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# Supplementary Materials for

## Gut dysbiosis contributes to amyloid pathology, associated with C/EBPβ/AEP signaling activation in Alzheimer's disease mouse model

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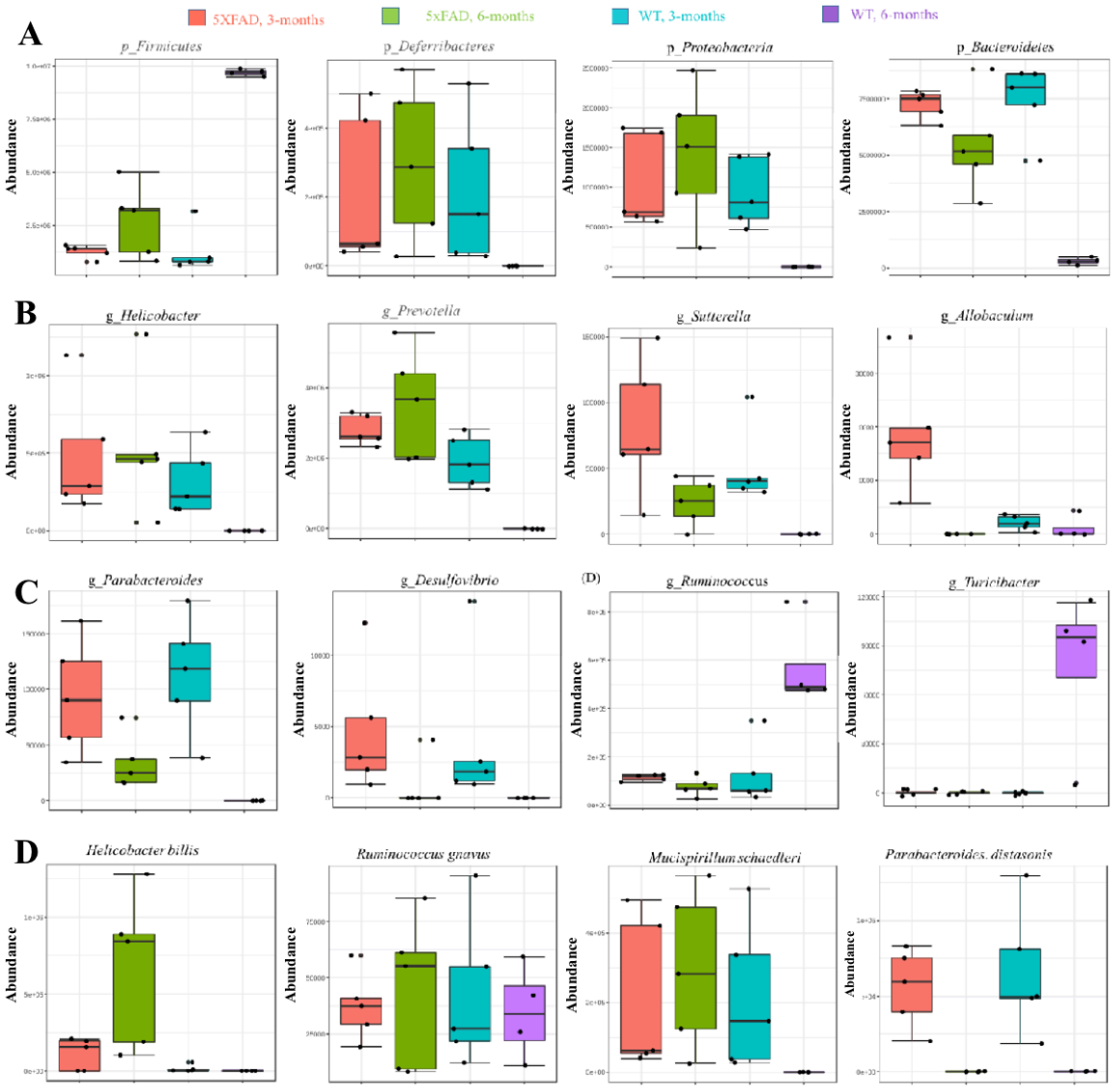
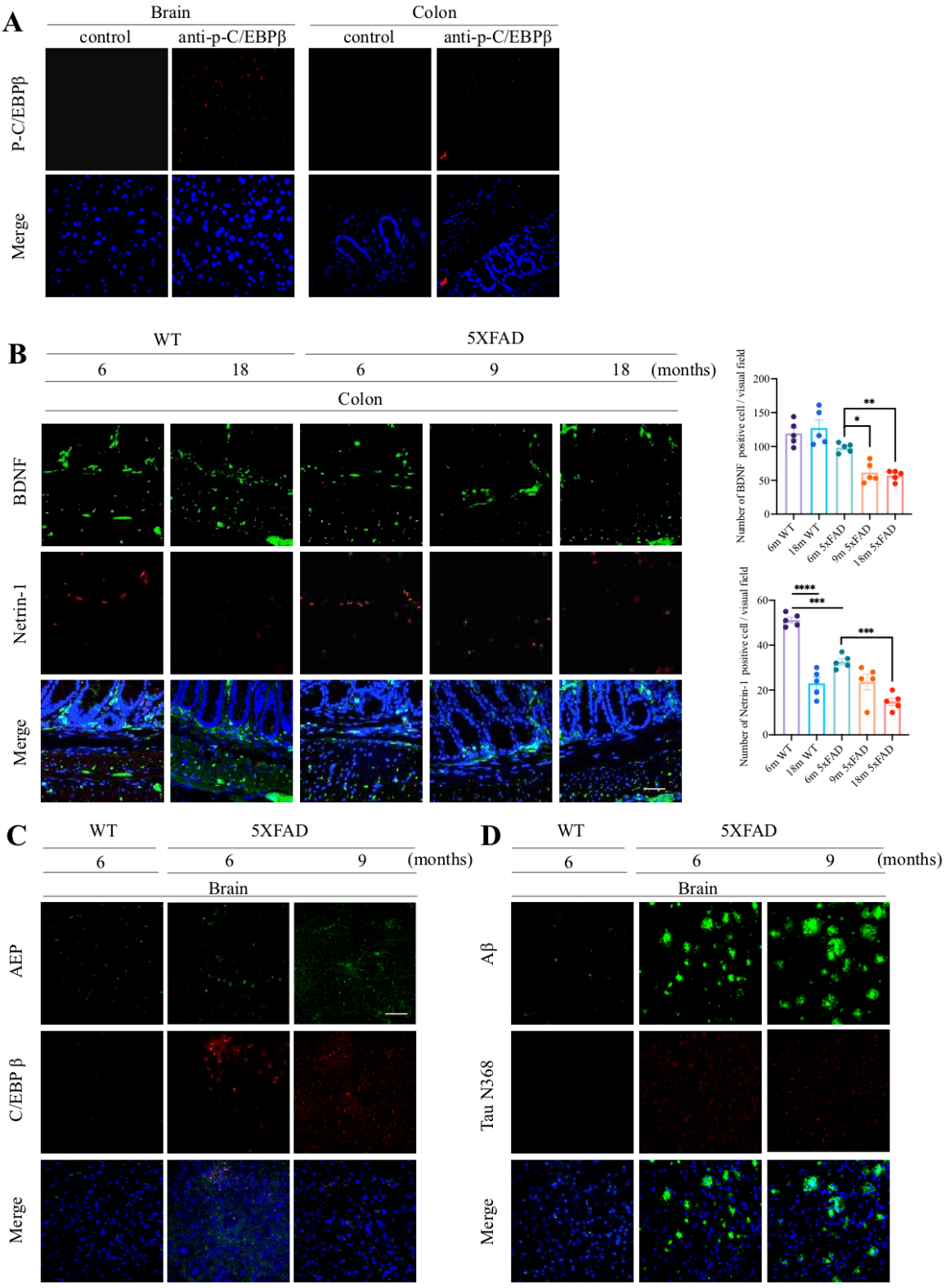
**This PDF file includes:**

## Supplementary Figures



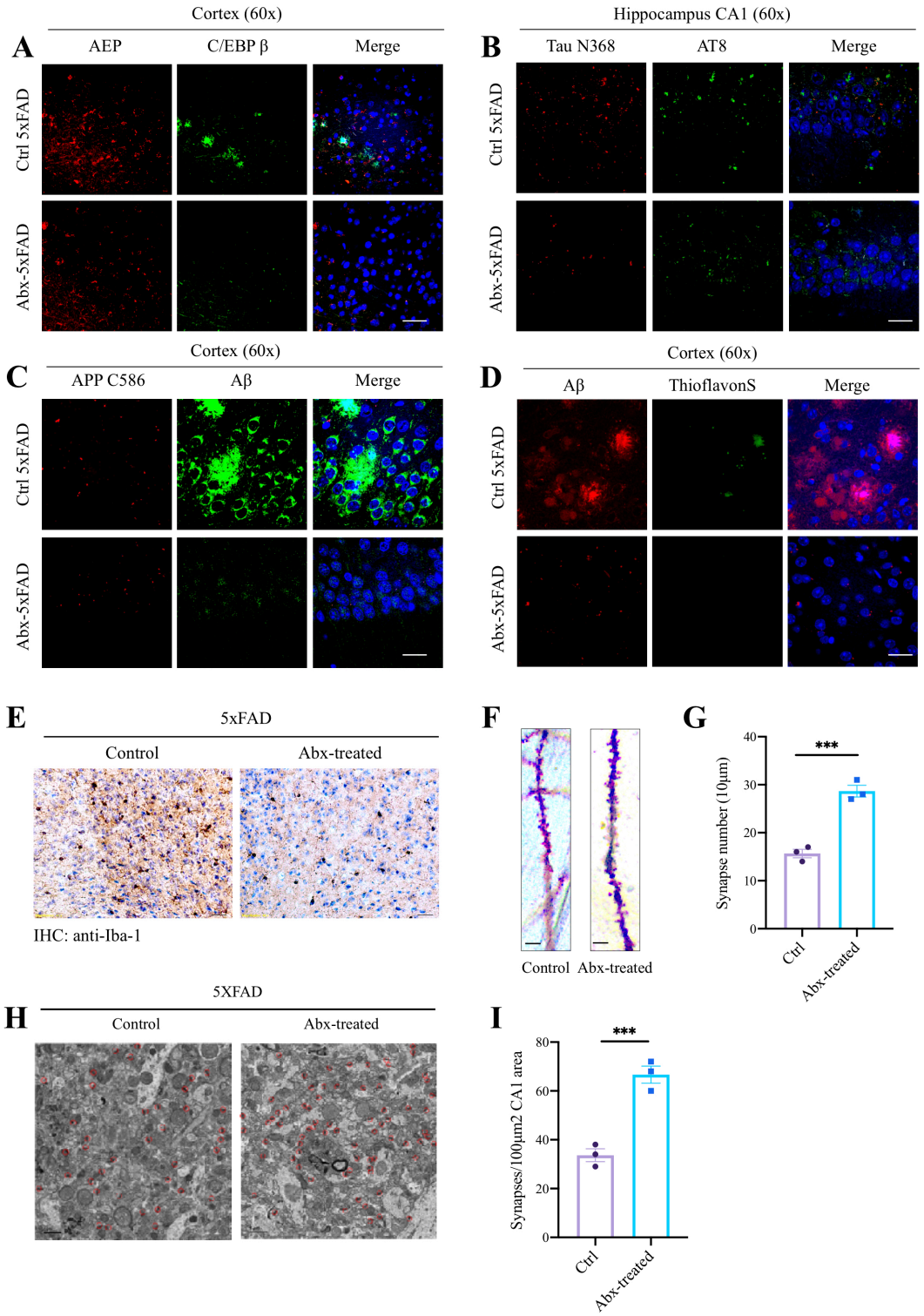
**Supplementary Figure 1. 5xFAD mice are associated with temporal alterations in gut microbiome composition.**

(A-D) Age-dependent disequilibrium in the taxonomic composition of the gut microbiome in 5XFAD. Boxplot showing mean abundance of bacterial phyla and genera determined by high-throughput sequencing analysis (n = 5).


A
Brain
Colon
control
anti-p-C/EBPβ
control
anti-p-C/EBPβ
P-C/EBPβ
Merge
B
WT
5XFAD
6
18
6
9
18
(months)
Colon
BDNF
Netrin-1
Merge
Number of BDNF positive cell / visual field
Number of Netrin-1 positive cell / visual field
6m WT
18m WT
6m 5xFAD
9m 5xFAD
18m 5xFAD
C
WT
5XFAD
6
6
9
(months)
Brain
AEP
C/EBP β
Merge
D
WT
5XFAD
6
6
9
(months)
Brain
Aβ
Tau N368
Merge

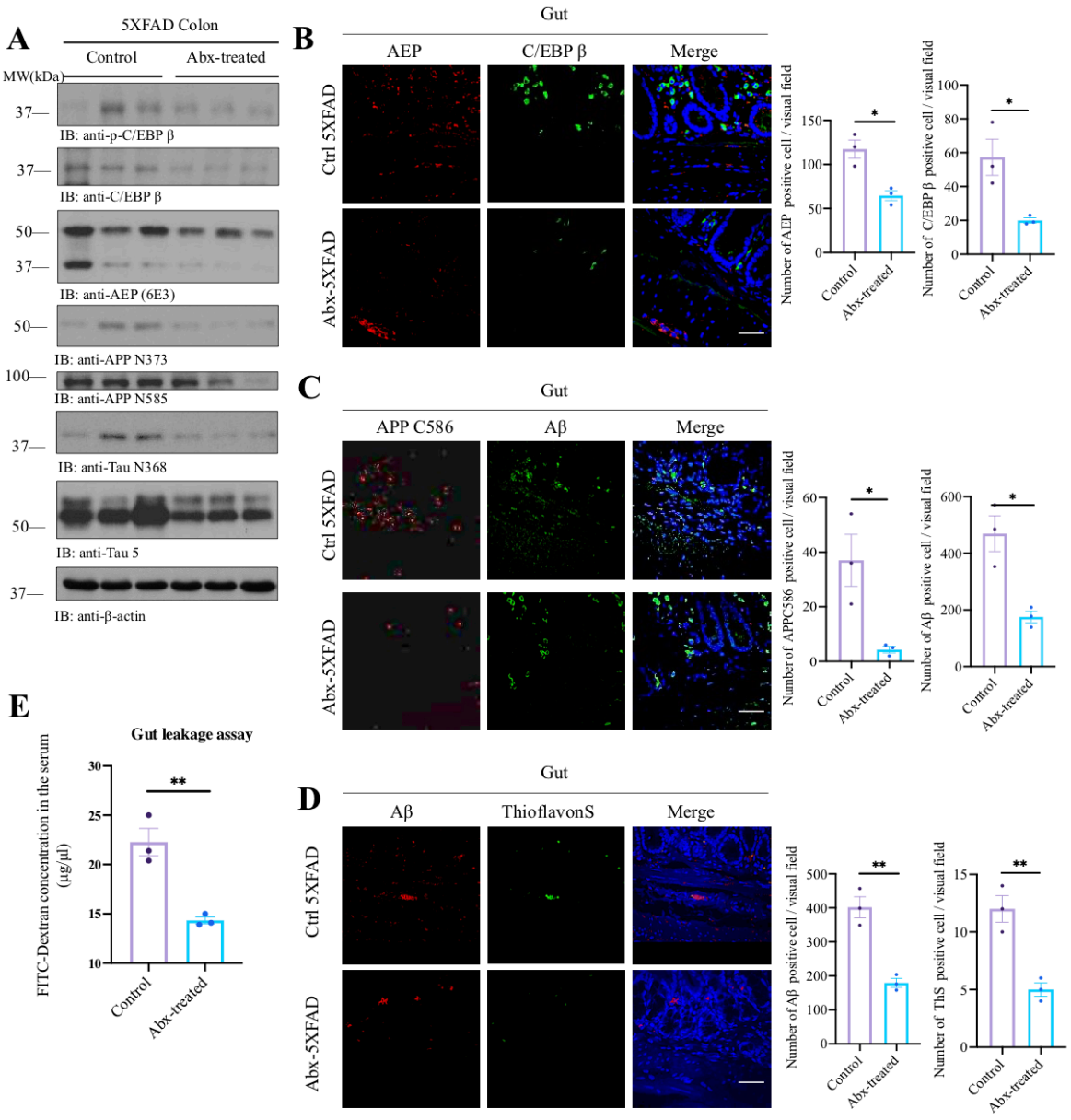
**Supplementary Figure 2. C/EBPβ/AEP pathway is escalated in an age-dependent way in 5xFAD mice brain.**

(A) Validation of the specific staining of p-CEBP/β antibody in both the brain and the colon sections. Immunofluorescent staining of BDNF and Netrin-1 (B) in the colon of 5xFAD mice and the quantification are included right panels, C/EBPβ and AEP (C), Aβ and cleaved Tau N368 (D) in the brain of 5xFAD mice. Scale bar: 20 μm.



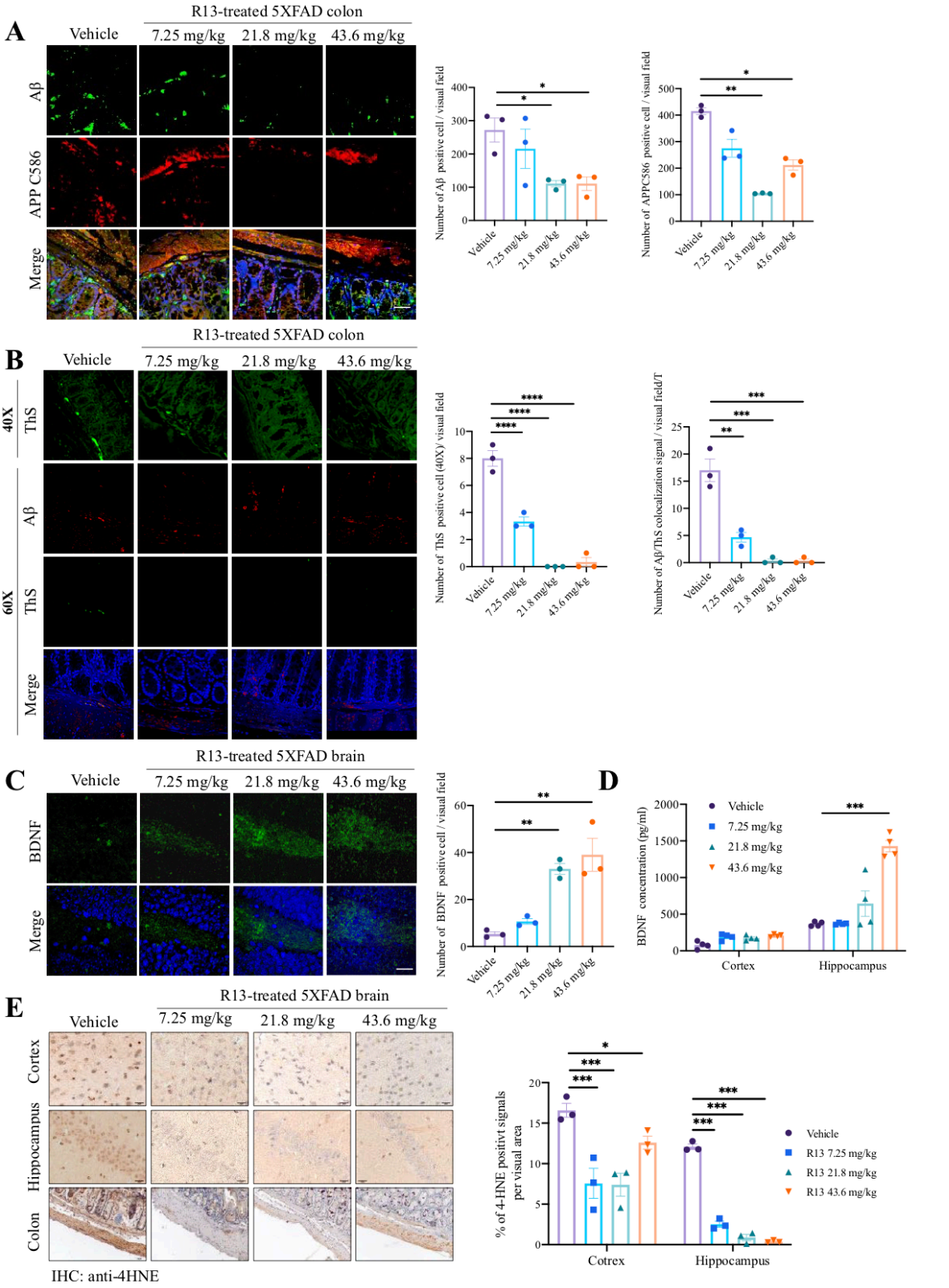

**Supplementary Figure 3. Antibiotic treatment represses C/EBPβ/AEP signaling and prevents the synaptic loss in hippocampal CA1 area of 5XFAD mice.**

Immunofluorescent staining of AEP and C/EBPβ (A), AT8 and cleaved Tau N368 (B), cleaved APP C586 and Aβ (C), Aβ and Thioflavin S co-staining (D) in the brains of 5xFAD mice. Scale bar: 40 μm. (E) Immunohistochemistry Staining of Iba-1 in the brains of 5xFAD mice. Scale bar: 50 μm. (F) The dendritic spines from the apical dendritic layer of the cerebral cortex region were analyzed by Golgi staining. (Scale bar: 5 μm). (G) Quantitative analysis of the spine density. The decreased spine densities in 5xFAD mice were reversed by ABX treatment. (n=3 in each group, Data are shown as mean ± SEM. ***P < 0.001). (H) Representative electron microscopy of the synaptic structures. Red circles indicate the synapses. (Scale bar: 1 μm). (I) Quantitative analysis of the synaptic densities in vehicle and ABX-treated 5xFAD mice. 5xFAD mice showed decreased synaptic densities, which was alleviated by ABX treatment. (n=3 in each group, Data are shown as mean ± SEM. ***P < 0.001).


A
5XFAD Colon
Control
Abx-treated
MW(kDa)
37—
IB: anti-p-C/EBP β
37—
IB: anti-C/EBP β
50—
37—
IB: anti-AEP (6E3)
50—
IB: anti-APP N373
100—
IB: anti-APP N585
37—
IB: anti-Tau N368
50—
IB: anti-Tau 5
37—
IB: anti-β-actin
B
Gut
AEP
C/EBP β
Merge
Ctrl 5XFAD
Abx-5XFAD
Number of AEP positive cell / visual field
Number of C/EBP β positive cell / visual field
Control
Abx-treated
C
Gut
APP C586
Aβ
Merge
Ctrl 5XFAD
Abx-5XFAD
Number of APPC586 positive cell / visual field
Number of Aβ positive cell / visual field
D
Gut
Aβ
ThioflavonS
Merge
Ctrl 5XFAD
Abx-5XFAD
Number of Aβ positive cell / visual field
Number of ThS positive cell / visual field
E
Gut leakage assay
FITC-Dextran concentration in the serum (μg/μl)
Control
Abx-treated

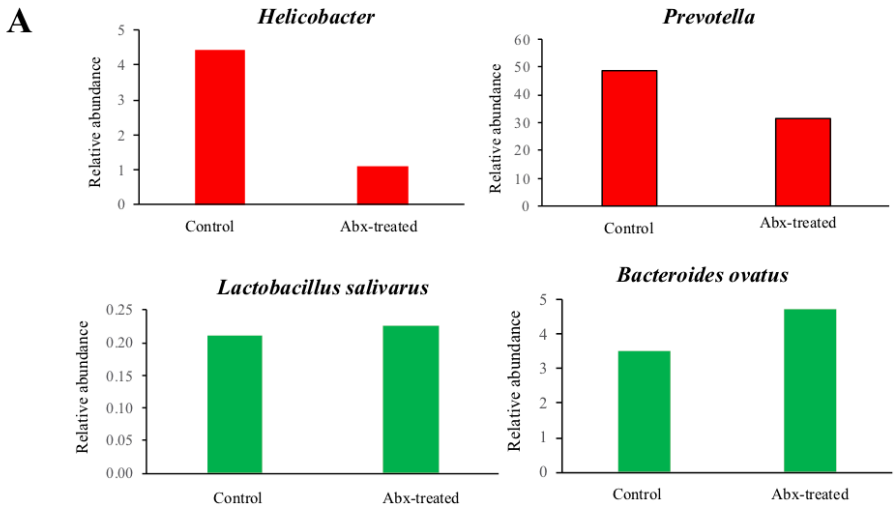
**Supplementary Figure 4. Antibiotic treatment represses C/EBP β/AEP signaling in the colon of 5XFAD mice.**

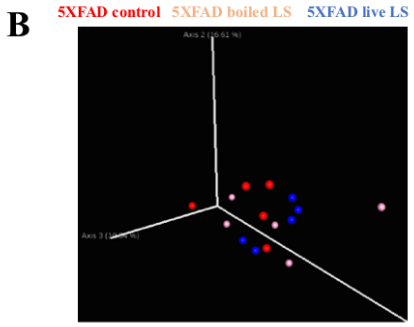
(A) Immunoblot showing p-C/EBPβ, C/EBPβ, AEP, APP, and Tau expression and processing in the mouse brains. (B-D) Immunofluorescent staining of AEP and C/EBPβ, cleaved APP C586 and Aβ, Aβ and Thioflavin S co-staining in the brains of 5xFAD mice. Scale bar: 20 μm. (E) Gastrointestinal permeability barrier defect as determined by FITC-dextran translocation in ABX-treated 5xFAD mice and control mice. Data represent the mean ± SEM; representative data of three samples; **P < 0.01 compared with control, one-way ANOVA.

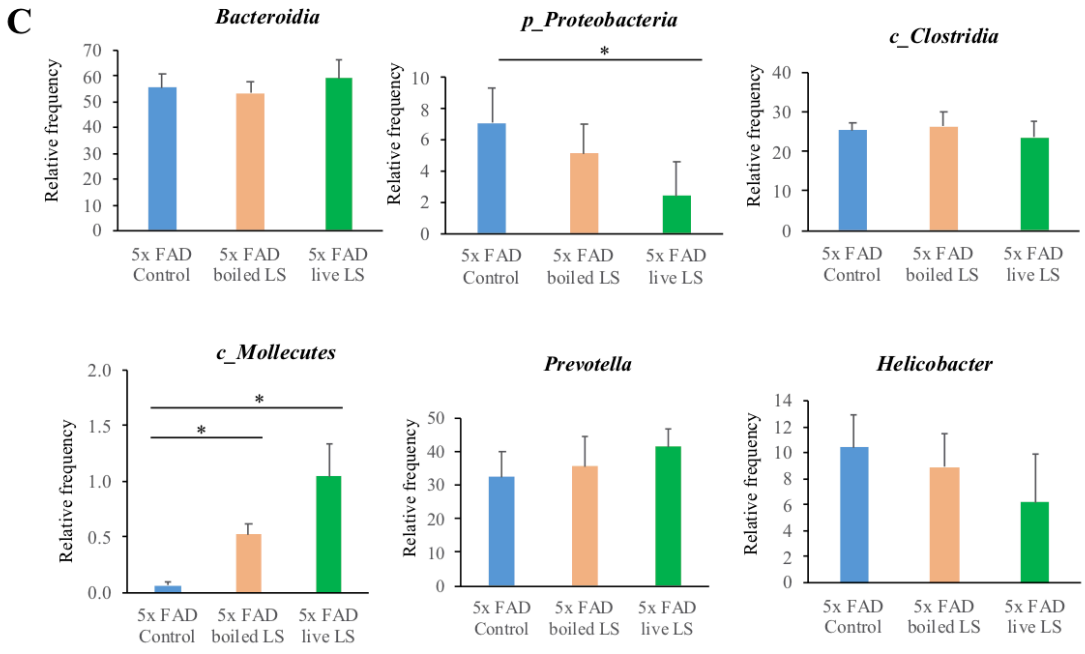

A
R13-treated 5XFAD colon
Vehicle
7.25 mg/kg
21.8 mg/kg
43.6 mg/kg
Aβ
APP C586
Merge
Number of Aβ positive cell / visual field
Number of APPC586 positive cell / visual field
B
40X
60X
ThS
Aβ
Number of ThS positive cell (40X)/ visual field
Number of Aβ/ThS colocalization signal / visual field/T
C
R13-treated 5XFAD brain
BDNF
Number of BDNF positive cell / visual field
D
BDNF concentration (pg/ml)
Cortex
Hippocampus
E
Cortex
Hippocampus
Colon
IHC: anti-4HNE
% of 4-HNE positivt signals per visual area
Cotrex
R13 7.25 mg/kg
R13 21.8 mg/kg
R13 43.6 mg/kg

**Supplementary Figure 5. R13 decreases AD pathology, alleviates oxidative stress and increases brain BDNF concentrations in 5xFAD mice.**

(A) Immunofluorescent staining of cleaved APP C586 and Aβ, (B) Immunofluorescent staining Aβ and Thioflavin S co-staining (B) in the gut sections, and BDNF (C) in the brain sections of 5xFAD mice treated with vehicle or different doses of R13. Scale bar: 20 μm (D) R13 treatment increased BDNF concentrations in the hippocampus. Data represent the mean ± SEM; representative data of four samples; ***P < 0.001 compared with vehicle, one-way ANOVA. (E) Oxidative stress evaluated by 4-hydroxynonenal (4-HNE) staining was selectively down-regulated in the R13-treated brains and gut of 5xFAD mice. Quantitative analysis of the 4-HNE positive signals per visual area. The increased 4-HNE in the brains of 5XFAD mice was mitigated by R13 treatment in a dose-dependent manner. (n=3 in each group, *P < 0.05, ***P < 0.001).

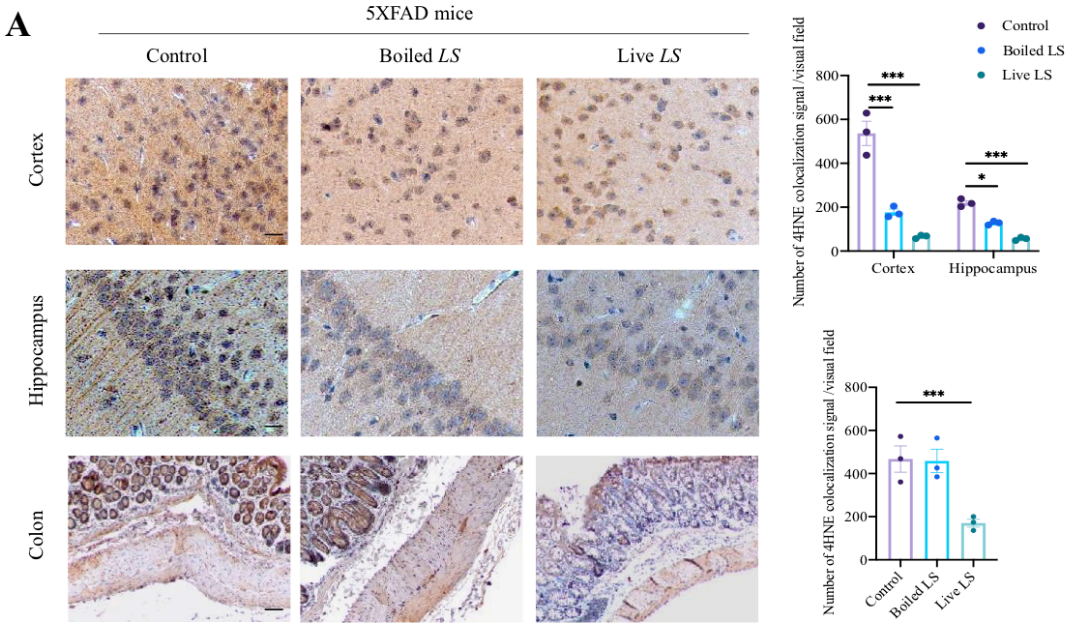

A
Helicobacter
Relative abundance
Control
Abx-treated
Prevotella
Relative abundance
Control
Abx-treated
Lactobacillus salivarus
Relative abundance
Control
Abx-treated
Bacteroides ovatus
Relative abundance
Control
Abx-treated

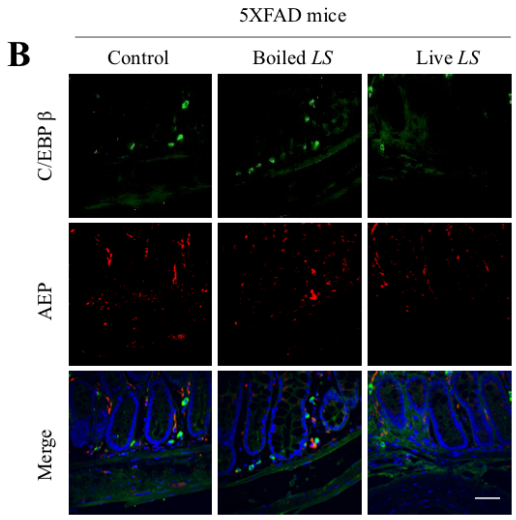

B
5XFAD control
5XFAD boiled LS
5XFAD live LS

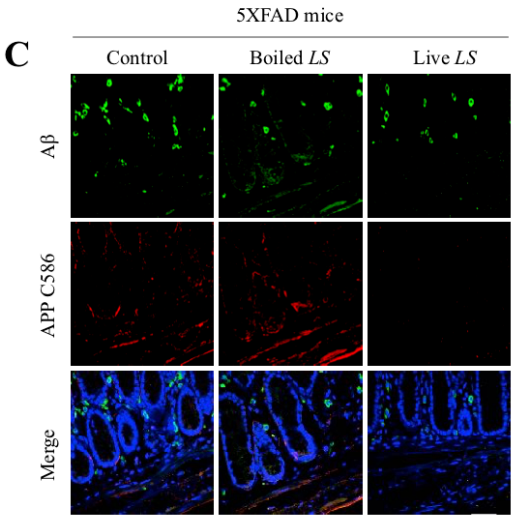

C
Bacteroidia
Relative frequency
5x FAD Control
5x FAD boiled LS
5x FAD live LS
p_Proteobacteria
Relative frequency
*
5x FAD Control
5x FAD boiled LS
5x FAD live LS
c_Clostridia
Relative frequency
5x FAD Control
5x FAD boiled LS
5x FAD live LS
c_Mollecutes
Relative frequency
*
*
5x FAD Control
5x FAD boiled LS
5x FAD live LS
Prevotella
Relative frequency
5x FAD Control
5x FAD boiled LS
5x FAD live LS
Helicobacter
Relative frequency
5x FAD Control
5x FAD boiled LS
5x FAD live LS

**Supplementary Figure 6. Gut microbiota analysis by 16S rRNA from the fecal samples of 5xFAD mice, chronically treated antibiotics or probiotics.**

Relative abundance of bacterial phyla determined by high-throughput sequencing analysis (n = 6). (A) *Mean bacteria abundance of bacterial genus and species in stool samples from ABX-treated and control 5xFAD mice.* (B) PCoA plot of microbiota community structure in vehicle and live *Lactobacillus salivarius* treated, boiled *Lactobacillus salivarius treated and control* 5xFAD mice. (C) Mean bacteria abundance of bacterial phylum, genus and class in stool samples from and live *Lactobacillus salivarius* treated, boiled *Lactobacillus salivarius treated and control* 5xFAD mice. Data represent the mean ± SEM; representative data five samples; *$P < 0.05$ compared with control, one-way ANOVA.


A
5XFAD mice
Control
Boiled LS
Live LS
Cortex
Hippocampus
Colon
IHC: anti-4HNE
Number of 4HNE colocalization signal /visual field
Control
Boiled LS
Live LS
***
***
***
*
Cortex
Hippocampus
Number of 4HNE colocalization signal /visual field
***
Control
Boiled LS
Live LS


B
5XFAD mice
Control
Boiled LS
Live LS
C/EBP β
AEP
Merge


C
5XFAD mice
Control
Boiled LS
Live LS
Aβ
APP C586
Merge

**Supplementary Figure 7. R13-induced probiotic *Lactobacillus salivarius* alleviates oxidative stress in both the brain and colon of 5xFAD mice and decreases C/EBPβ/AEP in 5xFAD mice colon.**

(A) Oxidative stress evaluated by 4-hydroxynonenal (4-HNE) staining was selectively reduced in live *Lactobacillus salivarius*-treated brains and gut of 5xFAD mice. Quantitative analysis of the 4-HNE positive signals per visual area. The increased 4-HNE in the brains of 5XFAD mice was reversed by probiotic treatment. (n=3 in each group, *P < 0.05, ***P < 0.001). Scale bar: 100 μm. (B-C) Immunofluorescent staining of C/EBPβ and AEP (B), cleaved APP C586 and Aβ (C) in the gut sections of 5xFAD mice. Scale bar: 20 μm