Supplemental informaion

Supplemental Figure 1. NE, DA, and DOPAL trigger Tau degradation *in vitro*.

A. Recombinant Tau (1 μg) was incubated with NE, DA, or DOPAL of 0, 2.5, 25, 125, or 250 μM in a 37 °C shaker for 24 h. Immunoblotting showed that NE, DA, and DOPAL dose-dependently induced Tau degradation. **B.** NE, DA, and DOPAL time-dependently stimulated Tau degradation from 2 to 24 h. **C.** Silver staining confirmed that Tau was dose- and time-dependently degraded by NE and DOPAL. **D.** Enhanced Tau fabrilization by DOPEGAL was demonstrated by electron microscopy. Scale bar = 100 nm.

Supplemental Figure 2. MAO-A or aberrant Tau overexpression induces AEP activation, Tau N368 cleavage, and cell death in SH-SY5Y cells.

SH-SY5Y cells were transfected with MAO-A or MAO-B. **A.** Western blot analysis was conducted on cell lysates using antibodies against AEP and different forms of Tau with β-actin as a control. **B**. The activation of MAO-A in transfected cells was confirmed by enzymatic assay. **C**. AEP enzymatic assay showed that the overexpression of MAO-A and MAO-B activated AEP. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$. **D**. DOPEGAL conversion by MAO-A overexpression was verified by HPLC analysis. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$. H_2O_2 triggered MAO-A and AEP activation. SH-SY5Y cells were pretreated with Clorgyline (10 μM), followed by treatment with H_2O_2 (100 μM) for 4 h. **E**. Western blot analysis showed that MAO-A and AEP were enhanced by H_2O_2, and the effects of H_2O_2 were attenuated by the specific MAO-A inhibitor clorgyline. **F**. Activation of MAO-A by H_2O_2 was confirmed by MAO-A enzymatic assay. **G**. AEP enzymatic assay showed that H_2O_2 activation of AEP was inhibited by clorgyline. Data are shown as mean ± SEM. N=3 each group. * $p<0.05$.

Inhibition of NE synthesis reduced H_2O_2-induced AEP activation. SH-SY5Y cells were transfected with siRNA for DBH or a control sequence, followed by treatment with H_2O_2 (100 μM) for 4 h. **H**. Western blot anaylsis showed that inhibition of NE synthesis by knocking down DBH reduced H_2O_2-induced AEP activation and Tau N368 cleavage. **I**. H_2O_2-induced AEP activity was inhibited by knocking down DBH. Data are shown as mean ± SEM. N=3 each group. * $p<0.05$. Tau cleavage by AEP induces SH-SY5Y cell death. SH-SY5Y cells were infected by AAV expressing Tau, Tau N368, Tau P301S or AEP-resistant Tau P301S/N255/368A. **J.** Representative images of cells co-stained for TH (red), Tau (blue) and TUNEL (green). Scale bar = 50 μm. **K & L**. Quantification of TUNEL+ cells and LDH assay showed that Tau-induced cell death was inhibited by AEP-resistant uncleavable Tau. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$, ** $p<0.01$. **M**. Western blot analysis showed that TH cell loss was inhibited by uncleavable Tau P301S/N255/368A.

Supplemental Figure 3. Tau-induced neurotoxicity in SH-SY5Y cells is enhanced by MAO overexpression and attenuated by DBH depletion.

A. SH-SY5Y cells were infected by AAV expressing Tau with MAO-A or MAO-B, or transfected with DBH siRNA. TUNEL was co-stained with TH and Tau. Scale bar = 50 μm. **B & C.** Quantification of TUNEL+ cells and LDH assay show that Tau-induced cell death is increased by MAO-A or MAO-B, and is reduced by knockdown of DBH. The activity change of MAO-A (**D**), MAO-B (**E**), and AEP (**F**) by AAV-MAO-A or siDBH was verified by enzymatic assay. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$, ** $p<0.01$. **G**. Western blot analysis shows that MAO-A overexpression induces AEP activation, Tau cleavage, and TH reduction in SH-SY5Y cells. **H**. Primary noradrenergic neurons were transfected with control

siRNA or DBH siRNA, and TUNEL was co-stained with TH. Scale bar = 50 μm. **I**. The reduced TUNEL+ neurons by knockdown of DBH were quantified. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$.

Supplemental Figure 4. DOPEGAL induces Tau neurotoxicity in SH-SY5Y cells.

A. SH-SY5Y cells were transfected with MAO-A or MAO-B, followed by treatment with the MAO-A inhibitor clorgyline (10 μM) or the MAO-B inhibitor rasagyline (10 μM). Western blot analysis showed that MAO overexpression induced AEP activation, Tau phosphorylation, and Tau cleavage, and MAO inhibitors blocked these events. **B-D**. The activation of MAO-A (**B**), MAO-B (**C**), and AEP (**D**) was confirmed by enzymatic assay. **E**. LDH assay showed that MAO-A or MAO-B overexpression in SH-SY5Y cells did not induce cell death on their own. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$. **F**. SH-SY5Y cells were transfected with MAO-A, followed by treatment with L-DOPA (200 μM), L-DOPS (200 μM), or Ascorbic acid (500 μM). Immunoblotting showed that MAO-A overexpression induced AEP activation, Tau phosphorylation, and Tau cleavage in the presence of NE precursors. **G & H**. AEP activity and cell death were increased by L-DOPA, L-DOPS, or Ascorbic acid with MAO-A overexpression. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$. **I.** SH-SY5Y cells were transfected with MAO-A and treated with Ascorbic acid (500 μM). And then cells were treated with vehicle, the aldehyde dehydrogenase inhibitor Daidzein (10 μM), the aldehyde reductase inhibitor Imirestat (1 μM), or their combination (D+I) for 24 h. Western blot analysis showed that inhibition of DOPEGAL metabolism induced AEP activation, Tau phosphorylation, and Tau cleavage following facilitation of NE production and MAO-A oxidation. **J & K**. AEP activity and cell death were increased by the treatment of Daidzein, Imirestat, or inhibitors combination

with MAO-A overexpression and ascorbic acid exposure. Data are shown as mean ± SEM. N=3 per group. * $p<0.05$.

Supplemental Figure 5. Tau cleavage by AEP is necessary for Tau pathology progression in LC region.

A. Tau P301S or AEP cleavage-resistant Tau P301S/N255/368A virus was injected into the LC of DBH +/- or DBH -/- mice. Immunofluorescent co-staining showed that Tau N368 and AT8 were increased in the LC of Tau P301S-injected mice 3 months after viral injection, and that these effects were inhibited by DBH knockout or blockade of Tau cleavage. Scale bar = 20 μm. **B**. Gallyas-Braak staining showed that Tau aggregation following infection of Tau P301S was inhibited in DBH -/- mice or by preventing Tau P301S cleavage. Scale bar = 100 μm. **C**. Quantification of aggregated cells from Gallyas-Braak stained images. Data are shown as mean ± SEM. N=4 per group. * $p<0.05$. **D.** Representative images of immunofluorescent staining for AT8 (red), ThioS (green), and DAPI (blue) in the HC 3, 6, or 9 months following viral injection. Scale bar = 20 μm. **E.** Control or MAO-A AAV were injected into the LC of wild-type (WT) or MAPT transgenic mice, and Tau pathology was assessed 2 months later. Shown are representative images of immunofluorescent staining for AT8 (red), ThioS (green), and DAPI (blue). Scale bar = 20 μm.

Supplemental Figure 6. Viral-mediated Tau expression in the LC drives propagation of pathology to the forebrain in 3xTg transgenic mice.

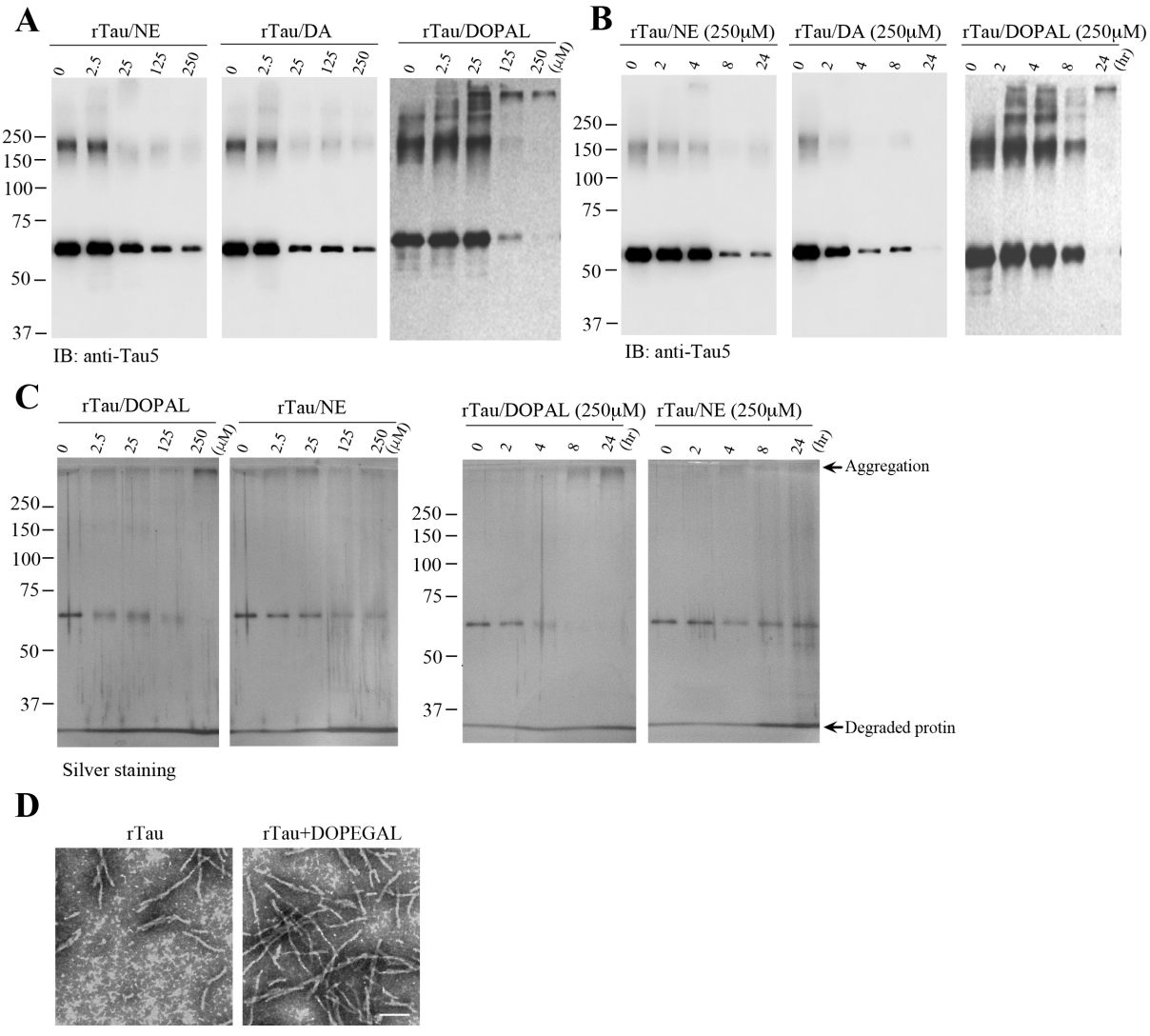
LC-specific AAV-PRSx8-Tau + AAV-PRSx8-mCherry or AAV-PRSx8-mCherry alone were injected into the LC of 3xTg mice, and cognition and pathology were assessed 3 months later. **A**.

Representative images of immunofluorescent staining of TH with AT8/ mCherry or N368/mCherry shows that AAV-Tau infection induces Tau phosphorylation and cleavage in LC. Scale bar = 20 μm. **B**. Representative images of immunofluorescent staining of TH with AT8/ mCherry or N368/mCherry in Entorhinal cortex (EC), Hippocampus (HC), and Cortex (Cx) sections. Scale bar = 20 μm. **C**. Gallyas-Braak staining shows that Tau aggregation induced by Tau is detected in LC, EC, HC, and Cx. Scale bar = 100 μm.

Supplemental Figure 7. Development of Tau pathology in the LC and propagation to the forebrain is dependent on AEP.

A. Primary LC neurons were prepared from neonatal AEP +/+ or AEP -/- mice and infected with LC specific AAV-PRSx8-Tau or control virus. Upper panels show representative immunofluorescent images for TH (green), total Tau or human Tau (red) in AEP +/+ neurons infected with AAV-PRSx8-Tau or control virus. Lower panels show representative immunofluorescent images for TH (green), AT8 or Tau N368 (red), and DAPI (blue) in AEP +/+ or AEP -/- neurons infected with AAV-PRSx8-Tau. LC neurons from AEP -/- mice were resistant to Tau phosphorylation and cleavage. Scale bar = 20 μm. **B.** Schematic summary maps of AT8 immunohistochemistry showing Tau pathology spreading from the LC to different brain regions in MAPT, 3XTG, AEP +/+, and AEP-/- mice.

Figure S1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0 2 4 8 24 (hr)
250
150
100
75
50
37
IB: anti-Tau5
C
rTau/DOPAL
rTau/NE
0 2.5 25 125 250 (μM)
rTau/DOPAL (250μM)
rTau/NE (250μM)
0 2 4 8 24 (hr)
Aggregation
Degraded protin
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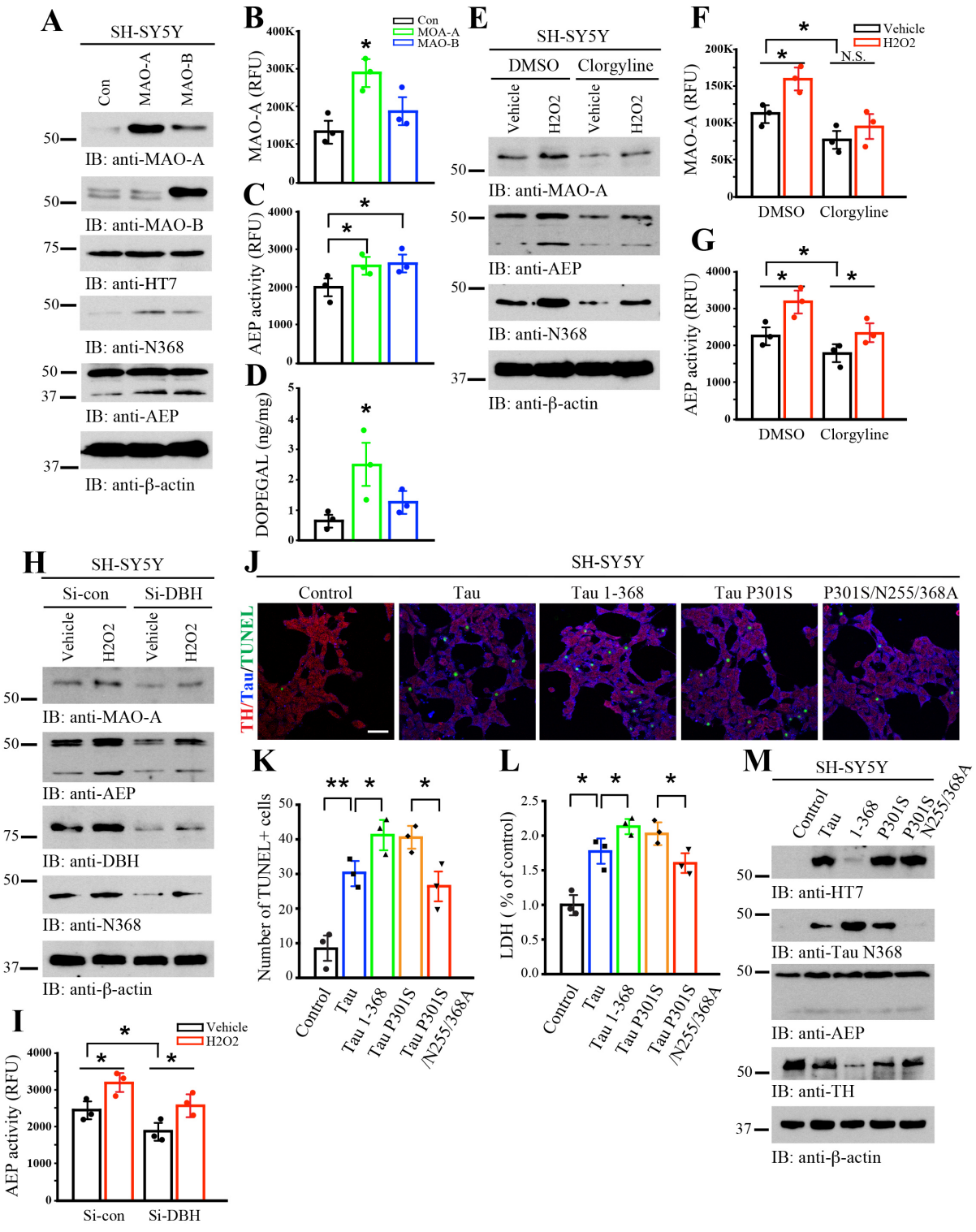
150
100
75
50
37
Silver staining
D
rTau
rTau+DOPEGAL

Figure S2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B
MAO-A (RFU)
C
AEP activity (RFU)
D
DOPEGAL (ng/mg)
E
SH-SY5Y
DMSO
Clorgyline
Vehicle
H2O2
F
MAO-A (RFU)
N.S.
G
AEP activity (RFU)
H
SH-SY5Y
Si-con
Si-DBH
IB: anti-DBH
I
AEP activity (RFU)
J
SH-SY5Y
Control
Tau
Tau 1-368
Tau P301S
P301S/N255/368A
TH/Tau/TUNEL
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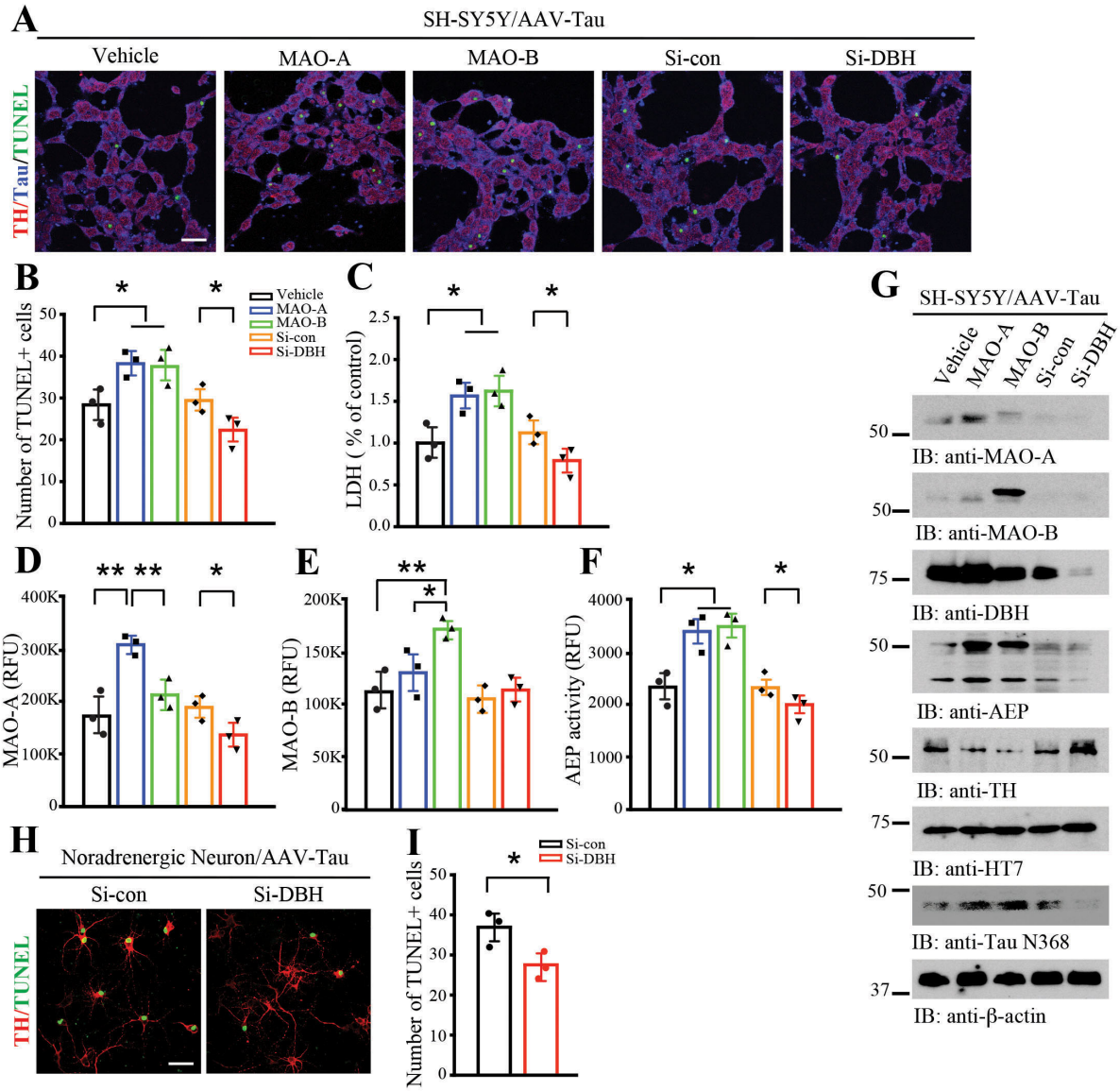
Number of TUNEL+ cells
L
LDH (% of control)
M
SH-SY5Y
IB: anti-Tau N368
IB: anti-TH

Figure S3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Vehicle
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MAO-B
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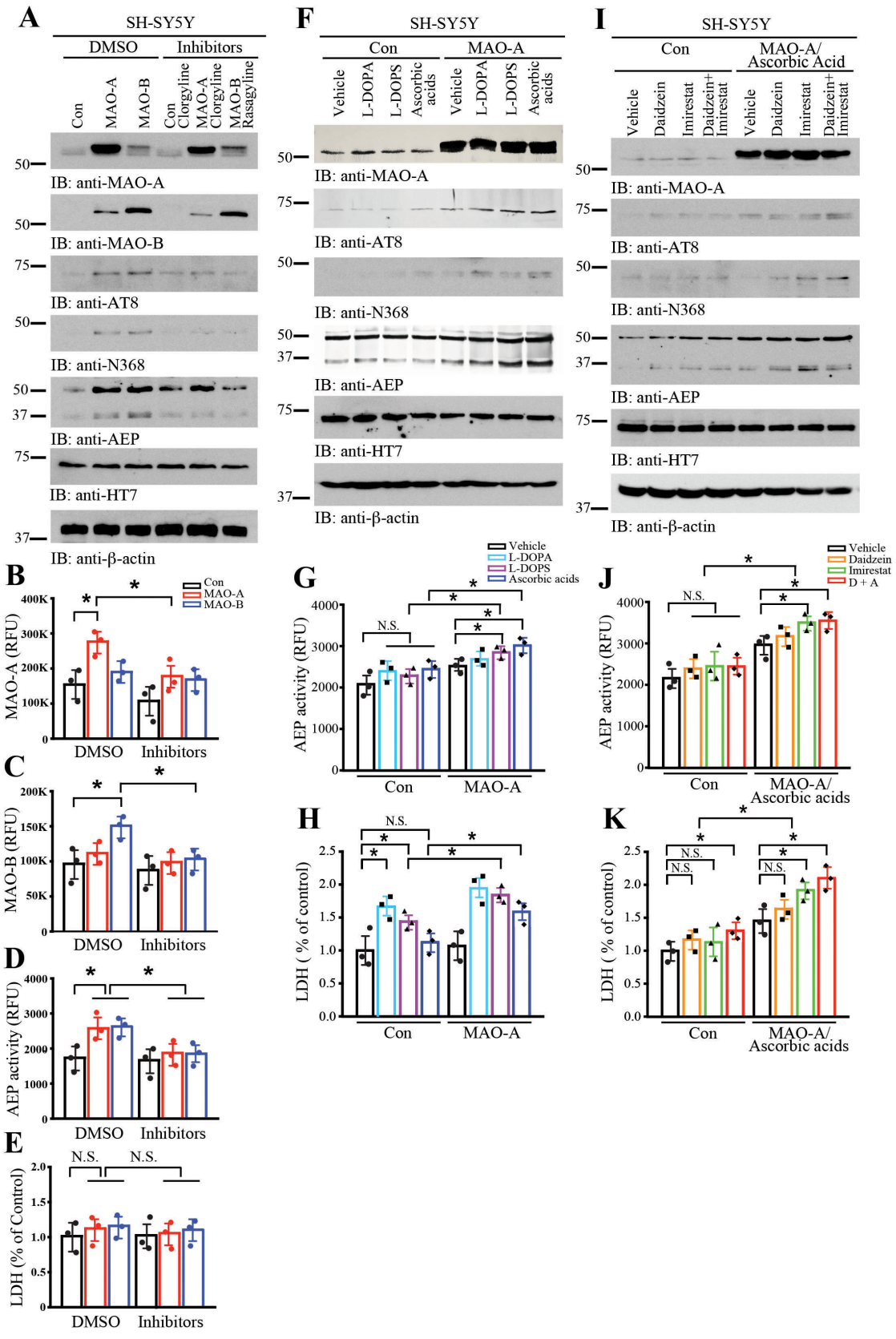
Si-DBH
TH/Tau/TUNEL
B
Number of TUNEL+ cells
Vehicle
MAO-A
MAO-B
Si-con
Si-DBH
C
LDH (% of control)
D
MAO-A (RFU)
E
MAO-B (RFU)
F
AEP activity (RFU)
G
SH-SY5Y/AAV-Tau
Vehicle
MAO-A
MAO-B
Si-con
Si-DBH
IB: anti-MAO-A
IB: anti-MAO-B
IB: anti-DBH
IB: anti-AEP
IB: anti-TH
IB: anti-HT7
IB: anti-Tau N368
IB: anti-β-actin
H
Noradrenergic Neuron/AAV-Tau
Si-con
Si-DBH
TH/TUNEL
I
Si-con
Si-DBH
Number of TUNEL+ cells

Figure S4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IB: anti-N368
IB: anti-AEP
IB: anti-HT7
IB: anti-β-actin
F
SH-SY5Y
Con
MAO-A
Vehicle
L-DOPA
L-DOPS
Ascorbic acids
I
SH-SY5Y
Con
MAO-A/ Ascorbic Acid
Daidzein
Imirestat
Daidzein+ Imirestat
B
MAO-A (RFU)
C
MAO-B (RFU)
D
AEP activity (RFU)
E
LDH (% of Control)
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J
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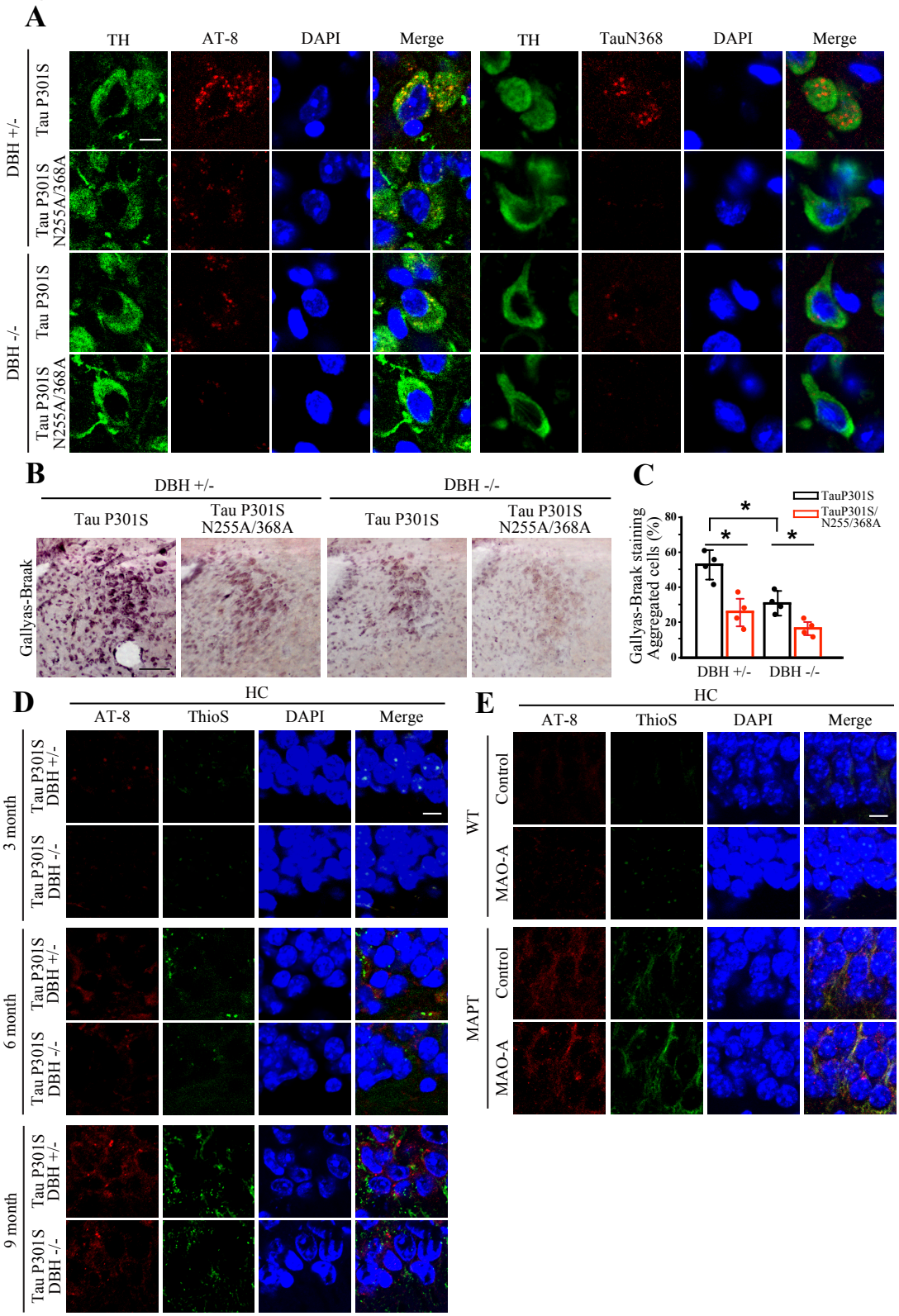
K
LDH (% of control)
N.S.

Figure S5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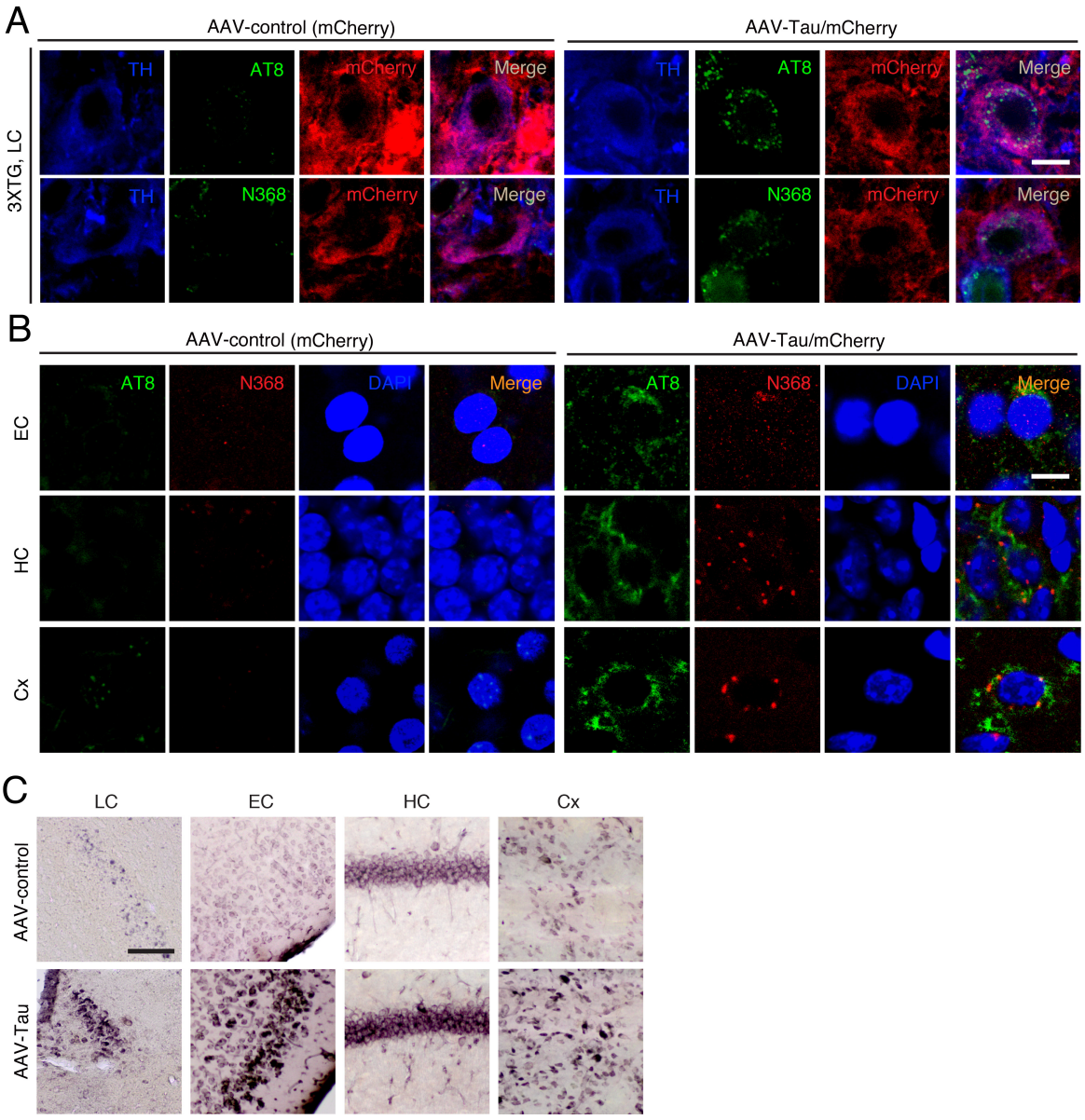
DAPI
Merge
TH
TauN368
DAPI
Merge
DBH +/-
DBH -/-
Tau P301S
Tau P301S N255A/368A
B
DBH +/-
DBH -/-
Tau P301S
Tau P301S N255A/368A
Gallyas-Braak
C
TauP301S
TauP301S/ N255/368A
Gallyas-Braak staining Aggregated cells (%)
DBH +/-
DBH -/-
D
HC
AT-8
ThioS
DAPI
Merge
3 month
6 month
9 month
E
WT
MAPT
Control
MAO-A

Figure S6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AAV-control (mCherry)
AAV-Tau/mCherry
3XTG, LC
TH
AT8
mCherry
Merge
TH
N368
mCherry
Merge
B
AAV-control (mCherry)
AAV-Tau/mCherry
EC
HC
Cx
AT8
N368
DAPI
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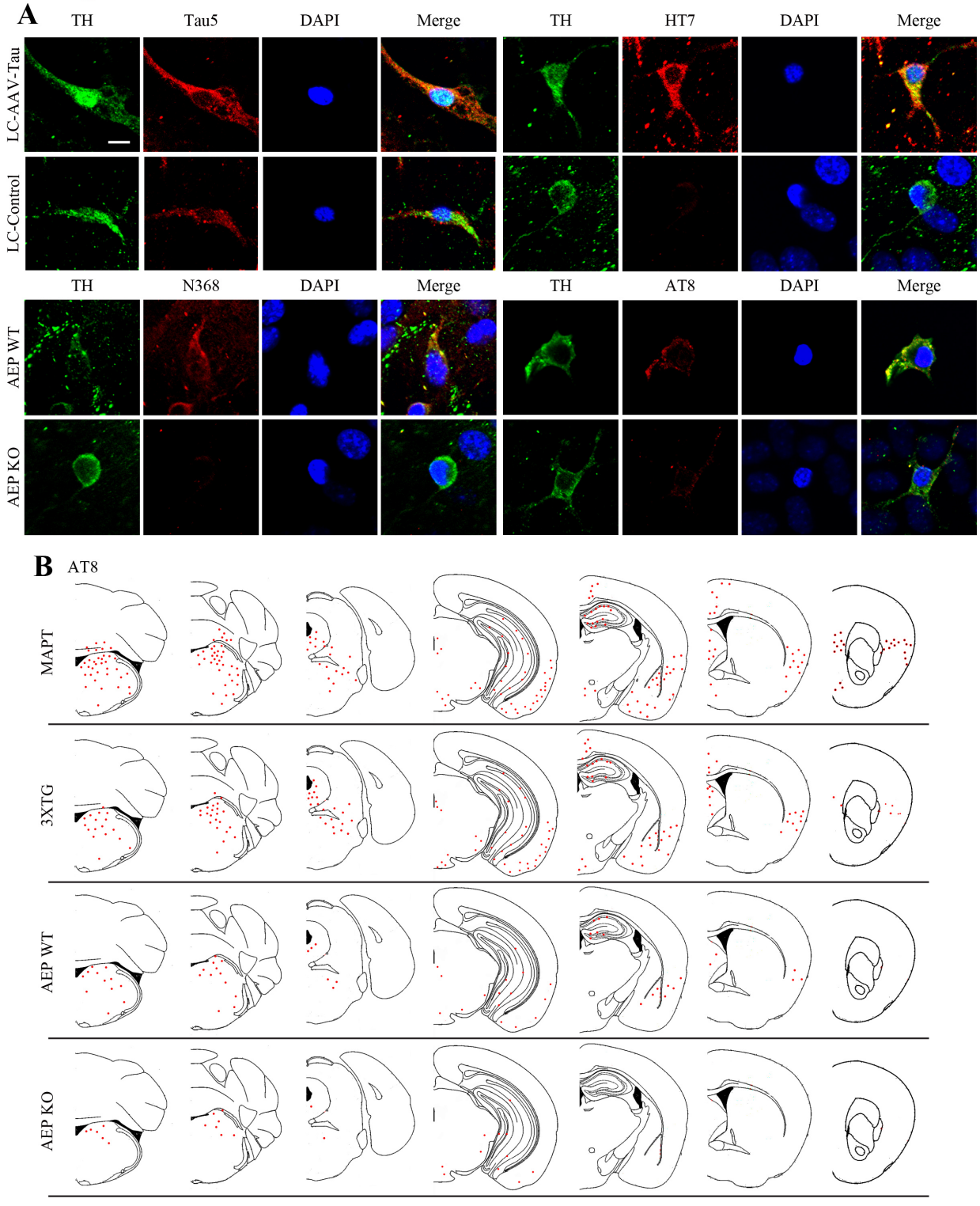
C
LC
EC
HC
Cx
AAV-control
AAV-Tau

Figure S7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AT8
DAPI
Merge
AEP WT
AEP KO
B
AT8
MAPT
3XTG
AEP WT
AEP KO