Supplemental material

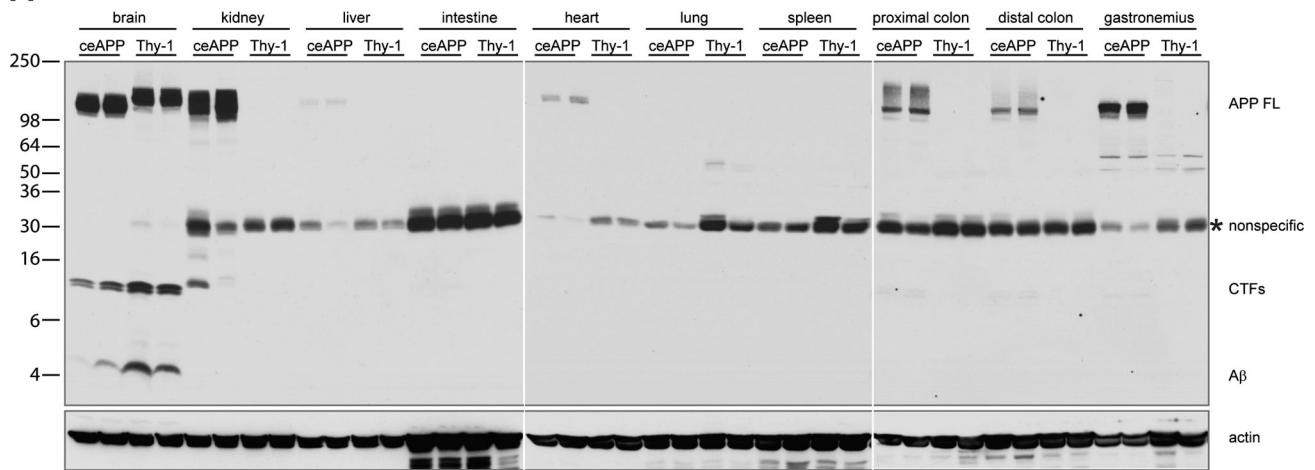**A** Western blot analysis using the human APP-specific 6E10 antibody

Figure S1. **APPswe is expressed specifically in the brains of APPPS1-21 mice that coexpress APP_SWE and PS1_L166P.** Western blot using human APP-specific 6E10 antibody to detect steady-state levels of full-length human APP-FL, β-CTF, and Aβ peptides in detergent-soluble tissue lysates of 3-mo-old APPPS1-21 mice compared with 8-mo-old APP_SWE/PS1_ΔE9 mice (Jankowsky et al., 2002) that were used in earlier studies (Minter et al., 2016, 2017). Note the levels of full-length APPswe are similar in the brains of APPPS1-1 and APP_SWE/PS1_ΔE9 mice (compare lanes 1 and 2 with 3 and 4). n = 2.
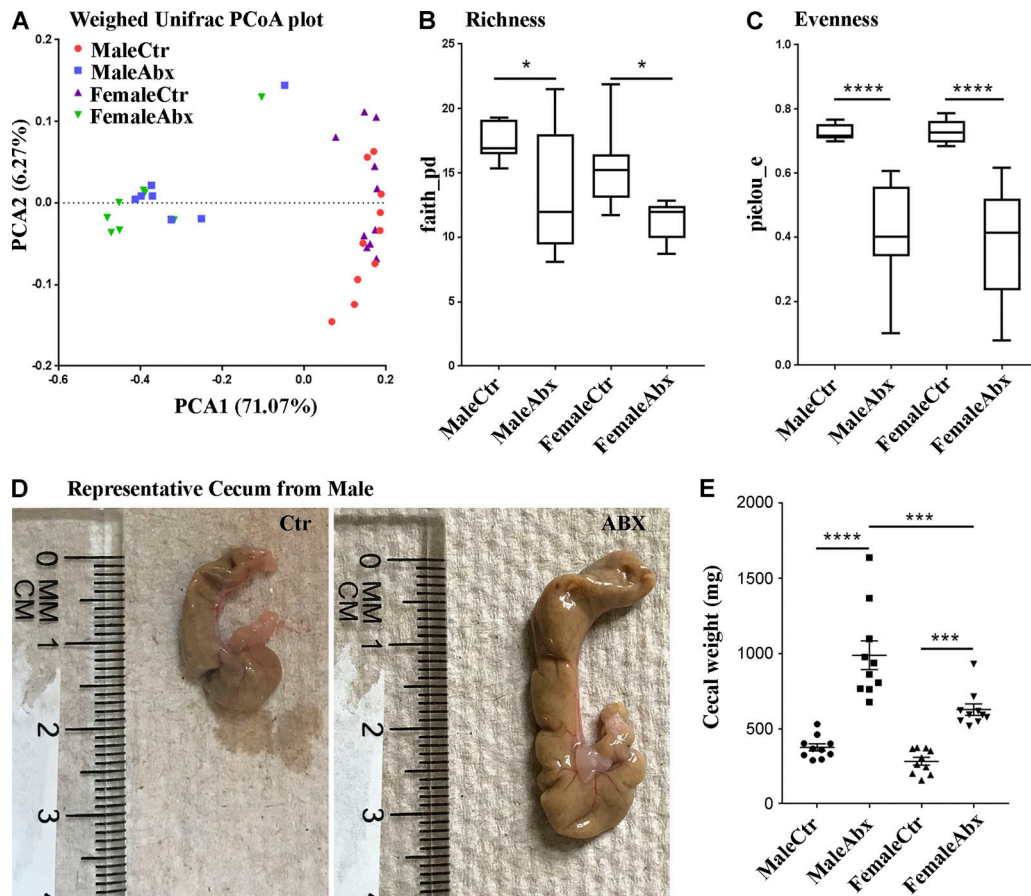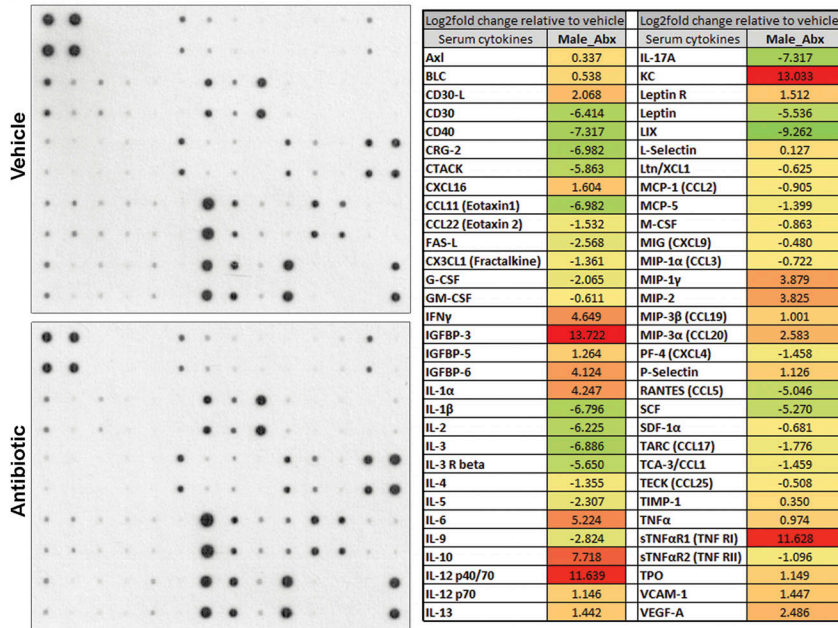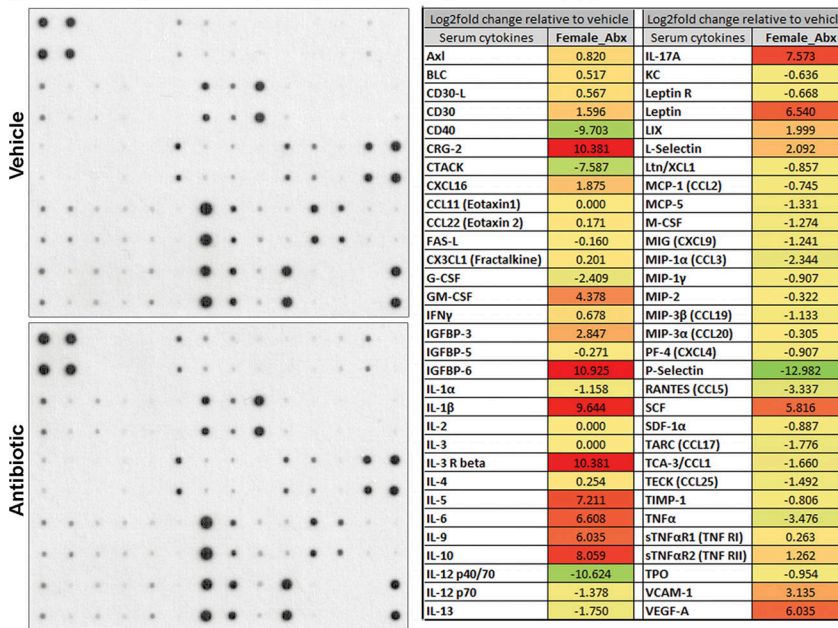
Figure S2. **Microbiome diversity at P22 and cecal weight analysis at week 7 in male and female APPPS1-21 mice (*n* = 10/group). (A)** PCoA plot at P22 generated by using weighted versions of the UniFrac distance metric. The two components explained 71.07% of the variance. **(B and C)** At P22, both α diversity values (species richness [B] and evenness [C]) were significantly lower in the ABX-treatment group compared with control (Ctr; Faith's phylogenetic diversity, two-way ANOVA: $F_{[1,34]}$ = 17.31, P = 0.0002; Pielou's evenness, two-way ANOVA: $F_{[1,34]}$ = 76.19, P < 0.0001). Sex or an interaction between sex and ABX treatment showed no significant difference in either richness or evenness (P > 0.05). Post hoc analysis showed significantly reduced richness and evenness in ABX-treated males (Faith's phylogenetic diversity: P = 0.0351; Pielou's evenness: P < 0.0001) and ABX-treated females (Faith's phylogenetic diversity: P = 0.0229, Pielou's evenness: P < 0.0001) compared with their vehicle-treated counterparts. **(D)** Representative cecal images from vehicle- or ABX-treated male mice at the time of necropsy. **(E)** Cecal weight analysis wherein two-way ANOVA showed significant effect of sex ($F_{[1, 36]}$ = 17.51, P = 0.0002), ABX ($F_{[1, 36]}$ = 77.39, P < 0.0001), and an interaction between sex and ABX ($F_{[1, 36]}$ = 6.104, P = 0.0184). Significantly larger ceca were observed in ABX-treated males compared with vehicle-treated males (P < 0.0001), ABX-treated females compared with vehicle-treated females (P = 0.0004), and ABX-treated males compared with ABX-treated females (P = 0.0002). Data are mean ± SEM. *, P < 0.05; ***, P < 0.001; ****, P < 0.0001. Ctr, Control; PCA1, principal coordinate 1; PCA2, principal coordinate 2.

A Plasma Cytokine Array Analysis - Male (Vehicle/Abx) @ 7 weeks

Serum cytokines	Male_Abx	Serum cytokines	Male_Abx
Axl	0.337	IL-17A	-7.317
BLC	0.538	KC	13.033
CD30-L	2.068	Leptin R	1.512
CD30	-6.414	Leptin	-5.536
CD40	-7.317	LIX	-9.262
CRG-2	-6.982	L-Selectin	0.127
CTACK	-5.863	Ltn/XCL1	-0.625
CXCL16	1.604	MCP-1 (CCL2)	-0.905
CCL11 (Eotaxin1)	-6.982	MCP-5	-1.399
CCL22 (Eotaxin 2)	-1.532	M-CSF	-0.863
FAS-L	-2.568	MIG (CXCL9)	-0.480
CX3CL1 (Fractalkine)	-1.361	MIP-1α (CCL3)	-0.722
G-CSF	-2.065	MIP-1γ	3.879
GM-CSF	-0.611	MIP-2	3.825
IFNγ	4.649	MIP-3β (CCL19)	1.001
IGFBP-3	13.722	MIP-3α (CCL20)	2.583
IGFBP-5	1.264	PF-4 (CXCL4)	-1.458
IGFBP-6	4.124	P-Selectin	1.126
IL-1α	4.247	RANTES (CCL5)	-5.046
IL-1β	-6.796	SCF	-5.270
IL-2	-6.225	SDF-1α	-0.681
IL-3	-6.886	TARC (CCL17)	-1.776
IL-3 R beta	-5.650	TCA-3/CCL1	-1.459
IL-4	-1.355	TECK (CCL25)	-0.508
IL-5	-2.307	TIMP-1	0.350
IL-6	5.224	TNFα	0.974
IL-9	-2.824	sTNFαR1 (TNF RI)	11.628
IL-10	7.718	sTNFαR2 (TNF RII)	-1.096
IL-12 p40/70	11.639	TPO	1.149
IL-12 p70	1.146	VCAM-1	1.447
IL-13	1.442	VEGF-A	2.486

B Plasma Cytokine Array Analysis - Female (Vehicle/Abx) @ 7 weeks

Serum cytokines	Female_Abx	Serum cytokines	Female_Abx
Axl	0.820	IL-17A	7.573
BLC	0.517	KC	-0.636
CD30-L	0.567	Leptin R	-0.668
CD30	1.596	Leptin	6.540
CD40	-9.703	LIX	1.999
CRG-2	10.381	L-Selectin	2.092
CTACK	-7.587	Ltn/XCL1	-0.857
CXCL16	1.875	MCP-1 (CCL2)	-0.745
CCL11 (Eotaxin1)	0.000	MCP-5	-1.331
CCL22 (Eotaxin 2)	0.171	M-CSF	-1.274
FAS-L	-0.160	MIG (CXCL9)	-1.241
CX3CL1 (Fractalkine)	0.201	MIP-1α (CCL3)	-2.344
G-CSF	-2.409	MIP-1γ	-0.907
GM-CSF	4.378	MIP-2	-0.322
IFNγ	0.678	MIP-3β (CCL19)	-1.133
IGFBP-3	2.847	MIP-3α (CCL20)	-0.305
IGFBP-5	-0.271	PF-4 (CXCL4)	-0.907
IGFBP-6	10.925	P-Selectin	-12.982
IL-1α	-1.158	RANTES (CCL5)	-3.337
IL-1β	9.644	SCF	5.816
IL-2	0.000	SDF-1α	-0.887
IL-3	0.000	TARC (CCL17)	-1.776
IL-3 R beta	10.381	TCA-3/CCL1	-1.660
IL-4	0.254	TECK (CCL25)	-1.492
IL-5	7.211	TIMP-1	-0.806
IL-6	6.608	TNFα	-3.476
IL-9	6.035	sTNFαR1 (TNF RI)	0.263
IL-10	8.059	sTNFαR2 (TNF RII)	1.262
IL-12 p40/70	-10.624	TPO	-0.954
IL-12 p70	-1.378	VCAM-1	3.135
IL-13	-1.750	VEGF-A	6.035

C Female Abx cytokines compared to Male Abx

Serum cytokines	Female_Abx	Serum cytokines	Female_Abx
Axl	0.087	IL-17A	7.442
BLC	0.054	KC	-13.026
CD30-L	-0.409	Leptin R	-0.419
CD30	-0.202	Leptin	5.662
CD40	-1.801	LIX	11.846
CRG-2	7.108	L-Selectin	4.134
CTACK	-1.140	Ltn/XCL1	0.458
CXCL16	-0.669	MCP-1 (CCL2)	0.512
CCL11 (Eotaxin1)	7.108	MCP-5	0.437
CCL22 (Eotaxin 2)	0.750	M-CSF	0.363
FAS-L	0.567	MIG (CXCL9)	0.029
CX3CL1 (Fractalkine)	0.015	MIP-1α (CCL3)	-0.992
G-CSF	-0.801	MIP-1γ	-2.032
GM-CSF	0.931	MIP-2	-3.562
IFNγ	-1.064	MIP-3β (CCL19)	-0.946
IGFBP-3	-0.278	MIP-3α (CCL20)	-2.084
IGFBP-5	-0.345	PF-4 (CXCL4)	2.136
IGFBP-6	-1.862	P-Selectin	-11.939
IL-1α	-2.498	RANTES (CCL5)	-3.072
IL-1β	6.921	SCF	5.396
IL-2	6.351	SDF-1α	0.596
IL-3	7.012	TARC (CCL17)	1.533
IL-3 R beta	5.776	TCA-3/CCL1	1.708
IL-4	0.319	TECK (CCL25)	1.186
IL-5	7.322	TIMP-1	0.434
IL-6	0.000	TNFα	-2.033
IL-9	8.506	sTNFαR1 (TNF RI)	0.137
IL-10	0.000	sTNFαR2 (TNF RII)	1.137
IL-12 p40/70	-11.164	TPO	-0.750
IL-12 p70	-0.765	VCAM-1	1.274
IL-13	-1.568	VEGF-A	5.699

Figure S3. **ABX-altered microbiome changes affect plasma cytokines/chemokines in a sex-specific manner at week 7 in APPPS1-21 mice. (A)** Immunoblots and heatmap analysis of fold-changes in the levels of plasma cytokines/chemokines in ABX-treated male APPPS1-21 mice relative to vehicle-treated APPPS1-21 male mice. **(B)** Immunoblots and heatmap analysis of fold-changes in the cytokine/chemokine levels in vehicle- and ABX-treated female APPPS1-21 mice relative to vehicle-treated female APPPS1-21 mice. **(C)** Heatmap comparing fold-changes in the cytokine/chemokine levels in ABX-treated female APPPS1-21 mice relative to ABX-treated male APPPS1-21 mice. *n* = 10 pooled samples per membrane.
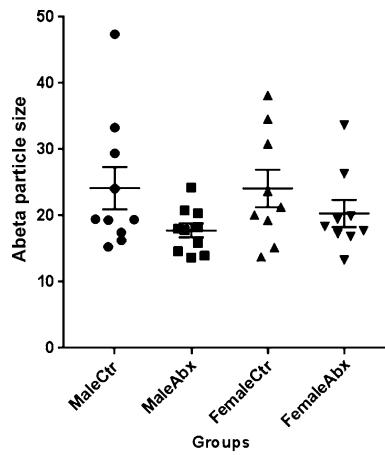
Figure S4. **Aβ plaque size analysis in the cortex of APPPS1-21 mice (*n* = 10/group).** Quantification of plaque size in vehicle- (Ctr) and ABX-treated APPPS1-21 mice using threshold-limited particle analysis of 3D6-positive staining from six sections per case. Two-way ANOVA showed significant effect of ABX treatment ($F_{[1,34]}$ = 4.424, P = 0.0429) but no effect of sex ($F_{[1,34]}$ = 0.274, P = 0.0604) or ABX treatment and sex interaction ($F_{[1,34]}$ = 0.2899, P = 0.5938). Post hoc comparison showed no significant differences among any groups, suggesting trends of reduction in Aβ plaque size with ABX-treated males compared with vehicle-treated males. Data are mean ± SEM.

Table S1. **ANCOM significant differences in fecal microbiota at P22 (FDR P < 0.05)**

	Higher in Male	Higher in Female
M_Vehicle vs. F_Vehicle		
ESV	**NS**	**NS**
L7 clustered taxa	NS	NS
M_ABX vs. F_ABX		
ESV	NS	NS
L7 clustered taxa	NS	NS
	Higher in M_ABX	**Lower in M_ABX**
M_Vehicle vs. M_ABX		
ESV	p_Proteobacteria; c_Gammaproteobacteria; f_Moraxellaceae; g_Acinetobacter	p_Bacteroidetes; c_Bacteroidia; f_S24-7; g_; s_ (×2)
	p_Proteobacteria; c_Alphaproteobacteria; f_mitochondria	p_Bacteroidetes; c_Bacteroidia; f_[Paraprevotellaceae]; g_[Prevotella]; s_
	p_Proteobacteria; c_Betaproteobacteria; f_Comamonadaceae	
	p_Proteobacteria; c_Gammaproteobacteria; o_Pseudomonadales; f_Pseudomonadaceae	
L7 clustered taxa	p_Bacteroidetes;c_Flavobacteriia;f_[Weeksellaceae]; g_Cloacibacterium;s_	p_Bacteroidetes;c_Bacteroidia;f_Bacteroidaceae; g_Bacteroides;s_ovatus
	p_Firmicutes;c_Bacilli;f_Lactobacillaceae;g_Lactobacillus;_	p_Bacteroidetes;c_Bacteroidia;f_Prevotellaceae;g_Prevotella; s_
	p_Firmicutes;c_Clostridia;f_Lachnospiraceae;g_;s_	p_Bacteroidetes;c_Bacteroidia;f_Rikenellaceae;g_AF12;s_
	p_Firmicutes;c_Clostridia;f_Ruminococcaceae;g_Oscillospira; s_	p_Bacteroidetes;c_Bacteroidia;f_[Paraprevotellaceae];g_[Prevotella];s_
	p_Proteobacteria;c_Alphaproteobacteria;f_mitochondria;_;_	
	p_Proteobacteria;c_Betaproteobacteria;f_Comamonadaceae; _;_	
	p_Proteobacteria;c_Gammaproteobacteria;f_Moraxellaceae; g_Acinetobacter;_	
	p_Proteobacteria;c_Gammaproteobacteria; f_Pseudomonadaceae;_;_	
	p_Proteobacteria;c_Gammaproteobacteria; f_Pseudomonadaceae;g_Pseudomonas;_	
	Higher in F_ABX	**Lower in F_ABX**
F_Vehicle vs. F_ABX		
ESV	p_Firmicutes; c_Bacilli; o_Lactobacillales; f_Lactobacillaceae; g_Lactobacillus	p_Bacteroidetes; c_Bacteroidia; f_S24-7; g_; s_ (×2)
	p_Proteobacteria; c_Alphaproteobacteria; o_Rickettsiales; f_mitochondria	p_Bacteroidetes; c_Bacteroidia; f_Prevotellaceae;
	p_Bacteroidetes; c_Flavobacteriia; o_Flavobacteriales; f_[Weeksellaceae]; g_Cloacibacterium; s_	p_Bacteroidetes; c_Bacteroidia; f_[Paraprevotellaceae]; g_[Prevotella]; s_
		p_Bacteroidetes; c_Bacteroidia; f_Porphyromonadaceae; g_Parabacteroides; s_
		p_Bacteroidetes; c_Bacteroidia; f_[Odoribacteraceae]; g_Odoribacter; s_

Table S1. **ANCOM significant differences in fecal microbiota at P22 (FDR P < 0.05) (*Continued*)**

ESV	NS	NS
L7 clustered taxa	p_Bacteroidetes;c_Flavobacteriia;f_[Weeksellaceae]; g_Cloacibacterium;s_	p_Bacteroidetes;c_Bacteroidia;f_Bacteroidaceae; g_Bacteroides;s_ovatus
	p_Cyanobacteria;c_Chloroplast;o_Streptophyta;f_;g_;s_	p_Bacteroidetes;c_Bacteroidia;f_Prevotellaceae;g_Prevotella; s_
	p_Proteobacteria;c_Alphaproteobacteria;f_mitochondria;_;_	p_Bacteroidetes;c_Bacteroidia;f_Rikenellaceae;g_;s_
	p_Proteobacteria;c_Gammaproteobacteria;f_Moraxellaceae; g_Acinetobacter;_	p_Bacteroidetes;c_Bacteroidia;f_Rikenellaceae;g_AF12;s_
		p_Bacteroidetes;c_Bacteroidia;f_S24-7;g_;s_
		p_Bacteroidetes;c_Bacteroidia;f_[Odoribacteraceae]; g_Odoribacter;s_
		p_Bacteroidetes;c_Bacteroidia;f_[Paraprevotellaceae];g_ [Prevotella];s_
		p_Firmicutes;c_Bacilli;f_Lactobacillaceae;g_Lactobacillus;_
		p_Firmicutes;c_Bacilli;f_Lactobacillaceae;g_Lactobacillus;s_
		p_Firmicutes;c_Clostridia;o_Clostridiales;_;_;_
		p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
		p_Firmicutes;c_Clostridiaf_Clostridiaceae;g_Candidatus Arthromitus;s_
		p_Firmicutes;c_Clostridia;f_Lachnospiraceae;_;_
		p_Firmicutes;c_Clostridia;f_Ruminococcaceae;g_Oscillospira;s_
		p_Firmicutes;c_Clostridia;f_Ruminococcaceae; g_Ruminococcus;s_
		p_Proteobacteria;c_Epsilonproteobacteria; f_Helicobacteraceae;g_Helicobacter;s_

To determine the taxa that described the most variance between groups, ANCOM analyses of 16S rRNA sequencing data at the DADA2-inferred ESV level (taxonomy assigned via Greengenes) and clustered species levels were performed between vehicle- or ABX-treated groups at P22 in a sex-dependent manner. F_ABX, ABX-treated female mice; F_Vehicle, vehicle-treated female mice; M_ABX, ABX-treated male mice; M_Vehicle, vehicle-treated male mice. *n* = 10/group.

References

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