Supplementary information

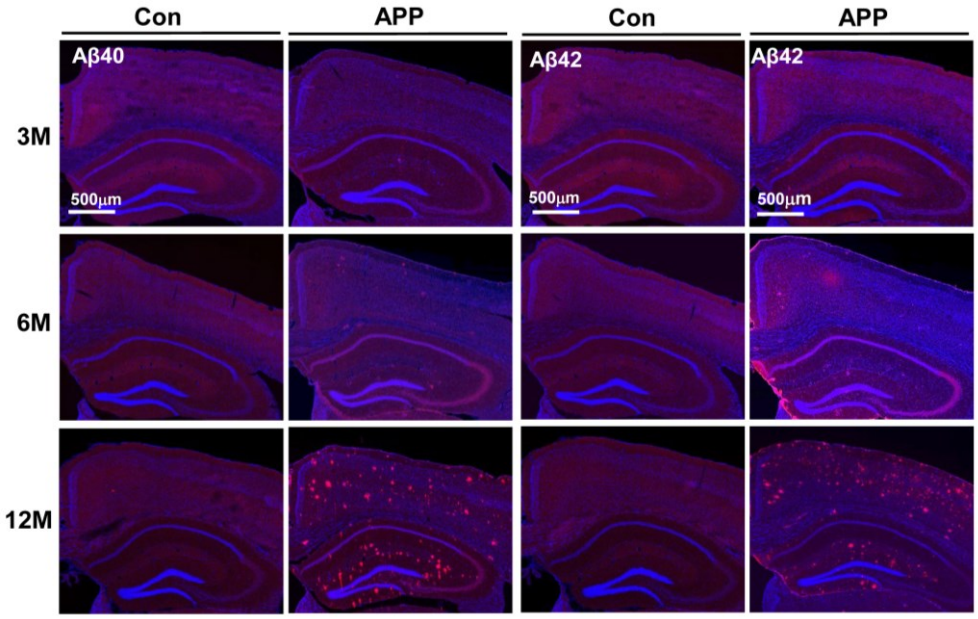

Fig.S1 Representative micrographs show Aβ deposition in the brain of APP/PS1 mice

Brain sections from APP/PS1 mice and the littermate controls (3-month, 6-month, 12-month) were immunostained with antibodies to Aβ42 (Biolegend:805501) and Aβ40 (Biolegend:805409). N=5/group. Sporadic Aβ deposition were observed in AD brain at 6 months, extensive Aβ deposition were found in the brain of 12-month-old APP/PS1 mice.

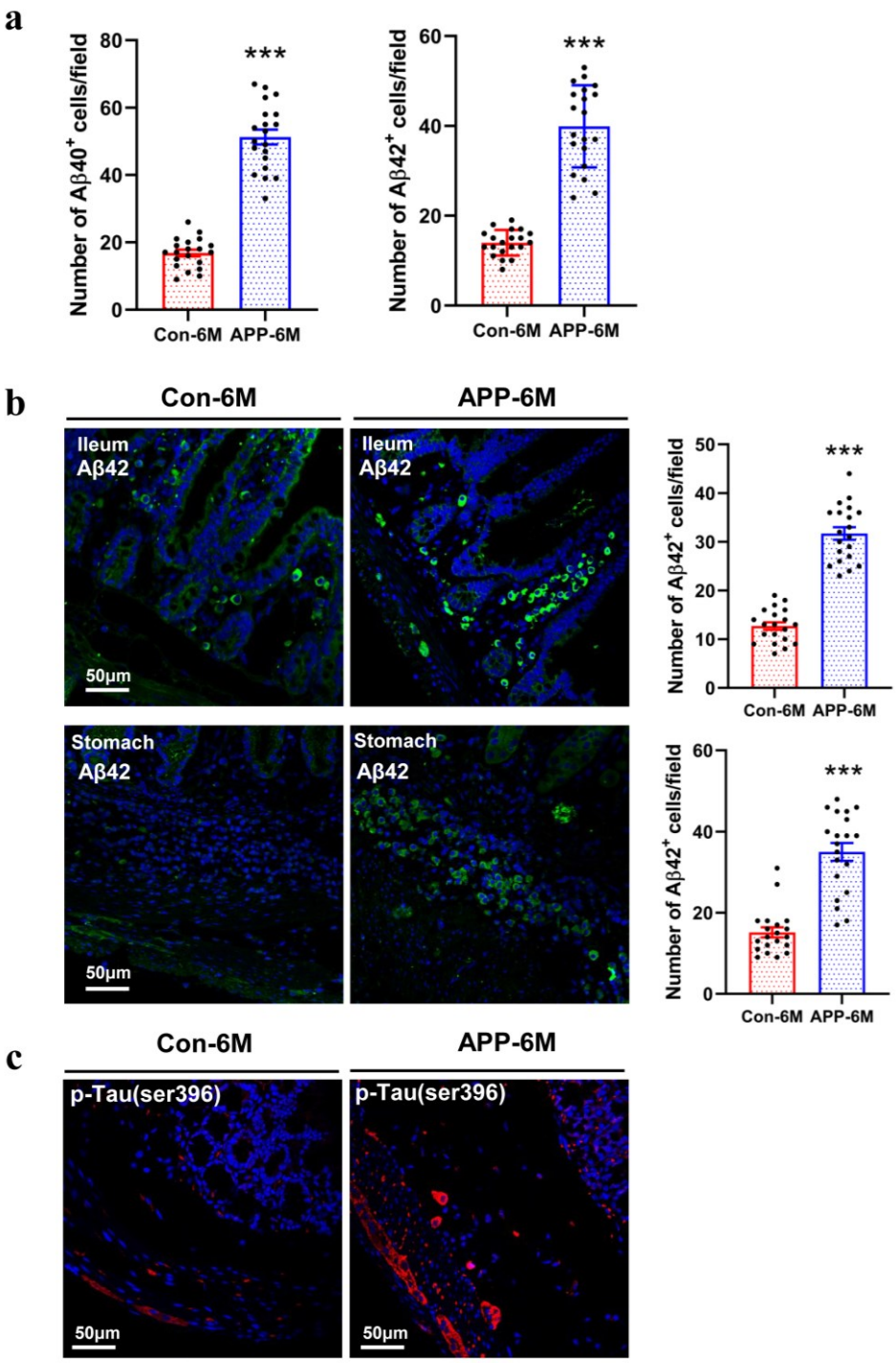

Fig.S2 Aβ deposition in the GI tract of APP/PS1 mice

(**a**) Quantitative analysis of the number of $A\beta40^+$ and $A\beta42^+$ cells in the colon of 6-month-old APP/PS1 mice and their littermate controls. Four fields per preparation, N=5/group. Data are presented as mean ± SEM, unpaired *t* test, ***P<0.001. (**b**) Representative micrographs and quantitative analysis of $A\beta42^+$ in the ileum and stomach of 6-month-old APP/PS1 mice and the littermate controls. The Anti-Aβ42

antibody (Biolegend:805501) was used. Four fields per preparation, N=5/group. Data are presented as mean ± SEM, unpaired *t* test, ***P<0.001. (**c**) Representative micrographs of phosphorylated tau (p-Tau) immunoreactivity in the colonic myenteric plexus of 6-month-old APP/PS1 mice and the littermate controls. N=5/group.

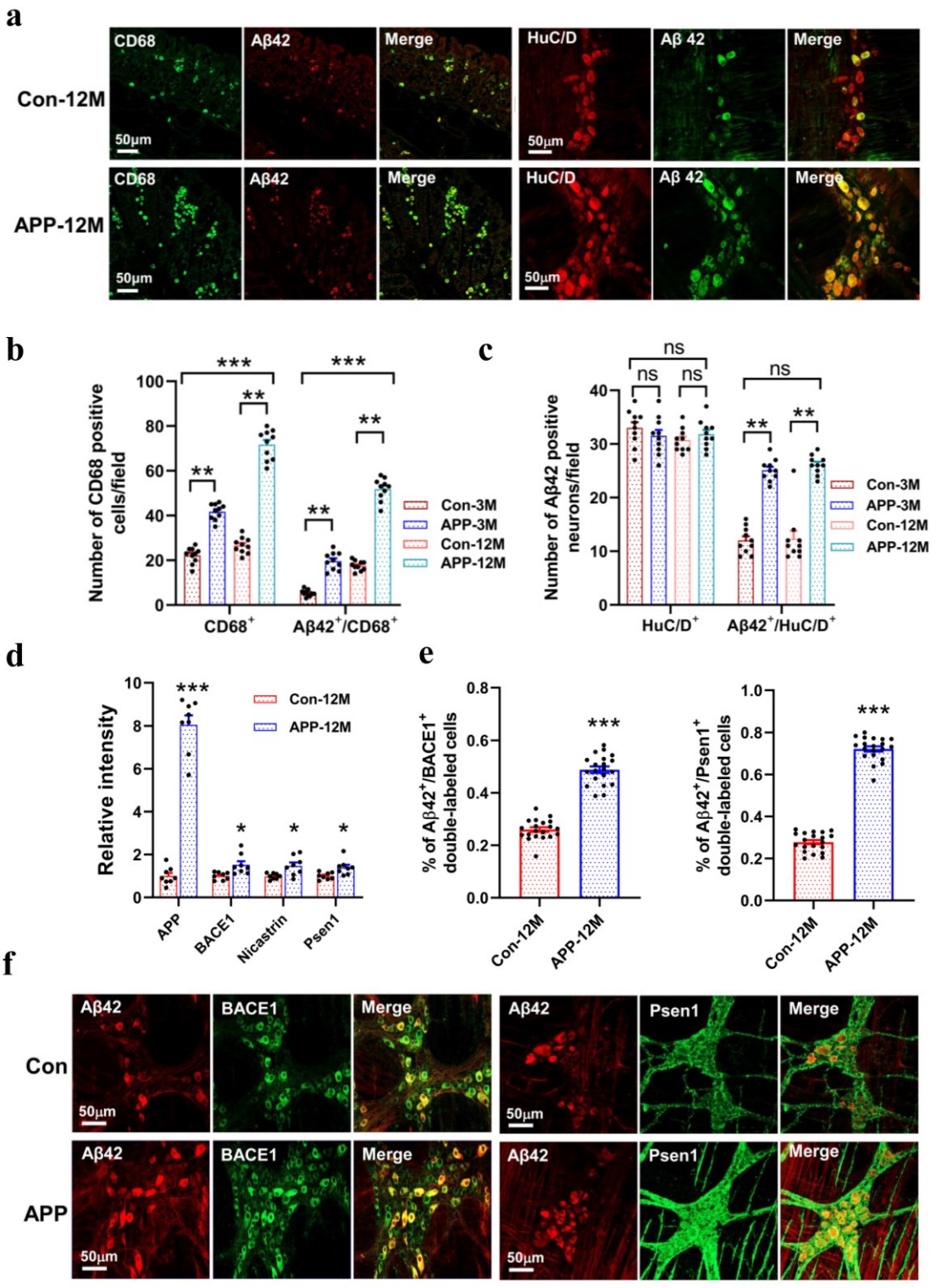

Fig.S3 Analysis of co-localization of Aβ42-positive cells in the colonic mucosal layer and myenteric plexus of APP/PS1 mice

(**a**) Immunofluorescence characterization of the $A\beta42^+$ cells from the colonic sections and plexus myenteric plexus strips in 12-month-old APP/PS1 mice and littermate controls. The Anti-Aβ42 antibody (Abcam: ab10148) was used. N=5/group. (**b**) Quantitative analysis of the $CD68^+$ cells and $A\beta42^+/CD68^+$ double-labeled cells from the colonic sections and plexus myenteric plexus strips. Two fields per preparation, N=5/group. Data are presented as mean ± SEM. A two-way ANOVA analysis revealed a significant effect of age on the number of $CD68^+$ cells ($F(1, 18) = 214.5$, $P<0.001$) and the number of $A\beta42^+/CD68^+$ cells ($F(1, 18) = 1038$, $P<0.001$), $^{**}P<0.01$, $^{***}P<0.001$. (**c**) Quantitative analysis of the HuC/D^+ cells and $A\beta42^+/HuC/D^+$ double-labeled cells from the colonic sections and plexus myenteric plexus strips. Two fields per preparation, N=5/group. Data are presented as mean ± SEM. A two-way ANOVA analysis revealed no significant effect of age on the number of HuC/D^+ cells ($F(1, 18) = 1.090$, $P=0.3102$) and the number of $A\beta42^+/HuC/D^+$ cells ($F(1, 18) = 0.5243$, $P=0.4783$), $^{**}P<0.01$, $^{***}P<0.001$. The "ns" represents no significance. (**d**) Quantitative analysis of the expression of proteins for Aβ generation in the colon of 12-month-old APP/PS1 mice and littermate controls. N=8/group. Data are presented as mean ± SEM, unpaired *t* test, $^{*}P<0.05$, $^{***}P<0.001$. (**e**) Quantitative analysis of the proportion of cells with Aβ42 deposition in $BACE1^+$ cells and $Psen1^+$ cells from the colonic myenteric plexus. Four fields per preparation, N=5/group. Data are presented as mean ± SEM, unpaired *t* test, $^{***}P<0.001$. (**f**) Representative micrographs show the intracellular Aβ42 deposition in $BACE1^+$ and $Psen1^+$ cells from the colonic myenteric plexus of APP/PS1 mice and the control group. The Anti-Aβ42 antibody (Biolegend:805501) was used. N=5/group.

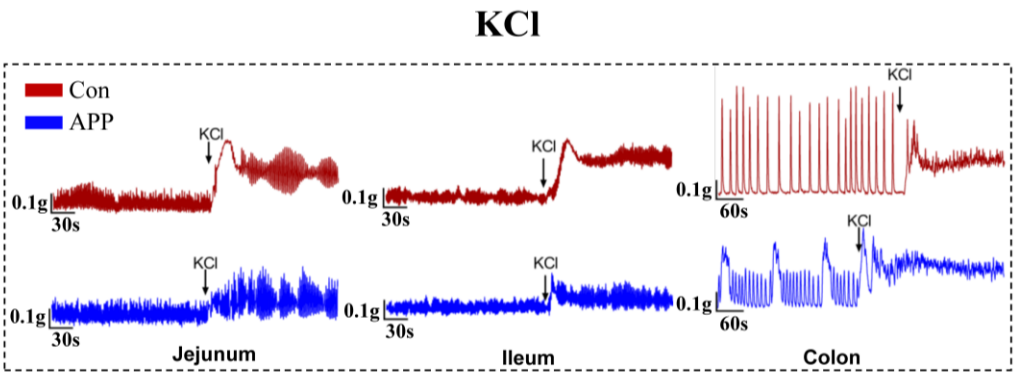

Fig.S4 The KCl-induced contraction of intestinal smooth muscle was impaired in the APP/PS1 mice

Representative tracing of KCl-induced muscle contraction in jejunal segments, ileal segments, and colonic segments from 3-month-old APP/PS1 and littermate controls. Changes in muscle tension 5-10 min before and after exposure to the drug were measured, 15 mmol/L of KCl.

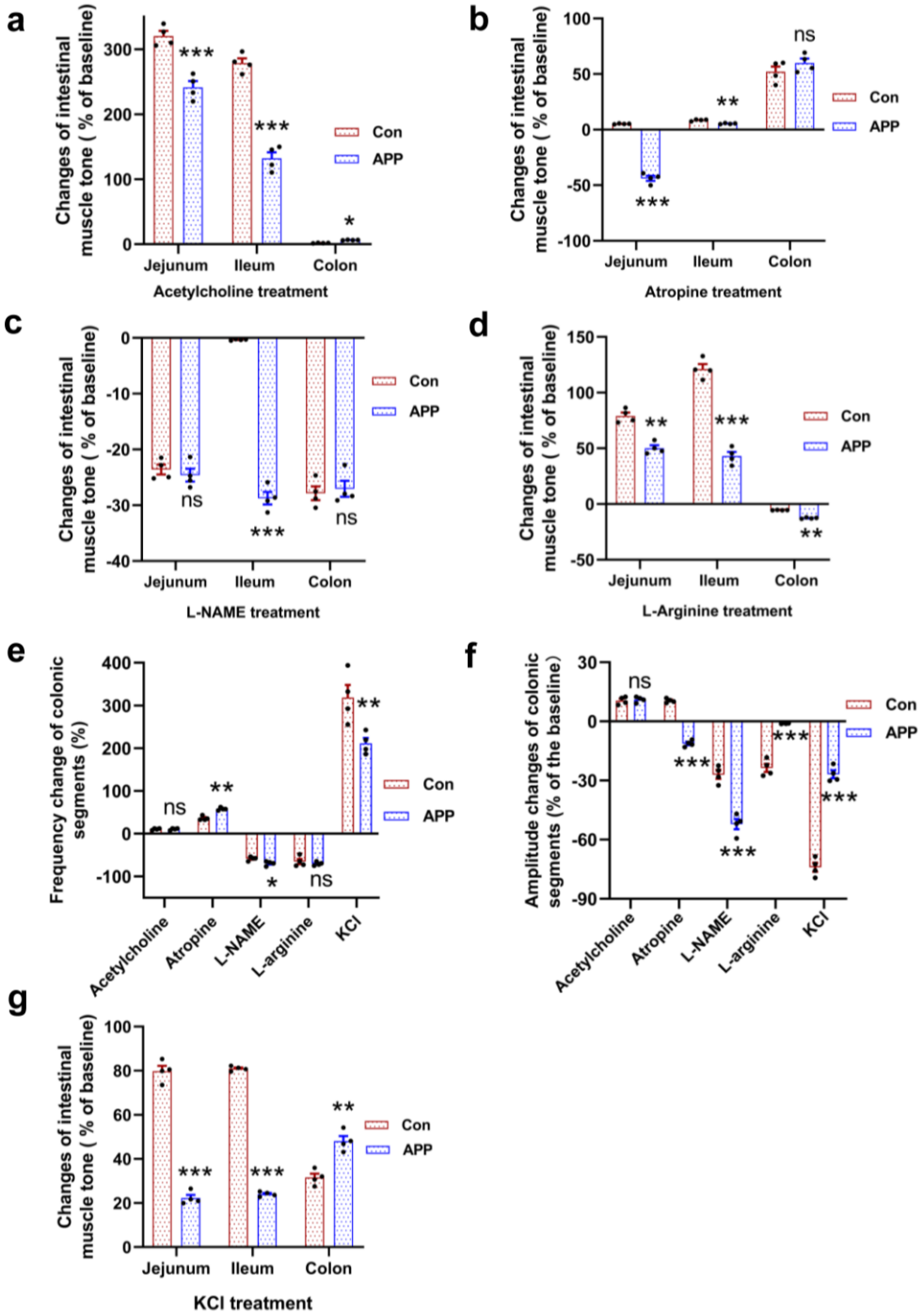

Fig.S5 Impaired contraction and relaxation in intestinal muscle strips of APP/PS1 mice

(**a-d**) Quantitative analysis of changes in intestinal muscle tone induced by (**A**) acetylcholine, (**b**) atropine, (**c**) L-NAME, (**d**) L-arginine in the 3-month-old APP/PS1 and littermate controls. (**e-f**) Quantitative analysis of frequency changes (**e**) and amplitude changes (**f**) in colonic segments induced by acetylcholine, atropine, L-NAME, L-arginine, and KCl. (**g**) Quantitative analysis of changes in intestinal

muscle tone induced by KCl. Changes in muscle tension 5-10 min before and after exposure to each drug were measured. Data are presented as mean ± SEM, **P*<0.05, ***P*<0.01, ****P*<0.001. The "ns" represents no significance. Drugs used in this study: 1 μmol/L of Acetylcholine; 50 μmol/L of Atropine; 10 mmol/L of L-NAME for jejunum and ileum; 2 mmol/L of L-NAME for colon; 15 mmol/L of L-arginine and KCl; 20 μmol/L of Aβ42 and Aβ40. All drug were prepared before being used.

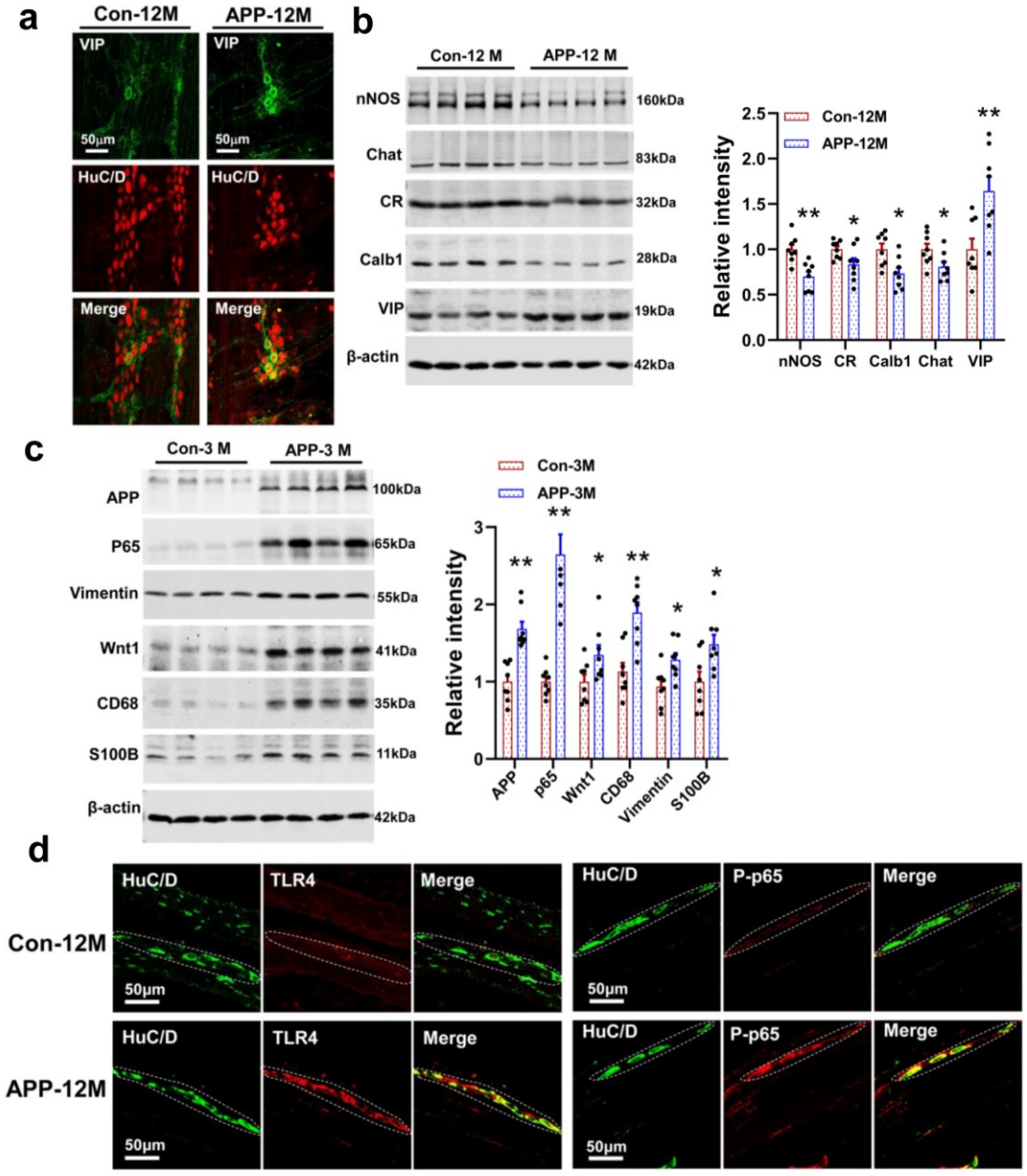

Fig.S6 Changes of neuron- and inflammation-related proteins in the APP/PS1

mice

(**a**) Representative micrographs of colonic whole mount staining for VIP in the 12-month-old APP/PS1 mice and littermate controls. N=5/group. (**b**) Western blot analyses the expression of nNOS, CR, VIP, Calb1 and Chat proteins in the colonic LMMP strips of the 12-month-old APP/PS1 mice and littermate controls. N=8/group. Data are presented as mean ± SEM, unpaired *t* test, **P*<0.05. (**c**) Western blot analysis of the expression of inflammation-associated proteins in the colonic LMMP strips of 3-month-old APP/PS1 mice and their littermate controls. N=8/group. Data are presented as mean ± SEM, unpaired *t* test, **P*<0.05, ***P*<0.01. (**d**) Representative micrographs show the immunoreactivity of TLR4 and phosphorylated p65 (p-P65) in the myenteric HuC/D^{+} neurons of APP/PS1 mice and the control group. N=5/group.

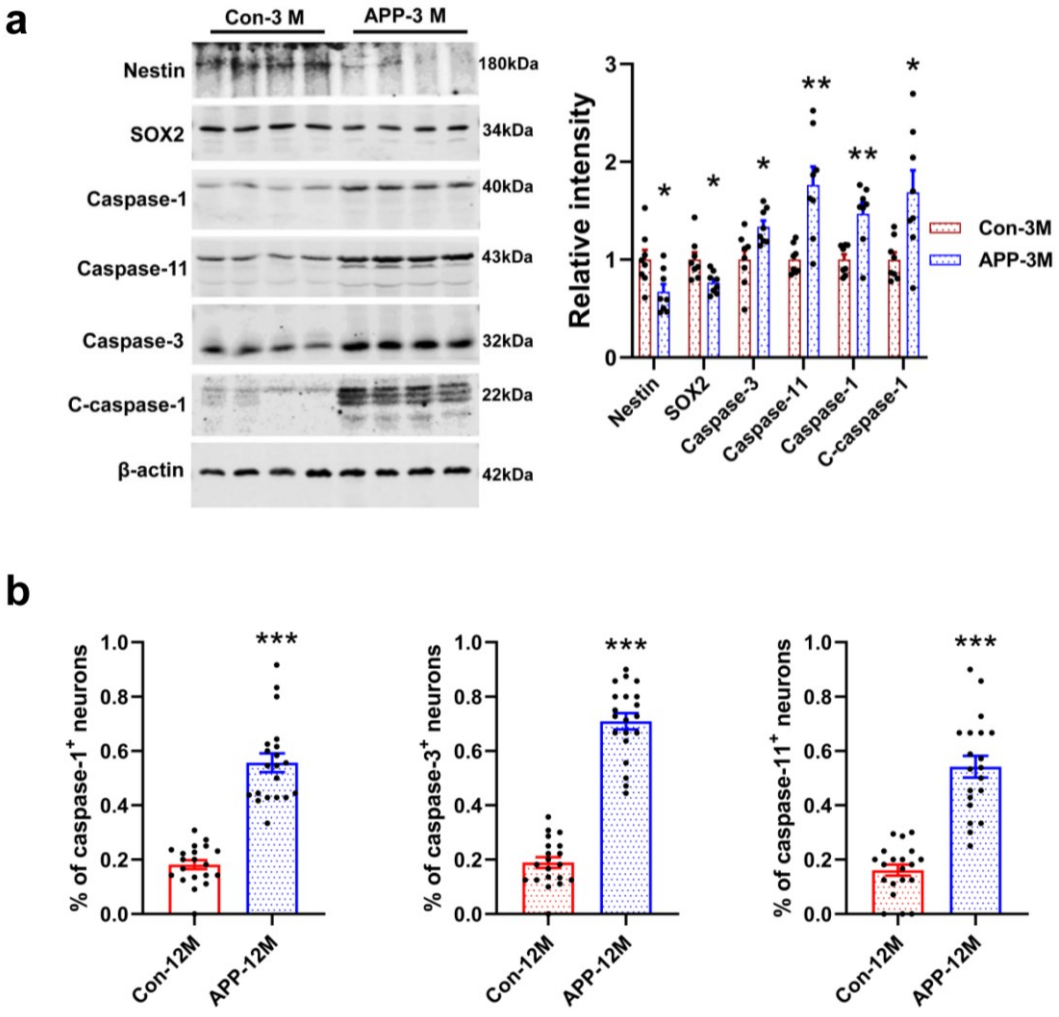

Fig.S7 Increased apoptosis and pyroptosis and impaired neurogenesis in the enteric neurons of APP/PS1 mice

(**a**) Western blot analysis shows that the expression of proteins involved in neurogenesis (SOX2 and Nestin), apoptosis (Caspase-3), and pyroptosis (Caspase-1 and Caspase-11) in the colonic LMMP strips of APP/PS1 mice and the littermate controls. Data are presented as mean ± SEM, **P*<0.05, ***P*<0.01, N=8/group. (**b**) Quantitative analysis of the relative proportion of Caspase-1, Caspase-3, and Caspase-11-positive neurons in colonic sections from 12-month-old APP/PS1 mice and the littermate controls. Four fields per preparation, N=5/group. Data are presented as mean ± SEM, unpaired *t* test, ****P*<0.001.

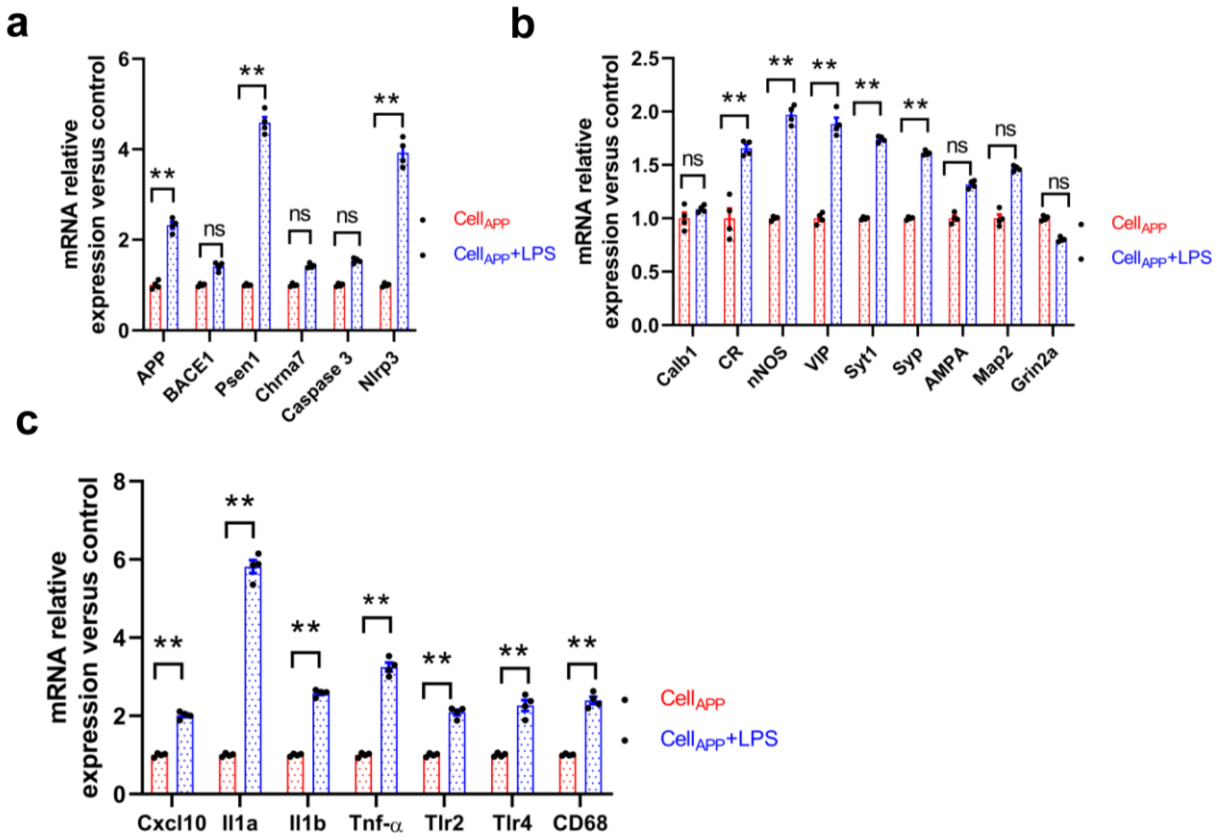

Fig.S8 Effects of LPS on the gene expression of cultured myenteric neurons from APP/PS1 mice

(**a-c**) Analysis of the effects of LPS treatment on the gene expression of cultured myenteric neurons from APP/PS1 mice by qRT-PCR. The longitudinal

muscle-myenteric plexuses from 3-month-old APP/PS1 mice were prepared and digested, cells were treated with 10 ng/ml lipopolysaccharide (LPS) for 72 h. After that, treatment cells were collected for real-time RT-PCR. Data are presented as mean ± SEM. Statistical significance was determined using unpaired *t* test, ***P*<0.01. The "ns" represents no significance.

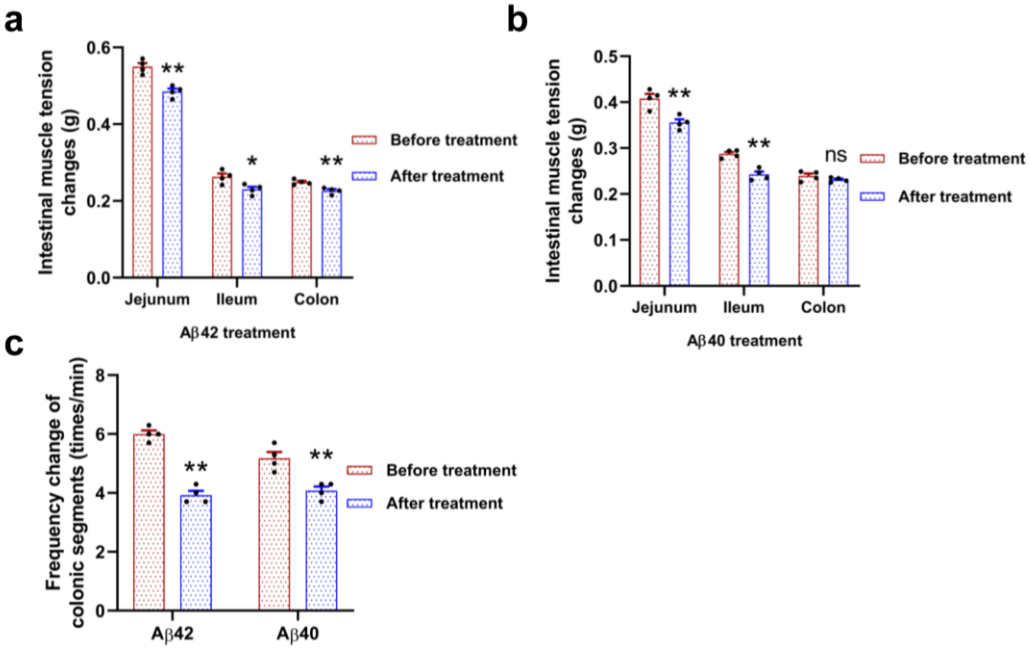

Fig.S9 Quantification of the effect of Aβ treatments on the spontaneous intestinal contraction

(**a-b**) Changes of intestinal muscle tension induced by Aβ42 treatments (**a**) and Aβ40 treatments (**b**). The 1.5-cm intestinal segments were removed from the 3-month-old WT mice, and changes in muscle tension 5-10 min before and after exposure to each drug were measured. N=4/group. Data are presented as mean ± SEM. Statistical significance was determined using unpaired *t* test, **P*<0.05, ***P*<0.01. The "ns" represents no significance. (**c**) The frequency changes of colonic segments indued by Aβ40 and Aβ42 treatments. The 1.5-cm intestinal segments were removed from the 3-month-old WT mice, and changes in muscle tension 5-10 min before and after exposure to each drug were measured. N=4/group. Data are presented as mean ± SEM. Statistical significance was determined using unpaired *t* test, ***P*<0.01.

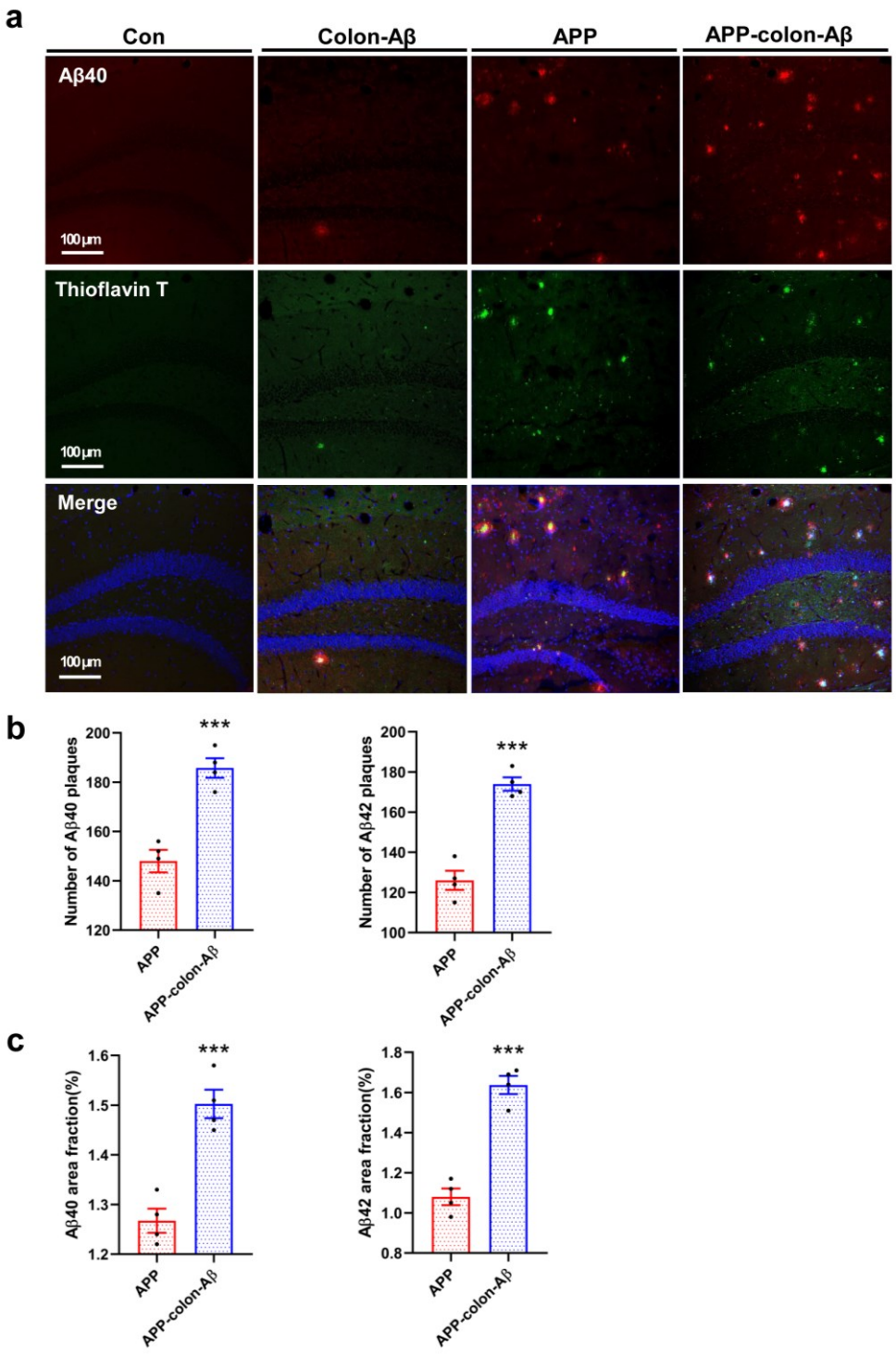

Fig.S10 The Aβ plaques in the hippocampus of Aβ-injected mice and control group

(**a**) Representative micrographs show the typical Aβ40 plaques in the hippocampus of 12-month-old Aβ-injected WT mice and Aβ-injected APP/PS1 mice. The 3-month-old mice were injected with Aβ42 oligomers in the proximal colon. Nine months later,

these 12-month-old mice subject to a series of tests. The nucleuses were stained with DAPI; the amyloid-β plaque was stained with anti-Aβ40 antibody (Biolegend:805409) and Thioflavin T. N=4/group. (**b**) Quantitative analysis of the number of Aβ40 and Aβ42 plaques in the hippocampus of the Aβ-injected APP/PS1 mice. Data are presented as mean ± SEM, unpaired *t* test, ***$P<0.001$. N=4/group. (**c**) Quantitative analysis of the area fraction of Aβ40 and Aβ42 plaques in the hippocampus of the Aβ-injected APP/PS1 mice. Data are presented as mean ± SEM, unpaired *t* test, ***$P<0.001$. N=4/group.

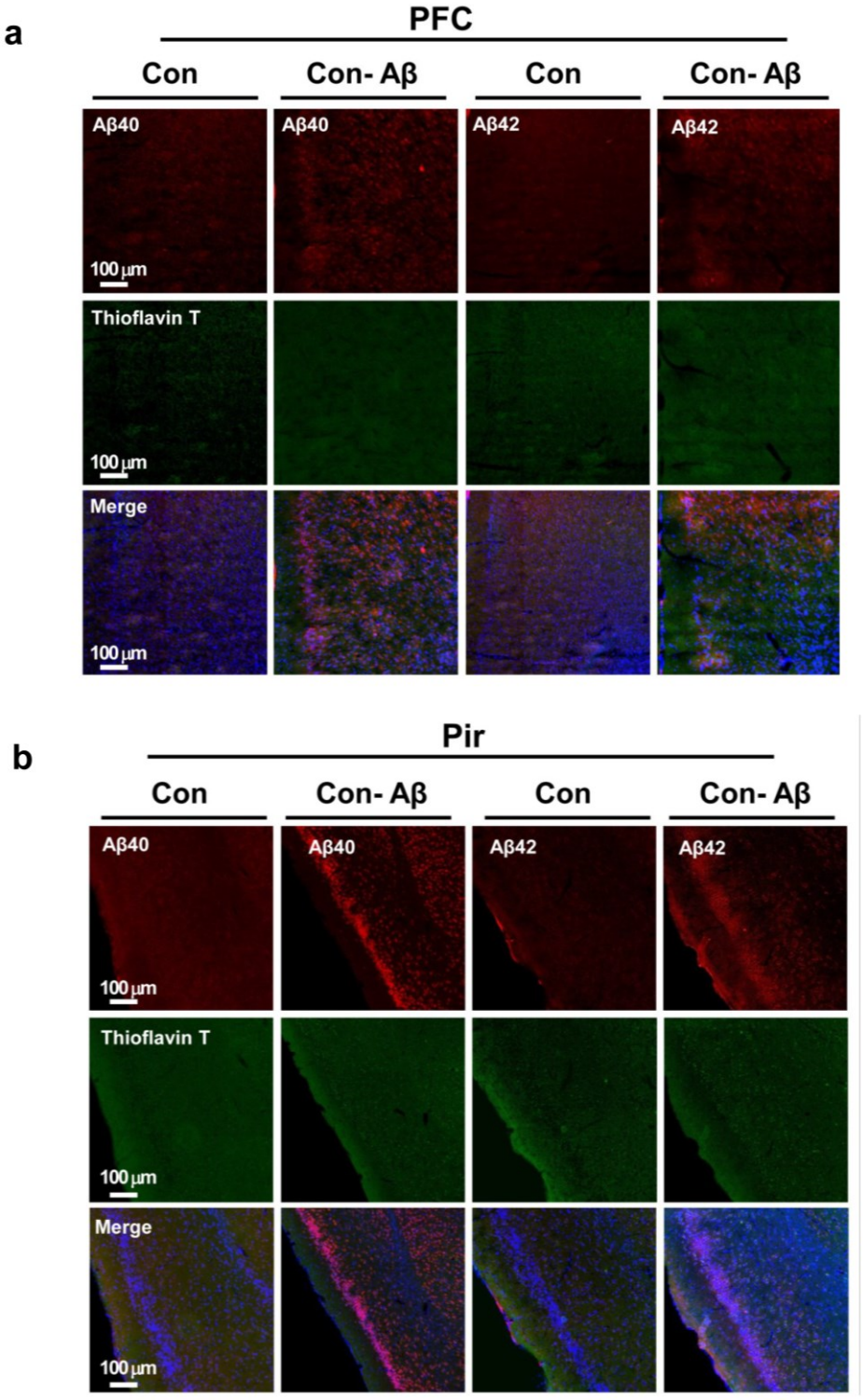

Fig.S11 The immunoreactivity of Aβ40 and Aβ42 in the PFC and Pir of the Aβ-injected mice and control group

(**a**) Representative micrographs show the intracellular Aβ40 (Biolegend:805409) and Aβ42 (Biolegend:805501) deposition in the prefrontal cortex (PFC) of the Aβ-injected mice and the control group. N=4/group. (**b**) Representative micrographs show the

intracellular Aβ40 (Biolegend:805409) and Aβ42 (Biolegend:805501) deposition in the piriform cortex (Pir) of the Aβ-injected mice and the control group. N=4/group. The nucleuses were stained with DAPI; the intraneuronal Aβ immunoreactivity was stained with anti-Aβ42 antibody and anti-Aβ40 antibody.

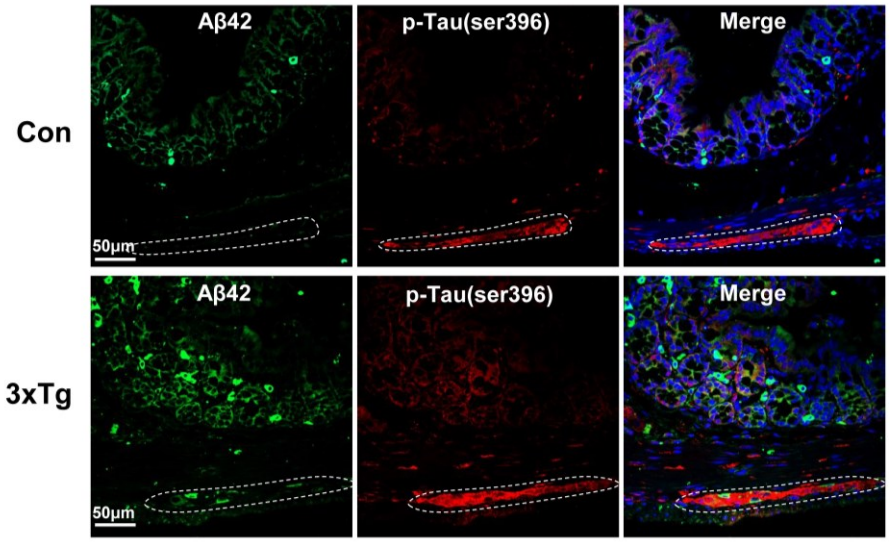

Fig.S12 The immunoreactivity of Aβ42 and phosphorylated tau in the colon of 3xTg mice

Representative micrographs showed the immunoreactivity of Aβ42 and phosphorylated tau (p-Tau) in the colonic myenteric plexus of 3-month-old 3xTg mice and littermate controls. N=4/group. The nucleuses were stained with DAPI; the Aβ immunoreactivity was stained with anti-Aβ42 antibody (Biolegend:805501); the phosphorylated tau immunoreactivity was stained with anti-phosphorylated Tau (ser396) polyclonal antibody.

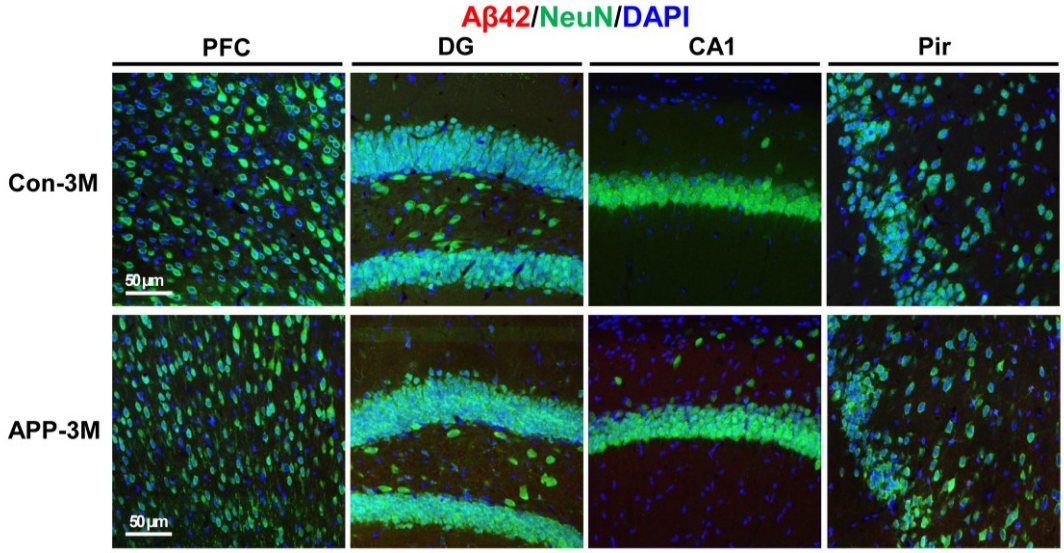

Fig.S13 The immunoreactivity of Aβ42 in the brain of 3-month-old APP/PS1 mice

Representative micrographs showed the immunoreactivity of Aβ42 and NeuN in the brain of 3-month-old APP/PS1 mice and the littermate controls. N=4/group. The nucleuses were stained with DAPI; the Aβ immunoreactivity was stained with anti-Aβ42 antibody (Biolegend:805501); the neurons in the brain were stained with anti-NeuN antibody. Prefrontal cortex: PFC. Dentate gyrus: DG. Piriform cortex: Pir.

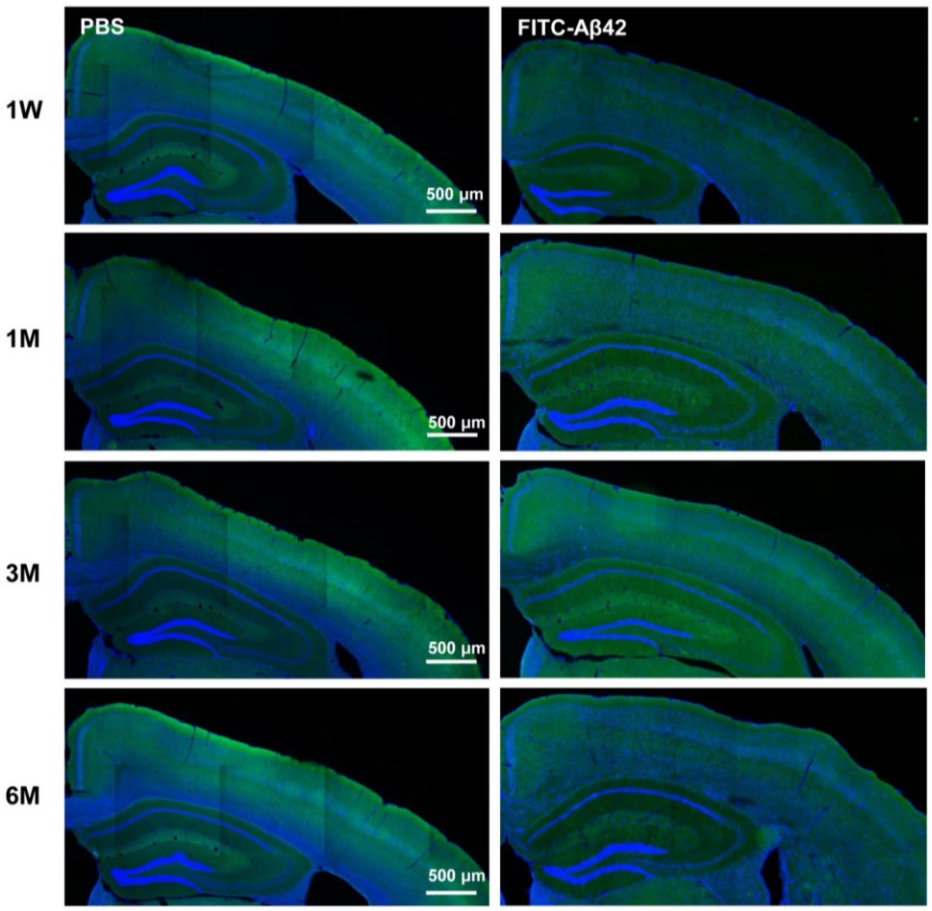

Fig.S14 No FITC fluorescence was observed in the brain of WT mice injected with FITC-Aβ42

Representative micrographs showed the fluorescence of FITC in the brain of wild-type (WT) mice that were injected with the FITC-labeled Aβ42 in the colon. The injections conducted using a pulled glass pipette into the wall (a depth of 1-2 mm) of the of proximal colon at 3 sites. A total of 5 μg of FITC-Aβ42 at each site was injected, the fluorescence of FITC in the brain was observed at different time points after injection. The nucleus was stained blue by DAPI. N=6/group. 1w: 1-week-old; 1M: 1-month-old; 3M: 3-month-old; 6M: 6-month-old.

Table S1: The antibody used in this study		
Antibody	Supplier	Dilution ratio
Mouse monoclonal antibody anti-HuC/D	Invitrogen:A21271	IF 1:200
Rabbit polyclonal antibody anti-β-amyloid 1-42	Abcam:ab10148	IF 1:200
Mouse monoclonal antibody anti-CD68	Abcam:ab31630	IF 1:200; WB 1:500
Mouse monoclonal antibody anti-β-amyloid 1-40	Biolegend:805409	IF 1:200
Mouse monoclonal antibody anti-β-amyloid 1-42	Biolegend:805501	IF 1:200
Rabbit polyclonal antibody anti-PSEN1	Proteintech:16163-1-AP	IF 1:200; WB 1:800
Rabbit polyclonal antibody anti-BACE1	Proteintech:12807-1-AP	IF 1:200; WB 1:500
Rabbit polyclonal antibody anti-Calbindin 1	Proteintech:14479-1-AP	IF 1:200; WB 1:500
Rabbit polyclonal antibody anti-nNOS	Invitrogen:61-7000	IF 1:200; WB 1:500
Rabbit polyclonal antibody anti-VIP	Servicebio:GB11279	IF 1:200; WB 1:500
Rabbit polyclonal antibody anti-Calretinin (CR)	Swant:7697	IF 1:200; WB 1:800
Rabbit polyclonal antibody anti-TLR4	Bioss:bs-1021R	IF 1:150; WB 1:500
Rabbit polyclonal antibody anti-p65 (phospho S536)	Servicebio:GB11142-1	IF 1:100
Rabbit polyclonal antibody anti-SOX2	Proteintech:11064-1-AP	IF 1:200; WB 1:800
Rabbit polyclonal antibody anti-Nestin	Proteintech:19483-1-AP	IF 1:200; WB 1:500
Mouse monoclonal antibody anti-NF-κB (p65)	ABclonal:A10609	IF 1:200; WB 1:800
Rabbit polyclonal antibody anti-Wnt1	Bioss:bs-1739R	WB 1:500
Rabbit polyclonal antibody anti-Vimentin	Bioss:bs-0756R	WB 1:500
Rabbit polyclonal antibody anti-S100B	ABclonal:A0676	WB 1:500
Rabbit monoclonal antibody anti-Caspase 3	Proteintech:19677-1-AP	IF 1:150; WB 1:800
Rabbit monoclonal antibody anti-Cleaved caspase 1	Cell Signaling Technology:89332	WB 1:500
Rabbit monoclonal antibody anti-Caspase 1	ABclonal:A18646	IF 1:100; WB 1:500
Rat monoclonal antibody anti-Caspase-11 (17D9)	Cell Signaling Technology:14340	IF 1:150; WB 1:500
Mouse monoclonal antibody anti-APP	Proteintech:60342-1-Ig	IF 1:150; WB : 1:500
Goat polyclonal antibody anti-Chat	Merck Millipore:AB144P	IF 1:100; WB 1:500
Rabbit polyclonal antibody anti-NLRP6	ABclonal:A15628	WB 1:500
Rabbit polyclonal antibody anti-phospho-Tau (Ser396)	Thermofisher: 44-752G	IF 1:200; WB 1:800
Rabbit polyclonal antibody anti-nNOS	Servicebio:GB11145	WB 1:800
Mouse monoclonal antibody anti-β-actin	Proteintech:66009-1-lg	WB 1:20000

Table S2: The primer used by real-time RT-PCR in this study

Gene	Primer sequence
Actb-F	CCACTGTCGAGTCGCGTCC
Actb-R	ATTCCCACCATCACACCCTGG
APP-F	TGCTCTGAACAAGCCGAGAC
APP-R	AAACTTTGGGTTGACACGCTG
BACE1-F	GGACTGCAAGGAGACGGAGAA
BACE1-R	AACAGTCGTCTTGGGACGTG
Psen1-F	GCCATACTGATCGGCCTGT
Psen1-R	GCTGCACAAGGTAATCCGTG
Chrna7-F	CCGTGCCCTTGATAGCACA
Chrna7-R	GGCATTTTGCCACCATCAGG
Caspase 3-F	GCTTGGAACGGTACGCTAA
Caspase 3-R	TCCGTACCAGAGCGAGATGA
Nlrp3-F	AGGCTGCTATCTGGAGGAACT
Nlrp3-R	GCAACGGACACTCGTCATCT
Cxcl10-F	GAAATCATCCCTGCGAGCCT
Cxcl10-R	AGGAGCCCTTTTAGACCTTTTT
IL-1α-F	CGCTTGAGTCGGCAAAGAAAT
IL-1α-R	CTTCCCGTTGCTTGACGTTG
IL-1β-F	TGCCACCTTTTGACAGTGATG
IL-1β-R	ATGTGCTGCTGCGAGATTTG
Tnf-α-F	AAGAGGCACTCCCCCAAAAG
Tnf-α-R	GTGGTTTGTGAGTGTGAGGGT
Tlr2-F	AAACCTCAGACAAAGCGTCAAAT
Tlr2-R	GCGTTTGCTGAAGAGGACTG
Tlr4-F	GTTCTCTCATGGCCTCCACT
Tlr4-R	TTAGGAACTACCTCTATGCAGGGAT
CD68-F	ACTTCGGGCCATGTTTCTCTT
CD68-R	GGGGCTGGTAGGTTGATTGT
Cx3cr1-F	CTTCCCATCTGCTCAGGACC
Cx3cr1-R	ACCAGACCGAACGTGAAGAC
Calb1-F	TTCATTTCGACGCTGACGGA
Calb1-R	TCCGGTGATAGCTCCAATCC
CR-F	TTTGATGCTGACGGAAATGGG
CR-R	AGCTCCGCCATCTCAATTTT
nNOS-F	AAGCGACCATTCTCTACGCC
nNOS-R	CACAGCCGAATTTCTCCCCA
VIP-F	AAGGAAACAGCCAAGGAGGC
VIP-R	GTGGTCCAAAGAGAGGCCAG
Syt1-F	TGCATAAAATCCCATTGCCACC
Syt1-R	GGGCCTGATCCTTCATGGTC
Syp-F	GGGCCAATGATGGACTTCCT
Syp-R	GCCTGTCTCCTTGAACACGA
Ampa2-F	GGGGACAAGGCGTGGAAATA
Ampa2-R	GTACCCAATCTTCCGGGGTC
Grin2a-F	TCTCCGCCTTTCCGATTTGG
Grin2a-R	GCGTCCAACTTCCCAGTTTT
Map2-F	TCCTCTCTACCTCCGCTTCC
Map2-R	TCACAGCTTACTCGCAGAGC