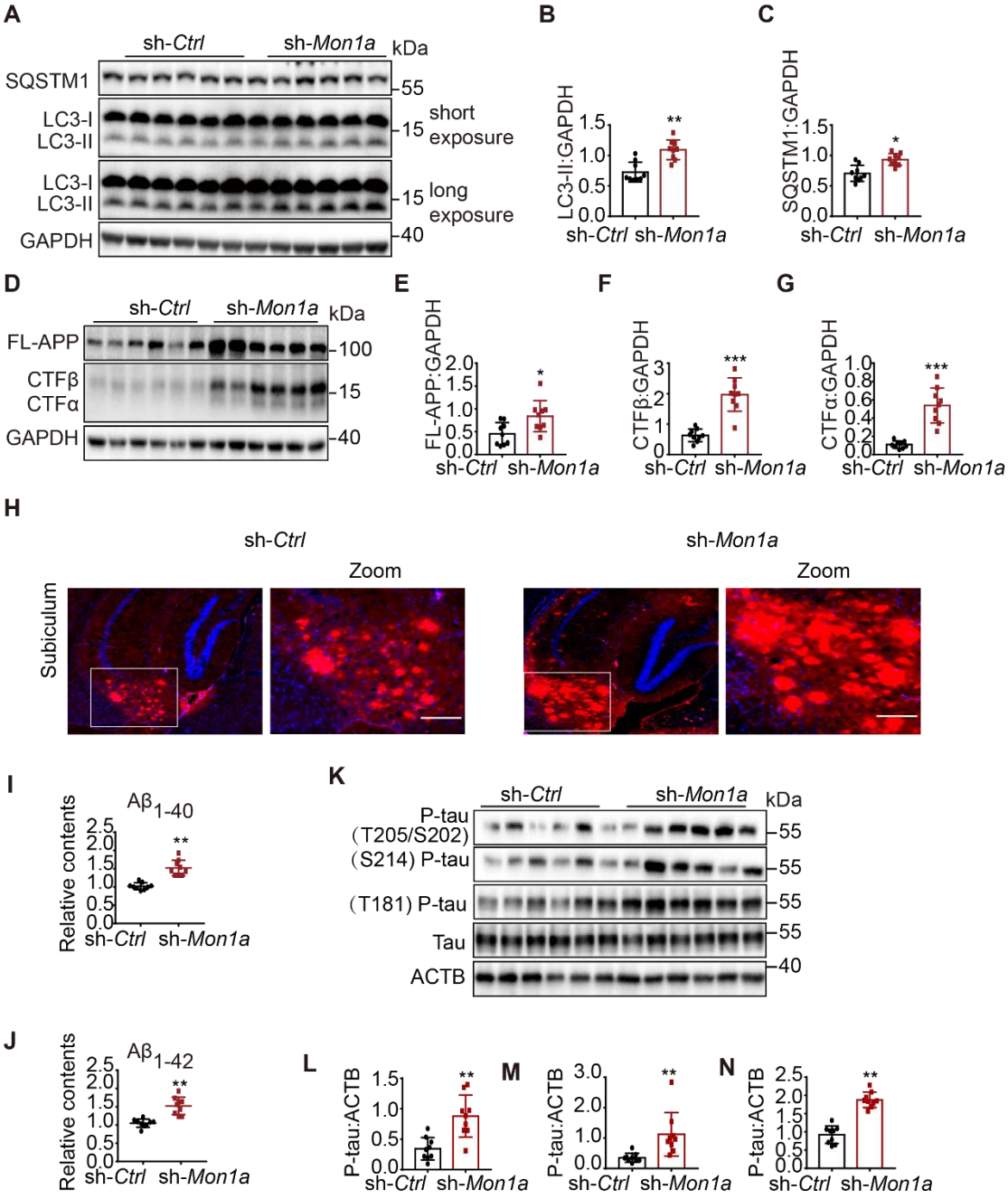

Figure S6. AAV-mediated CCZ1-MON1A knock down in hippocampus exacerbated autophagy impairment, APP/CTFs accumulation and Aβ deposits in 3xTg AD mice. (**A-G**) 2 months after control AAV or AAV-sh*Mon1a* injection, the hippocampus tissues of mice were dissected and subjected to immunoblotting analysis of LC3-II, SQSTM1, FL-APP and APP-CTFs. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. (**H**) Representative images show the Aβ labeled with biotinylated 4G8 antibody. Data are

quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. bar: 200 μm. (**I-J**) Intracellular Aβ1-40 and Aβ1-42 in mice hippocampus were measured by ELISA. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. (**K-N**) The phosphorylated tau in mice hippocampal lysates were determined by immunoblotting using antibodies indicated on the figure. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01 *vs.* the relative control.

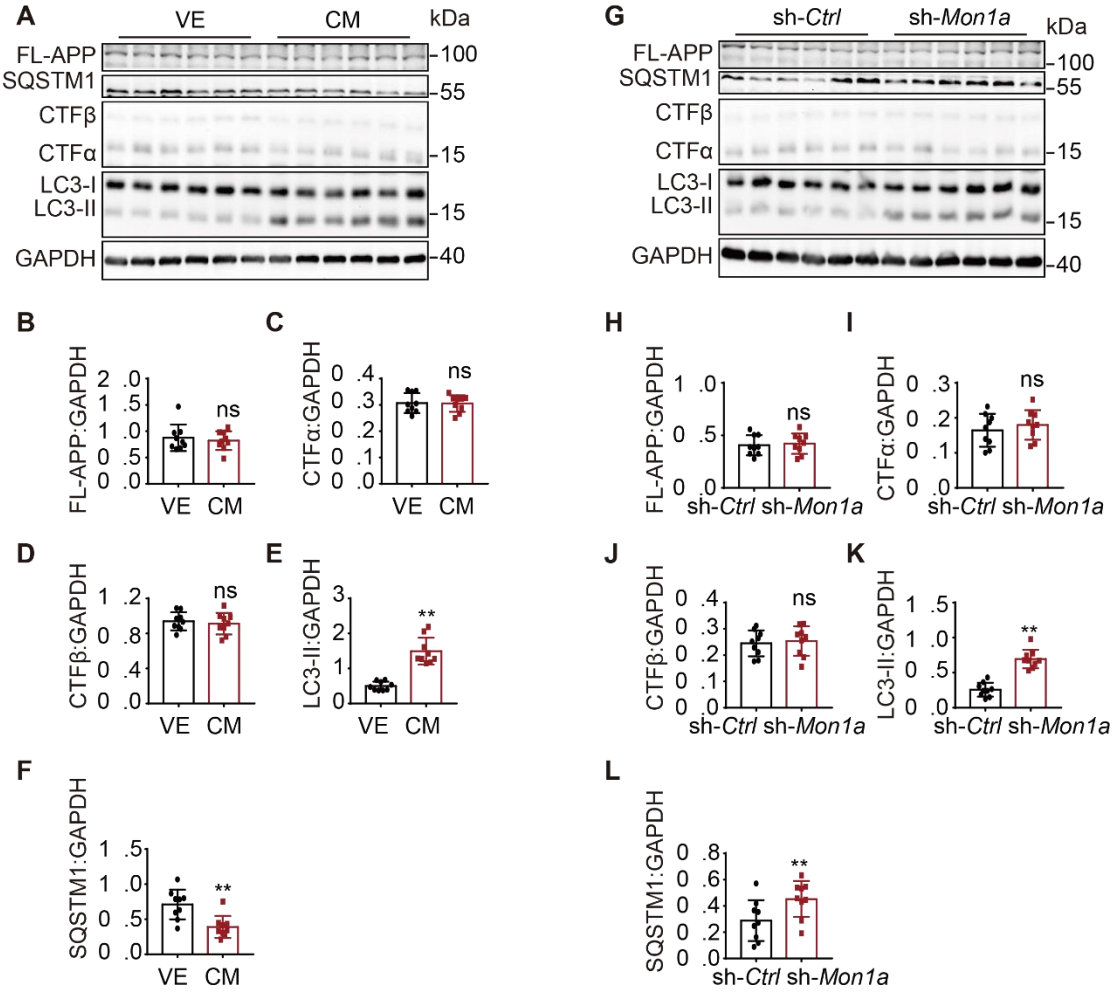

Figure S7. (A-F) 2 months after control AAV or CCZ1-MON1A AAV injection, the hippocampus tissues of mice were dissected and subjected to immunoblotting analysis of LC3-

II, SQSTM1, FL-APP and APP-CTFs. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs*. the relative control. ns, not significant. **(G-L)** 2 months after control AAV or AAV-sh*Mon1a* injection, the hippocampus tissues of mice were dissected and subjected to immunoblotting analysis of LC3-II, SQSTM1, FL-APP and APP-CTFs. Data are quantified as mean ± SEM (n = 9). *P < 0.05, **P <0.01, *vs.* the relative control.