Supplementary Figure 1. Microbiome analysis in 3xTg mouse stool reveals an age-dependent alteration in the microbial community.

(A) Relative abundance of bacterial phyla determined by high throughput sequencing analysis (n=5).

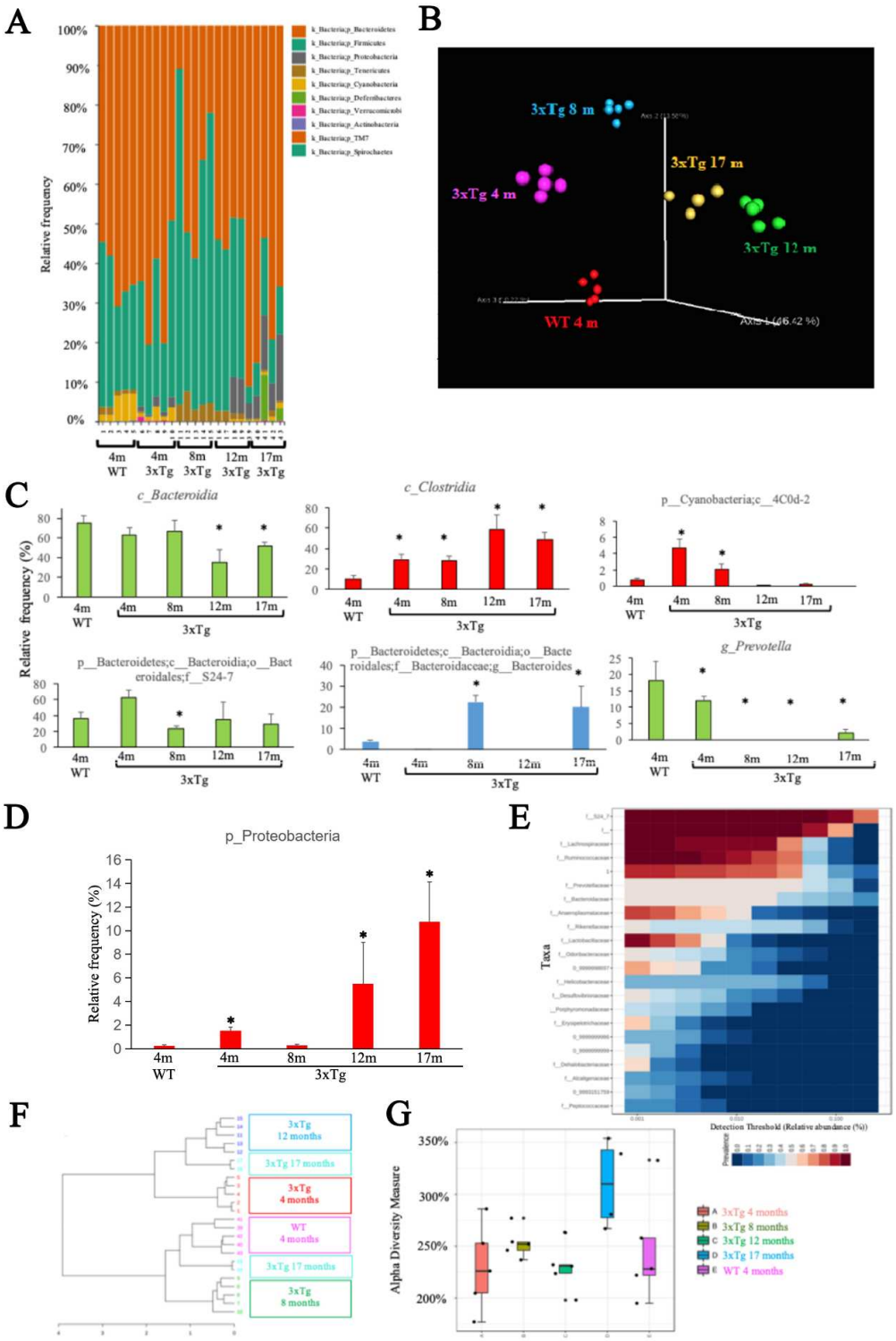
(B) Principal coordinate plot (PCoA) of microbial community structure in an age-dependent manner in 3xTg and WT mouse stool.

(C) Mean relative frequency of bacterial species. Data represent the means ± SEM; *P < 0.05 compared with control (WT 4 months), one-way ANOVA.

(D) Mean relative frequency of proteobacterial phylum. Data represent the means ± SEM; *P < 0.05 compared with control (WT 4 months), one-way ANOVA.

(E and F) Hierarchical clustering of the core microbial taxa shows alterations of abundances across different age groups of 3xTg and WT mouse.

(G) Boxplot of α-diversity pattern (Chao1) of the microbiota across different age groups in the 3xTg and WT mouse stool.

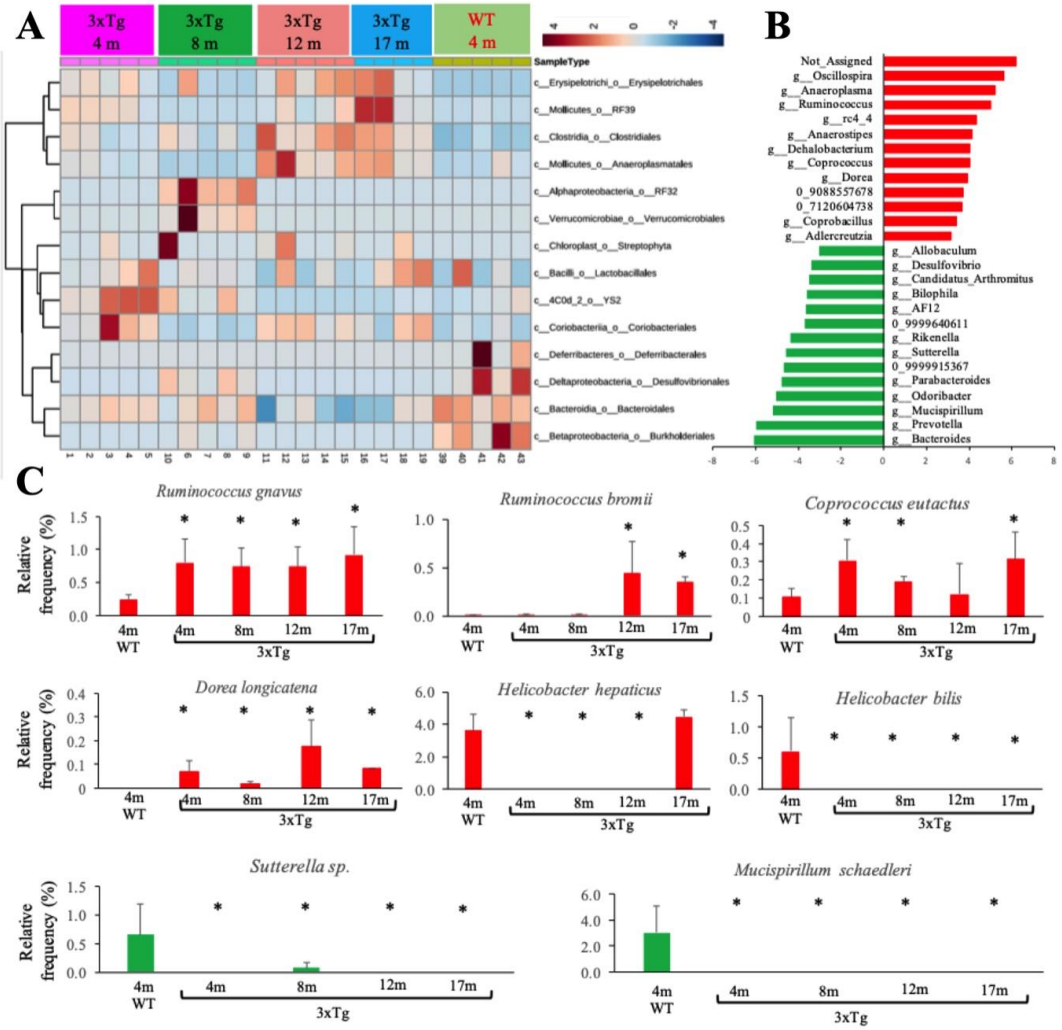

A
B
C
D
E
F
G
Relative frequency
3xTg 8 m
3xTg 17 m
3xTg 4 m
3xTg 12 m
WT 4 m
c_Bacteroidia
c_Clostridia
p__Cyanobacteria;c__4C0d-2
p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7
p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides
g_Prevotella
p_Proteobacteria
Relative frequency (%)
4m WT
4m 3xTg
8m 3xTg
12m 3xTg
17m 3xTg
3xTg
Taxa
Detection Threshold (Relative abundance (%))
Prevalence
Alpha Diversity Measure
3xTg 12 months
3xTg 17 months
3xTg 4 months
WT 4 months
3xTg 8 months
A 3xTg 4 months
B 3xTg 8 months
C 3xTg 12 months
D 3xTg 17 months
E WT 4 months

Supplementary Figure 2. Comparative and biomarker discovery analysis using relative taxonomic abundances reveal microbiome disequilibrium between 3xTg and wild-type mouse stool.

(A) Hierarchical clustering and heatmap analysis using taxonomic abundances.

(B) Biomarker analysis of the microbial genera in 3xTg and WT mouse stool.

(C) Mean relative abundance of bacterial phyla determined by high-throughput sequencing analysis (n = 5).


A
3xTg 4 m
3xTg 8 m
3xTg 12 m
3xTg 17 m
WT 4 m
SampleType
c__Erysipelotrichi_o__Erysipelotrichales
c__Mollicutes_o__RF39
c__Clostridia_o__Clostridiales
c__Mollicutes_o__Anaeroplasmatales
c__Alphaproteobacteria_o__RF32
c__Verrucomicrobiae_o__Verrucomicrobiales
c__Chloroplast_o__Streptophyta
c__Bacilli_o__Lactobacillales
c__4C0d_2_o__YS2
c__Coriobacteriia_o__Coriobacteriales
c__Deferribacteres_o__Deferribacterales
c__Deltaproteobacteria_o__Desulfovibrionales
c__Bacteroidia_o__Bacteroidales
c__Betaproteobacteria_o__Burkholderiales
B
Not_Assigned
g__Oscillospira
g__Anaeroplasma
g__Ruminococcus
g__rc4_4
g__Anaerostipes
g__Dehalobacterium
g__Coprococcus
g__Dorea
0_9088557678
0_7120604738
g__Coprobacillus
g__Adlercreutzia
g__Allobaculum
g__Desulfovibrio
g__Candidatus_Arthromitus
g__Bilophila
g__AF12
0_9999640611
g__Rikenella
g__Sutterella
0_9999915367
g__Parabacteroides
g__Odoribacter
g__Mucispirillum
g__Prevotella
g__Bacteroides
C
Ruminococcus gnavus
Ruminococcus bromii
Coprococcus eutactus
Dorea longicatena
Helicobacter hepaticus
Helicobacter bilis
Sutterella sp.
Mucispirillum schaedleri
Relative frequency (%)
4m WT
4m 8m 12m 17m
3xTg

Supplementary Figure 3. Characterization of Germ-free 3xTg mice

(A) Body weights of Germ-free 3xTg mice and SPF 3xTg mice at the age of 7.5 months. Data represent the mean ± SEM; representative data of eight samples; **P < 0.01 compared with control, unpaired t tests.

(B) Representative pictures of cecum from Germ-free 3xTg mice and SPF 3xTg mice. Germ-free 3xTg mice have enlarged cecum compared with SPF 3xTg mice.

(C) Representative pictures of gastrointestinal tract from both male and female Germ-free 3xTg mice and SPF 3xTg mice.

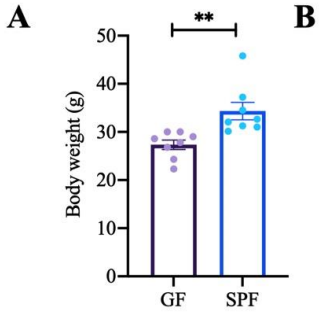
(D) Quantification analysis of gastrointestinal tract length from Germ-free 3xTg mice and SPF 3xTg mice. Data represent the mean ± SEM; representative data of three to four samples, unpaired t tests.

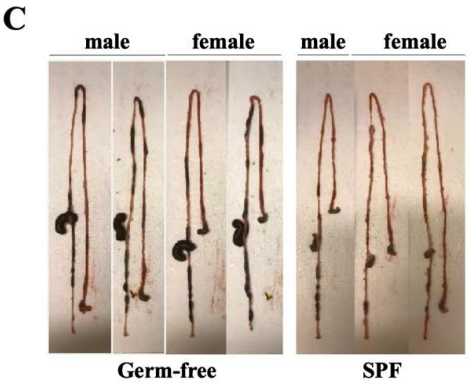
(E&F) *In vitro* culture of bacteria from fecal pallets of Germ-free 3xTg mice and SPF 3xTg mice

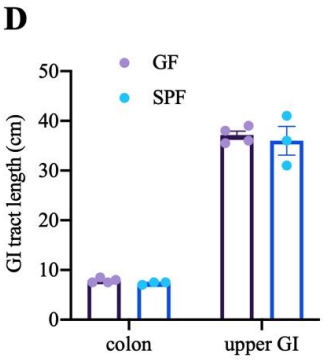
(G) BDNF concentrations in the brains of Germ-free 3xTg mice and SPF 3xTg mice. Data represent the mean ± SEM; representative data of five samples; unpaired t tests.

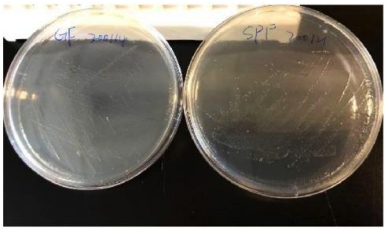
(H) The dendritic spines from the apical dendritic layer of the hippocampus region were analyzed by Golgi staining. Scale bar: 5 μm.

(I) Quantitative analysis of the spine density. (n = 5 in each group, Data are shown as mean ± SEM. ****P < 0.0001).

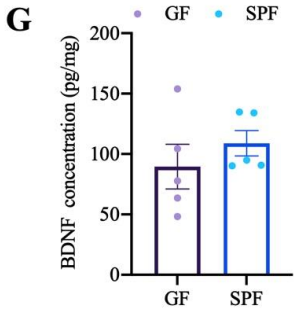


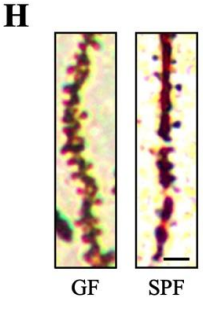


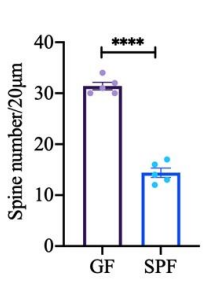




	OD_{60}
Control	0.051
SPF	0.522
GF1 1.5m	0.066
GF2 7.5m	0.070







Supplementary Figure 4. Germ-free 3xTg mice exhibit diminished AD pathologies and AA-associated inflammation.

(A) Immunofluorescent staining of Iba-1 (red) and Aβ (green) in the hippocampus CA1 region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

(B) Immunofluorescent staining of AEP (red) and C/EBPβ (green) in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

(C) Immunofluorescent staining of TauN368 (red) and AEP (green) in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

(D) Immunofluorescent staining of TauN368 (red) and AT8 (green) in the hippocampus CA1 region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

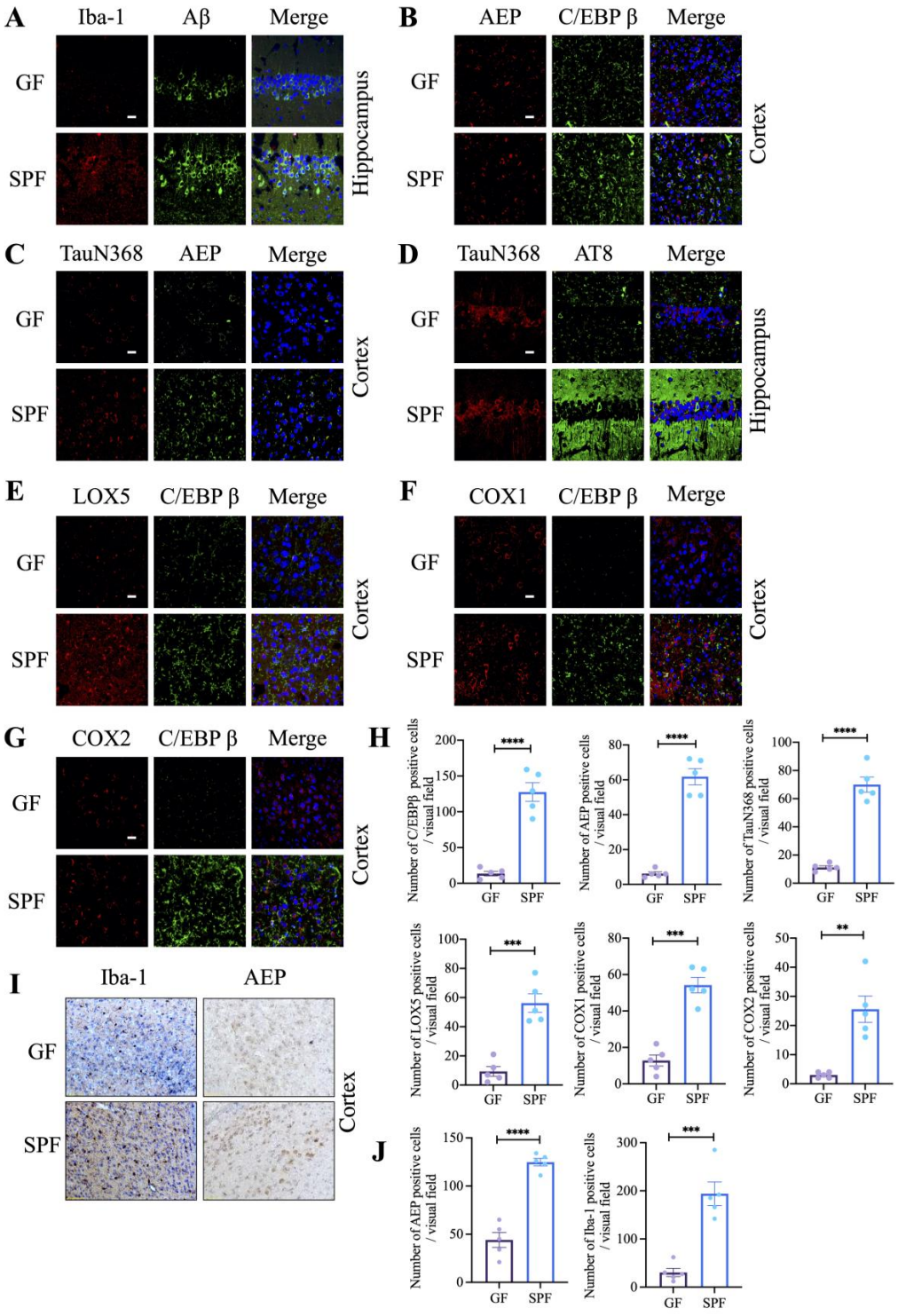
(E) Immunofluorescent staining of LOX5 (red) and C/EBPβ (green) in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

(F) Immunofluorescent staining of COX1 (red) and C/EBPβ (green) in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

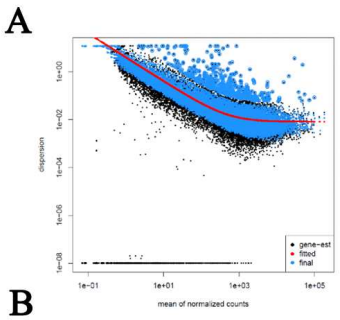
(G) Immunofluorescent staining of COX2 (red) and C/EBPβ (green) in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.

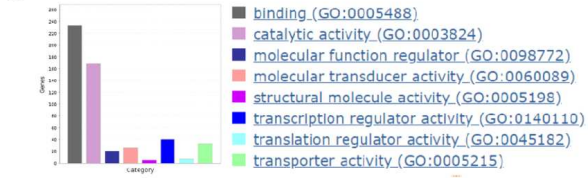
(H) Quantitative analysis of C/EBPβ positive cells, AEP positive cells, TauN368 positive cells, LOX5 positive cells, COX1 positive cells, and COX2 positive cells, respectively. The densities of C/EBPβ, AEP, TauN368, LOX5, COX1 and COX2 positive cells were significantly increased in SPF 3xTg mice brain. Hippocampus: A and D; Cortex: B, C, E, F and G. (n = 5 in each group, Data are shown as mean ± SEM. **P< 0.01, ***P< 0.001, ****P< 0.0001 compared with control, unpaired t tests).

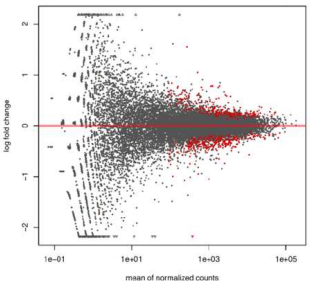
(I) Immunohistochemistry staining of Iba-1 and AEP in the cortex region of the brains from Germ-free 3xTg mice and SPF 3xTg mice.


A
Iba-1
Aβ
Merge
GF
SPF
Hippocampus
B
AEP
C/EBP β
Merge
Cortex
C
TauN368
AEP
D
AT8
E
LOX5
F
COX1
G
COX2
H
Number of C/EBPβ positive cells / visual field
Number of AEP positive cells / visual field
Number of TauN368 positive cells / visual field
Number of LOX5 positive cells / visual field
Number of COX1 positive cells / visual field
Number of COX2 positive cells / visual field
I
J
Number of Iba-1 positive cells / visual field

(J) Quantitative analysis of AEP positive cells and Iba-1 positive cells in mice cortex. The densities of AEP, and Iba-1 positive cells were significantly increased in SPF 3xTg mice brain. (n = 5 in each group, Data are shown as mean ± SEM. ***P< 0.001, ****P< 0.0001 compared with control, unpaired t tests).

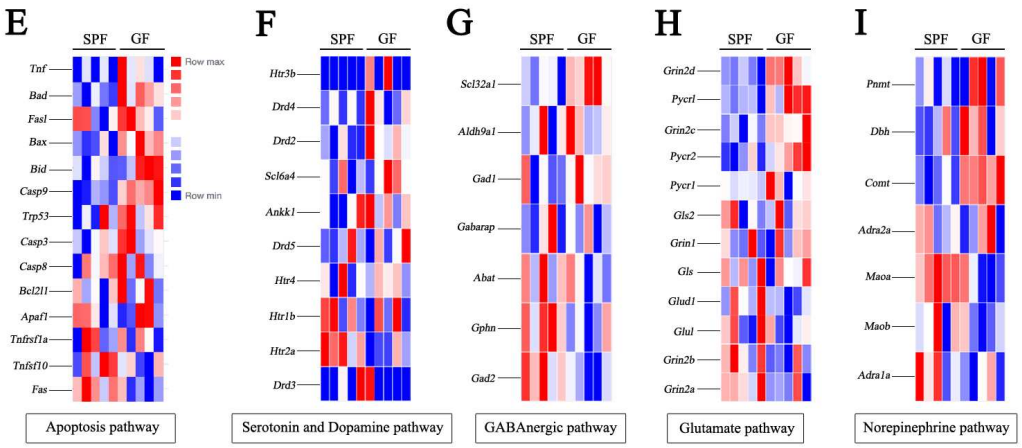


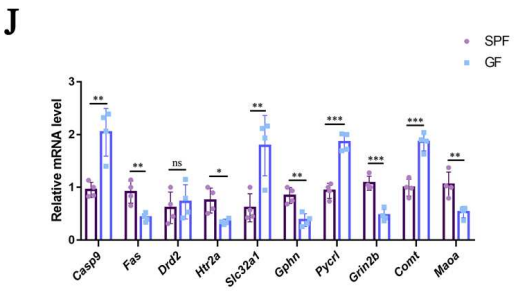


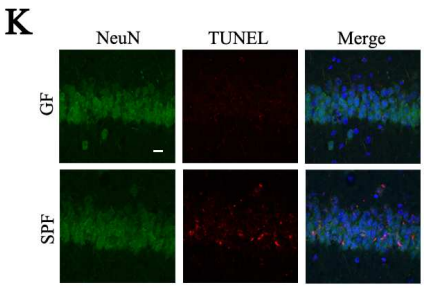


D

	Mus musculus (REF)	Client Text Box Input					
PANTHER Pathways	#	#	expected	Fold Enrichment	+/-	raw P value	FDR
Insulin/IGF pathway-protein kinase B signaling cascade	39	9	1.45	6.22	+	4.40E-05	2.43E-03
PI3 kinase pathway	52	10	1.93	5.18	+	6.47E-05	2.15E-03
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	35	6	1.30	4.62	+	3.18E-03	3.29E-02
Histamine H1 receptor mediated signaling pathway	45	7	1.67	4.19	+	2.41E-03	2.85E-02
Ras Pathway	70	10	2.60	3.85	+	5.56E-04	1.03E-02
p53 pathway feedback loops 2	50	7	1.85	3.77	+	4.08E-03	3.76E-02
Endothelin signaling pathway	82	11	3.04	3.62	+	4.84E-04	1.15E-02
B cell activation	70	9	2.60	3.47	+	2.02E-03	2.57E-02
Ubiquitin proteasome pathway	66	8	2.45	3.27	+	4.87E-03	4.04E-02
Angiogenesis	169	20	6.27	3.19	+	1.56E-05	2.58E-03
Cytoskeletal regulation by Rho GTPase	79	9	2.93	3.07	+	4.21E-03	3.68E-02
EGF receptor signaling pathway	136	15	5.05	2.97	+	3.49E-04	9.66E-03
FGF signaling pathway	120	13	4.45	2.92	+	9.84E-04	1.36E-02
PDGF signaling pathway	143	15	5.31	2.83	+	5.66E-04	9.39E-03
CCKR signaling map	159	16	5.90	2.71	+	5.70E-04	8.60E-03
Inflammation mediated by chemokine and cytokine signaling pathway	258	25	9.57	2.61	+	3.32E-05	2.75E-03
Integrin signalling pathway	190	16	7.05	2.27	+	3.65E-03	3.57E-02
Gonadotropin-releasing hormone receptor pathway	236	19	8.76	2.17	+	3.10E-03	3.43E-02
Wnt signaling pathway	311	25	11.54	2.17	+	5.48E-04	1.14E-02







Supplementary Figure 5. Summary of transcriptome sequencing results of mRNA expression in the hippocampal samples from germ-free and SPF 3xTg mice.

(A) Dispersion plot showing empirical (black dots) and fitted (red lines) dispersion values plotted against the mean of normalized counts.

(B) MA-plot of normalized mean versus log2 fold change for the contrast Germ-free versus SPF 3xTg mice.

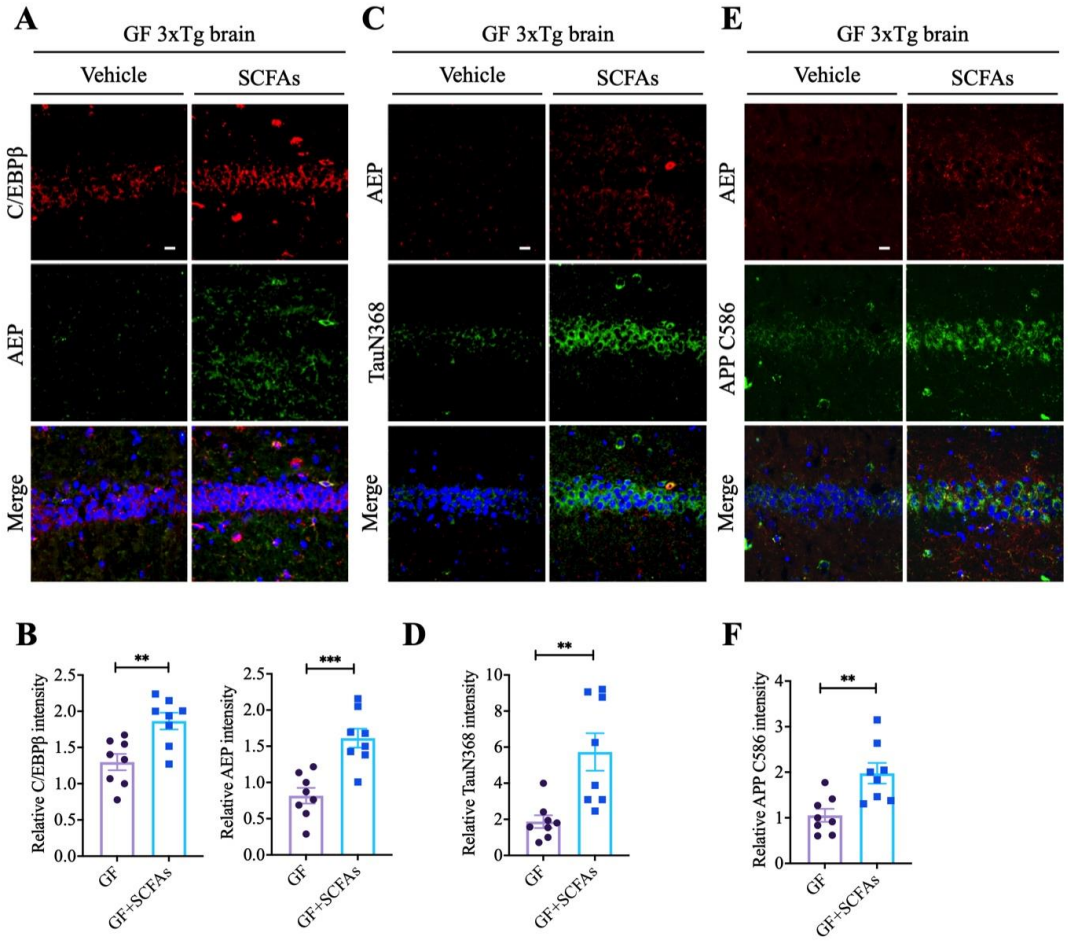
(C)PANTHER pathway analysis of differential genes.

(D) Table showing PANTHER analysis of enriched pathways.

(E-I) Heatmap showing differential genes of apoptotic, serotonin and dopamine, GABAnergic, glutamate, and norepinephrine pathways.

(J) qRT-PCR analysis of representative genes in apoptotic, serotonin and dopamine, GABAnergic, glutamate, and norepinephrine pathways.

(K) Immunofluorescent staining of NeuN (green) and TUNEL (red) in the hippocampus CA1 region of the brains from Germ-free 3xTg mice and SPF 3xTg mice. Scale bar: 20 μm.


A
GF 3xTg brain
Vehicle
SCFAs
C/EBPβ
AEP
Merge
C
GF 3xTg brain
Vehicle
SCFAs
AEP
TauN368
Merge
E
GF 3xTg brain
Vehicle
SCFAs
AEP
APP C586
Merge
B
Relative C/EBPβ intensity
Relative AEP intensity
GF
GF+SCFAs
D
Relative TauN368 intensity
GF
GF+SCFAs
F
Relative APP C586 intensity
GF
GF+SCFAs

Supplementary Figure 6. SCFAs stimulate AD pathologies in Germ-free 3xTg mice.

(A) Immunofluorescent staining of C/EBPβ (red) and AEP (green) in the hippocampus CA1 region of the brains from vehicle treated Germ-free 3xTg mice and SCFAs treated Germ-free 3xTg mice. Scale bar: 20 μm.

(B) Quantitative analysis of C/EBPβ positive cells and AEP positive cells. The fluorescence intensity of C/EBPβ positive cells and AEP positive cells in the brain of Germ-free 3xTg mice increased with SCFAs treatment. (n = 8 in each group, Data are shown as mean ± SEM. **P< 0.01, ***P< 0.001 compared with control, unpaired t tests)

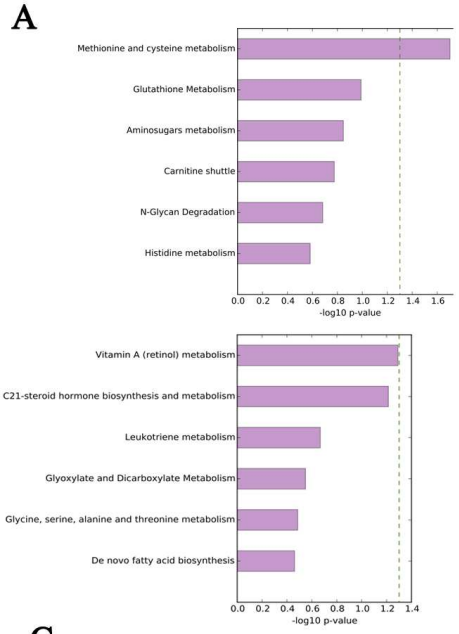
(C) Immunofluorescent staining of AEP (red) and TauN368 (green) in the hippocampus CA1 region of the brains from vehicle-treated Germ-free 3xTg mice and SCFAs-treated Germ-free 3xTg mice. Scale bar: 20 μm.

(D) Quantitative analysis of TauN368 positive cells. The fluorescence intensity of TauN368 positive cells in the brain of Germ-free 3xTg mice increased with SCFAs treatment. (n = 8 in each group, Data are shown as mean ± SEM. **P< 0.01 compared with control, unpaired t tests)

(E) Immunofluorescent staining of AEP (red) and APPC586 (green) in the hippocampus CA1 region of the brains from vehicle-treated Germ-free 3xTg mice and SCFAs-treated Germ-free 3xTg mice. Scale bar: 20 μm.

(F) Quantitative analysis of APPC586 positive cells. The fluorescence intensity of TauN368 positive cells in the brain of Germ-free 3xTg mice increased with SCFAs treatment. (n = 8 in each group, Data are shown as mean ± SEM. **P< 0.01 compared with control, unpaired t tests)

A



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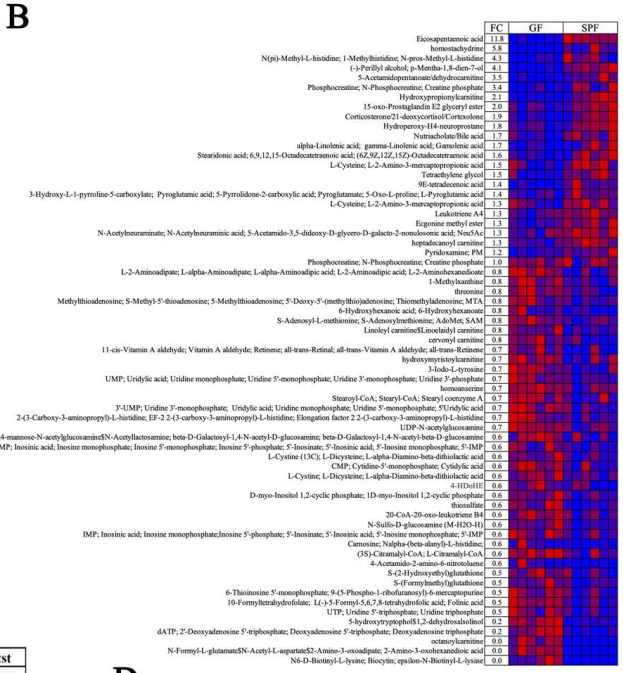


C

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change SPF/GF
Phenylalanine Metabolism	phenylalanine	1.14
	N-acetylphenylalanine	1.19
	1-carboxyethylphenylalanine	1.64
Tyrosine Metabolism	tyrosine	1.29
	1-carboxyethyltyrosine	1.72
	phenol sulfate	2.14
	dopamine	0.42
	3-methoxytyrosine	1.57
	N-formylphenylalanine	1.49
Tryptophan Metabolism	5-hydroxyindoleacetate	0.84
	indoleacetate	0.61
	3-indoxyl sulfate	6.85
Vitamin A Metabolism	retinol(Vitamin A)	1.22
Vitamin B6 Metabolism	pyridoxamine	1.14
	pyridoxal	1.28
Benzoate Metabolism	hippurate	4.68
	catechol sulfate	1.79
	p-cresol sulfate	3.23

D

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change SPF/GF
Glycolysis, Gluconeogenesis, and	glucose	0.67
	pyruvate	0.78
Pentose Metabolism	ribonate	0.81
	sedopeptulose	1.17
TCA Cycle	citrateaconitate	0.90
	isocitrate	0.91
	succinylcarnitine(C4-DC)	1.17
	succinate	1.28
	malate	1.17
	trigonelline (N'-methylnicotinate)	0.96
Nitcotinate and Nicotinamide Metabolism	N1-Methyl-2-pyridone-5-carboxamide	1.81
	adenosine	0.71
	adenosine 5'-monophosphate (AMP)	0.76

E

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change SPF/GF
Dihydrosphingomyelins	behenoyl dihydrosphingomyelin(d18:0/22	1.01
	sphingomyelin(d18:0/18:0,d19:0/17:0)	0.89
	sphingomyelin(d18:0/20:0,d16:0/22:0)	0.87
Sphingomyelins	behenoyl sphingomyelin(d18:1/22:0)	0.94
	tricosanoyl sphingomyelin(d18:1/23:0)	0.92
	lignoceroyl sphingomyelin(d18:1/24:0)	0.95
	sphingomyelin (d17:1/16:0,d18:1/15:0)	1.22
	sphingomyelin (d18:2/16:0,d18:1/16:1)	1.12
	sphingomyelin (d18:1/18:1,d18:2/18:0)	1.08
	sphingomyelin (d18:1/19:0,d19:1/18:0)	0.97
	sphingomyelin (d18:1/20:0,d16:1/22:0)	0.98
	sphingomyelin (d18:2/24:1,d18:1/24:2)	0.92

F

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change SPF/GF
Urea Cycle; Arginine and Proline Metabolism	ornithine	1.33
	2-oxo arginine	0.86
	homocitrulline	0.86
	proline	1.11
	N-delta-acetylornithine	3.71

G

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change SPF/GF
Methinonine, Cysteine, SAM and Taurine Metabolism	S-adenosylhomocysteine(SAH)	1.11
	cysteine	0.83
	hypotaurine	0.85
	N-acetyltaurine	1.08
	cyano-alanine	0.71
Glutathione Metabolism	glutathione, reduced(GSH)	0.67
	glutathione, oxidized(GSSG)	1.11
	cysteinylglycine	0.81
	2-hydroxybutyrate/2-hydroxyisobutyrate	0.70
	S-(1,2-dicarboxyethyl)glutathione	0.86
	4-hydroxy-nonenal-glutathione	1.27
	3'-dephospho-CoA-glutathione	1.18
Gamma-glutamyl Amino Acid	gamma-glutamyl-epsilon-lysine	0.73
	gamma-glutamylphenylalanine	1.17

H

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change SPF/GF
Glutamate Metoblism	glutamate	0.98
	glutamine	1.09
	alpha-ketoglutaramate	1.32
	N-acetylglutamate	0.94
	N-acetylglutamine	1.24

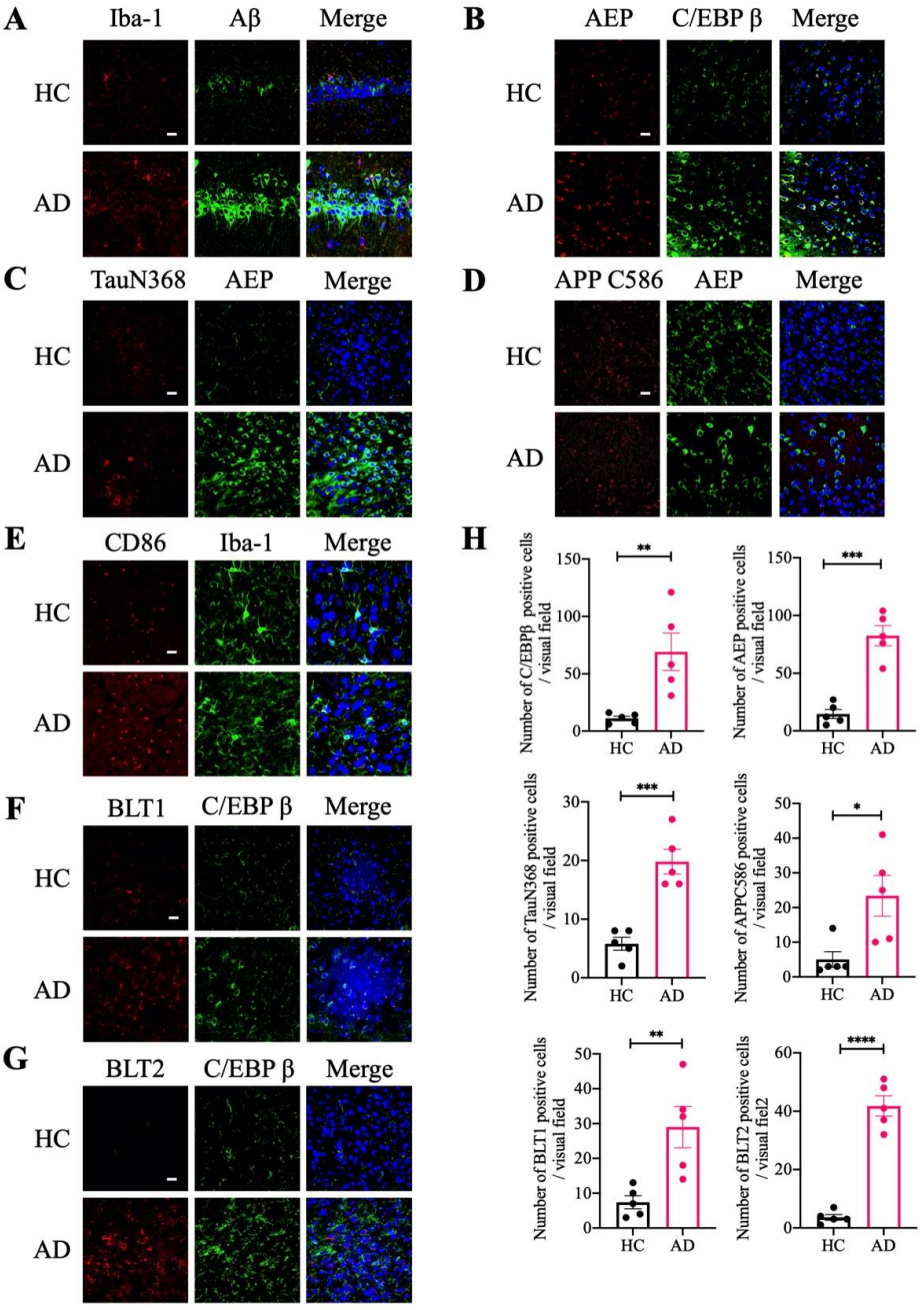
Supplementary Figure 7. Metabolomics analysis of the brains from Germ-free and SPF 3xTg mice.

(A) Differential metabolism pathway in brains from Germ-free 3xTg mice versus SPF 3xTg mice.

(B) Heatmap showing differential metabolites in Germ-free 3xTg mice versus SPF 3xTg mice.

(C-H) Metabolomics analysis of the brains from SPF versus GF 3xTg mice. The differences in microbiomes related metabolites from amino acids (C); carbohydrate and energy (D); lipids (E); arginine (F); oxidative stress (G) and glutamate metabolism (H).

(Red and green shaded cells indicate $p \leq 0.05$ (red indicates the fold change values are significantly higher for that comparison; green values significantly lower). Light red and light green shaded cells indicate $0.05 < p < 0.10$ (light red indicates the fold change values trend higher for that comparison; light green values trend lower)).


A
Iba-1
Aβ
Merge
HC
AD
B
AEP
C/EBP β
Merge
HC
AD
C
TauN368
AEP
Merge
HC
AD
D
APP C586
AEP
Merge
HC
AD
E
CD86
Iba-1
Merge
HC
AD
F
BLT1
C/EBP β
Merge
HC
AD
G
BLT2
C/EBP β
Merge
HC
AD
H
Number of C/EBPβ positive cells / visual field
Number of AEP positive cells / visual field
Number of TauN368 positive cells / visual field
Number of APPC586 positive cells / visual field
Number of BLT1 positive cells / visual field
Number of BLT2 positive cells / visual fiel2
HC
AD

Supplementary Figure 8. AD fecal humanized ex-Germ-free mice exhibit augmented AD pathologies and escalated inflammatory AA metabolic genes.

(A) Immunofluorescent staining of Iba-1 (red) and Aβ (green) in the hippocampus CA1 region of the brains from HC humanized ex-GF 3xTg mice and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(B) Immunofluorescent staining of AEP (red) and C/EBPβ (green) in the cortex region of brains from HC humanized ex-GF 3xTg mice and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(C) Immunofluorescent staining of TauN368 (red) and AEP (green) in the cortex region of the brains from HC and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

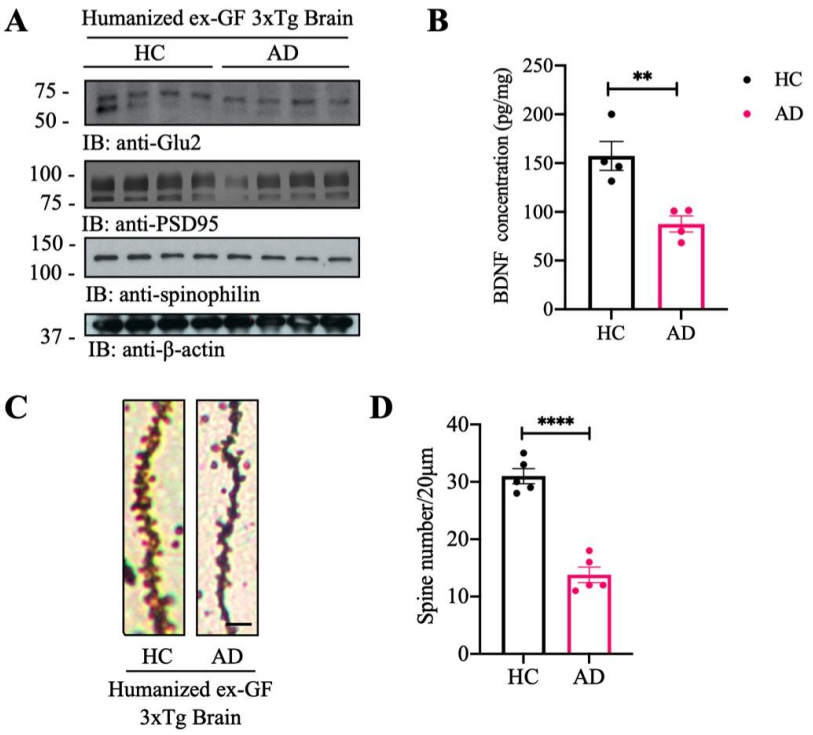
(D) Immunofluorescent staining of APPC586 (red) and AEP (green) in the cortex region of the brains from HC humanized and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(E) Immunofluorescent staining of CD86 (red) and Iba-1 (green) in the cortex region of the brains from HC and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(F) Immunofluorescent staining of BLT1 (red) and C/EBPβ (green) in the cortex region of the brains from HC and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(G) Immunofluorescent staining of BLT2 (red) and C/EBPβ (green) in the cortex region of brains from HC and AD humanized ex-GF 3xTg mice. Scale bar: 20 μm.

(H) Quantitative analysis of C/EBPβ positive cells, AEP positive cells, TauN368 positive cells, APPC586 positive cells, BLT1 positive cells, and BLT2 positive cells, respectively. The densities of C/EBPβ, AEP, TauN368, APPC586, BLT1 and BLT2 positive cells were significantly increased in AD humanized ex-GF 3xTg mice brains. (n = 5 in each group, Data are shown as mean ± SEM. *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001 compared with control, unpaired t tests).


A
Humanized ex-GF 3xTg Brain
HC
AD
75 -
50 -
IB: anti-Glu2
100 -
75 -
IB: anti-PSD95
150 -
100 -
IB: anti-spinophilin
37 -
IB: anti-β-actin
B
BDNF concentration (pg/mg)
250
200
150
100
50
0
**
HC
AD
HC
AD
C
HC
AD
Humanized ex-GF
3xTg Brain
D
Spine number/20μm
40
30
20
10
0
****
HC
AD

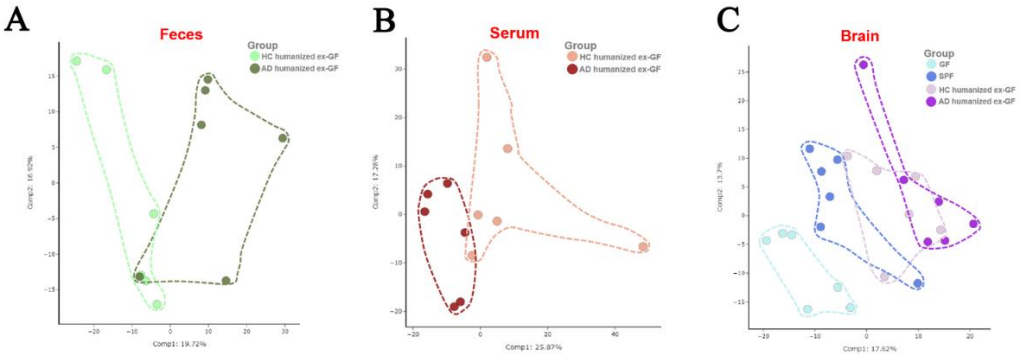
Supplementary Figure 9. AD patient gut microbiome humanized ex-Germ-free mice decrease dendritic spines and synaptic proteins.

(A) Immunoblotting analysis of synaptic markers in the brain homogenates from HC and AD humanized ex-GF 3xTg mice. The expression of synaptic markers decreased in AD humanized ex-GF 3xTg mice.

(B) BDNF concentrations in the brains of HC and AD humanized ex-GF 3xTg mice. Data represent the mean ± SEM; representative data of four samples; unpaired t tests.

(C) The dendritic spines from the apical dendritic layer of the hippocampus region were analyzed by Golgi staining. Scale bar: 5 μm.

(D) Quantitative analysis of the spine density. (n = 5 in each group, Data are shown as mean ± SEM. ****P < 0.0001).



D

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Phenylalanine Metabolism	N-acetylphenylalanine	1.82
	phenyllactate(PLA)	3.90
Tyrosine Metabolism	N-formylphenylalanine	1.62
Tryptophan Metabolism	oxindolylalanine	1.79
	kynurenine	1.40
	N-formylanthranilic acid	1.38
	indolelactate	4.99
	indole-3-carboxylate	4.78
Secondary Bile Acid Metoblism	deoxycholic acid 3-sulfate	2.73
	6-beta-hydroxylithocholate	0.53
	taurolithocholate 3-sulfate	0.22
	tauroursodeoxycholate	0.08
	7,12-diketolithocholate	0.25
	taurohyodeoxycholic acid	0.08
	3-dehydrocholate	0.21
	7-ketodeoxycholate	0.06
	taurohyodeoxycholic acid(7 or 27 isi	0.19
Ascorbate and Aldarate Metabolism	ascorbic acid 3-sulfate	2.22
	2-O-methylascorbic acid	0.16
Thiamine Metabolism	thiamin(Vitamin B1)	1.73
	thiamin monophosphate	2.63
Vitamin B6 Metabolism	pyridoxal	1.33
Benzoate Metabolism	benzoate	0.60
	2-(4-hydroxyphenyl)propionate	50.79

E

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Phenylalanine Metabolism	phenylpyruvate	1.92
Tyrosine Metoblism	tyrosine	1.24
	N-acetyltyrosine	1.38
	4-hydroxyphenylpyruvate	2.00
	3-(4-hydroxyphenyl)lactate	1.37
	4-methoxyphenol sulfate	1.91
	dopamine 3-O-sulfate	1.57
	4-hydroxycinnamate sulfate	2.20
	3,4-dihydroxyphenylacetate sulfate	1.63
	3-hydroxyphenylacetate sulfate	2.74
Tryptophan Metabolism	N-formylanthranilic acid	1.45
	anthranilate	1.70
	xanthurenate	1.90
	picolinate	1.60
	indoleacetate	0.41
	3-indoxyl sulfate	1.82
Secondary Bile Acid Metabolism	tauroursodeoxycholate	3.14
	taurohyodeoxycholic acid	5.87
Vitamin B6 Metabolism	pyridoxal	1.42
Benzoate Metabolism	3-methylcatechol sulfate(1)	1.65
	4-ethylcatechol sulfate	2.96
	4-acetylphenyl sulfate	1.87
	4-ethylphenyl sulfate	506.70
	4-vinylcatechol sulfate	0.77
	3-methoxycatechol sulfate(1)	3.10
	3-methoxycatechol sulfate(2)	2.02
	2-(4-hydroxyphenyl)propionate	6.85

F

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Phenylalanine Metabolism	phenylalanine	1.00
	N-acetylphenylalanine	1.05
	1-carboxyethylphenylalanine	1.05
Tyrosine Metabolism	tyrosine	1.13
	1-carboxyethyltyrosine	1.18
	phenol sulfate	1.77
	dopamine	0.44
	3-methoxytyrosine	0.73
	N-formylphenylalanine	0.83
Tryptophan Metabolism	5-hydroxyindoleacetate	0.92
	indoleacetate	0.55
	3-indoxyl sulfate	1.96
Vitamin A Metabolism	retinol(Vitamin A)	1.02
Vitamin B6 Metabolism	pyridoxamine	1.05
	pyridoxal	1.00
Benzoate Metabolism	hippurate	2.16
	catechol sulfate	2.09
	p-cresol sulfate	1.08

G

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Pentose Metabolism	ribonate	2.22
	ribulose/xylulose	1.66
	arabitol/xylitol	1.33
	sedoheptulose	2.24
	lyxonate	2.91
TCA Cycle	malate	2.05
	tricarballylate	2.38
	2-methylcitrate/homocitrate	1.16
Nitcotinate and Nicotinamide Metabolism	quinolinate	0.12
	nicotinate	1.60
	nicotinamide riboside	2.12

H

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	pyruvate	1.26
Pentose Metabolism	arabitol/xylitol	1.68
TCA Cycle	citrate	1.24
	alpha-ketoglutarate	2.05
	fumarate	1.54
	itaconate	0.48
Nitcotinate and Nicotinamide Metabolism	quinolinate	2.47
	nicotinamide riboside	1.49
	N1-Methyl-2-pyridone-5-carboxamide	0.59

I

Sub Pathway	Biochemical Name	Welch's t-test Fold of Change AD humanized ex-GF / HC humanized ex-GF
Glycolysis, Gluconeogenesis, and Pyruvate	glucose	0.71
	pyruvate	1.10
Pentose Metabolism	ribonate	0.96
	sedoheptulose	1.05
TCA Cycle	citrateaconitate	1.12
	isocitrate	1.16
	succinylcarnitine(C4-DC)	0.99
	succinate	1.19
	malate	0.95
	trigonelline (N'-methylnicotinate)	1.10
Nitcotinate and Nicotinamide Metabolism	N1-Methyl-2-pyridone-5-carboxamide	1.01
	adenosine	0.82
	adenosine 5'-monophosphate (AMP)	1.02

Supplementary Figure 10. Metabolomics analysis of the brains, serum samples and feces from HC humanized ex-GF 3xTg mice and HC humanized ex-GF 3xTg mice, and the brains from SPF versus GF mice.

(A-C) Principal Component Analysis (PCA). PCA of feces and serum dataset from AD and HC humanized ex-GF groups (A&B). Within the feces dataset, the HC humanized ex-GF group segregated loosely to the left, while the AD humanized ex-GF group segregated loosely to the right of component 1, with a little overlapping in the center (left, compare light green and dark green circles) (A). Within the serum dataset, the HC humanized ex-GF group segregated to the right, while the AD humanized ex-GF group segregated to the left of component 1, with some overlap in the center (middle, compare pink and maroon circles) (B). PCA analysis of brain dataset from GF/SPF and AD/HC inoculated ex-GF groups (C). Within the brain dataset, the GF group segregated away from the other groups, while the SPF, HC humanized ex-GF, and AD humanized ex-GF groups each segregated loosely throughout component 2 (right, compare light blue, dark blue, light purple, and dark purple circles).

(D-F) Differences in microbiome-related metabolites in feces (D), serum (E) and brain (F) from AD humanized ex-GF groups versus HC humanized ex-GF groups.

(G-I) Differences in carbohydrate and energy metabolites in feces (G), serum (H), and brain (I) from AD humanized ex-GF groups versus HC humanized ex-GF groups.

Red and green shaded cells indicate $P \leq 0.05$ (red indicates the fold change values are significantly higher for that comparison; green values significantly lower). Light red and light green shaded cells indicate $0.05 < P < 0.10$ (light red indicates the fold change values trend higher for that comparison; light green values trend lower).

A

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Phosphatidyleth anolamine(PE)	1-palmitoy-2-oleoyl-GPE(16:0/18:1)	5.64
	1-palmitoy-2-linoleoyl-GPE(16:0/18:2)	2.21
	1-palmitoy-2-arachidonoyl-GPE(16:0/20:4)	3.23
	1-stearoyl-linoleoyl-GPE(18:0/18:2)	2.28
Short Chain Fatty Acid	butyrate/isobutyrate (4:0)	0.46
	valerate (5:0)	0.87

B

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Long Chain Monounsaturated Fatty Acid	myristoleate(14:1n5)	0.38
	palmitoleate(16:1n7)	0.36
	10-heptadecenoate(17:1n7)	0.45
	oleate/vaccenate(18:1)	0.51
	10-nonadecenoate(19:1n9)	0.52
Long Chain polyunsaturated Fatty Acid (n3 or n6)	tetradecadienoate(14:2)	0.45
	heneicosapentaenoate(21:5n3)	0.57
	docosapentaenoate(n3 DPA;22:5n3)	0.57
	hexadecadienoate(16:2n6)	0.44
	dihomo-linoleate(20:2n6)	0.58
	dihomo-linolenate(20:3n3 or n6)	0.60
	docosapentaenoate(n6 DPA;22:5n6)	0.58
	docosadienoate(22:2n6)	0.55
Fatty Acid metabolism(Acyl Carnitine，Long Chain Saturated)	myristoylcarnitine(C14)	0.48
	pentadecanoylcarnitine(C15)	0.44
	palmitoylarnitine(C16)	0.45
	margaroylcarnitine(C17)	0.52
Fatty Acid Metabolism(Acyl Carnitine，Monounsaturated)	myristoleoylcarnitine(C14:1)	0.45
	palmitoleoylcarnitine(C16:1)	0.29
	oleoylcarnitine(C18:1)	0.35
	eicosenoylcarnitine(C20:1)	0.40
Fatty Acid Metabolism(Acyl Carnitine，Polyunsaturated)	linoleoylcarnitine(C18:2)	0.40
	linoleoylcarnitine(C18:3)	0.42
	dihomo-linoleoylcarnitine(C20:2)	0.39
	arachidonoylcarnitine(C20:4)	0.64
	dihomo-linoleoylcarnitine(C20:3n3 or 6)	0.53
	docosahexaenoylcarnitine(C22:6)	0.50
Fatty Acid Metabolism(Acyl Choline)	palmitoylcholine	0.30
	oleoycholine	0.36
	stearoycholine	0.31
Short Chain Fatty Acid	butyrate/isobutyrate (4:0)	0.65
Eicosanoid	thromboxane B2	1.13
	12-HHTrE	1.31

C

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Dihydrosphingomyelins	behenoyl dihydrosphingomyelin(d18:0/22:0	1.32
	sphingomyelin(d18:0/18:0,d19:0/17:0)	1.24
	sphingomyelin(d18:0/20:0,d16:0/22:0)	1.31
Sphingomyelins	behenoyl sphingomyelin(d18:1/22:0)	1.21
	tricosanoyl sphingomyelin(d18:1/23:0)	1.16
	lignoceroyl sphingomyelin(d18:1/24:0)	1.20
	sphingomyelin (d17:1/16:0,d18:1/15:0)	0.99
	sphingomyelin (d18:2/16:0,d18:1/16:1)	1.02
	sphingomyelin (d18:1/18:1,d18:2/18:0)	1.03
	sphingomyelin (d18:1/19:0,d19:1/18:0)	1.15
	sphingomyelin (d18:1/20:0,d16:1/22:0)	1.18
	sphingomyelin (d18:2/24:1,d18:1/24:2)	1.12
Eicosanoid	Prostaglandin F2alpha	1.14
	12-HHTrE	1.10

D

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Urea Cycle; Arginine and Proline Metabolism	argininosuccinate	2.23
	ornithine	4.17
	3-amino-2-piperidone	1.85
	citrulline	1.75
	dimethylarginine(SDMA+ADMA)	1.46
	N-delta-acetylornithine	1.34
	N-alpha-acetylornithine	3.55
	argininate	2.93

E

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Urea Cycle; Arginine and Proline Metabolism	arginine	1.37
	2-oxo arginine	1.46
	homocitrulline	0.84
	N-acetylproline	1.83
	pro-hydroxy-pro	0.71
	argininate	1.66

F

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Urea Cycle; Arginine and Proline Metabolism	ornithine	1.35
	2-oxo arginine	1.12
	homocitrulline	0.71
	proline	1.10
	N-delta-acetylornithine	0.90

G

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Methinonine, Cysteine, SAM and Taurine Metabolism	N-acetylmethionine	1.61
	S-adenosylhomocysteine(SAH)	2.97
	cysteine s-sulfate	1.88
Glutathione Metabolism	5-oxoproline	0.66
	ophthalmate	1.41
Gamma-glutamyl Amino Acid	gamma-glutamylleucine	1.29
	gamma-glutamyl-alpha-lysine	1.24
	gamma-glutamylmethionine	2.89
	gamma-glutamylphenylalanine	1.39
	gamma-glutamylthreonine	1.45
	gamma-glutamylcitrulline	2.03
	gamma-glutamyl-2-aminobutyrate	2.03

H

Sub Pathway	Biochemical Name	Welch's t-tst Fold of Change AD humanized ex-GF / HC humanized ex-GF
Methinonine, Cysteine, SAM and Taurine Metabolism	S-adenosylhomocysteine(SAH)	1.11
	cysteine	0.98
	hypotaurine	1.06
	N-acetyltaurine	0.99
	cyano-alanine	1.09
Glutathione Metabolism	glutathione, reduced(GSH)	1.00
	glutathione, oxidized(GSSG)	0.96
	cysteinylglycine	0.99
	2-hydroxybutyrate/2-hydroxyisobutyrate	0.99
	S-(1,2-dicarboxyethyl)glutathione	1.10
	4-hydroxy-nonenal-glutathione	1.12
	3'-dephospho-CoA-glutathione	0.97
Gamma-glutamyl Amino Acid	gamma-glutamyl-epsilon-lysine	0.97
	gamma-glutamylphenylalanine	1.02

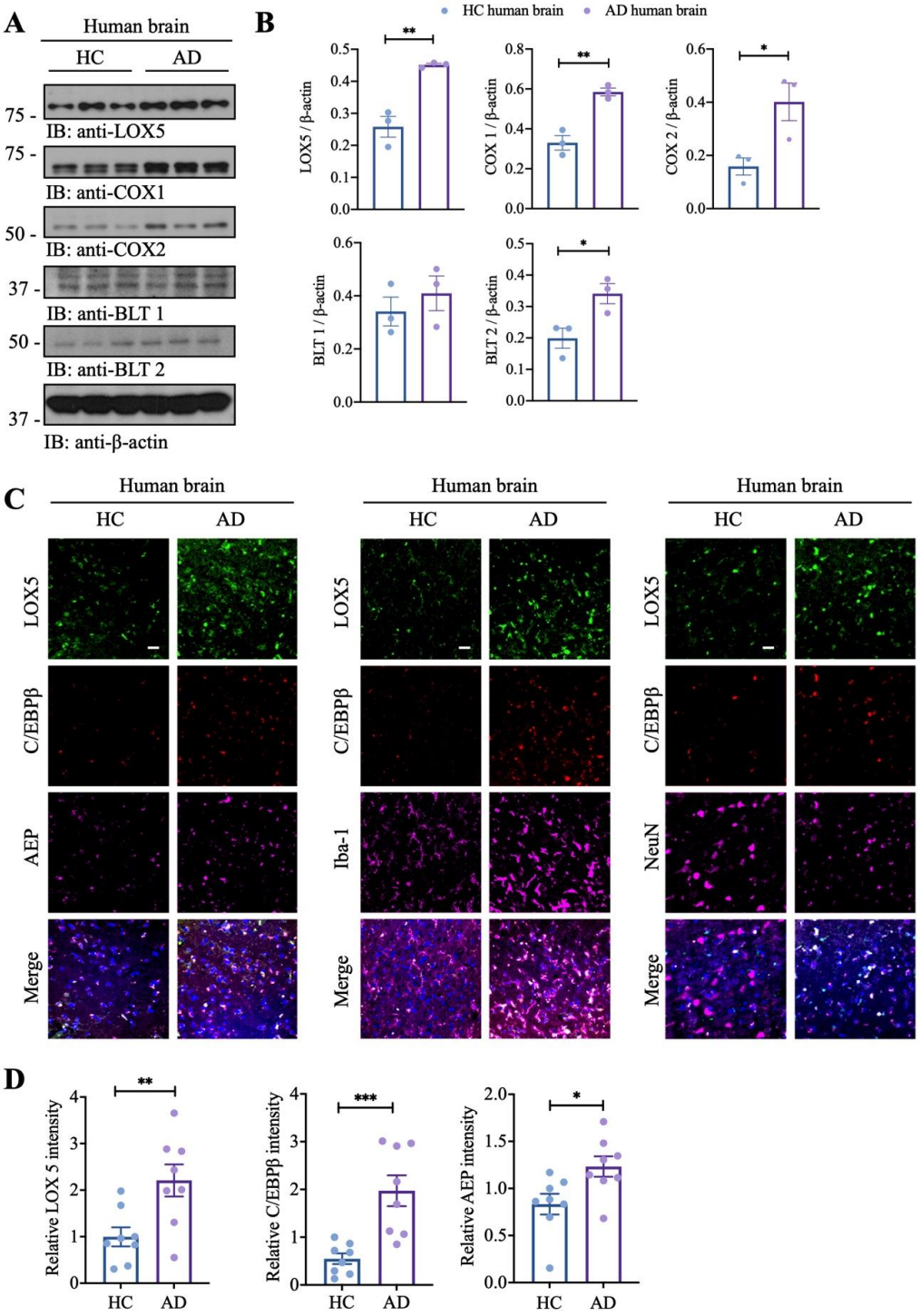
Supplementary Figure 11. Metabolomics analysis of the brains, serum samples and feces from AD humanized ex-GF 3xTg mice and HC humanized ex-GF 3xTg mice.

(A-C) Differences in lipid metabolism in feces (A), serum (B) and brain (C). The SCFAs, especially butyrate, were reduced in the feces and serum in AD humanized ex-GF 3xTg mice, whereas AA-associated metabolites including Thromboxane B2, Prostaglandin F2α, 12-HHTrE were increased in the serum and the brain.

(D-F) Differences in arginine metabolism in feces (D), serum (E) and brain (F).

(G&H) Differences in oxidative stress metabolites in serum (G) and brain (H).

Red and green shaded cells indicate $P \leq 0.05$ (red indicates the fold change values are significantly higher for that comparison; green values significantly lower). Light red and light green shaded cells indicate $0.05 < P < 0.10$ (light red indicates the fold change values trend higher for that comparison; light green values trend lower).


A
Human brain
HC
AD
75 -
IB: anti-LOX5
75 -
IB: anti-COX1
50 -
IB: anti-COX2
37 -
IB: anti-BLT 1
50 -
IB: anti-BLT 2
37 -
IB: anti-β-actin
B
HC human brain
AD human brain
LOX5 / β-actin
COX 1 / β-actin
COX 2 / β-actin
BLT 1 / β-actin
BLT 2 / β-actin
C
Human brain
HC
AD
LOX5
C/EBPβ
AEP
Iba-1
NeuN
Merge
D
Relative LOX 5 intensity
Relative C/EBPβ intensity
Relative AEP intensity

Supplementary Figure 12. AD patient brains demonstrate enhanced AA-associated LOX/COX1/2 pathway, correlated with elevated C/EBPβ/AEP signaling.

(A) Immunoblotting analysis of Arachidonic acid metabolism in the brain homogenates from AD patients and age-related healthy controls.

(B) Quantitative analysis of immunoblot. The bands of C/EBPβ, LOX5, COX1, COX2, BLT1 and BLT2 were measured with Image J and normalized with β-actin. (n = 3 in each group. The expression of Arachidonic acid metabolism related protein increased in AD patients' brains. Data are shown as mean ± SEM, *P<0.05, **P<0.01 compared with control, unpaired t tests).

(C) Immunofluorescent staining of LOX5, C/EBPβ, AEP, Iba-1 and NeuN in the cortex region of the brains from Alzheimer's patients and age-related healthy controls. Scale bar: 20 μm.

(D) Quantitative analysis of LOX5, C/EBPβ and AEP positive cells. The fluorescence intensities of LOX5, C/EBPβ and AEP positive cells in the brains of Alzheimer's patients were increased compared with age-related healthy controls. (n = 8 in each group, Data are shown as mean ± SEM. *P< 0.05, **P< 0.01, ***P< 0.001 compared with control, unpaired t tests)

Supplementary Table 1 Clinical files of the human feces donors

Sample ID	Age	Gender	Dementia Rating of 2	Antibiotics past 6 months	Hospitalized past 6 months	Taking PPI	Any Acid reducing medication	Atypical Antipsychotics	malnutrition indicator score	Clinical Frailty Score >=7	5 or more daily mdications	Medications
AD_1	85	F	Yes	No	No	No	No	No	2	Y	Y	gabapentin, acetaminophen, miralax, atenolol, aspirin, trazodone, namenda, escitalopram, milk of magnesia, lorazepam, miralax, loratadine, donepezil, ativan, calcium supplement, ativan
AD_2	85	F	Yes	No	No	No	No	No	2	Y	Y	Vitamin D with calcium, children's chewable multivitamin, docusate sodium, levothyroxine, senna, simvastatin, vitamin C, ferrous sulfate, polyethylene glycol,
AD_3	94	F	Yes	No	No	No	No	No	2	Y	Y	hydrochlorothiazide, lisinopril, metoprolol tartrate, miralax, senokot, timolol maleate, tylenol,
HC_1	91	F	No	No	No	No	No	No	2	Y	Y	Glucernia, Melatonin, Vitamin D, Glizipide, Celexa, Simvastatin, Levothyroxine, multivitamin, actulose maalox maximum strength,
HC_2	94	F	No	No	No	No	No	No	2	Y	Y	Calcitonin, citalopram, furosemide, feri max, guaifenesin, lisinopril, milk of magnesia, multivitamin, salonpas, senna, prednisone, polyethylene glycol, acetominophen, coumadin, Vitamin D3
HC_3	93	F	No	No	No	No	No	No	2	Y	Y	acetaminophen, aspirin, children's chewable multivitamin, Vitamin D, Flomax, Lasix, milk of magnesia, Neurontin, remeron, senokot