Supporting Information

Ellagic acid ameliorates alcohol-induced cognitive and social dysfunction through the gut microbiota-mediated CCL21-CCR7 axis

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Table S1 Key resources in the current study

Reagent or resource	Source	Identifier
Antibodies		
IBA-1	Abcam	Cat# ab178847; RRID:AB_2832244
CCL21	Thermo Fisher	Cat# 500-P114BT; RRID:AB_2929279
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP	Thermo Fisher Scientific	Cat# a16078; RRID:AB_2534752
Chemicals		
Ellagic acid	Nanjing DASF Biotechnology	CAS NO 476-66-4
Metronidazole	Dalian Meilun biological Technology Co	CAS NO 443-48-1
Ampicillin	Dalian Meilun biological Technology Co	CAS NO 7177-48-2

Vancomycin hydrochloride	Dalian Meilun biological Technology Co	CAS NO 1404-93-9
Neomycin Sulfate	Dalian Meilun biological Technology Co	CAS NO 1405-10-3
Hematoxylin	Poly-scientific	S212
Eosin	StatLab	SL98-1
TB Green™ Premix Ex Taq™ II	TaKaRa	RR820Q
Sterile ethanol liquid diet	TROPHIC Animal Feed High-tech Co.	AIN-93M
Software and Algorithms		
ImageJ v2.3.0	National Institutes of Health	RRID:SCR_003070
Super Maze	XR-Xmaze	http://www.softmaze.com/
R (v4.2.1)	R Team	https://www.r-project.org/

Table S2 Primer sequences for qRT-PCR

Gene	Forward Primer	Reverse Primer
Tnfa	CTCATGCACCACCATCAAGG	ACCTGACCACTCTCCCTTTG

Il6	CCTCTGGTCTTCTGGAGTAC C	ACTCCTTCTGTGACTCCAGC
Il10	ATAACTGCACCCACTTCCCA	GGGCATCACTTCTACCAGGT
Iba1	TGACGGACCCCAAAAGATGA	TCTCCACAGCCACAATGAGT
Ccl21a	ATCCCGGCAATCCTGTTCTC	CCCTTGGAGCCCTTTCCTTT
Ccl21b	CTGGTGGTAACGAGGCTCA	AATGGTGTCCCAGTTGCCTC
Ccl21c	GTGGTAACGAGGCTCAC	CCAGCCTAAGATCCTGCCTT
Ccl21d	CATCCCGGCAATCCTGTTCT	TTCTCTTGCAGCCCTTGGAG
Tnfrsf17	CAGCTTGACGGATCGGCT	CCCCTTGGGTTTGCTCTTGA
Total bacteria	ACTCCTACGGGAGGCAGCA G	GGACTACHVGGGTWTCTAA T
Bace2	AGTAACTTCGCTGTGGCAGG	AGAGAGCTTGATGGCTTGG C
Fos	AGAGCGGGAATGGTGAAGA C	AGTTGATCTGTCTCCGCTTG G
Npas4		
Rab3a	TTTGCAGGACGTCAGCTAGG	CTGTGGCGGAAGCCATCTTA

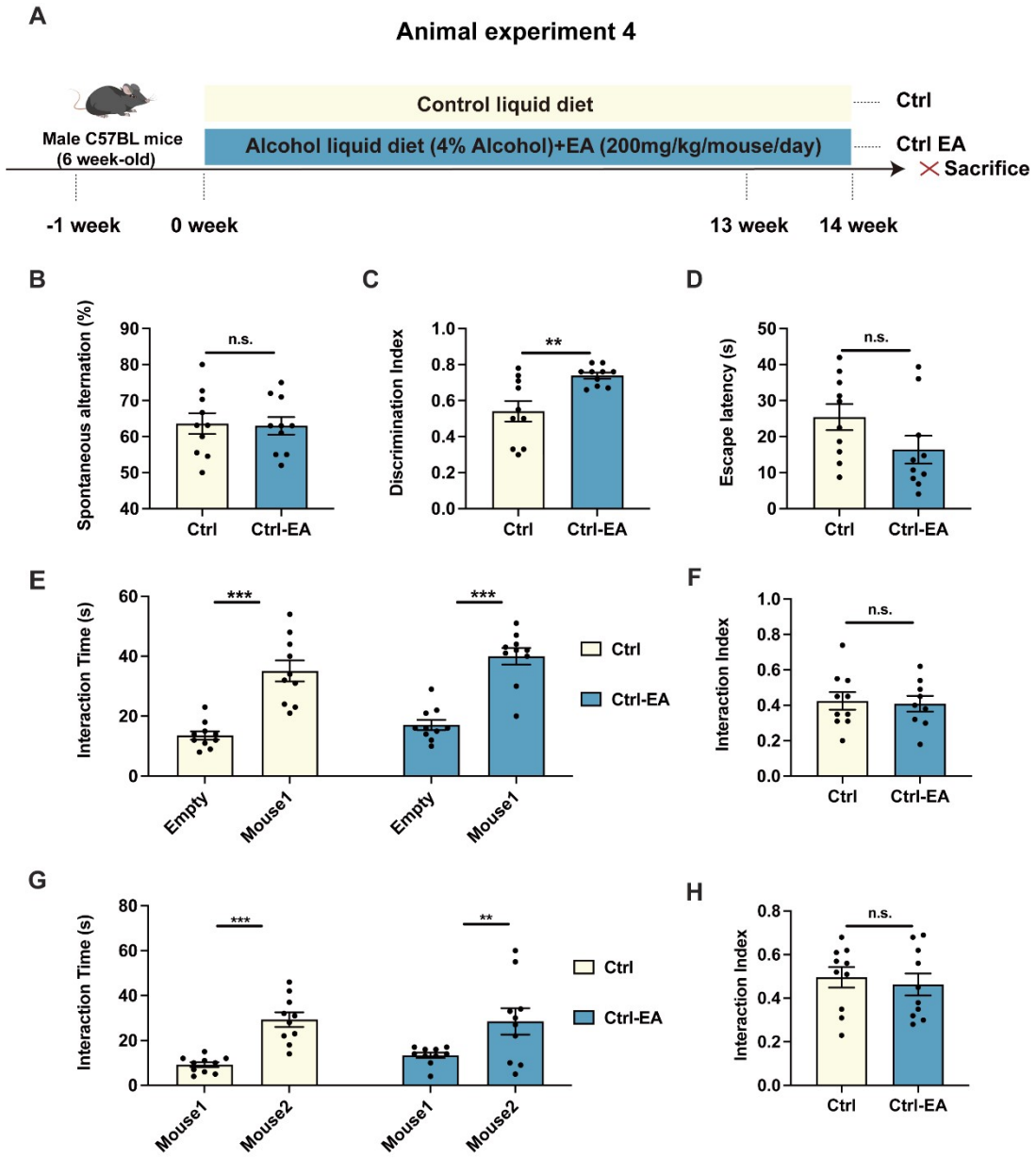

Fig. S1. Effects of UA on cognitive and social functions in non-alcohol diet mice.

(A) Timeline of animal experiment 4 depicting the non-alcohol diet with EA treatment.

(B) For the Y-maze, spontaneous alternations were recorded. E

(C) For the Barnes maze, escape latency was recorded.

(D) The discrimination index between the novel and familiar objects was calculated for the novel object recognition test.

(E) In the sociability test, the interaction index was calculated.

(F) The sociability test recorded the time spent interacting with a mouse or an empty wire cage.

(G) In the social novelty test, the interaction index was calculated.

(H) In the social novelty test, the time spent interacting with a novel versus a familiar mouse was recorded.

Data presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Alc group. Significant differences between mean values were determined by one-way ANOVA with Tukey's multiple comparisons test.

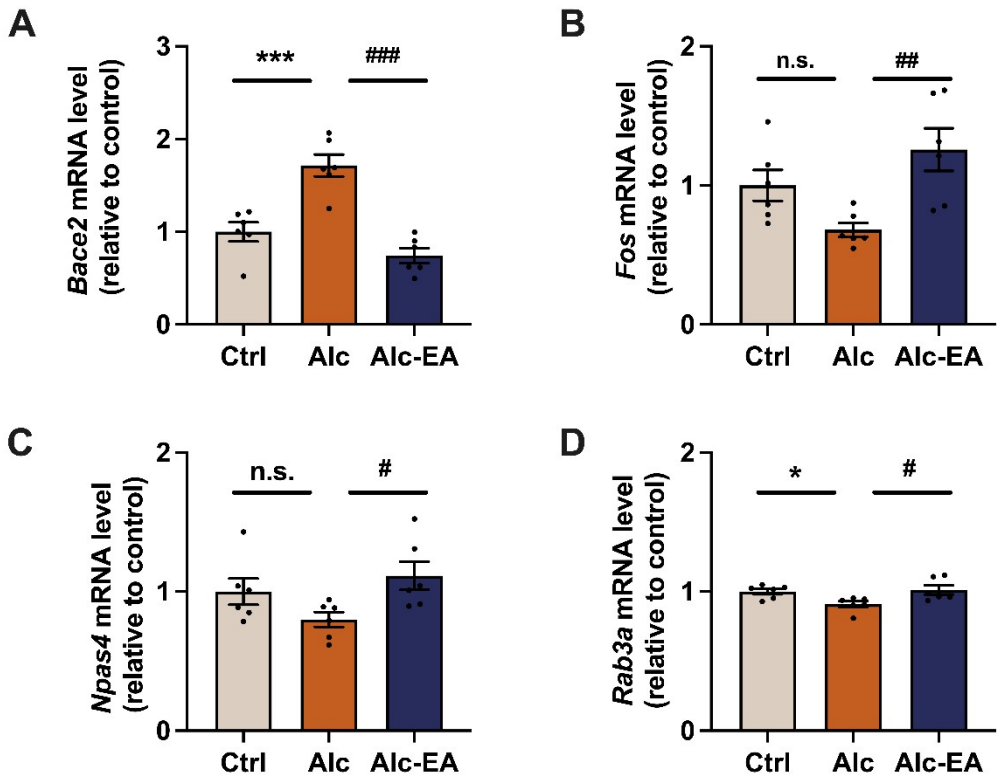

Fig. S2. The mRNA expression levels of cognitive-related genes were analyzed based on RNA sequencing data from the hippocampus of mice.

(A) The mRNA expression level of the *Bace2* gene in the hippocampus.

(B) The mRNA expression level of the *Fos* gene in the hippocampus.

(C) The mRNA expression level of the *Npas4* gene in the hippocampus.

(D) The mRNA expression level of the *Rab3a* gene in the hippocampus.

Data presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Ctrl group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared with the Alc group. Significant differences between mean values were determined by one-way ANOVA with Tukey's multiple comparisons test.

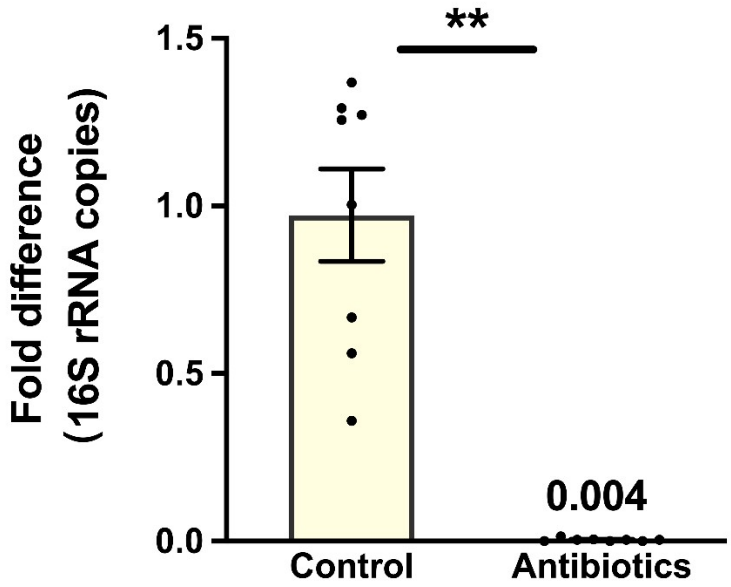

Fig. S3. Removing gut microbiome effect diagram.

Data were represented as mean ± SEM ($n \geq 8$); *means compared to the "Control" group, *$P < 0.05$, **$P < 0.01$