

Supplemental Results:

Results of 16S rRNA analysis after the filtering procedure of taxonomic data

One sample was removed after NG-Tax pipeline, because of its low number of final total reads. The NG-Tax sequencing results were represented by a total of 1 700 925 reads corresponding to 2 186 OTUs which represented 176 genera (Figure S1). After filtering, the taxonomic data was represented by 1 659 853 reads corresponding to 1 965 OTUs within 77 genera. Removed genera (56.25%) were represented by 2.4% of the total reads and 0.18% of total relative abundance. One sample was removed due to having less than 10% of the total observed genera.

Sequencing depth comparison

After filtering of the taxonomic data, the total reads were compared between the groups. This comparison showed that the sequencing depth was evenly distributed among all groups; ADHD (mean $\text{rank}_{\text{ADHD}}=52.51$; Median_{ADHD}=12 888), controls (mean $\text{rank}_{\text{controls}}=51.81$; Median_{controls}=13 189) and subthreshold ADHD (mean $\text{rank}_{\text{subthreshold}}=51.20$; Median_{subthreshold}=10 503; $H=0.25$, $P=0.988$) (Table S1).

Supplemental Tables and Figures

Figure S1. Filtering procedure applied in our study.

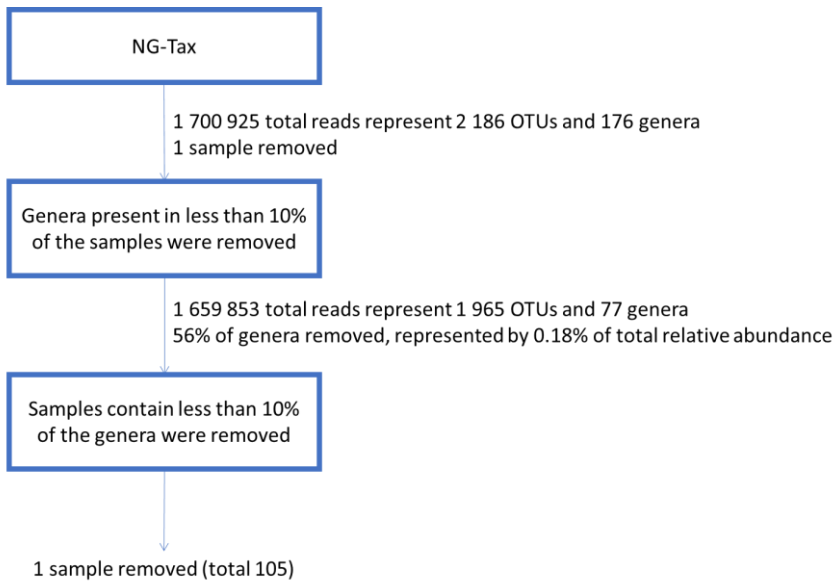


Figure S2. Alpha diversity comparison of the gut microbiome between participants with ADHD and controls. Alpha diversity was indicated by observed OTUs (P=0.08), Shannon index (P=0.16) and Phylogenetic diversity (P=0.15). Box plots represent median with whiskers on ± 1.5 IQR.

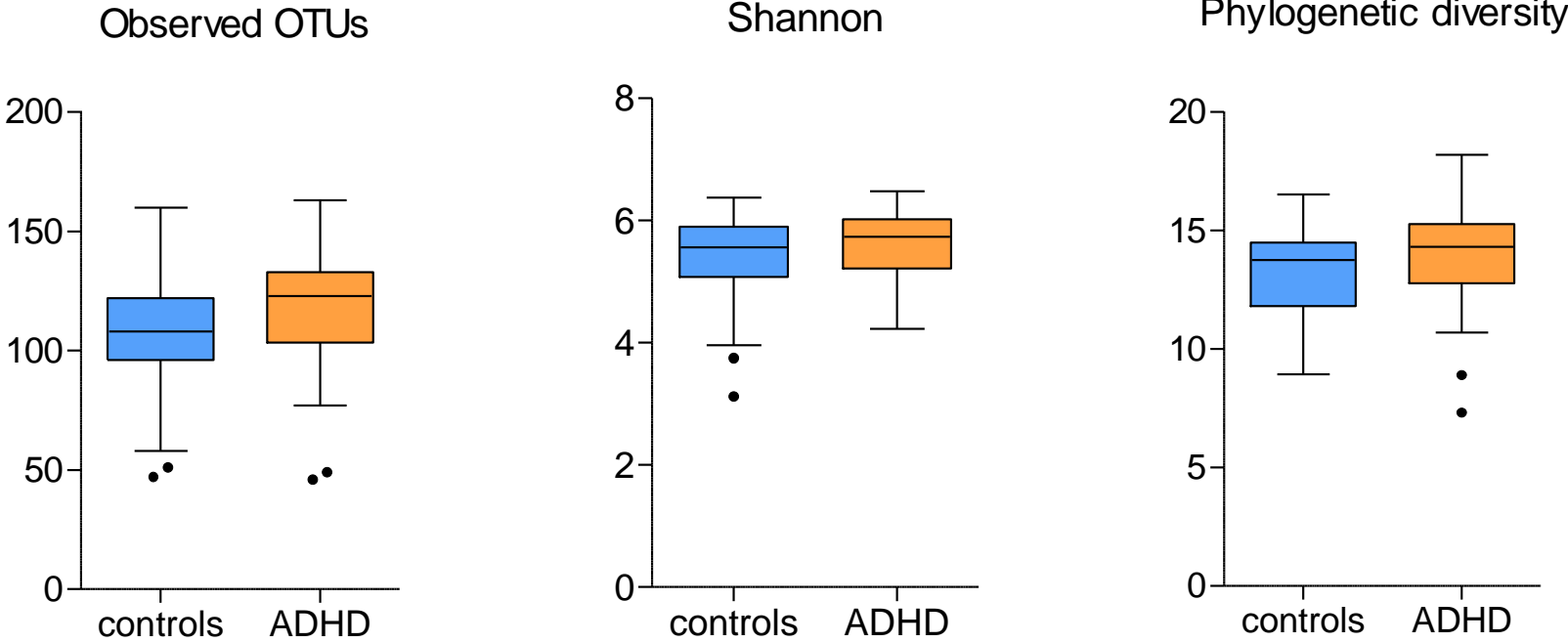


Figure S3. Principal coordinates analysis (PCoA) of weighted UniFrac distances representing microbial composition of participants with ADHD and controls. The first two components are plotted with the percentage of the variance explained by each principal component. Each point represents an individual sample.

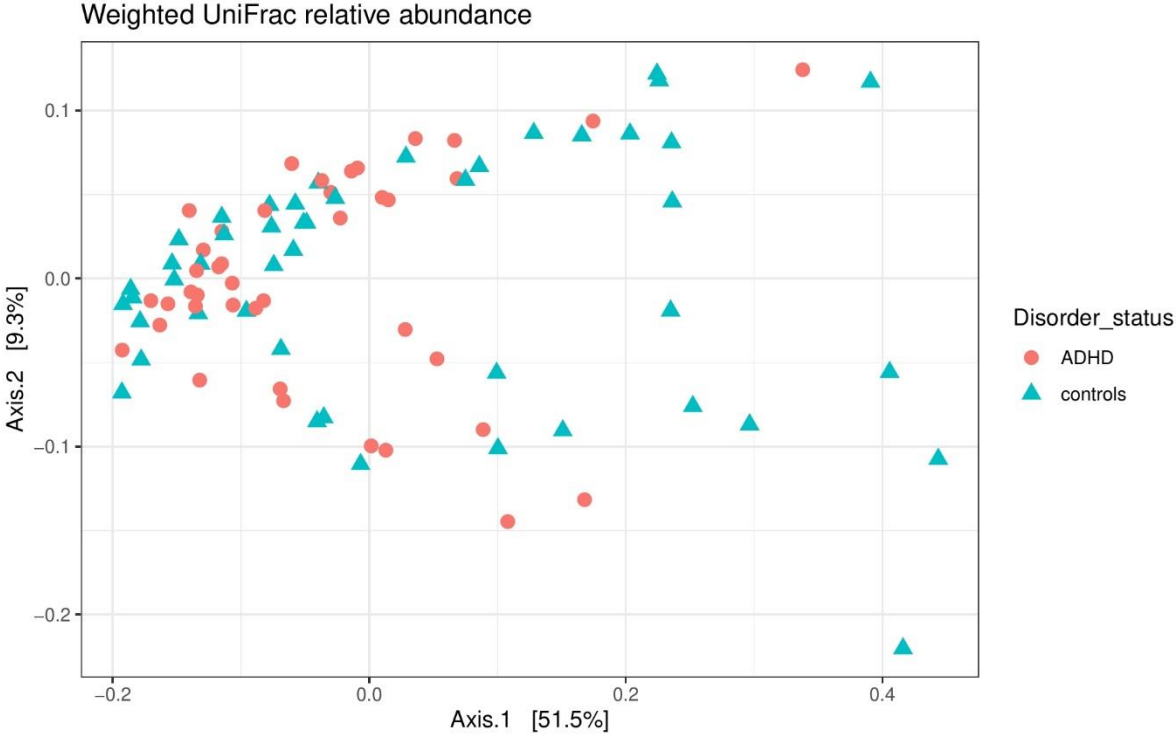
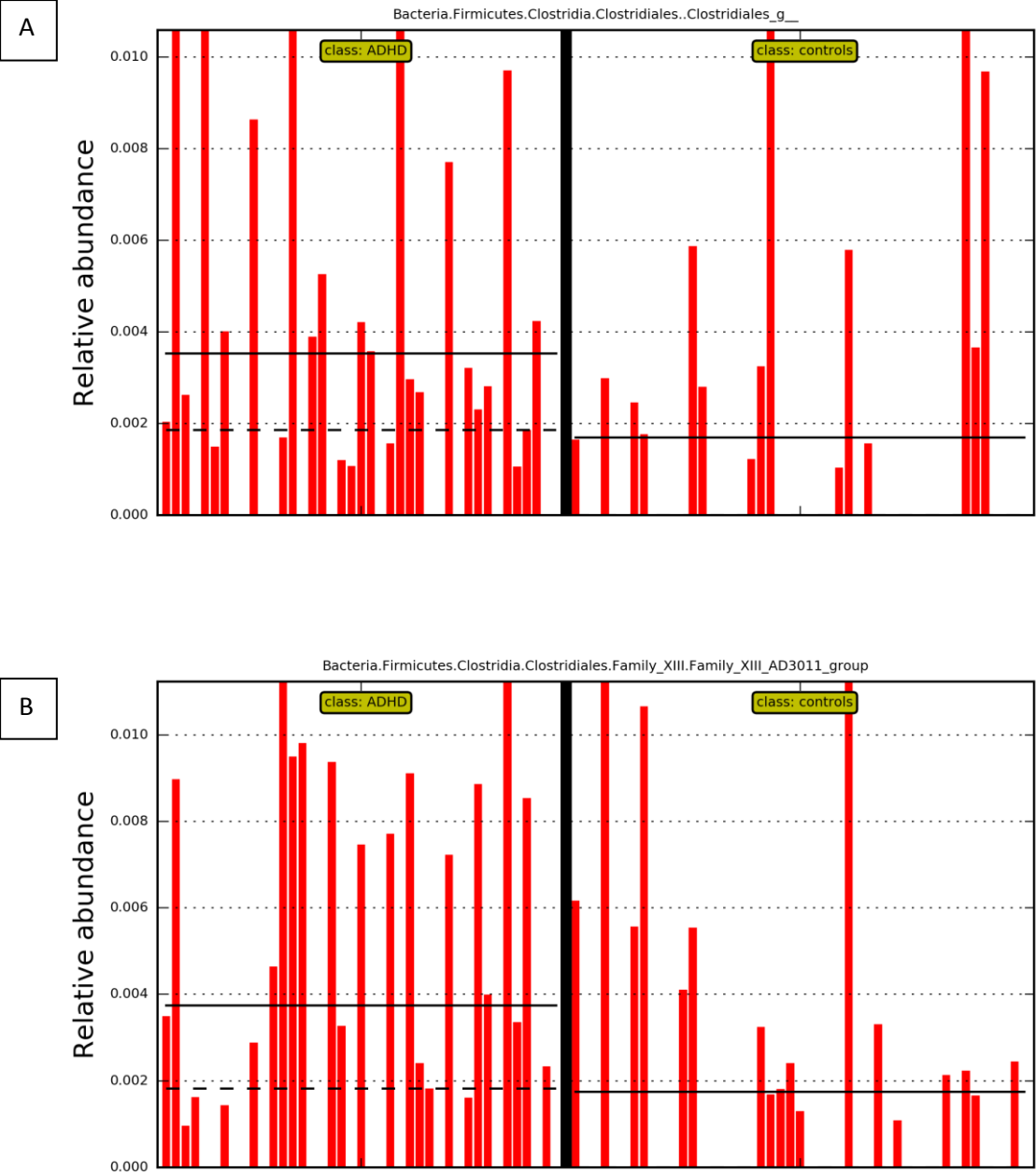
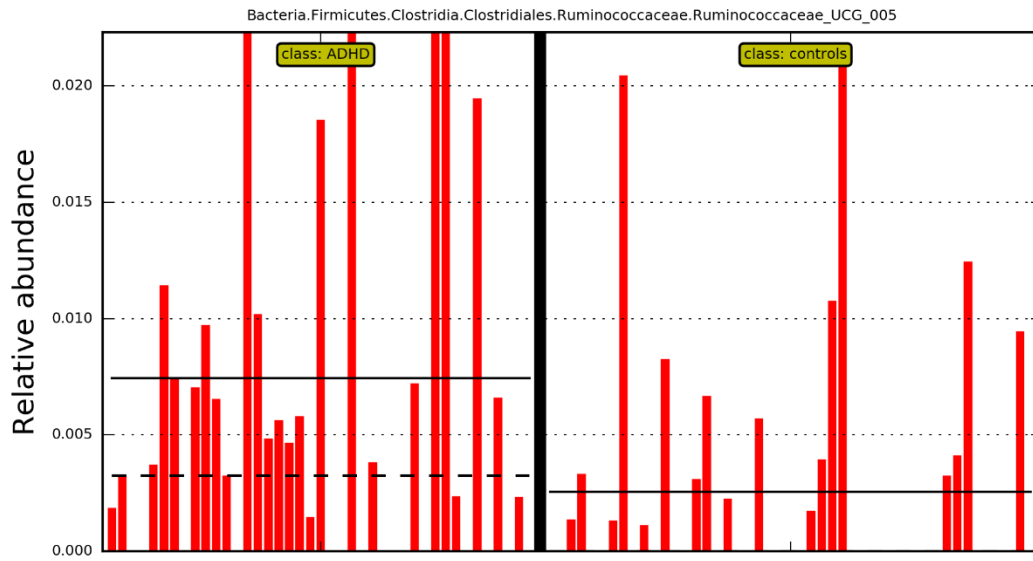


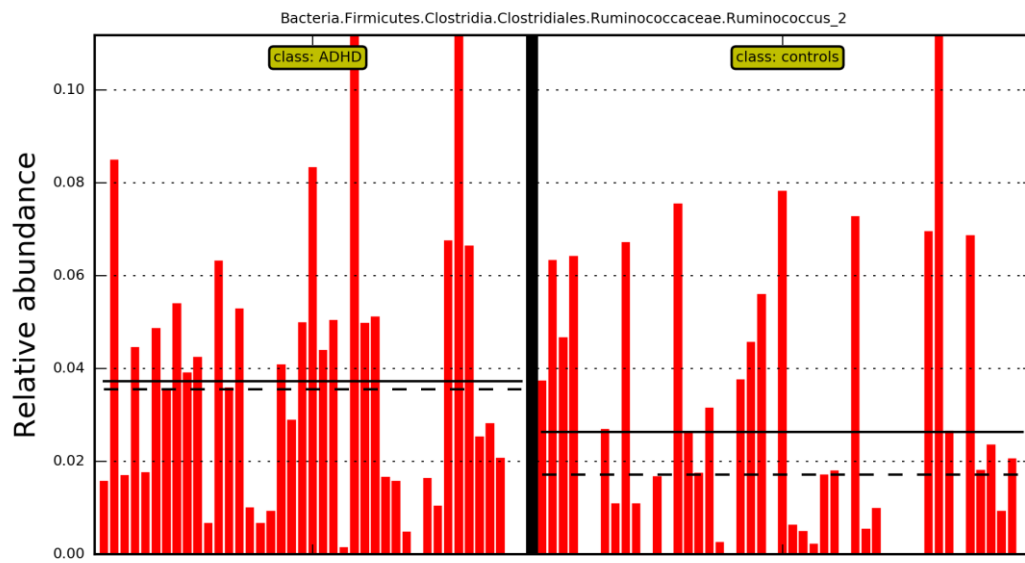
Figure S4. Distribution of the selected genera for behavioral analysis for each group (ADHD samples on the left and controls on the right). Barplots of *Clostridiales_g__* (A), *Family_XIII_AD3011_group* (B), *Ruminococcaceae_UCG_005* (C), *Ruminococcus_2* (D), *Ruminococcaceae_g__* (E), *Ruminococcaceae_NK4A214_group* (F) and *Ruminococcaceae_UCG_004* (G). Each bar represents the sum to 1 of the taxa relative abundance for an individual sample; straight line represents mean and dotted line represents median.

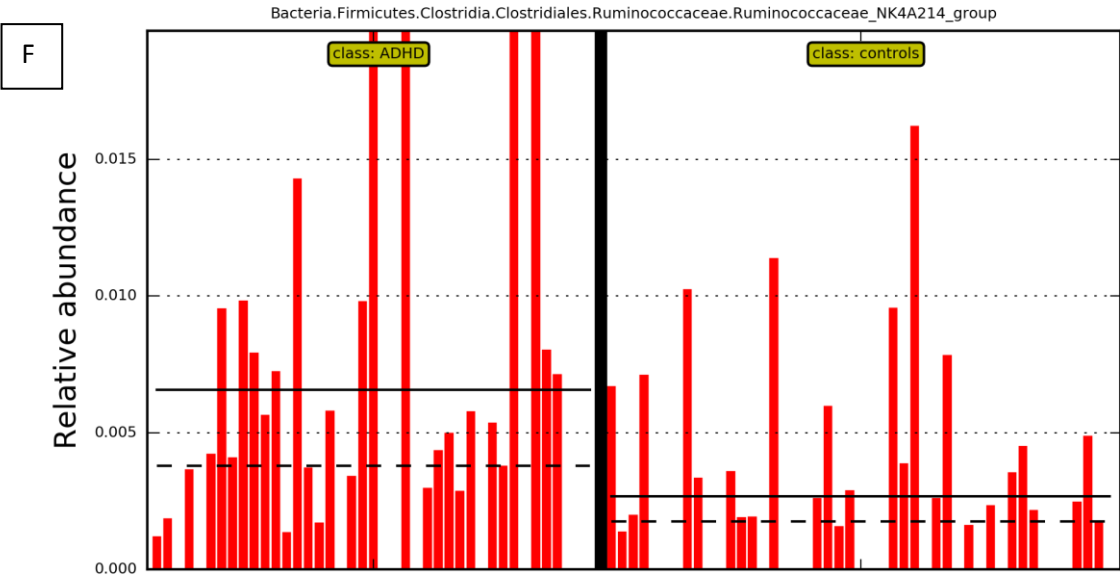
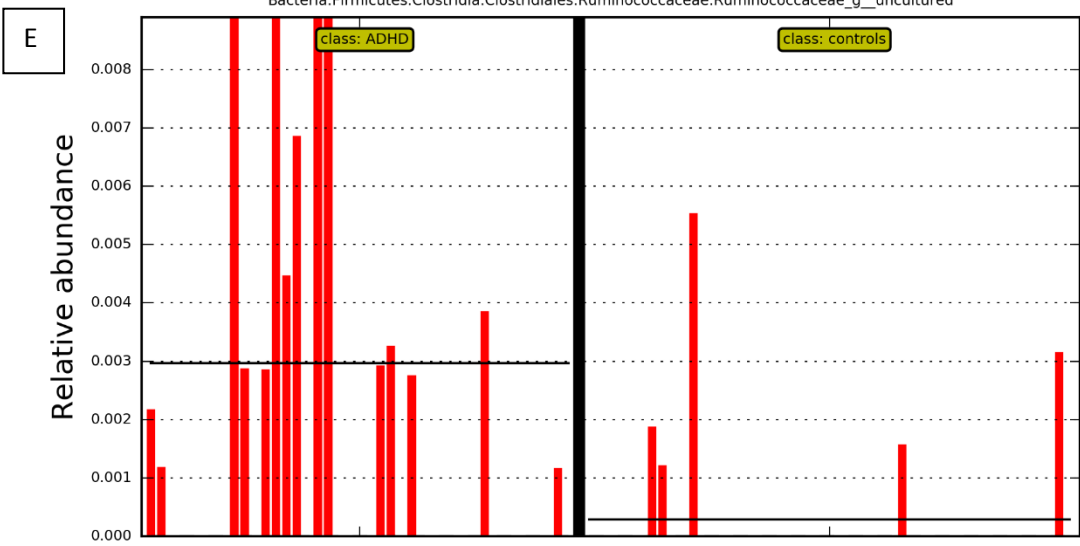


C



D





G

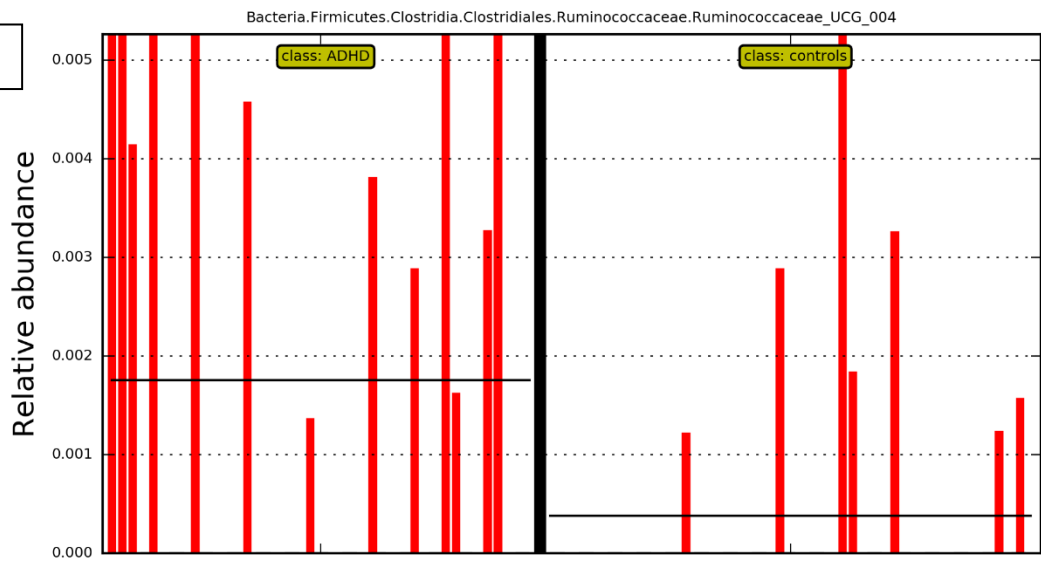
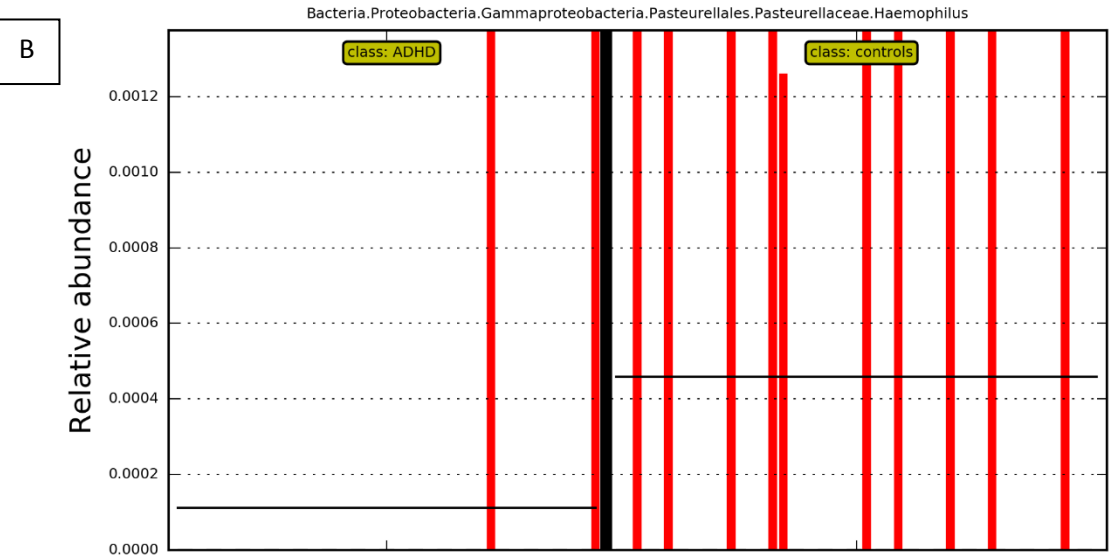
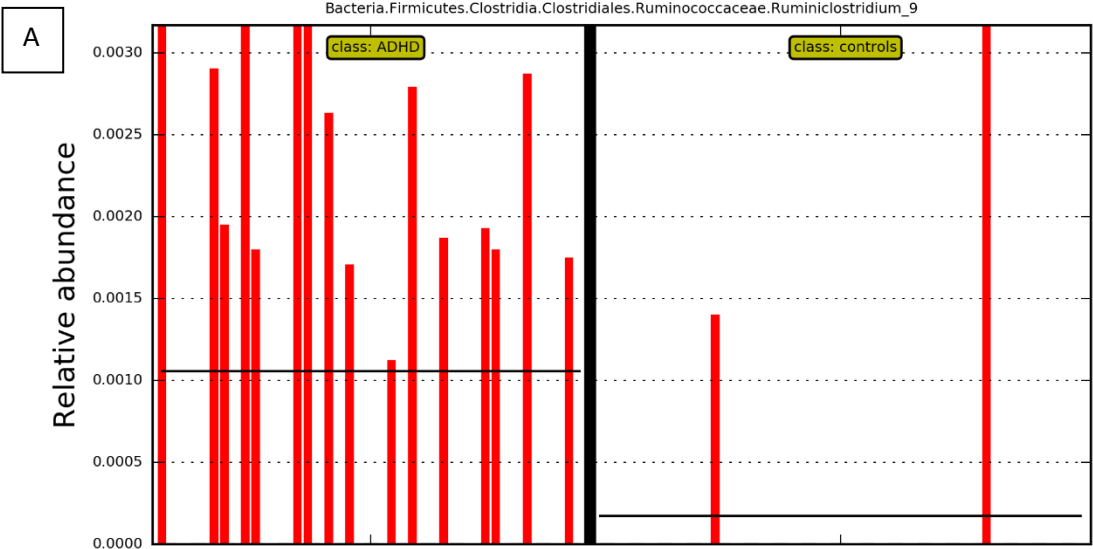


Figure S5. Distribution of the non-selected genera for behavioral analysis for each group (ADHD samples on the left and controls on the right). Barplots of *Ruminiclostridium_9* (A), *Haemophilus* (B) and *Ruminococcaceae_UCG-003* (C). Each bar represents the sum to 1 of the taxa relative abundance for an individual sample; straight line represents mean and dotted line represents median.



C

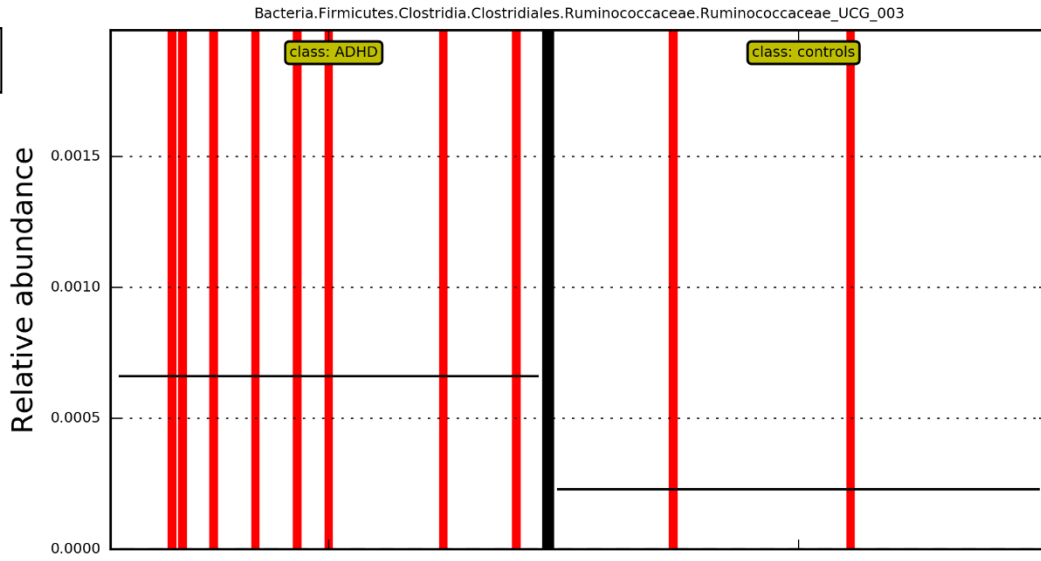


Figure S6. Boxplot of two genera relative abundance being different between medicated and non-medicated participants with ADHD. Box plots represent median with whiskers on ± 1.5 IQR.

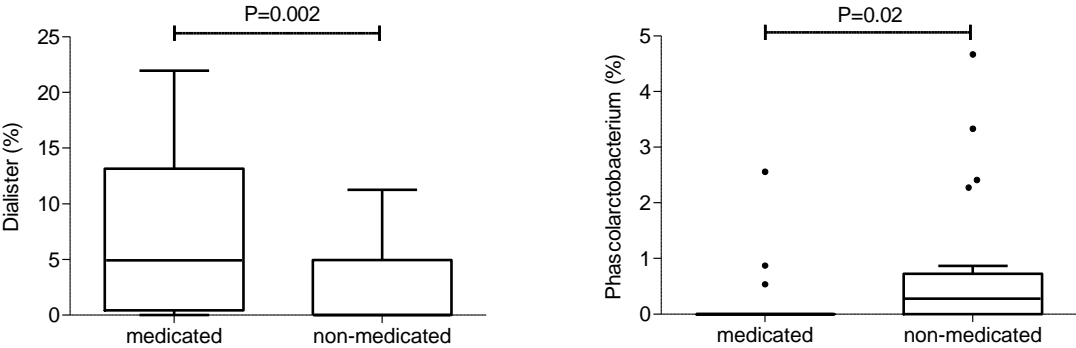


Figure S7. Spearman's rank correlation matrix of the selected bacterial taxa. The selection of the genera was done prior to correlation analysis and it was done based on their prevalence (see the method section). The matrix represents only nominal significant ($P < 0.05$) correlations.

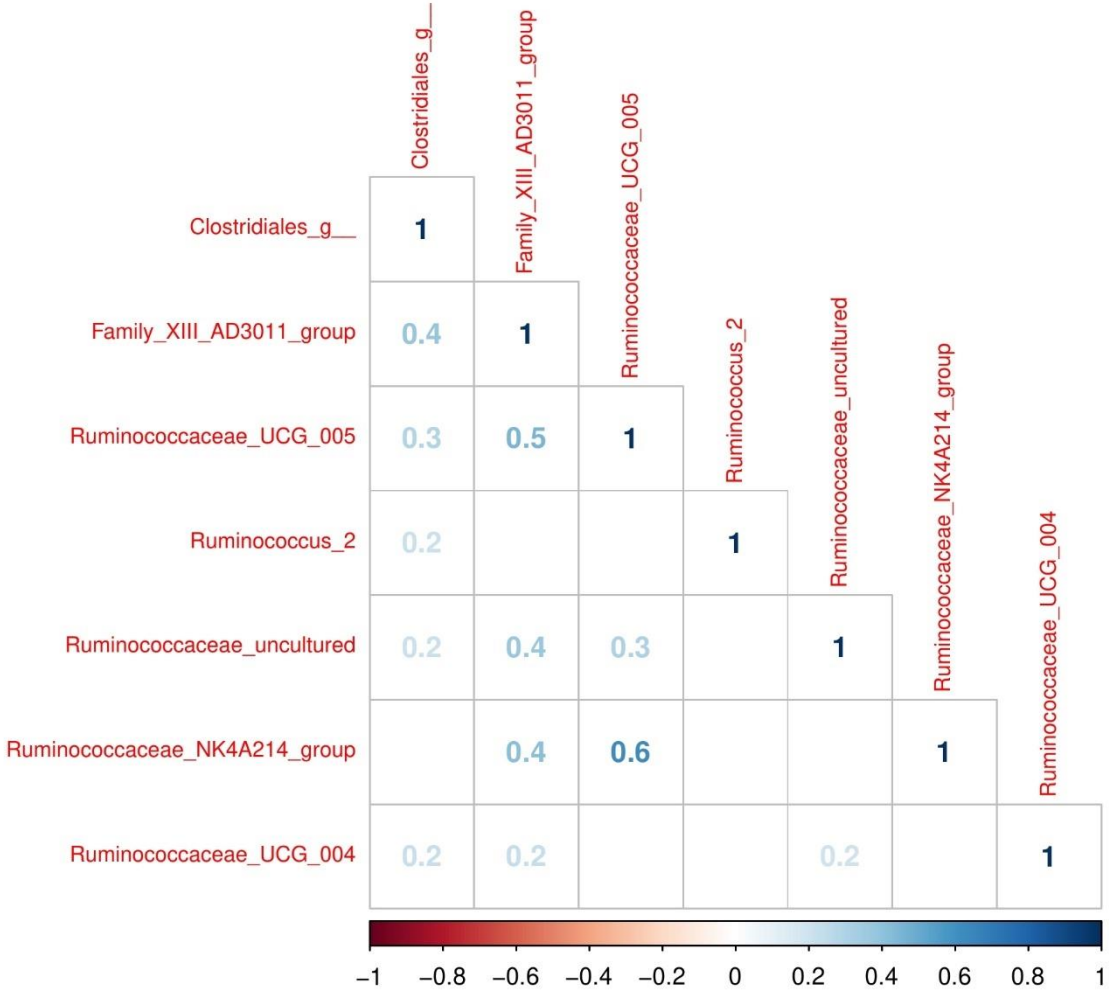


Table S1. Total number of reads of participants with ADHD, controls and subthreshold ADHD.

	Total number of reads			
	N	Median (IQR)	Mean rank ^a	P-value ^a
ADHD	41	12888 (6041 – 21569)	52.51	
controls	47	13819 (7605 – 21413)	51.81	.988
subthreshold ADHD	15	10503 (6280 -21290)	51.20	

^a calculated based on the Kruskal-Wallis Test

Table S2. Relative abundance of bacterial phyla of all participants in our study.

Phylum's relative abundance and FBR					
	ADHD median (IQR)	ADHD Mean rank ^a	controls median (IQR)	controls Mean rank ^a	P-values ^a
<i>Firmicutes</i>	77.25 (71.63 – 87.06)	49.63	78.85 (52.19 – 83.04)	40.02	0.078
<i>Bacteroidetes</i>	16.91 (8.56 – 25.63)	40.37	18.21 (10.93 – 47.49)	48.11	0.156
<i>Actinobacteria</i>	0.83 (.25 – 2.35)	45.98	0.71 (0.12 – 2.61)	43.21	0.612
<i>Proteobacteria</i>	0.14 (0 – 0.64)	46.06	0.18 (0 – .43)	43.14	0.579
<i>Verrucomicrobia</i>	0.11 (0 – 0.33)	44.26	0.11 (0 – .51)	44.71	0.929
FBR	4.61 (2.84 – 9.78)	47.70	4.44 (1.10 – 7.89)	40.85	0.208

N=88; ^a calculated based on the Mann-Whitney U test; FBR = *Firmicutes* to *Bacteroidetes* ratio; IQR = interquartile range

Table S3. Multiple regression model with the selected genera on inattention.

	Inattention			Results taken from Table3 ^b	
	B (S.E.)	95% CI	P-value	B (S.E.)	P-value
<i>Clostridiales_g__</i>	-4.312 (3.571)	-11.143 - 3.037	0.231	-1.467 (3.077)	0.634
<i>Family_XIII_AD3011_group</i>	-0.269 (4.663)	-8.888 – 8.423	0.954	5.323 (2.779)	0.059
<i>Ruminococcaceae_UCG_005</i>	-1.509 (3.053)	-7.363 - 4.205	0.622	1.495 (1.647)	0.367
<i>Ruminococcus_2</i>	1.525 (0.454)	0.675 - 2.362	0.001	1.098 (0.445)	0.016
<i>Ruminococcaceae_g__uncultured</i>	6.081 (6.305)	-5.601 - 18.013	0.338	12.241 (5.011)	0.017
<i>Ruminococcaceae_NK4A214_group</i>	4.279 (2.651)	-0.805 - 9.481	0.111	3.392 (1.860)	0.072
<i>Ruminococcaceae_UCG_004</i>	43.920 (13.348)	19.372 - 69.095	0.001	39.291 (12.296)	0.002

The model includes all selected genera in one model; The identified outliers were removed from this analysis, N=89; The model was adjusted for age, sex, BMI, diff_days and a random factor for family relatedness; ^b Results from simple regression models (taken from Table 3 for the purpose of easier comparison).