

Supporting Information

***Kluyveromyces marxianus* supplementation ameliorates alcohol-induced liver injury associated with the modulation of gut microbiota in mice**

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Supplementary materials

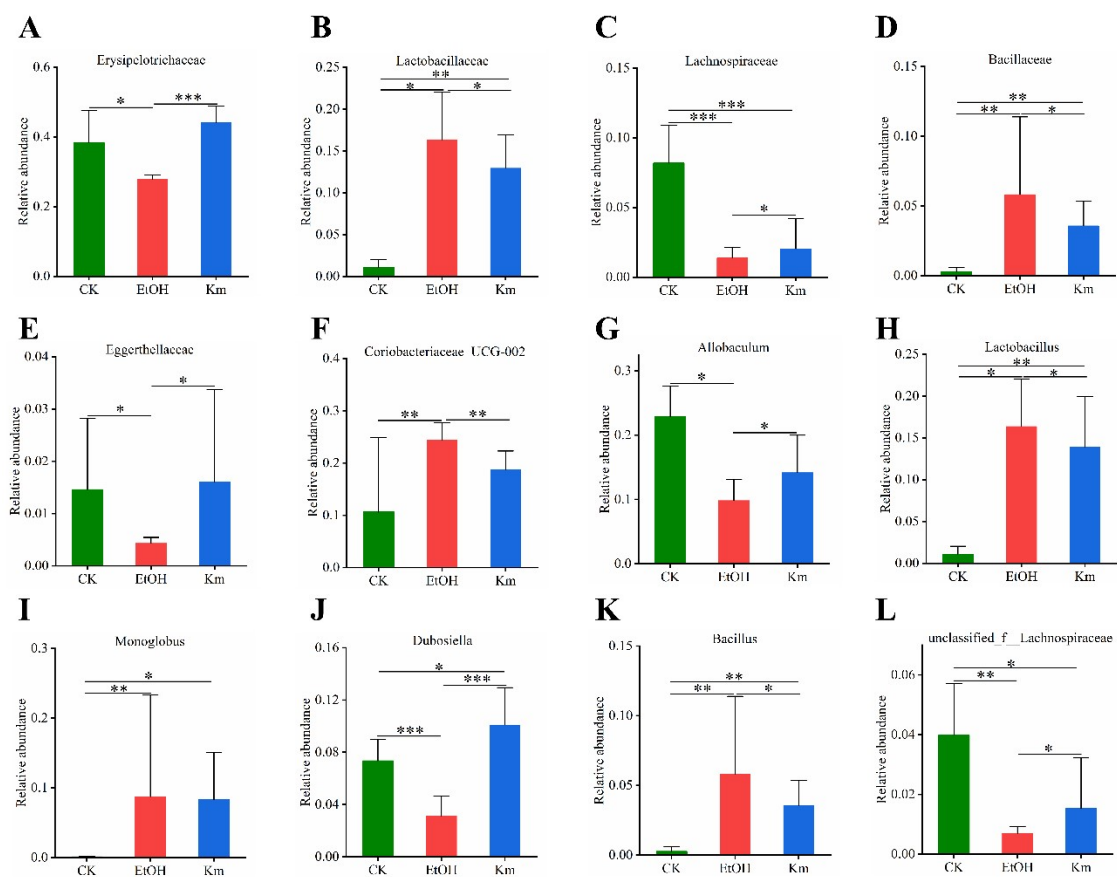


Figure S1 *K. marxianus* regulated specific gut bacterial microbiota. Relative abundance of (A) Erysipelotrichaceae, (B) Lactobacillaceae, (C) Lachnospiraceae, (D) Bacillaceae and (E) Eggerthellaceae at the family level. Relative abundance of (F) *Coriobacteriaceae*_UCG-002, (G) *Allobaculum*, (H) *Lactobacillus*, (I) *Monoglobus*, (J) *Dubosiella*, (K) *Bacillus* and (L) *unclassified_f_Lachnospiraceae* at the genus level. $n = 6$; Data are presented as mean \pm standard deviation (SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ according to Wilcoxon rank sum test. CK, control fed mice; EtOH, ethanol fed mice; Km, ethanol + *K. marxianus* fed mice.

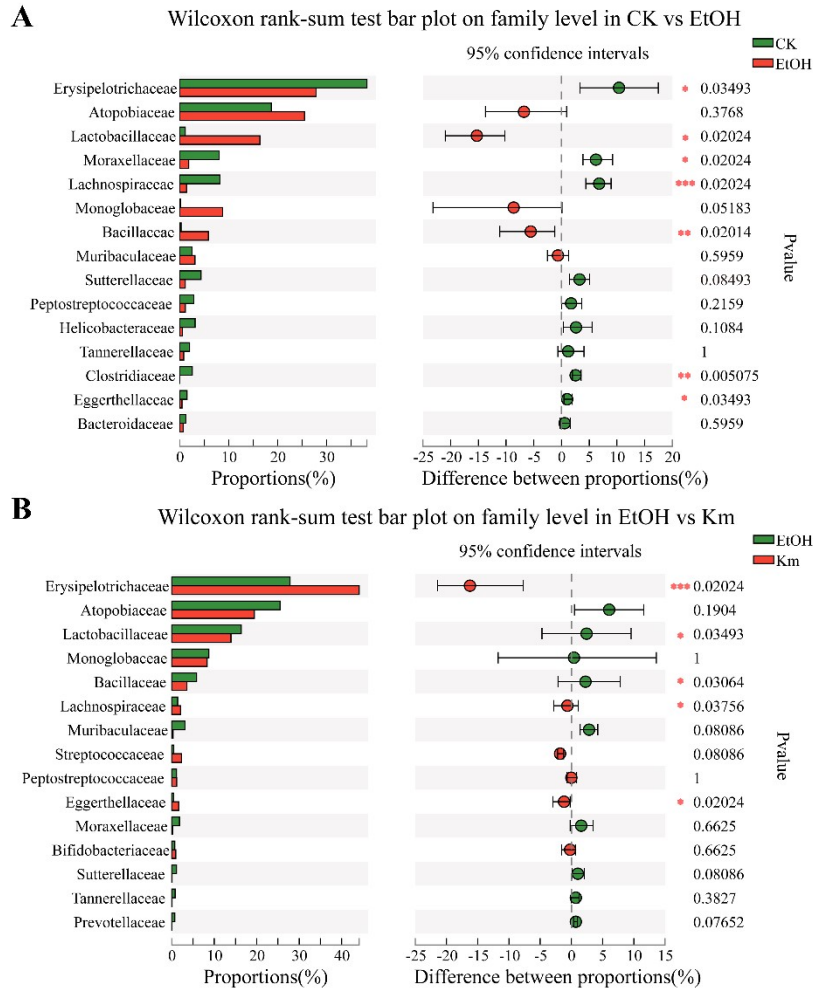


Figure S2 Variation analysis of gut bacterial microbiota on family level in CK vs EtOH (A) and EtOH vs Km (B) according to Wilcoxon rank sum test. $n = 6$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CK, control fed mice; EtOH, ethanol fed mice; Km, ethanol + *K. marxianus* fed mice.

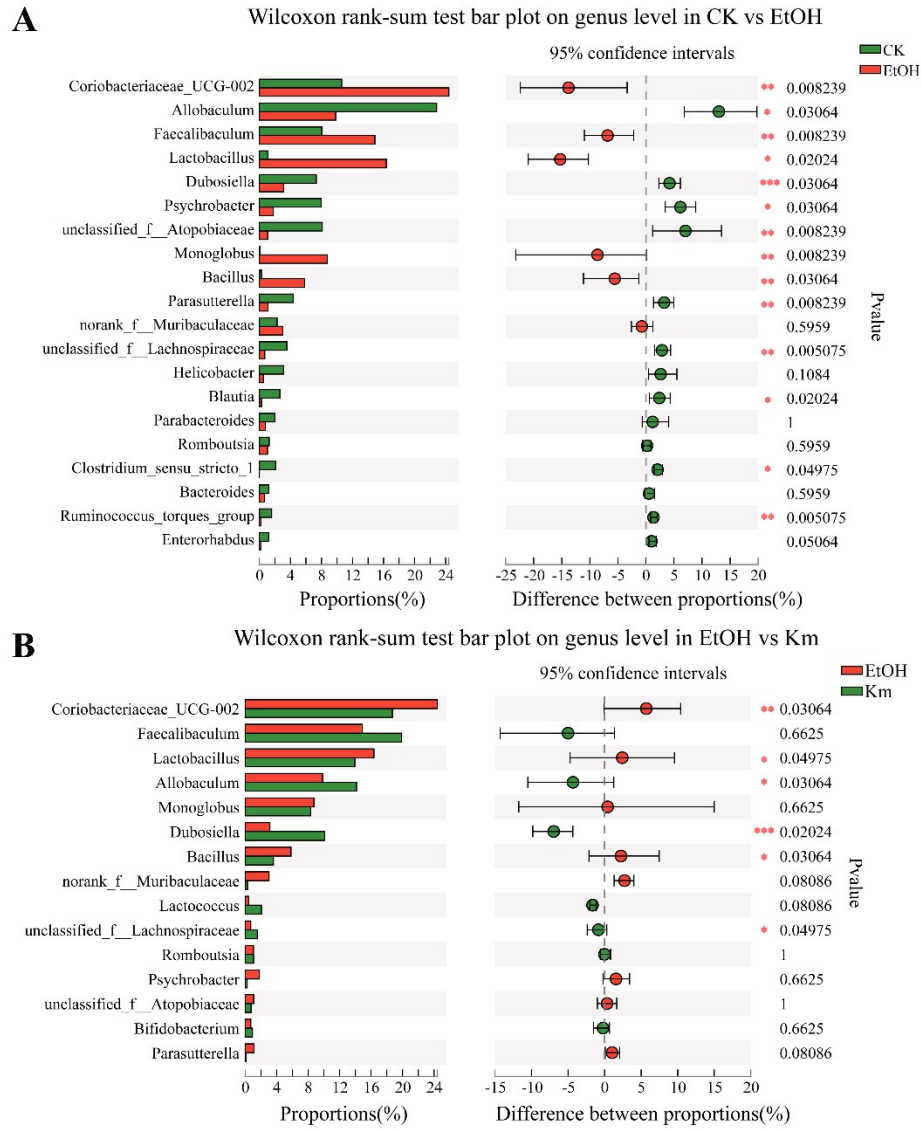


Figure S3 Variation analysis of gut bacterial microbiota on genus level in CK vs EtOH (A) and EtOH vs Km (B) according to Wilcoxon rank sum test. n = 6; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CK, control fed mice; EtOH, ethanol fed mice; Km, ethanol + *K. marxianus* fed mice.

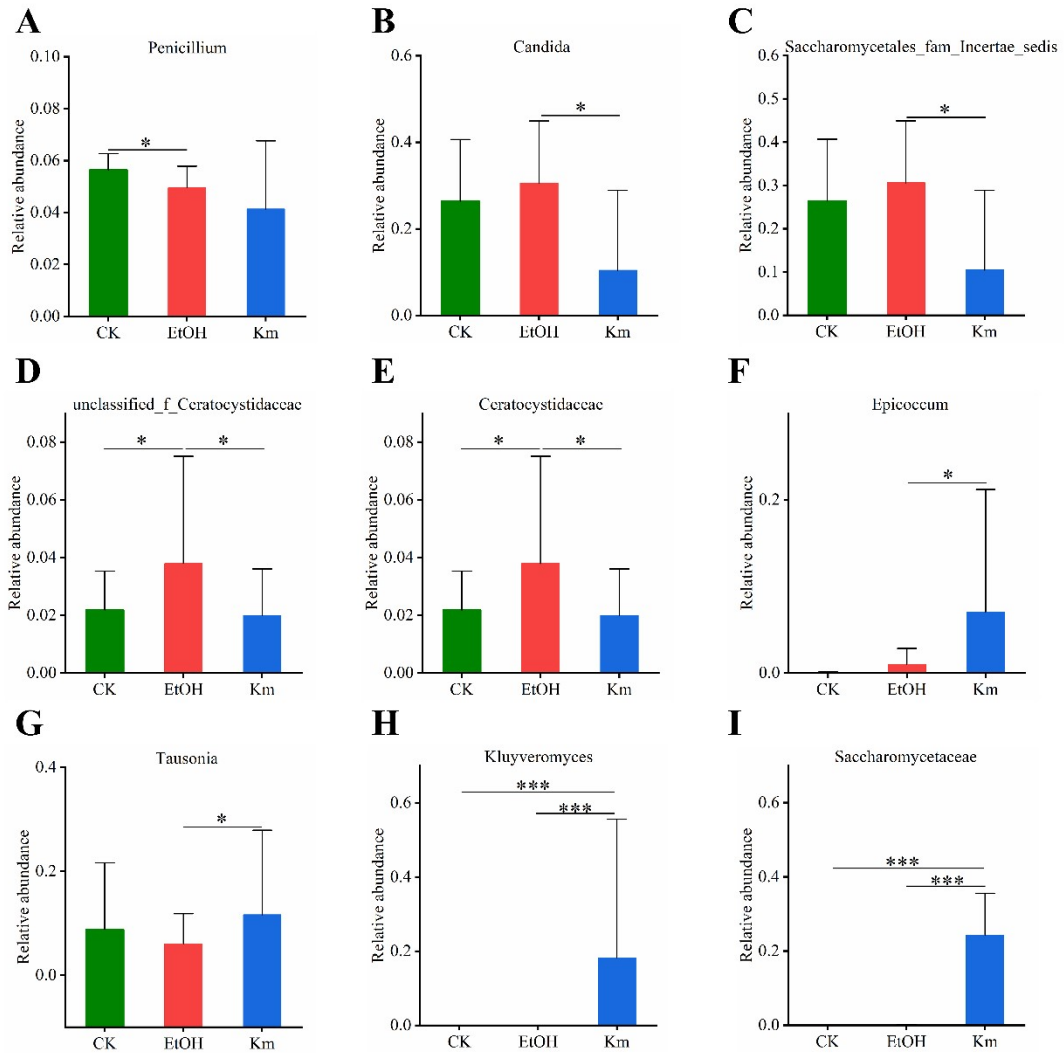


Figure S4 *K. marxianus* regulated specific gut fungal microbiota. Relative abundance of (A) *Penicillium*, (B) *Candida*, (C) *Saccharomycetales_fam_Incertae_sedis*, (D) *unclassified_f_Ceratocystidaceae*, (E) *Ceratocystidaceae*, (F) *Epicoccum*, (G) *Tausonia*, (H) *Kluyveromyces*, (I) *Saccharomycetaceae*. n = 6; Data are presented as mean \pm standard deviation (SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ according to Wilcoxon rank sum test. CK, control fed mice; EtOH, ethanol fed mice; Km, ethanol + *K. marxianus* fed mice.

Table S1 Ingredients and calories of Lieber DeCarli (LDC) diet

Ingredient	LDC diet (L10016A)		Control diet		Ethanol diet	
	g	kcal	g	kcal	g	kcal
Casein	41.4	166	41.4	166	41.4	166
DL-Methionine	0.3	1	0.3	1	0.3	1
L-Cystine	0.5	2	0.5	2	0.5	2
Maltodextrin	25.6	102	25.6+89.6	461	25.6+ (--)	--
Cellulose	10	0	10	0	10	0
Xantham Gum	3	0	3	0	3	0
Corn oil	8.5	77	8.5	77	8.5	77
Olive oil	28.4	256	28.4	256	28.4	256
Safflower oil	2.7	24	2.7	24	2.7	24
Minerals	8.75	0	8.75	0	8.75	0
Vitamins	2.5	9	2.5	9	2.5	9
Choline Bitartrate	0.53	0	0.53	0	0.53	0
Ethanol, 100%					1% up to 4%	--
H ₂ O			778.22		--	
Total	132.18	637	1000	996	1000	996

Table S2 Primer sequences used in qRT-PCR for cytokine mRNA quantification^{1,2}

Gene	Primer sequence (5'→3')
β-actin	F: GCTCTGGCTCCTAGCACCAT
	R: GCCACCGATCCACACAGAGT
IL-6	F: GTTGCCTTCTTGGGACTGATGCT
	R: GCCTCCGACTTGTGAAGTGGTATAG
IL-1β	F: GCAACTGTTCTGAACTCAACT
	R: ATCTTTTGGGGTCCGTCAACT
TNF-α	F: CCCTCACACTCAGATCATCTTCT
	R: GCTACGACGTGGGCTACAG

Reference

1. X. Zeng, X. Li, Y. Yue, X. Wang, H. Chen, Y. Gu, H. Jia, Y. He, Y. Yuan and T. Yue, Ameliorative Effect of *Saccharomyces cerevisiae* JKSP39 on *Fusobacterium nucleatum* and Dextran Sulfate Sodium-Induced Colitis Mouse Model, *J Agric Food Chem*, 2022, **70**, 14179-14192.
2. X. Zeng, H. Jia, Y. Shi, K. Chen, Z. Wang, Z. Gao, Y. Yuan and T. Yue, *Lactobacillus kefirifaciens* JKSP109 and *Saccharomyces cerevisiae* JKSP39 isolated from Tibetan kefir grain co-alleviated AOM/DSS induced inflammation and colorectal carcinogenesis, *Food & Function*, 2022, **13**, 6947-6961.