Supplementary materials for:

Modulation of the gut microbiota impacts non-alcoholic fatty liver disease: a potential role for bile acids

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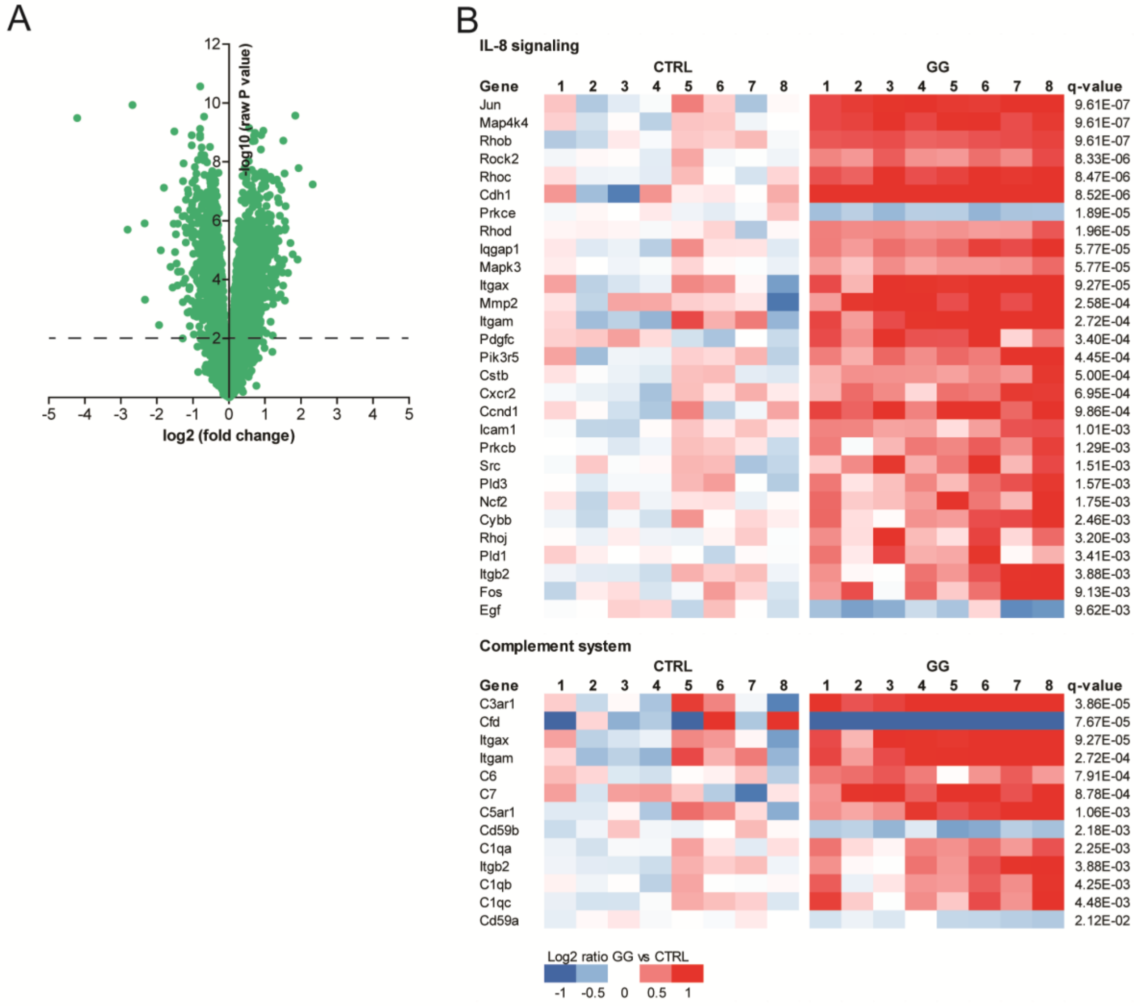
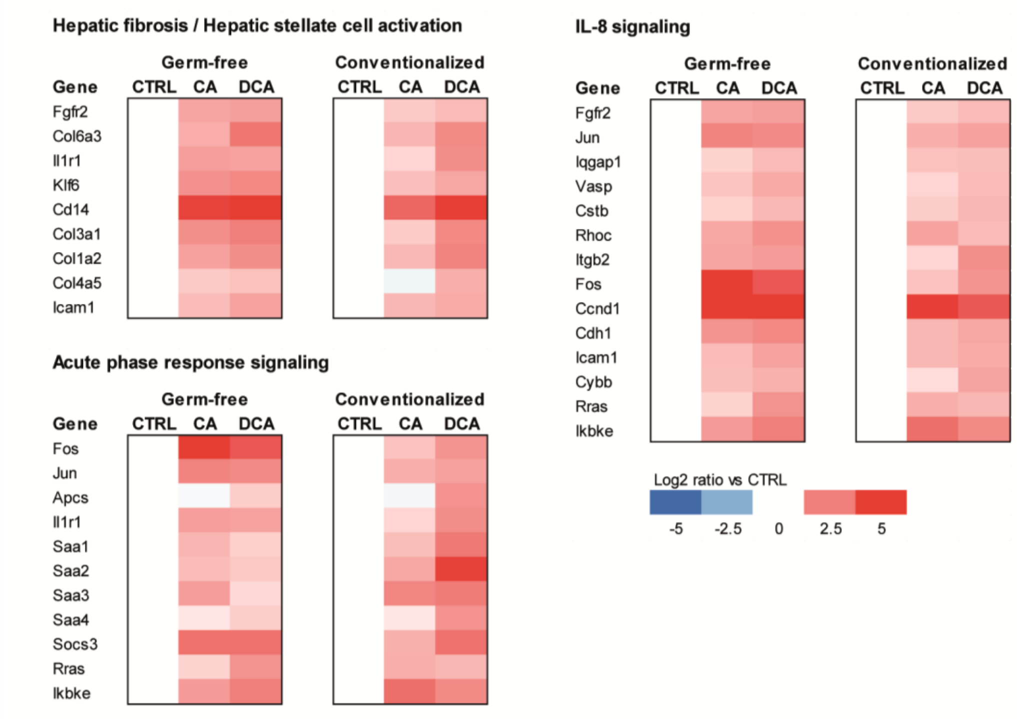


Supplemental figure 1. Guar gum alters IL-8 signaling and complement system activation

(A) Volcano plot displaying relative changes in expression of 21115 genes (x axis) plotted against statistical significance (y axis) upon GG feeding. Dotted line represents cut-off for p<0.01. **(B)** Heat map showing significant changes in expression of genes involved in IL-8 signaling and complement system activation in the livers of CTRL and GG mice. The Log2 expression signals of the CTRL group was arbitrarily set at 0.



Supplemental figure 2. Effect of cholic acid and deoxycholic acid on hepatic genome-wide expression

Heat maps showing changes in expression of genes involved in hepatic fibrosis/hepatic stellate cell activation, IL-8 signaling and acute phase response signalling in the livers of germ-free and conventionalized mice fed either a CTRL diet or supplemented with 0.5% CA or 0.5% DCA for two weeks. The Log2 expression signals of CTRL was arbitrarily set at 0.

Supplemental Table 1. Macronutrient and ingredient composition of various diets based on formula D12451 study 1

	Control		Resistant starch		Guar Gum	
	g%	kcal%	g%	kcal%	g%	kcal%
Protein	24	20	24	21	24	20
Carbohydrate	41	35	41	32	41	35
Fat	24	45	24	47	24	45
Total		100		100		100
kcal/g	4.7		4.7		4.7	
Ingredient	**g**	**kcal**	**g**	**kcal**	**g**	**kcal**
Casein, 30 Mesh	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn Starch	72.8	291	0	0	0	0
Resistant Starch	0	0	86.7	173.4	0	0
Guar Gum	0	0	0	0	86.7	346.8
Maltodextin 10	100	400	86.1	344.4	86.1	344.4
Sucrose	172.8	691	172.8	691	172.8	691
Cellulose, BW200	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225
Safflower Oil	177.5	1598	177.5	1598	177.5	1598
Cholesterol	8.66	0	8.66	0	8.66	0
Mineral Mix S10026	10	0	10	0	10	0
DiCalcium Phosphate	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0
Patassium Citrate	16.5	0	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
Total	**866.8**	**4057**	**866.8**	**3884**	**866.8**	**4057**

Supplemental Table 2. Primer sequences used for qPCR

	Primer Sequence	
Name	Forward	Reverse
m*36b4*	ATGGGTACAAGCGCGTCCTG	GCCTTGACCTTTTCAGTAAG
mβ-actin	GATCTGGCACCACACCTTCT	GGGGTGTTGAAGGTCTCAAA
m*Cd68*	CCAATTCAGGGTGGAAGAAA	CTCGGGCTCTGATGTAGGTC
m*F4/80*	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
m*Cd11c*	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
m*Cd206*	GCTTCCGTCACCCTGTATGC	GTGTGTCATTCTTACACTCCC
m*Mcp-1*	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG
m*Tnfα*	CAACCTCCTCTCTGCCGTCAA	TGACTCCAAAGTAGACCTGCCC
m*Il-10*	CTGGACAACATACTGCTAACCG	GGGCATCACTTCTACCAGGTAA
m*Col1α1*	TGTGTGCGATGACGTGCAAT	GGGTCCCTCGACTCCTACA
m*αSma*	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
m*Timp1*	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT
m*Cyp7a1*	CAGGGAGATGCTCTGTGTTCA	AGGCATACATCCCTTCCGTGA
m*Cyp27a1*	GCCTTGCACAAGGAAGTGACT	CGCAGGGTCTCCTTAATCACA
m*Cyp8b1*	CCTCTGGACAAGGGTTTTGTG	GCACCGTGAAGACATCCCC
m*Slc51b*	AGATGCGGCTCCTTGGAATTA	TGGCTGCTTCTTTCGATTTCTG
mNtcp	ATGACCACCTGCTCCAGCTT	GCCTTTGTAGGGCACCTTGT
mBsep	CTGCCAAGGATGCTAATGCA	CGATGGCTACCCTTTGCTTCT
mFgf15	GCTCTGAAGACGATTGCCATC	TTCCTCCCTGAAGGTACAGTC
mAsbt	ATGGCGACATGGACCTCAGT	CCCGAGTCAACCCACATCTTG
mIbabp	CTTCCAGGAGACGTGATTGAAA	CCTCCGAAGTCTGGTGATAGTTG
mSlc51a	GTTCCAGGTGCTTGTCATCC	CCACTGTTAGCCAAGATGGAGAA

Supplemental Table 3. Relative abundance of microbiota in colonic luminal content study 1

Phylum	Class	Order	Family	Genus	Species	CTRL (%)	RS (%)	GG (%)	FC RS*	P-value RS**	FC GG*	P-value GG**
Actinobacteria						0,26	1,54	15,63	5,9	0,012	59,2	p<0,001
	Actinobacteria					0,26	1,54	15,63	5,9	0,012	59,2	p<0,001
		Bifidobacteriales				0,26	1,54	15,63	5,9	0,012	59,2	p<0,001
			Bifidobacteriaceae			0,26	1,54	15,63	5,9	0,012	59,2	p<0,001
				Bifidobacterium		0,26	1,54	15,63	5,9	0,012	59,2	p<0,001
Bacteroidetes						4,51	17,16	14,49	3,8	0,002	3,2	p<0,001
	Bacteroidia					4,51	17,16	14,49	3,8	0,002	3,2	p<0,001
		Bacteroidales				4,51	17,16	14,49	3,8	0,002	3,2	p<0,001
			Unidentified			2,18	5,70	0,52	2,6	0,038	-4,2	0,002
			Paraprevotellaceae			0,12	0,09	3,24	-1,3	0,046	27,2	p<0,001
				Prevotella		0,12	0,09	3,24	-1,3	0,046	27,2	p<0,001
			Bacteroidaceae			1,21	3,94	5,26	3,2	0,01	4,3	0,002
				Bacteroides		1,21	3,94	5,26	3,2	0,01	4,3	0,002
			S24-7			0,99	7,43	5,47	7,5	0,001	5,5	p<0,001
				Unidentified		0,99	7,43	5,47	7,5	0,001	5,5	p<0,001
Deferribacteres						4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
	Deferribacteres					4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
		Deferribacterales				4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
			Deferribacteraceae			4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
				Mucispirillum		4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
					Mucispirillum schaedleri	4,95	1,71	1,12	-2,9	0,038	-4,4	0,006
Firmicutes						84,63	74,76	55,43	-1,1	0,016	-1,5	p<0,001
	Bacilli					9,99	1,88	0,62	-5,3	0,012	-16,1	p<0,001
		Lactobacillales				9,99	1,88	0,62	-5,3	0,012	-16,1	p<0,001
			Lactobacillaceae			9,99	1,88	0,62	-5,3	0,012	-16,1	p<0,001
				Lactobacillus		9,99	1,88	0,62	-5,3	0,012	-16,1	p<0,001
	Clostridia					26,58	9,61	9,94	-2,8	0,002	-2,7	0,001
		Clostridiales				26,58	9,61	9,94	-2,8	0,002	-2,7	0,001
			Unidentified			17,87	3,99	6,34	-4,5	0,001	-2,8	0,006
			Clostridiaceae			4,09	3,03	0,00	-1,3	ns	-2275,9	0,001
				SMB53		4,09	3,03	0,00	-1,3	ns	-2275,9	0,001
			Lachnospiraceae			1,70	1,56	3,19	-1,1	ns	1,9	ns
				Unidentified		1,40	1,28	2,16	-1,1	ns	1,5	ns
				Ruminococcus		0,30	0,27	1,03	-1,1	ns	3,4	ns
					Ruminococcus gnavus	0,30	0,27	1,03	-1,1	ns	3,4	ns
			Ruminococcaceae			2,92	1,04	0,41	-2,8	ns	-7,2	p<0,001
				Oscillospira		2,92	1,04	0,41	-2,8	ns	-7,2	p<0,001
	Erysipelotrichi					48,06	63,27	44,87	1,3	ns	-1,1	ns
		Erysipelotrichales				48,06	63,27	44,87	1,3	ns	-1,1	ns
			Erysipelotrichaceae			48,06	63,27	44,87	1,3	ns	-1,1	ns
				Allobaculum		48,06	63,27	44,87	1,3	ns	-1,1	ns
Proteobacteria						5,51	4,81	5,10	-1,1	ns	-1,1	ns
	Deltaproteobacteria					5,51	4,81	5,10	-1,1	ns	-1,1	ns
		Desulfovibrionales				5,51	4,81	5,10	-1,1	ns	-1,1	ns
			Desulfovibrionaceae			5,51	4,81	5,10	-1,1	ns	-1,1	ns
				Bilophila		3,08	2,22	1,53	-1,4	ns	-2,0	ns
				Desulfovibrio		2,43	2,59	3,56	1,1	ns	1,5	ns
					Unidentified	2,42	2,47	1,60	1,0	ns	-1,5	ns
					Desulfovibrio C21_c20	0,00	0,12	1,97	83,2	ns	1308,8	0,002
Verrucomicrobia						0,14	0,01	8,23	-17,3	ns	60,8	p<0,001
	Verrucomicrobiae					0,14	0,01	8,23	-17,3	ns	60,8	p<0,001
		Verrucomicrobiales				0,14	0,01	8,23	-17,3	ns	60,8	p<0,001
			Verrucomicrobiaceae			0,14	0,01	8,23	-17,3	ns	60,8	p<0,001
				Akkermansia		0,14	0,01	8,23	-17,3	ns	60,8	p<0,001
					Akkermansia muciniphila	0,14	0,01	8,23	-17,3	ns	60,8	p<0,001

Abundance treshold for presentation in the table >0.5% in at least one of the three diet groups

* fold change (FC) compared with CTRL

** significance according to Kruskal-Wallis test followed by unpaired Wilcoxon rank-sum test to test significance compared with CTRL