Figure S1

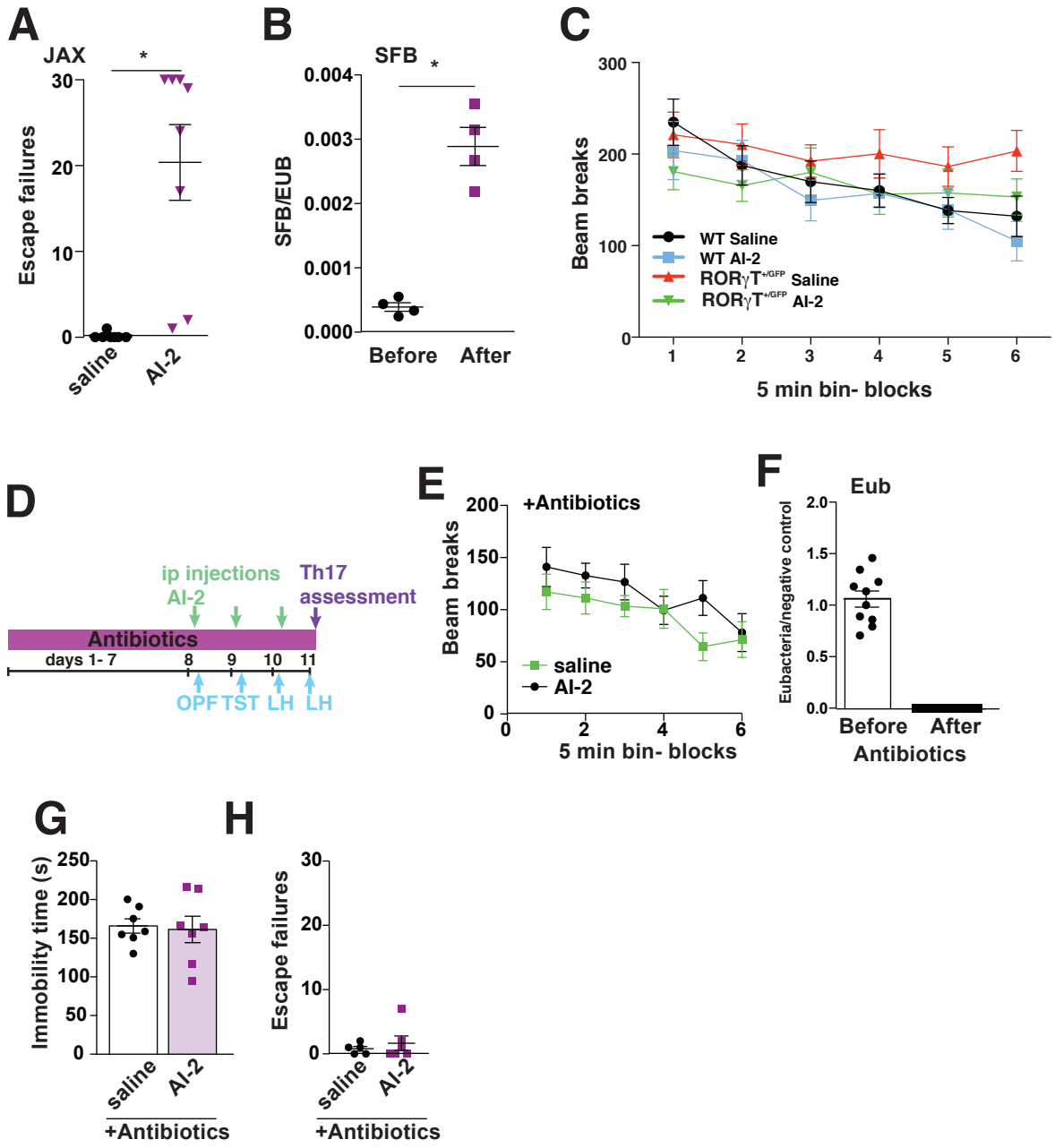

Figure S1: AI-2 promotes depressive-like behavior. A, JAX mice were injected with 5 nmoles/-mouse of AI-2 i.p. or saline daily for 3 days, and on the third day of injection they were subjected to the learned helplessness paradigm. Escape failures were recorded. Data are means+/-SEM. n=7-8 mice/group. Mann and Whitney *p<0.05, U=0.5. Levels of SFB (B), were measured before AI-2 treatment (Before), and after AI-2 treatment and learned helplessness (After). Each symbol represent a mouse. Data are means+/-SEM. n=4 mice/group. Mann and Whitney *p<0.05, U= 0. C, Wild-type or RORγT$^{+/GFP}$ mice were injected with 5 nmoles/mouse of AI-2 i.p. or saline daily for 3 days as indicated, and on the third day of injection locomotor activity was measured in an open field. Data are means+/-SEM. n=5-18 mice/group. D-H, TAC and JAX mice were pretreated with antibiotics for 7 days, and injected with 5 nmoles/mouse of AI-2, or saline i.p. daily for 3 days while remaining on antibiotics treatment, as indicated in D, and behaviors were assessed: openfield (OPF) (E), TST (G) and learned helplessness (LH) (H). Eubacteria 16s was evaluated before and after antibiotics treatment, to ensure the depletion of the microbiota (F). Data are means+/-SEM. n=7-8 mice/group. (F-H) Each symbol represent a mouse.

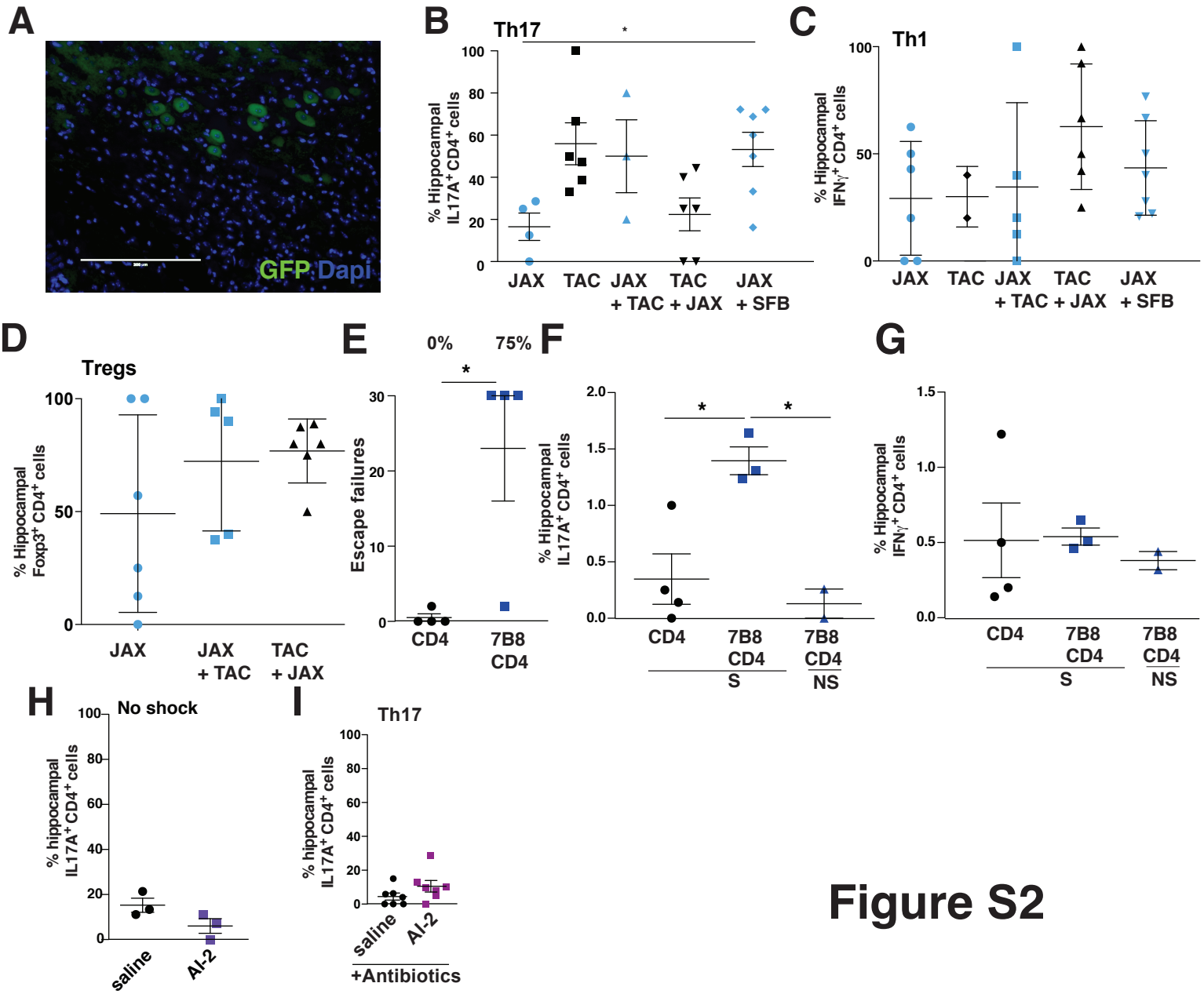

Figure S2

Figure S2: Hippocampal Th17 cells increase in JAX mice receiving SFB. A, GFP-labelled Th17 cells (~140,000 cells/mouse) were adoptively transferred to Rag2$^{-/-}$ mice and subjected to the learned helplessness paradigm, and brain sections were stained for GFP and Dapi. A represnetative image is shown in A at a magnification of 20X. JAX and TAC mice were housed with or without mice from the other source for 2 weeks, or JAX mice were gavaged with SFB, and then mice were subjected to the learned helplessness paradigm. Th17 (A), Th1 (B) and Tregs (C) were analyzed after learned helplessness in the hippocampus. Cells are gated on CD4^{+} cells. Each symbol represents a mouse. Data are means+/-SEM. n=2-7 One-way ANOVA, F(4,27)=3.202, *p<0.05 Bonferroni post-hoc test. SFB^{+}Rag2$^{-/-}$ mice were adoptively transferred with either CD4 cells from 7B8 mice or wild-type littermate mice, and subjected to the reduced paradigm of learned helplessness. D, Escape failures were recorded and means±SEM of the escape failures are displayed, and the percent of mice exhibiting learned helplessness (failing to escape >15 out of the 30 trials) is shown above each group. Each symbol represents an individual mouse. n=4, Mann-Whitney U= 0.5. Th17 (E) and Th1 (F) were analyzed after learned helplessness (S) or in non shocked mice (NS) in the hippocampus. Cells are gated on lymphocytes cells. Each symbol represents a mouse. Data are means+/-SEM. n=2-4 One-way ANOVA, F(2,8)=10.69, *p<0.05 Bonferroni post-hoc test. G,TAC mice were injected with 5 nmoles/mouse of AI-2, or saline i.p. daily for 3 days, and sacrificed 1 h after the last injection. Th17 cells in the hippocampus were analyzed by flow cytometry. Each symbol represents an individual mouse. Data are means+/-SEM. n=3 mice/group. H, Mice were pretreated with antibiotics for 7 days, and injected with 5 nmoles/mouse of AI-2, or saline i.p. daily for 3 days while remaining on antibiotics treatment, as indicated in Suppl Fig 1D, and hippocampal Th17 cells were analyzed by flow cytometry after learned helplessness. Data are means+/-SEM. n=7-8 mice/group. Each symbol represent a mouse.

Figure S3

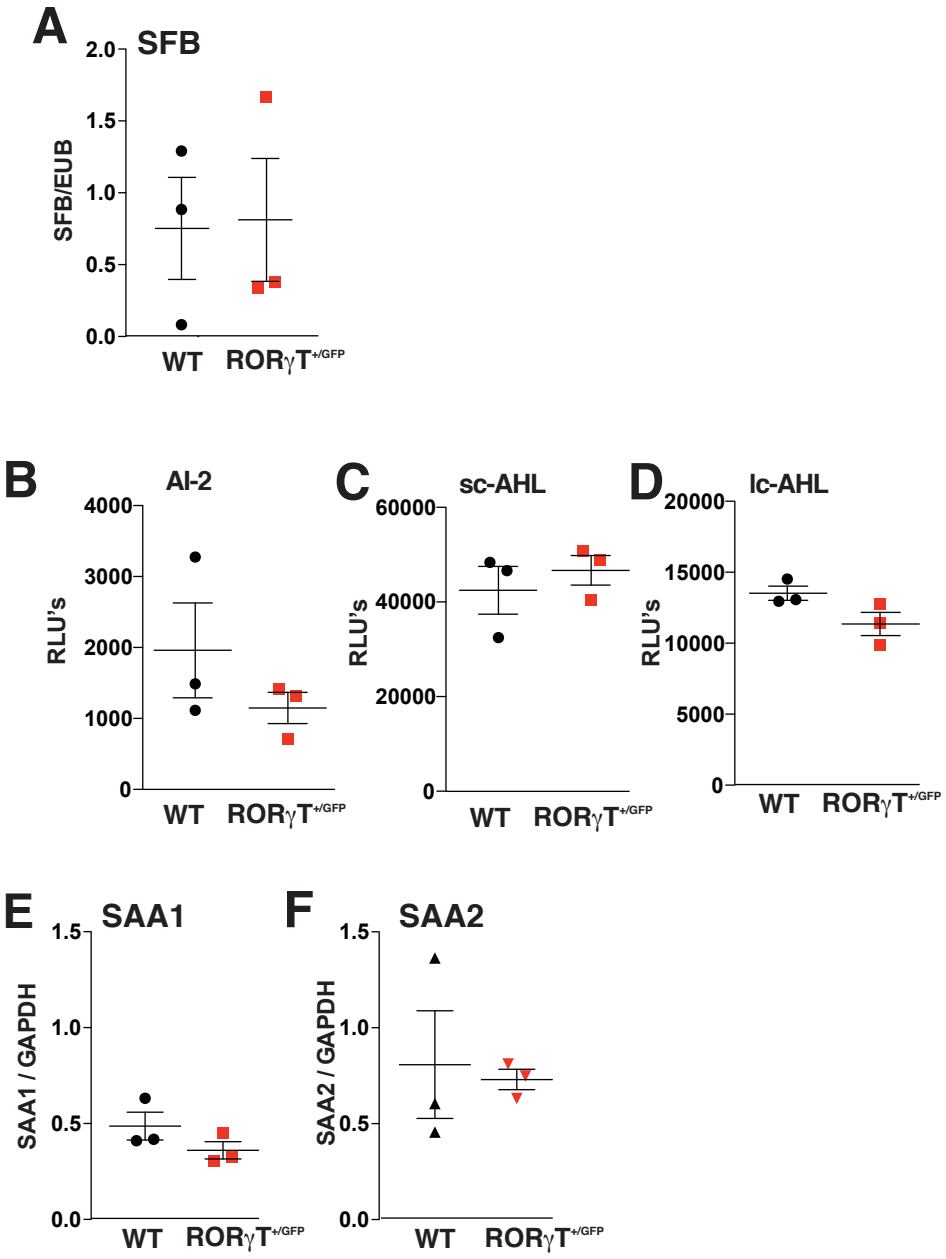

Figure S3: Levels of SFB (A), AI-2 (B), sc-AHL (C), lc-AHL (D), SAA1 (E) and SAA2 (F) were measured in wild-type and RORγT$^{+/GFP}$ litermates. Each symbol represent a mouse. Data are means+/- SEM. n=3 mice/group.

Figure S4

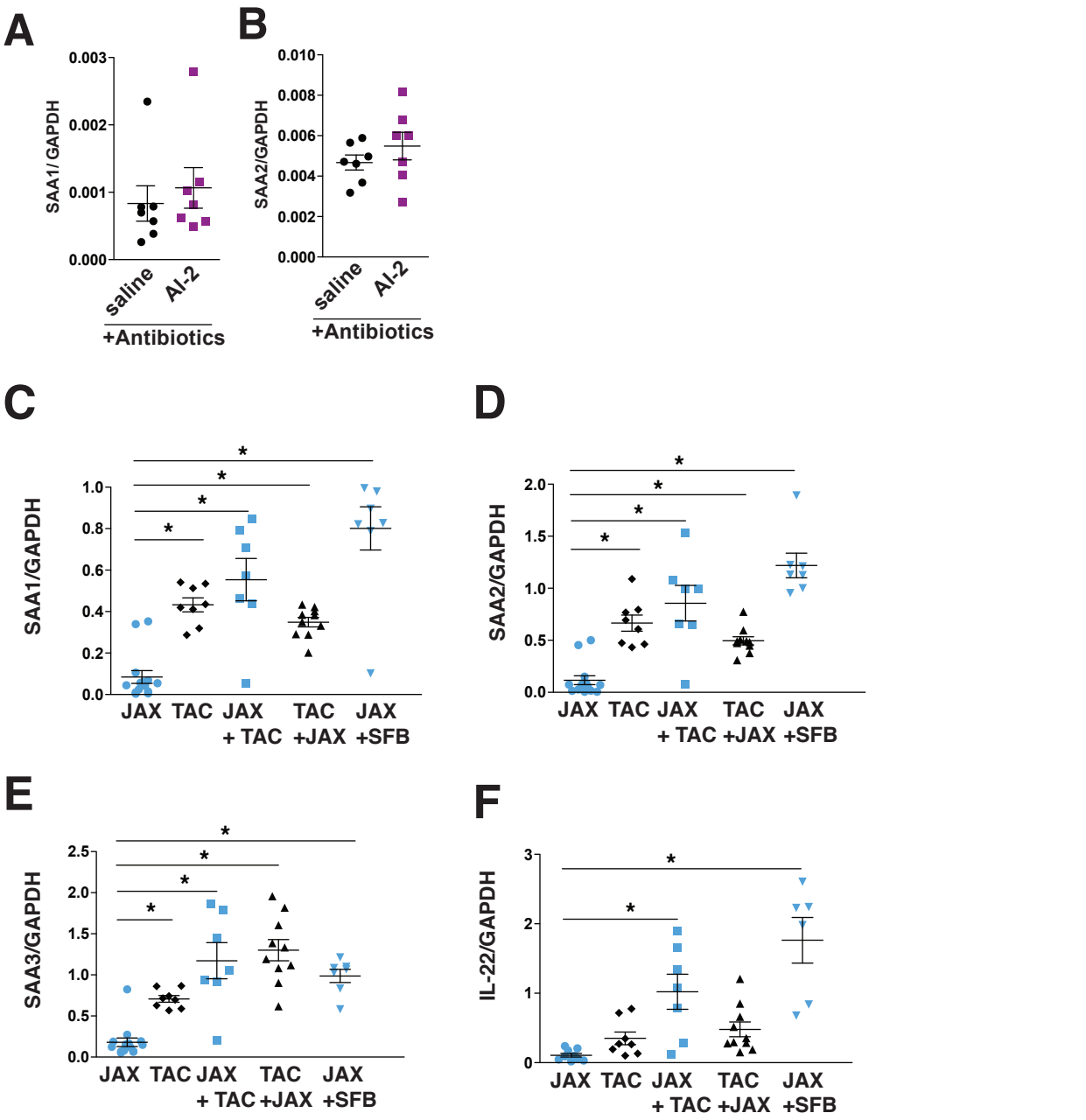

Figure S4: SFB increases SAA1 and SAA2 levels. A-B, TAC and JAX mice were pretreated with antibiotics for 7 days, and injected with 5 nmoles/mouse of AI-2, or saline i.p. daily for 3 days while remaining on antibiotics treatment, as indicated in Suppl Fig 1D, and SAA1 and SAA2 were analyzed by qRT-PCR in the small intestine. Data are means+/-SEM. n=7-8 mice/group. Each symbol represents a mouse.C-F, JAX mice were housed with or without TAC mice for 2 week, or were gavaged with SFB and subjected to the learned helplesness, and intestinal levels of SAA1 (C), SAA2 (D), SAA3 (E) and IL-22 (F) were measured by qRT-PCR. Each symbol represents a mouse. Data are means+/-SEM. n=7-12 mice/group, one way ANOVA, $F(4,42)=22.78$ (SAA1), $F(4,41)=25.53$ (SAA2), $F(4,41)=21.39$ (SAA3), $F(4,35)=14.17$ (IL-22), *$p<0.05$ Bonferroni's post-hoc.

Figure S5

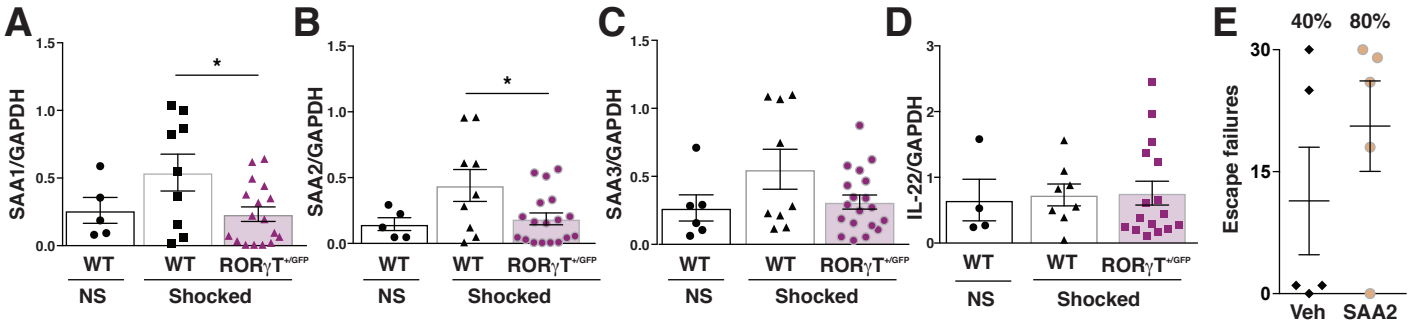

Figure S5: SAA1 and 2 levels are reduced in RORγT$^{+/GFP}$ mice after footshocks. Wild-type and RORγT$^{+/GFP}$ littermate mice were subjected to the learned helplessness paradigm and sacrificed after the last shocks, and intestinal levels of SAA1 (A), SAA2 (B), SAA3 (C) and IL-22 (D) were measured by qRT-PCR. Each symbol represent a mouse. Data are means+/-SEM. A, n=8-18 mice/ group, one way ANOVA, F(2,28)=3.536 (SAA1), F(2,29)=3.794 (SAA2), *p<0.05 Bonferroni's post-hoc. E, TAC mice were treated with 5 μg of SAA2 and subjected to the reduced intensity learned helplessness paradigm and escape failures were recorded. Each symbol represent a mouse. Data are means+/-SEM (n=5).

Figure S6

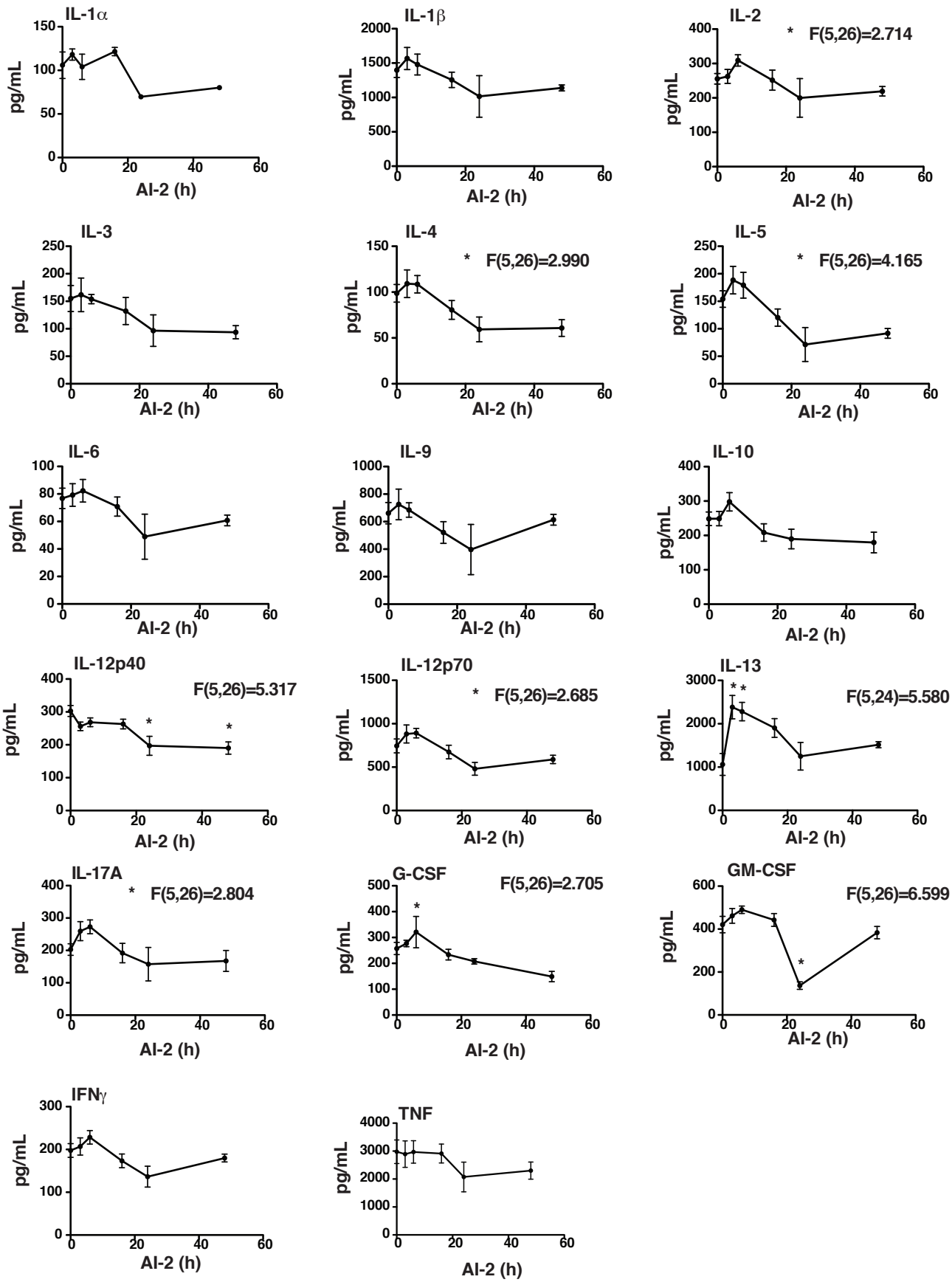
IL-1α
IL-1β
IL-2
* F(5,26)=2.714
IL-3
IL-4
* F(5,26)=2.990
IL-5
* F(5,26)=4.165
IL-6
IL-9
IL-10
IL-12p40
F(5,26)=5.317
IL-12p70
* F(5,26)=2.685
IL-13
F(5,24)=5.580
IL-17A
* F(5,26)=2.804
G-CSF
F(5,26)=2.705
GM-CSF
F(5,26)=6.599
IFNγ
TNF
pg/mL
AI-2 (h)

Figure S6 *cont'*

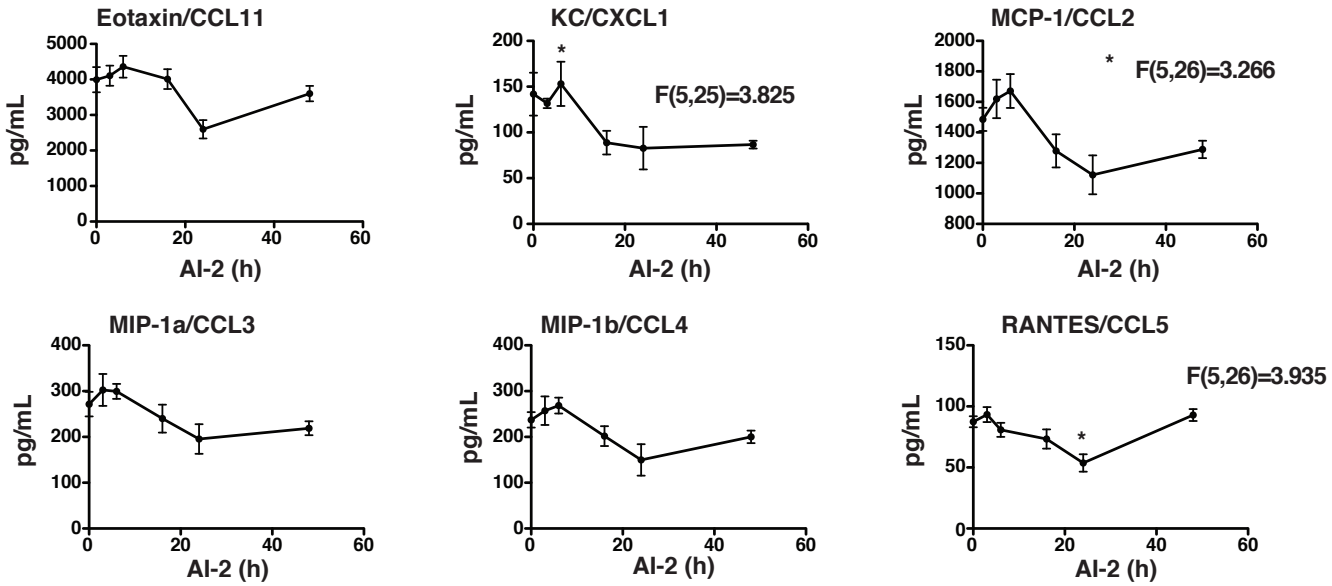

Figure S6: AI-2 induces cytokine and chemokine changes. TAC mice were injected with 5 nmoles/mouse of AI-2 or vehicle i.p. and sacrificed as indicated after 3, 6, 16, 24 and 48 h, and cytokine and chemokine levels were measured by multiplex. Data are means+/-SEM. n=3-8 mice/group, one-way ANOVA, F(5,26)=2.714 (IL-2), F(5,26)=2.990 (IL-4), F(5,26)=4.165 (IL-5), F(5,26)=5.317 (IL-12p40), F(5,26)=2.685 (IL-12p70), F(5,24)=5.580 (IL-13), F(5,26)=2.804 (IL-17A), F(5,26)=2.705 (G-CSF), F(5,26)=6.599 (GM-CSF), F(5,25)=3.825 (KC/CXCL1), F(5,26)=3.266 (MCP-1/C-CL2), F(5,26)=3.935 (RANTES/CCL5), *p<0.05, Bonferroni's post-hoc.

Figure S7

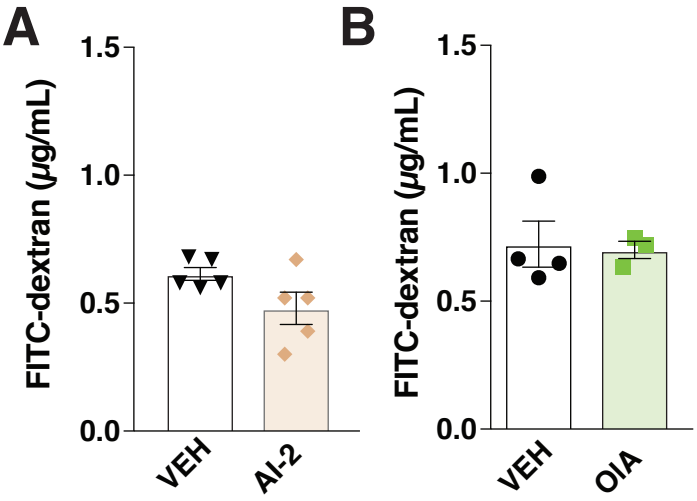

Figure S7: AI-2 or OIA did not affect gut permeability. Wild-type mice were injected with 5 nmoles/mouse of AI-2 or oleic acid (OIA) or wehicle (VEH) i.p. daily for 3 days (AI-2) or 2 weeks (OIA), and on the third day of injection, mice were subjected to the reduced intensity learned helplessness paradigm and gavaged with 4 kDa FITC-dextran and sacrificed 4 hours later. Fluorescence in the blood was assessed with a plate reader. Each symbol represents a mouse. Data are means+/-SEM. A, n=3-5 mice/group.

Table S1. Demographics of the human stool sample cohorts.

Variable	MDD	Healthy Control	Test	p-value
Age, mean±sd, Years	47.2 ±12.8	39.6 ±13.4	t-test	p=0.211
Gender, n (%)			Fisher's exact	p=0.999
Male	2 (20%)	2 (20%)		
Female	8 (80%)	8 (80%)		
Race			Fisher's exact	p=0.314
White	4 (40%)	6 (60%)		
African American	3 (30%)	3 (30%)		
Native American	0 (0%)	1 (10%)		
Other	3 (30%)	0 (0%)		
Ethnicity			Fisher's exact	p=0.303
Hispanic	4 (40%)	1 (10%)		
Non-Hispanic	6 (60%)	9 (90%)		
BMI, mean±sd	33.9±6.7	28.7±7.1	t-test	p=0.111
QIDS, mean±sd	15.0±1.8	1.7±1.5	t-test	p<0.001