

Table S1. Oligonucleotide primers used in real-time PCR in cell line and mice.

Target gene	Primer designation	Oligonucleotide sequence (5'-3')	Product size (bp)
<b>m-TLR-2</b>	Tlr <sub>2</sub> -F	TCCTGCGAACTCCTATCC	151
	Tlr <sub>2</sub> -R	CCTGGTGACATTCCAAGAC	
<b>m-TLR-4</b>	Tlr <sub>4</sub> -F	GCCTTTCAGGGAATTAAGCTCC	114
	Tlr <sub>4</sub> -R	GATCAACCGATGGACGTGTAAA	
<b>m-TNF-<math>\alpha</math></b>	Tnf-F	AACAACACTACTCAGAAACACAAG	130
	Tnf-R	GCAGAACTCAGGAATGGA	
<b>m-TGF-<math>\beta</math></b>	Tgfb-F	AATTCCTGGCGTTACCTT	116
	Tgfb-R	TGTATTCCGTCTCCTTGG	
<b>m-<math>\alpha</math>-SMA</b>	Sma-F	CTGCCGAGCGTGAGATTG	122
	Sma-R	AGGCAGTTCGTAGCTCTTCT	
<b>m-Col1a1</b>	Col1-F	GGGGCAAGACAGTCATCGAA	144
	Col1-R	GGGTGGAGGGAGTTTACACG	
<b>m-TIMP1</b>	Timp-R	AGTGTTTCCCTGTTTATCTATCC	105
	Timp-R	AAGTGACGGCTCTGGTAG	
<b>m-PDGF</b>	Pdgf-F	CACCACCGCAGTGTCAAG	116
	Pdgf-R	AGGTTGGAGGTTCGCACAT	
<b>m-RPL-19</b>	Rpl-F	TCAGCCACAACATTCTCA	138
	Rpl-R	GCACCTCCAACAGTAAGT	
<b>m-HPRT</b>	Hprt-F	TCAGTCAACGGGGGACATAAA	142
	Hprt-R	GGGGCTGTACTGCTTAACCAG	
<b>TLR-2</b>	Tlr <sub>2</sub> -F	TTATCCAGCACACGAATACACAG	160
	Tlr <sub>2</sub> -R	AGGCATCTGGTAGAGTCATCAA	
<b>TLR-4</b>	Tlr <sub>4</sub> -F	AGACCTGTCCCTGAACCCTAT	147
	Tlr <sub>4</sub> -R	CGATGGACTTCTAAACCAGCCA	
<b>TGF-<math>\beta</math></b>	Tgfb-F	GCACAACCTCCGGTGACATCAA	86
	Tgf-R	CAATTCCTGGCGATACCTCAG	
<b><math>\alpha</math>-SMA</b>	Sma-F	ATGATGCTGTTGTAGGTG	142
	Sma -R	TTGAGAAGAGTTACGAGTTG	
<b>Col1a1</b>	Col1-F	TTACACAAGGAACAGAACA	91
	Col1-R	CGACAAAGCAGAAACATC	

<b>TIMP1</b>	Timp-F	CTATCAGCCACAGCAACAACAGG	82
	Timp-R	GCCCAGAGAGACACCAGAGAAC	
<b>GAPDH</b>	GAPDH-F	TGGAAGATGGTGATGGGATT	211
	GAPDH-R	TCAACGGATTTGGTCGTATTG	

**Table S2.** Oligonucleotide primers used for the gut microbiota analysis.

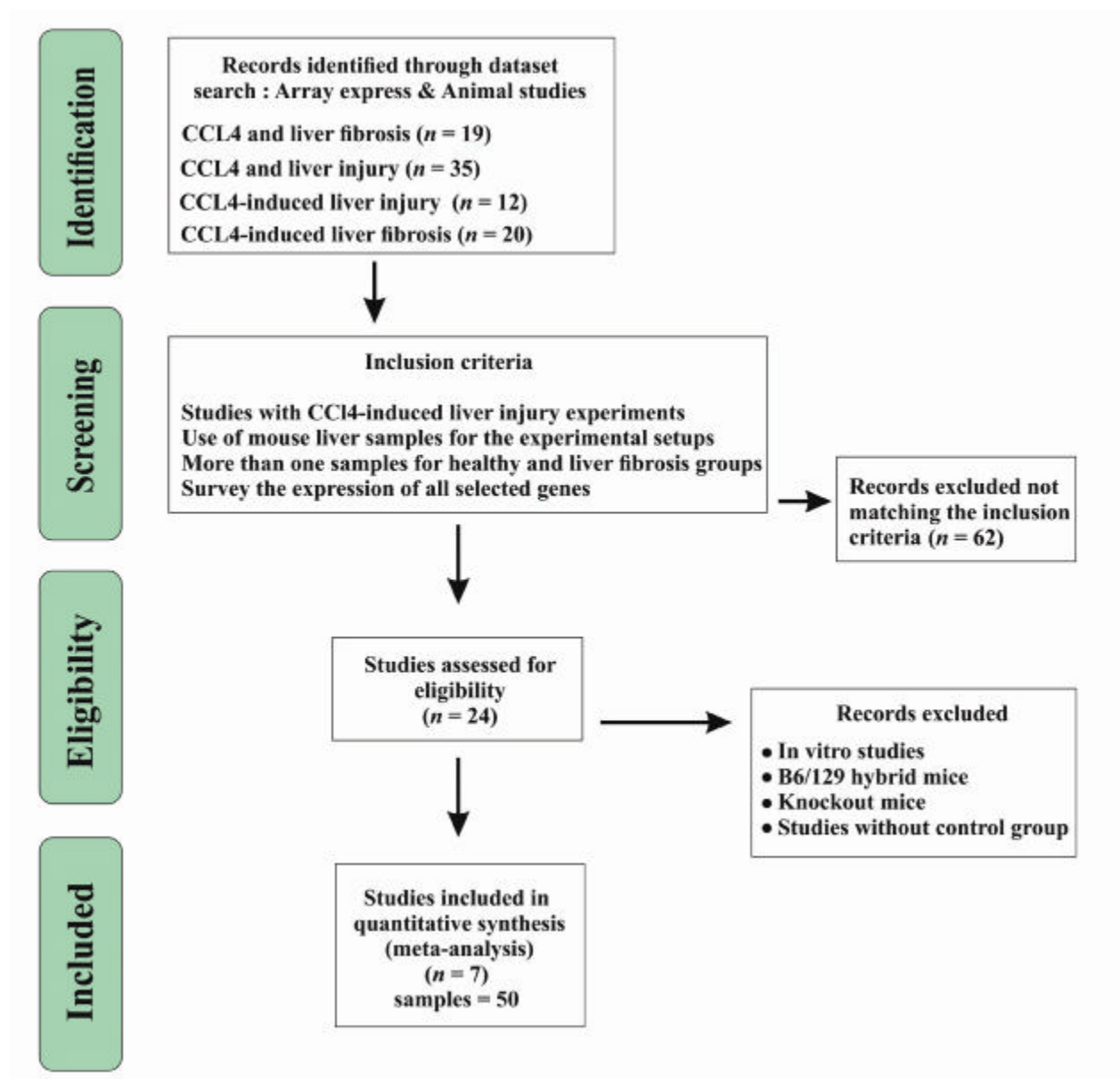
Target gene	Primer designation	Oligonucleotide sequence (5'-3')	Product size (bp)
<b>Universal</b>	Uni-F	ACTCCTACGGGAGGCAGCAGT	1
	Uni-R	ATTACCGCGGCTGCTGGC	
<b>Firmicute</b>	Firm-F	GGAGYATGTGGTTTAATTCGAAGCA	2
	Firm-R	AGCTGACGACAACCATGCAC	
<b>Bacteroidetes</b>	Bactero-F	GTTTAATTCGATGATACGCG	2
	Bactero-R	TTAAGCCGACACCTCACG	
<b>Fusobacteria</b>	Fuso-F	GATCCAGCAATTCTGTGTG	3
	Fuso-R	CGAATTTACCTCTACACTTG	
<b>Actinobacteria</b>	Actino-F	GCGACCTATCAGCTTGTT	3
	Actino-R	CCGCCTACGAGCTCTTTACGC	
<b>Clostridia</b>	Ccl-F	AAAGGAAGATTAATACCGCATA	4
	Ccl-R	TTCTTCTAATCTCTACGCA	
<b><math>\gamma</math>-Proteobacteria</b>	Gamma-F	CATGCCGCGTGTGTGAA	5
	Gamma -R	ACTCCCCAGGCGGTCDACTTA	
<b><math>\alpha</math>-Proteobacteria</b>	Alpha-F	GGTAAGGTTCTGCGCGTT	5
	Alpha-R	GGTAAGGTTCTGCGCGTT	
<b><math>\epsilon</math>-Proteobacteria</b>	Epsilon-R	TGGTGTAGGGGTAATAATCCG	3
	Epsilon-R	AGGTAAGGTTCTTCGYGTATC	
<b><i>Enterobacteriaceae</i></b>	Enterob-F	CGTCGCAAGMMCAAAGAG	3
	Enterob-R	TTACCGCGGCTGCTGGCAC	
<b><i>Ruminococcaceae</i></b>	Rum-F	GGCGGCYTRCTGGGCTTT	6
	Rum-R	CCAGGTGGATWACTTATTGTGTTAA	
<b><i>Peptostreptococcus</i></b>	Pepto-F	ATAGGAGGAAGCCCTGGCTAAA	This study
	Pepto-R	CTCCACGCTTTGACACCTGA	
<b><i>Prevotellaceae</i></b>	Prevo-F	CACCAAGGCGACGATCA	7
	Prevo-R	GGATAACGCCTGGACCT	
<b><i>Methanobrevibacter spp.</i></b>	Methano-F	CGATGCGGACTTGGTGTG	8
	Methano-R	TGTCGCCTCTGGTGAGATGTC	

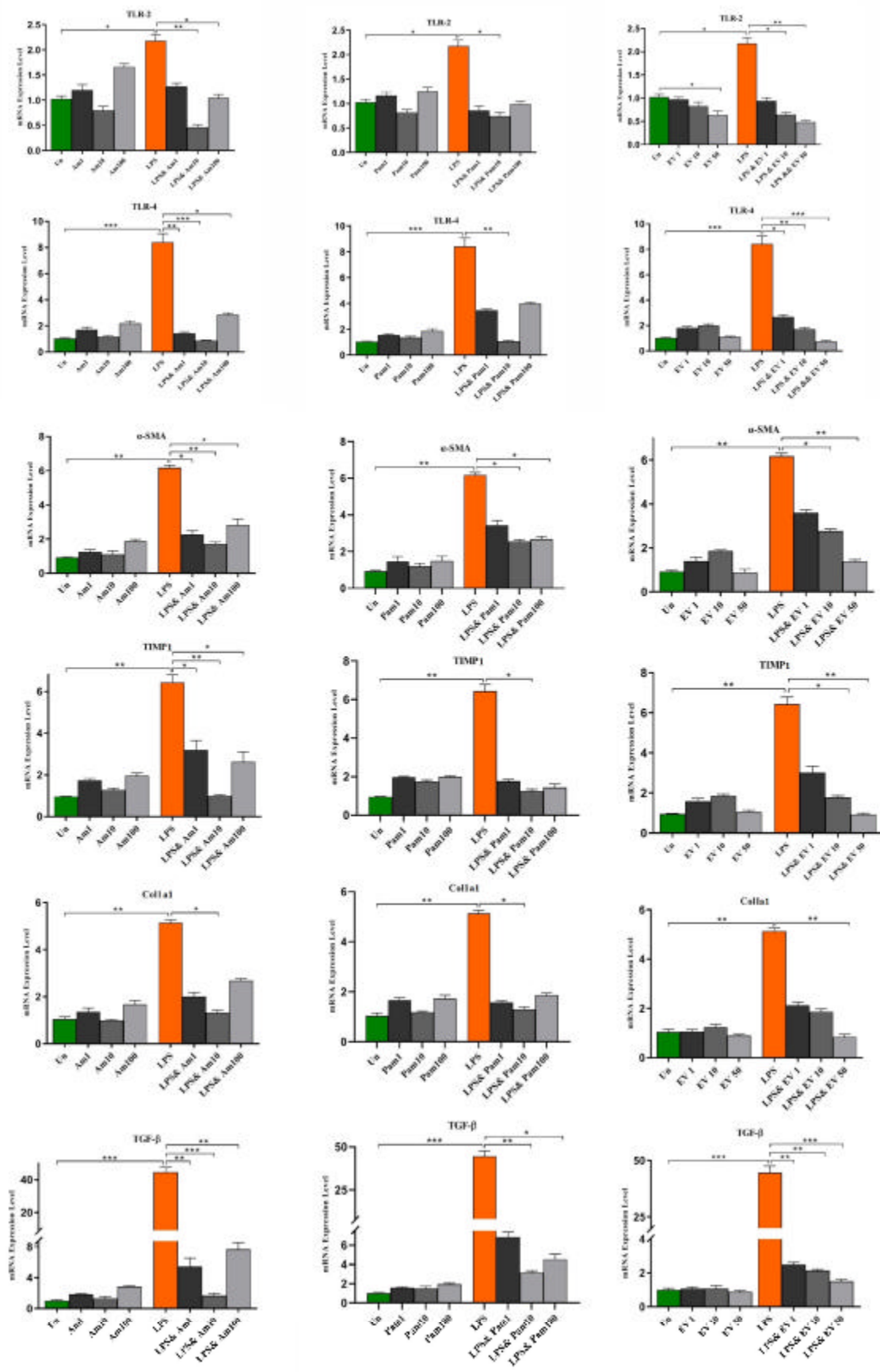
<i>A. muciniphila</i>	Am-F	CAGCACGTGAAGGTGGGGAC	9
	Am-R	CCTTGCGGTTGGCTTCAGAT	
<i>Alistipes spp.</i>	Alis-F	TTAGAGATGGGCATGCGTTGT	10
	Alis-R	TGAATCCTCCGTATT	
<i>Veillonella spp.</i>	Veillo-F	ACAACCTGCCCTTCAGA	11
	Veillo-R	CGTCCCATTAAACAGAGCTT	
<i>Enterococcus spp.</i>	Enteroc-F	CCCTTATTGTTAGTTGCCATCATT	12
	Enteroc-R	ACTCGTTCTACTTCCCATTGT	
<i>F. prausnitzii</i>	Fp-F	GATGGCCTCGCGTCCGATTAG	13
	Fp-R	CCGAAGACCTTCTTCCTCC	
<i>E. coli</i>	GAPDH-F	TGGAAGATGGTGATGGGATT	14
	GAPDH-R	TCAACGGATTTGGTTCGTATTG	
<i>Bifidobacterium spp.</i>	Bifido-F	GGGATGCTGGTGTGGAAGAG	12
	Bifido-R	TGCTCGCGTCCACTATCCAG	
<i>Lactobacillus spp.</i>	Lacto-F	TGGATGCCTTGGCACTAG	12
	Lacto-R	AAATCTCCGGATCAAAGCTTAC	
<i>Roseburia spp.</i>	Rose-F	TACTGCATTGGAAACTGTCTG	7
	Rose-R	CGGCACCGAAGAGCAAT	

The nucleotides in bold type represent: Y, C or T; K, G or T; M, A or C; D, A or G or T; R, A or G.

Figure S1. Flow chart for database search.

Figure S2. Gene expression of liver fibrosis markers in LX-2 cells upon treatment with different MOIs of live, pasteurized *A. muciniphila* and its EVs. Relative gene expression of fibrosis markers were markedly increased after treatment of LX-2 cells with LPS in comparison with quiescence condition. The mRNA level of an anti-fibrosis factors were decreased in LPS stimulated LX-2 cells treated with different supplementation of *A. muciniphila*. Data are shown as the mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and by post hoc one-way ANOVA statistical analysis.





## Reference

- 1 Moraes, J. G. d., Motta, M. E. F. d. A., Beltrão, M. F. d. S., Salviano, T. L. & Silva, G. A. P. d. Fecal microbiota and diet of children with chronic constipation. *International journal of pediatrics*. 2016; **2016**, p. 6787269.
- 2 Yang, Y.-W. *et al.* Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in mouse feces. *Applied and environmental microbiology*. 2015; **81**, p.6749-6756.
- 3 Hermann-Bank, M. L., Skovgaard, K., Stockmarr, A., Larsen, N. & Mølbak, L. The Gut Microbiotassay: a high-throughput qPCR approach combinable with next generation sequencing to study gut microbial diversity. *BMC genomics*. 2013; **14**, p.788.
- 4 Matsuki, T. *et al.* Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Applied and environmental microbiology*. 2002; **68**, p.5445-5451.
- 5 De Gregoris, T. B., Aldred, N., Clare, A. S. & Burgess, J. G. Improvement of phylum-and class-specific primers for real-time PCR quantification of bacterial taxa. *Journal of microbiological methods*. 2011; **86**, p.351-356.
- 6 Omar, J. M., Chan, Y.-M., Jones, M. L., Prakash, S. & Jones, P. J. Lactobacillus fermentum and Lactobacillus amylovorus as probiotics alter body adiposity and gut microflora in healthy persons. *Journal of functional foods*. 2013; **5**, p.116-123.
- 7 Larsen, N. *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS one*. 2010; **5**, e9085.
- 8 Verma, R., Verma, A. K., Ahuja, V. & Paul, J. Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. *Journal of clinical microbiology*. 2010; **48**, p.4279-4282.
- 9 Schneeberger, M. *et al.* Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Scientific reports*. 2015; **5**, p.16643.
- 10 Vignæs, L. K., Brynskov, J., Steenholdt, C., Wilcks, A. & Licht, T. R. Gram-negative bacteria account for main differences between faecal microbiota from patients with ulcerative colitis and healthy controls. *Beneficial microbes*. 2012; **3**, p.287-297.
- 11 Rinttilä, T., Kassinen, A., Malinen, E., Krogius, L. & Palva, A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of applied microbiology*. 2004; **97**, p.1166-1177.

- 12 Wang, I.-K. *et al.* Real-time PCR analysis of the intestinal microbiotas in peritoneal dialysis patients. *Applied and environmental microbiology*. 2012; **78**, p.1107-1112.
- 13 Fitzgerald, C. B. *et al.* Comparative analysis of *Faecalibacterium prausnitzii* genomes shows a high level of genome plasticity and warrants separation into new species-level taxa. *BMC genomics*. 2018; **19**, p.931.
- 14 Bartosch, S., Fite, A., Macfarlane, G. T. & McMurdo, M. E. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and environmental microbiology*. 2004; **70**, p.3575-3581.