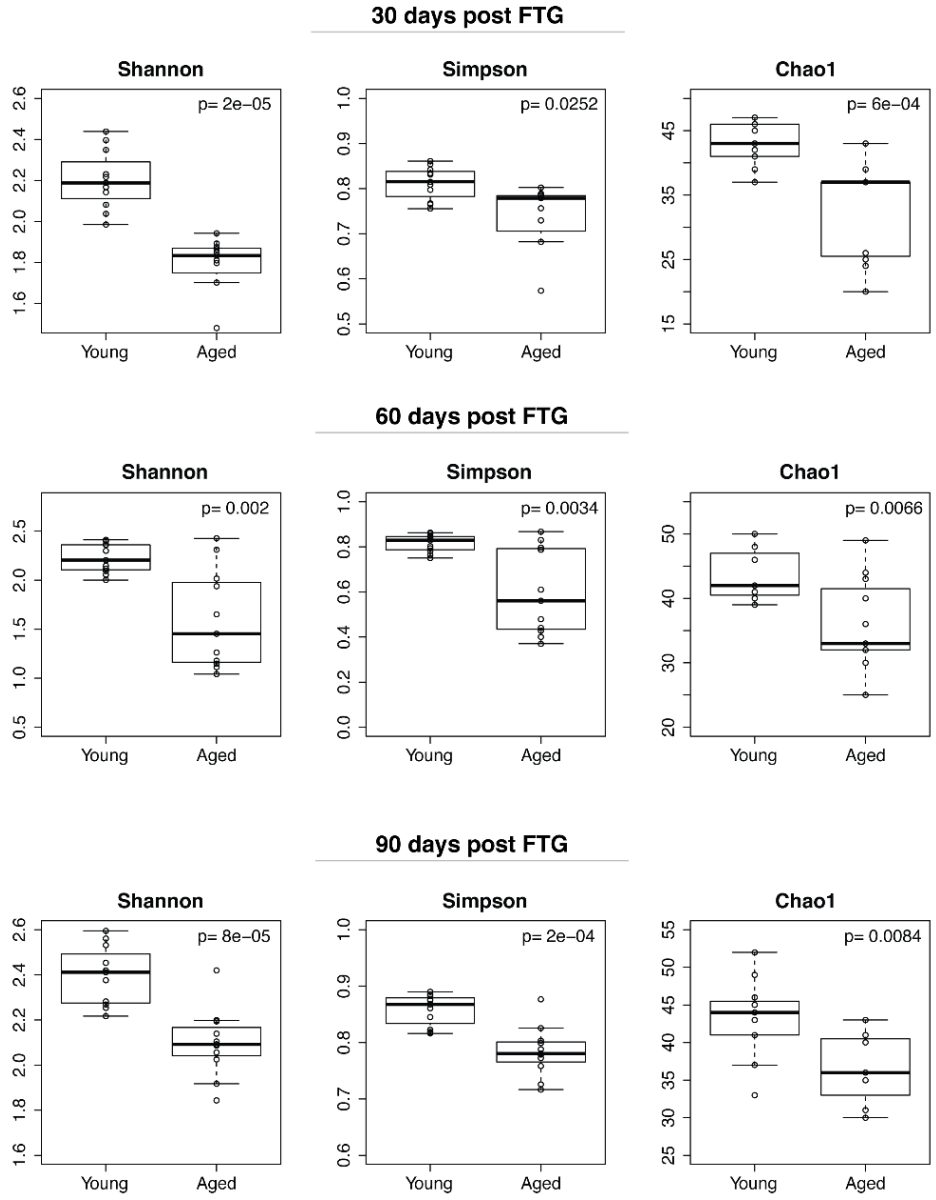
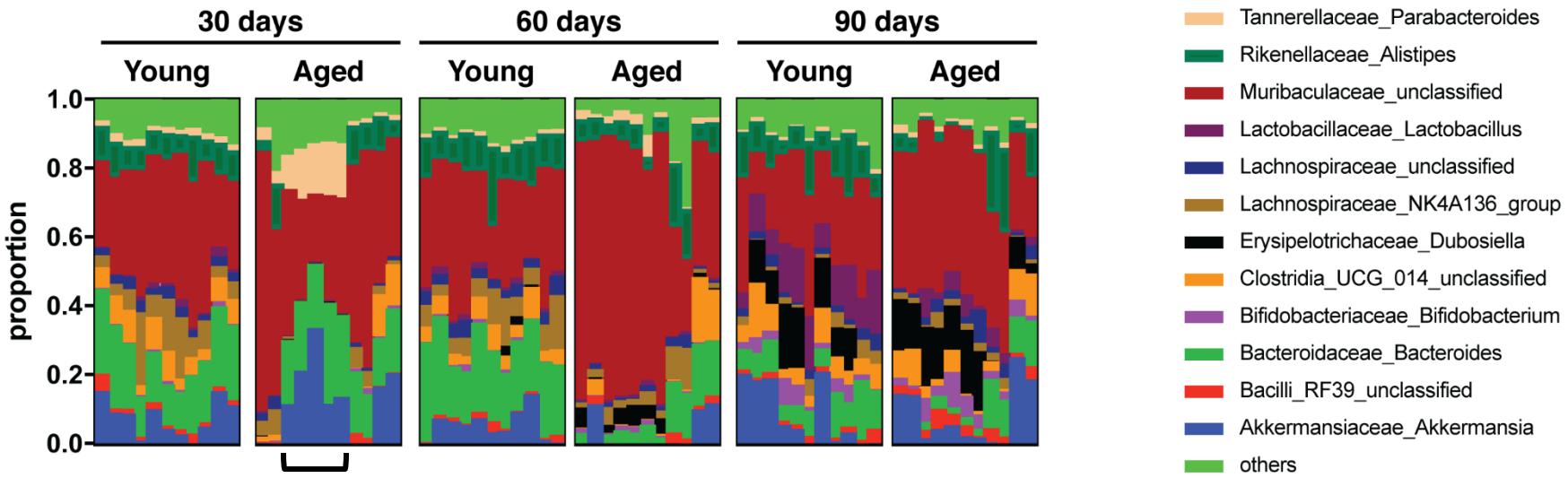


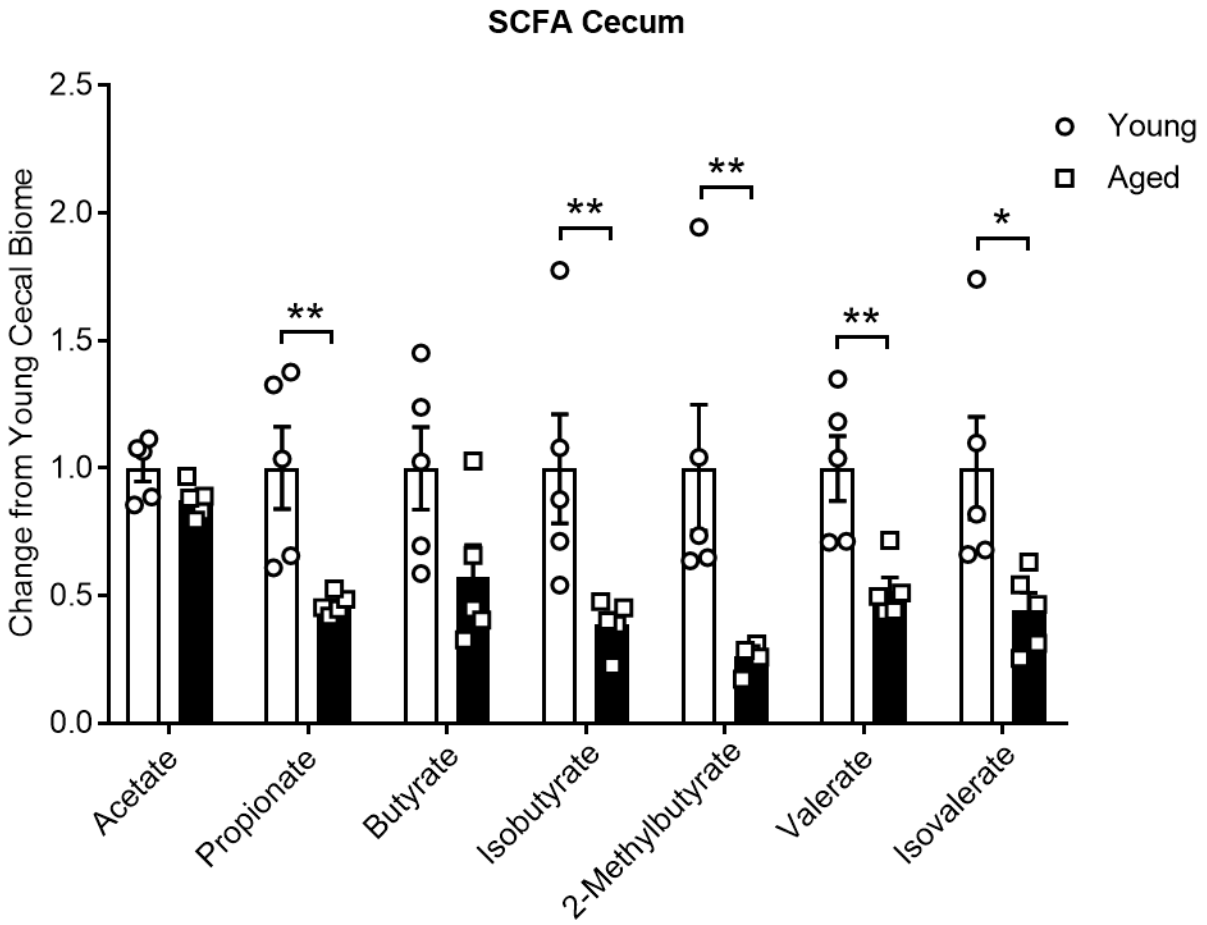
Supplemental Figure I. Alpha diversities (Shannon, Simpson, Chao1) at 30, 60, and 90 post-fecal transfer gavage (FTG). Data was analyzed using the t test with the adjusted P values in the upper right corner of each graph (N=11 per group). The Shannon and Simpson indices take into account both richness and evenness; Chao1 index only takes diversity into account.



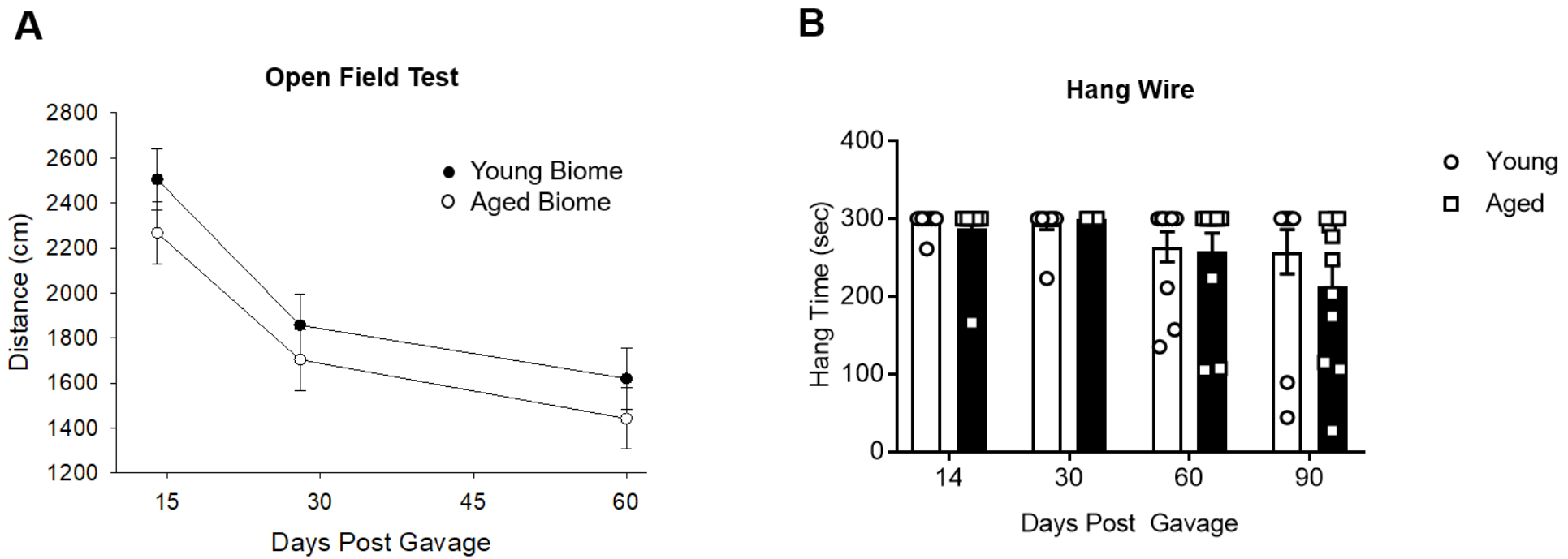
Supplemental Figure II: Stacked bar plots of taxa relative abundance for individual mice. Only taxa having >1% abundance in at least one group were included. The bracket below the aged group at 30 days represents a subset that separates from the others individuals in the same group (see text).

		Main Effects of 2 way ANOVA		
Figure	Analysis for	Microbiome	Days after FTG	Microbiome x Days after FTG
Figure 5	Acetate	$F_{1,20} = 9.989$, P=0.005	$F_{1,20} = 0.511$, P=0.483	$F_{1,20} = 0.0975$, P=0.758
Figure 5	Propionate	$F_{1,20} = 25.026$, P<0.001	$F_{1,20} = 0.990$, P=0.332	$F_{1,20} = 0.472$, P=0.500
Figure 5	Butyrate	$F_{1,20} = 8.501$, P = 0.009	$F_{1,20} = 5.173$, P = 0.034	$F_{1,20} = 4.207$, P = 0.054
Figure 5	Isobutyrate	$F_{1,20} = 3.695$, P = 0.069	$F_{1,20} = 0.0214$, P = 0.885	$F_{1,20} = 2.095$, P = 0.163
Figure 5	2-methylbutyrate	$F_{1,20} = 3.526$, P = 0.075	$F_{1,20} = 0.911$, P = 0.351	$F_{1,20} = 1.839$, P = 0.190
Figure 5	Valerate	$F_{1,20} = 3.641$, P= 0.071	$F_{1,20} = 4.282$, P = 0.052	$F_{1,20} = 14.482$, P = 0.001
Figure 5	Isovalerate	$F_{1,20} = 0.644$, P = 0.432	$F_{1,20} = 0.840$, P = 0.370	$F_{1,20} = 1.031$, P = 0.322
Figure 6	Novel Object Recognition	$F_{1,39} = 57.342$, P < 0.001	$F_{1,39} = 1.531$, P = 0.299	$F_{1,39} = 6.770$, P = 0.003
Figure 7A	Tail suspension	$F_{1,60} = 8.804$, P = 0.008	$F_{1,60} = 7.817$, P <0.001	$F_{1,60} = 2.470$, P = 0.071
Figure 7B	Barnes Maze	$F_{1,10} = 6.343$, P = 0.030	$F_{1,10} = 7.252$, P = 0.023	$F_{1,10} = 0.517$, P = 0.489
Supplemental Figure IVA	Open field	$F_{1,40} = 1.394$, P = 0.252	$F_{1,40} = 45.020$, P <0.001	$F_{1,40} = 0.108$, P = 0.898
Supplemental Figure IVB	Hang Wire	$F_{1,40} = 0.496$, P = 0.489	$F_{1,60} = 7.112$, P <0.001	$F_{1,60} = 1.057$, P = 0.374

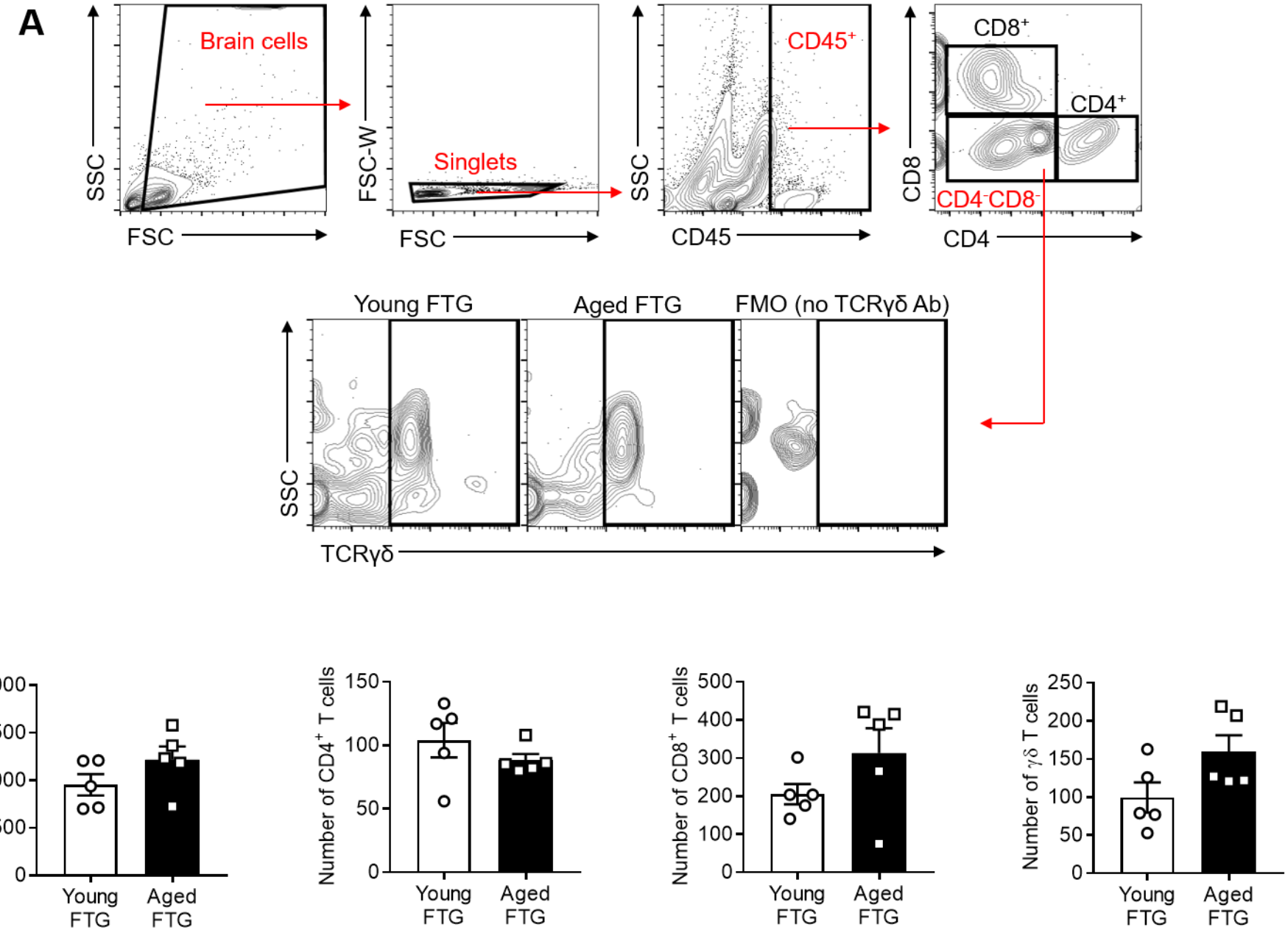
Supplemental Table I. Statistical table for all 2-way ANOVA in this study. F statistic and P value for all main effects [Microbiome, Days after FTG (fecal transfer gavage), and Microbiome X Days after FTG]. Subscript following F represents the degrees of freedom for the variables and the residuals respectively.



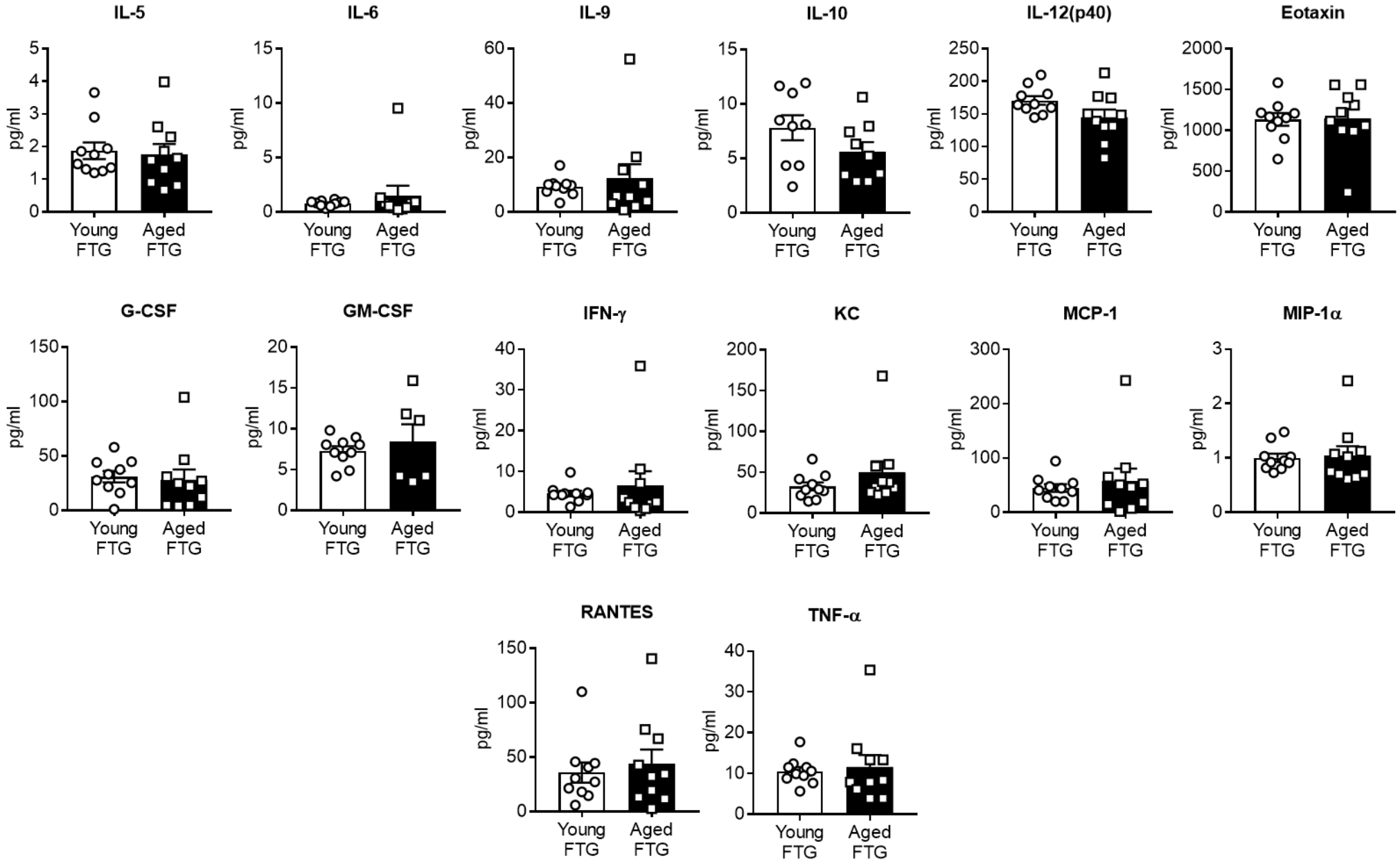
Supplemental Figure III. Changes in SCFA concentrations in cecal samples of mice with aged microbiomes relative to the concentrations in mice with young microbiomes at 90 days after initial FTG. *, ** P<0.05 and <0.01 respectively compared the young microbiome (t test, N=5 per group).



Supplemental Figure IV. (A) Open field test and (B) hang wire test. There were no significant differences between mice with a young or aged microbiome at any time point (N=11). In the Open field test there were no differences in either the total moving time or the velocity of movement between groups at any time point. See Supplemental Table I for more statistical detains.


A
Brain cells
SSC
FSC
Singlets
FSC-W
FSC
CD45+
SSC
CD45
CD8+
CD4+
CD4-CD8-
CD8
CD4
Young FTG
Aged FTG
FMO (no TCRγδ Ab)
SSC
TCRγδ
B
Number of CD45+ leukocytes
2000
1500
1000
500
0
Young FTG
Aged FTG
Number of CD4+ T cells
150
100
50
0
Young FTG
Aged FTG
Number of CD8+ T cells
500
400
300
200
100
0
Young FTG
Aged FTG
Number of γδ T cells
250
200
150
100
50
0
Young FTG
Aged FTG

Supplemental Figure V. (A) Gating strategy for flow cytometry studies from brains of mice that were removed and digested 90 days after the initial fecal transfer gavage. (B) There were no statistical differences in the number of $CD45^+$ cells (leukocyte), $CD4^+$ helper T cells, $CD8^+$ cytotoxic T cells, or γδ T cells ($CD45^+CD4^-CD8^-$TCR $\gamma\delta^+$) in brains from mice with a young and aged microbiome (n=5 per group).


IL-5
IL-6
IL-9
IL-10
IL-12(p40)
Eotaxin
G-CSF
GM-CSF
IFN-γ
KC
MCP-1
MIP-1α
RANTES
TNF-α
pg/ml
Young FTG
Aged FTG

Supplemental Figure VI. Plasma cytokine profiles at 90 days after the initial fecal transfer gavage in mice with a young or aged microbiome (N=6-10 per group). There were no significant differences in IL-5, IL-6, IL-9, IL-10, IL-12(p40), eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, keratinocyte chemoattractant (KC, also known as CXCL1) or, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), RANTES or TNF-α.