

## Supplementary Figure legends

### Supplementary Figure S1. Relative fungal abundance based on taxa levels.

ITS sequencing of fecal samples from 11 non-alcoholic controls, 15 patients with alcohol use disorder and 59 alcoholic hepatitis patients. The graphs demonstrate the mean relative abundance of sequence reads for each group based on the (A) Phylum level, (B) Class level, (C) Order level and (D) Family level. 0-1 corresponds to 0-100% abundance. Shown are only taxa that cover in total at least 95% abundance of all genera. In (A-D), the standard error of the mean is shown as error bar. Kruskal-Wallis test for nonparametric data and Dunn post-hoc test,  $*p < 0.05$ .

### Supplementary Figure S2. Mycobiome and ASCA among different disease stages in alcohol use disorder

Alcohol use disorder patients were grouped according to their non-invasive FIB-4 Index in low (FIB-4  $< 1.45$ ,  $n=4$ ), intermediate (FIB-4  $1.45-3.25$ ,  $n=6$ ) and high (FIB-4  $> 3.25$ ,  $n=4$ ) fibrosis subgroups. (A-C) Fungal diversity. Kruskal-Wallis test for nonparametric data and Dunn post-hoc test,  $*p < 0.05$   $p > 0.01$ . (D-E) Heat map representing color-coded spearman's correlations of clinical parameters. Red color indicates positive- and blue color negative correlation. All variables are coded from low to high. In (E) and (F), 38 alcohol use disorder patients were included. (G) The graphs demonstrate the mean relative abundance of sequence reads in each genus for each group. 0-1 corresponds to 0-100% abundance. A total of 81 different genera were detected. In (H), the standard error of the mean is shown as error bar. In (G-H), 4/6/4 patients are included in the groups, respectively. ASCA, anti-*Saccharomyces cerevisiae* antibodies. INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl-transferase; AP, alkaline phosphatase; BMI, body mass index; FIB-4, fibrosis-4 Index; ns, not significant.

### Supplementary Figure S3. LPS-BP is increased in alcoholic hepatitis patients

(A) LPS-BP levels were compared and correlated with ASCA levels (C) in 23 non-alcoholic controls, 40 patients with alcohol use disorder and 156 alcoholic hepatitis

patients. (B) Longitudinal analysis of LPS-BP in 24 alcoholic hepatitis patients, measurement at day zero (D0, after admission) and around ninety-day follow-up visit (D90, follow-up measurement around ninety days after day zero, 18 of the 24 samples were collected at day-90, 6 had a longer follow up (between 90 and 154 days)). LPS-PB, lipopolysaccharide binding protein; ns, not significant.

**Supplementary Figure S4. Longitudinal intestinal microbiota changes in alcoholic hepatitis patients.**

Seven alcoholic hepatitis patients provided feces samples at 90 days following their initial presentation. Each point represents one patient. D0, measurement at day zero (after admission), D90, follow-up measurement ninety days after day zero. Paired t-test. \* $p < 0.05$   $p > 0.01$ , \*\* $p < 0.01$   $p > 0.001$ . (A) Albumin. (B) AST. (C) MELD score. (D) Fecal *Candida* (mean relative abundance, 0-1 corresponds to 0-100% abundance). (E) Shannon-Index. (F) Simpson-Index. AST, aspartate aminotransferase, MELD, Model for End-stage Liver Disease, ns, not significant.

## Supplementary tables

**Supplementary Table S1:** Comparison of the principal components

Principal Component	<i>p</i> values	Post hoc <i>p</i> values		
		Ctrl vs AH	Ctrl vs AUD	AH vs AUD
PC1	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.539
PC2	<b>0.014</b>	<b>0.005</b>	<b>0.010</b>	0.711
PC3	<b>0.004</b>	<b>0.006</b>	<b>&lt;0.001</b>	0.145
PC4	0.559			

One-Way ANOVA with Tukey's post-hoc test. Bold font indicates significance (*p* value <0.05). PC, principal component; Ctrl, non-alcoholic controls; AUD, alcohol use disorder; AH, alcoholic hepatitis.

**Supplementary Table S2: Spearman correlation of fungal genera and clinical variables in alcoholic hepatitis patients**

Variable	Genus	r value	p value	Variable	Genus	r value	p value	
<b>Demographics</b>				Platelet Count	<i>Alternaria</i>	0.359	0.005	
Age	<i>Alternaria</i>	-0.281	0.031		<i>Aureobasidium</i>	0.039	0.039	
	<i>Filobasidium</i>	-0.267	0.041		<i>Fusarium</i>	0.033	0.033	
Gender Female	<i>Alternaria</i>	0.306	0.018	Sodium	Chao Richness	0.014	0.014	
	<i>Bipolaris</i>	0.294	0.024		<i>Filobasidium</i>	0.035	0.035	
	<i>Cryptococcus</i>	0.289	0.026	Creatinine	<i>Nakaseomyces</i>	0.001	0.001	
	<i>Fusarium</i>	0.297	0.022		<i>Saccharomyces</i>	0.015	0.015	
	<i>Phoma</i>	0.364	0.005		<i>Sporobolomyces</i>	0.034	0.034	
	<i>Stemphylium</i>	0.294	0.024		<b>Liver Histology</b>			
<b>Outcome and Clinical Scores</b>				Stage of Fibrosis	<i>Diaporthe</i>	-0.338	0.047	
30 Day Mortality Rate	<i>Catenulostroma</i>	0.43	0.001		<i>Fusarium</i>	-0.338	0.047	
	<i>Sclerotinia</i>	0.43	0.001		Shannon Index	0.346	0.042	
MELD	<i>Sporobolomyces</i>	-0.286	0.029		<i>Aspergillus</i>	0.344	0.04	
	<i>Wallemia</i>	-0.345	0.008		<i>Doratomyces</i>	0.354	0.034	
	Simpson Index	-0.305	0.02		<i>Pichia</i>	0.388	0.019	
MELDNa	<i>Kluyveromyces</i>	-0.276	0.036	Lobular Fibrosis	Chao Richness	-0.346	0.045	
	<i>Sporobolomyces</i>	-0.281	0.033		<i>Diaporthe</i>	-0.411	0.016	
	<i>Wallemia</i>	-0.35	0.007		<i>Flammulina</i>	-0.411	0.016	
	Simpson Index	-0.323	0.013		<i>Fusarium</i>	-0.411	0.016	
<b>Laboratory Parameters</b>				Pericellular Fibrosis	<i>Candida</i>	0.403	0.017	
Albumin	<i>Alternaria</i>	-0.295	0.027		<i>Rhizomucor</i>	-0.477	0.004	
	<i>Epicoccum</i>	-0.278	0.038		<i>Wickerhamomyces</i>	-0.477	0.004	
	<i>Torulaspota</i>	0.275	0.04		Shannon Index	-0.338	0.047	
ALT	<i>Clavispora</i>	-0.259	0.047	Inflammatory Grade	<i>Alternaria</i>	-0.391	0.018	
	<i>Kluyveromyces</i>	0.262	0.045		<i>Aspergillus</i>	-0.391	0.018	
	<i>Wallemia</i>	0.323	0.013		<i>Cladosporium</i>	-0.342	0.041	
AST	<i>Phoma</i>	-0.294	0.024		<i>Malassezia</i>	-0.371	0.026	
	<i>Wallemia</i>	0.337	0.009		<i>Nakaseomyces</i>	-0.377	0.023	
GGT	<i>Wallemia</i>	0.447	0.017		<i>Penicillium</i>	-0.341	0.042	
	Simpson Index	0.457	0.015		<i>Pichia</i>	-0.379	0.022	
Alkaline Phosphatase	<i>Kluyveromyces</i>	0.258	0.049		Mallory Bodies	<i>Bionectria</i>	-0.376	0.028
	Simpson Index	0.287	0.027			<i>Cyberlindnera</i>	-0.376	0.028
INR	<i>Colletotrichum</i>	0.311	0.018	<i>Kluyveromyces</i>		-0.376	0.028	
	<i>Diaporthe</i>	0.277	0.035	<i>Malassezia</i>		-0.441	0.009	
	<i>Kluyveromyces</i>	-0.296	0.024	<i>Penicillium</i>		-0.361	0.036	
	<i>Wallemia</i>	-0.407	0.002	<i>Sporobolomyces</i>		-0.376	0.028	
Prothrombin Time	<i>Flammulina</i>	-0.286	0.047	<i>Wallemia</i>		-0.376	0.028	

	<i>Kluyveromyces</i>	-0.313	0.029	Giant Mitochondria	<i>Yamadazyma</i>	0.372	0.039
	<i>Wallemia</i>	-0.397	0.005	PMN Infiltration	<i>Cladosporium</i>	-0.341	0.045
					<i>Trichosporon</i>	-0.383	0.023

Significant Spearman Rho's correlations of clinical parameters and fungi. INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl-transferase; BMI, body mass index. MELD, Model for End-stage Liver Disease, MELDNa, Sodium Model for End-stage Liver Disease; PMN, polymorphonuclear infiltration.

**Supplementary Table S3:** Univariate linear regression log (ASCA) and clinical variables

Dependent: log (ASCA)		Estimates (univariable, SD, <i>p</i> value)	Estimates (multiivariable, SD, <i>p</i> value)
Lobular fibrosis	0	-	
	1	0.56 (-0.01-1.13, <i>p</i> =0.055)	0.92 (0.19-1.65, <b><i>p</i>=0.015</b> )*
	2	0.73 (0.06-1.40, <b><i>p</i>=0.034</b> )	0.71 (-0.17-1.59, <i>p</i> =0.109)*
	3	0.74 (0.24-1.23, <b><i>p</i>=0.004</b> )	0.85 (0.26-1.45, <b><i>p</i>=0.006</b> )*
Bilirubinostasis	0	-	
	1	-0.26 (-0.55-0.03, <i>p</i> =0.077)	-0.40 (-0.79- -0.01, <b><i>p</i>=0.046</b> )*
	2	-0.75 (-1.31- -0.20, <b><i>p</i>=0.009</b> )	-0.72 (-1.44-0.01, <i>p</i> =0.054)*
	3	0.04 (-0.32-0.41, <i>p</i> =0.812)	
Liver cirrhosis	No	-	
	Yes	0.39 (0.13-0.65, <b><i>p</i>=0.004</b> )	0.52 (0.19-0.86, <b><i>p</i>=0.003</b> )*
Antibiotic use	No	-	
	Yes	0.22 (0.02-0.43, <b><i>p</i>=0.032</b> )	0.22 (-0.00-0.44, <i>p</i> =0.051)**

\**p* value adjusted for MELD, infection, antibiotics, steroids, pentoxifylline.

\*\* *p* value adjusted for MELD, steroids, pentoxifylline. Bold font indicates significance (*p* value <0.05). Lobular fibrosis, 0 no fibrosis, 1 zone 3 (centrilobular) fibrosis, 2 zone 2+3 (midzonal) fibrosis, 3 panlobular fibrosis. Bilirubinostasis, 0 no, 1 hepatocanalicular, 2 cholangiolar, 3 both. ASCA, anti-*Saccharomyces cerevisiae* antibodies; SD, standard deviation; MELD, Model for End-stage Liver Disease.

**Supplementary Table S4:** Patient characteristics for longitudinal mycobiome analysis of alcoholic hepatitis patients (n=7)

<b>Clinical parameter</b>			
Age, years, n=7	59 (41-66)		
Body Mass Index (BMI), kg/m <sup>2</sup> , n=6	26.8 (23-32)		
Gender (male), n, n=7	6		
Abstinent at day 90, n=7			
Completely abstinent, n	6		
Reduced alcohol consumption, n	1		
Continued alcohol abuse, n	0		
<b>Histology</b>			
Liver biopsy available, n	3		
Fibrosis stage, 0/1/2/3/4, n	1/0/0/0/2		
<b>Treatment received within the 90 days</b>			
Steroids, n, n=7	3		
Pentoxifylline, n, n=7	0		
Steroids and pentoxifylline, n, n=7	0		
Antibiotics, n, n=7	1		
Prophylactic antibiotics, n, n=7	3		
Proton pump inhibitors, n, n=4	0		
Antifungals n, n=4	0		
	<b>Day-0</b>	<b>Day-90</b>	<b>p value</b>
<b>Laboratory parameter</b>			
Albumin (g/dl), n=7	2.2 (1.1-3.1)	2.9 (2.0-4.0)	<b>0.019</b>
ALT (U/l), n=7	30 (21-65)	28 (16-43)	0.17
AST (U/l), n=7	132 (57-267)	51 (29-86)	<b>0.0034</b>
Total bilirubin (mg/dl), n=7	9.6 (4.5-11.9)	2.2 (0.6-6.2)	<b>0.0047</b>
<b>Clinical score</b>			
Model for end-stage liver disease (MELD), n=7	22 (13-28)	15 (5-20)	<b>0.0094</b>

Values are presented as median and range in brackets. The number of patients for which the respective data was available is indicated in the first column. Paired t-test. Bold font indicates significance ( $p$  value <0.05). Fibrosis stage, 0 no fibrosis, 1 portal fibrosis, 2 expansive periportal fibrosis, 3 bridging fibrosis, 4 cirrhosis. INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Supplementary Table S5:** Patient characteristics for longitudinal ASCA analysis of alcoholic hepatitis patients (n=24)

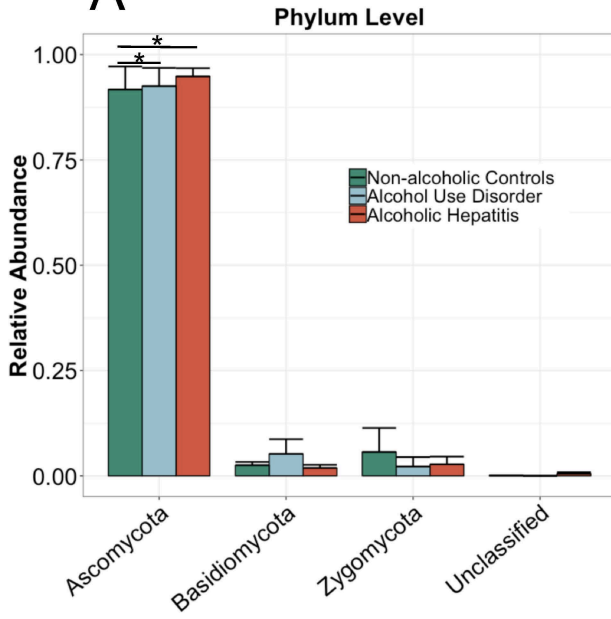
<b>Clinical parameter</b>			
Age, years, n=24	48 (29-65)		
Body Mass Index (BMI), kg/m <sup>2</sup> , n=22	26.3 (19-39)		
Gender (male), n, n=24	16		
Abstinent at day 90, n=22			
Completely abstinent, n	21		
Reduced alcohol consumption, n	1		
Continued alcohol abuse, n	0		
<b>Histology</b>			
Liver biopsy available, n,	19 (79)		
Fibrosis stage, 0/1/2/3/4, n	7/0/3/5/9		
<b>Treatment received within the 90 days</b>			
Steroids, n, n=22	2		
Pentoxifylline, n, n=20	1		
Steroids and pentoxifylline, n, n=20	0		
Antibiotics, n, n=22	4		
Prophylactic antibiotics, n, n=24	9		
Proton pump inhibitors, n, n=12	3		
Antifungals n, n=12	0		
	<b>Day-0</b>	<b>Day-90</b>	<b>p value</b>
<b>Laboratory parameter</b>			
Albumin (g/dl), n=24	2.5 (1.1-3.8)	3.6 (2.0-4.8)	<b>&lt;0.001</b>
ALT (U/l), n=21	47.5 (21-168)	38 (16-56)	0.0093
AST (U/l), n=21	125 (38-267)	59 (35-103)	<b>&lt;0.001</b>
Total bilirubin (mg/dl), n=21	13 (3.2-33)	2.2 (0.4-24.3)	<b>&lt;0.001</b>
<b>Clinical score</b>			
Model for end-stage liver disease (MELD), n=7	23 (16-34)	16 (5-23)	<b>&lt;0.001</b>

Values are presented as median and range in brackets. The number of patients for which the respective data was available is indicated in the first column. Paired t-test. Bold font indicates significance ( $p$  value <0.05). Fibrosis stage, 0 no fibrosis, 1 portal fibrosis, 2 expansive periportal fibrosis, 3 bridging fibrosis, 4 cirrhosis. INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

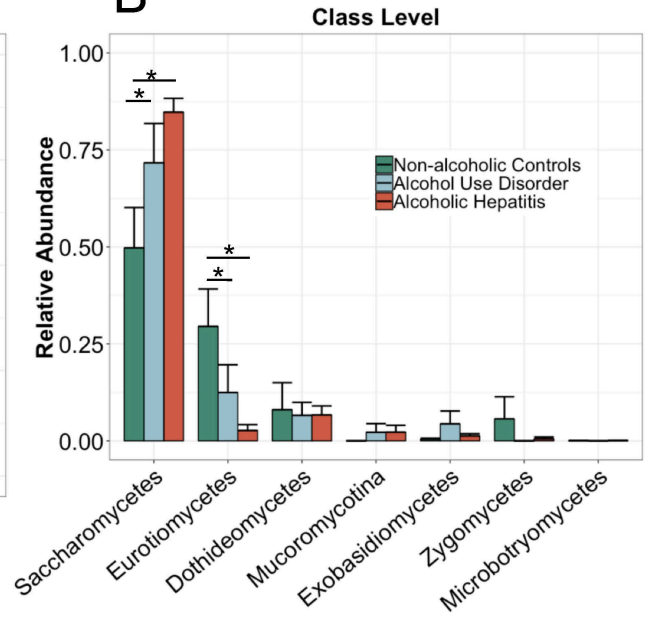


# Supplementary Figure S1

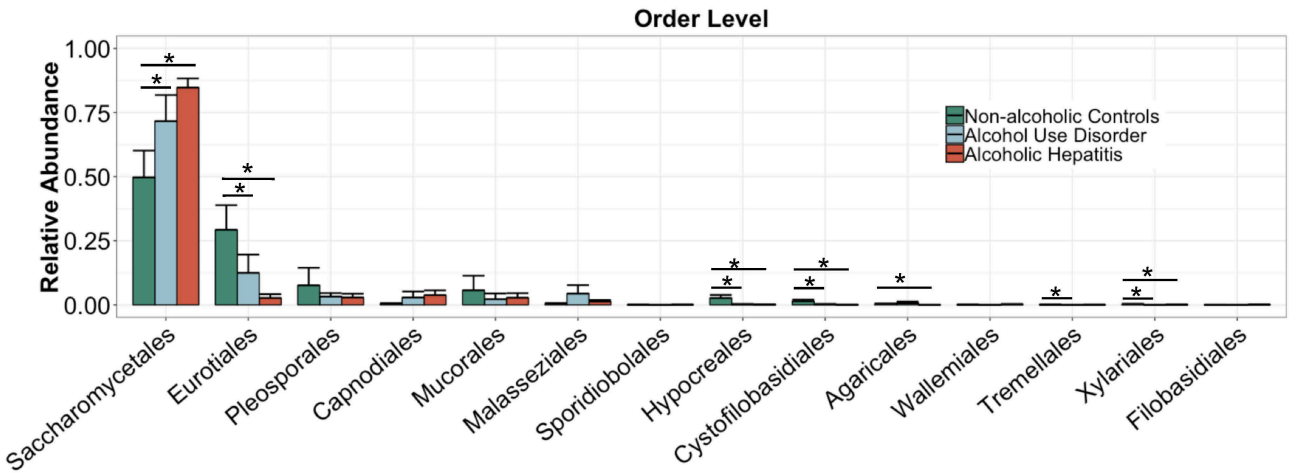
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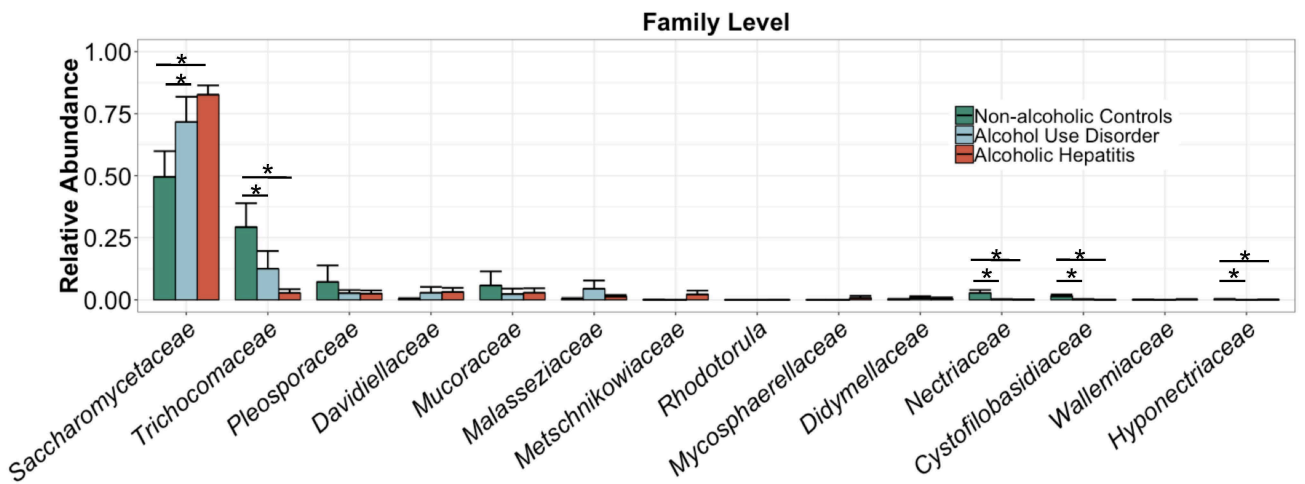
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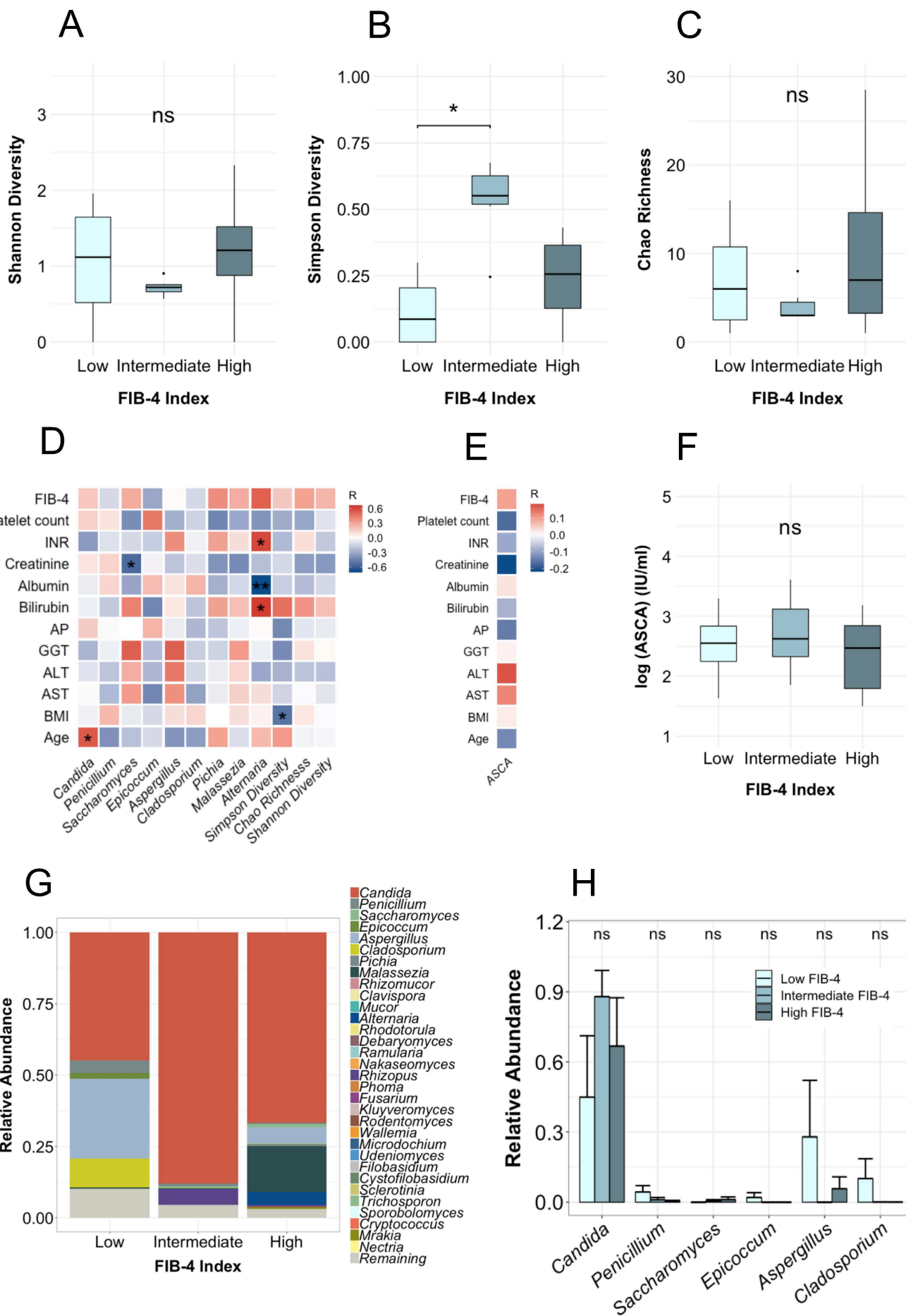
## D



## C

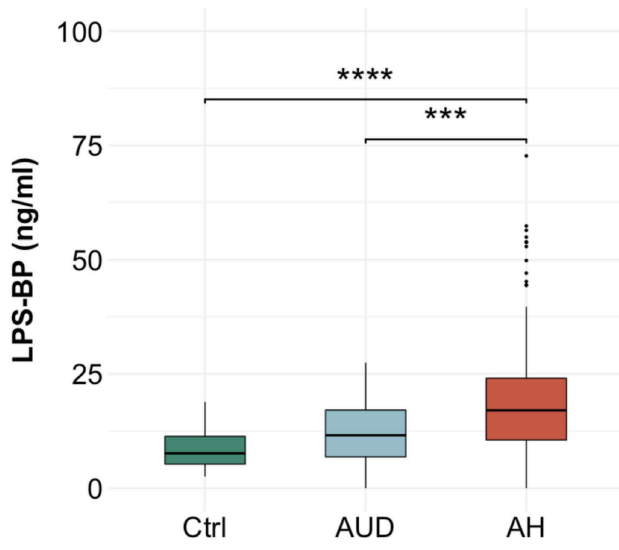


# Supplementary Figure S2

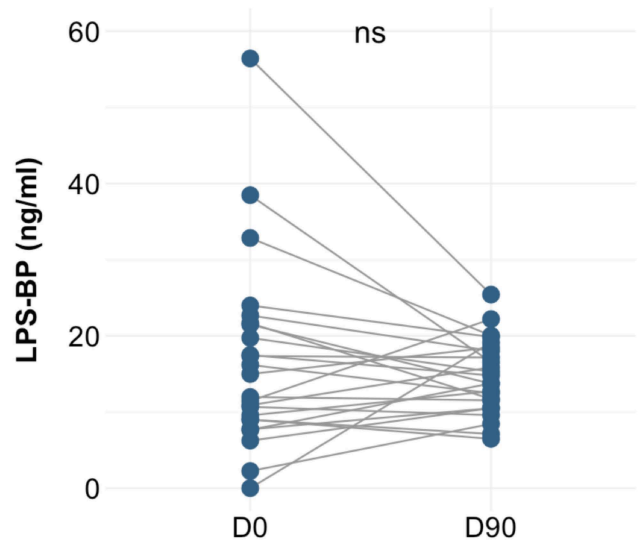


# Supplementary Figure S3

## A



## B



# Supplementary Figure S4

