

Supplementary Material

Menthacarin, a proprietary combination of peppermint and caraway oil alters cultured human fecal microbiota composition resulting in increased SCFA production

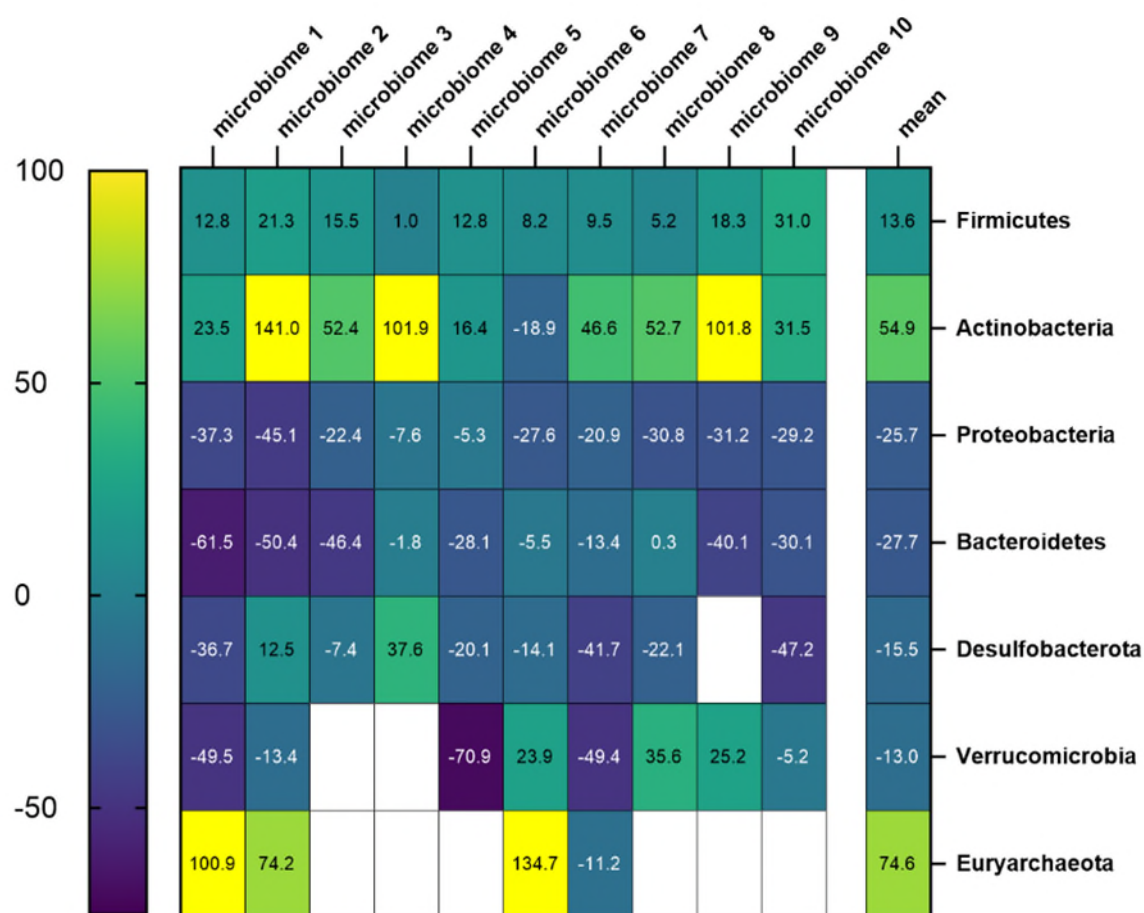
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Supplementary Table S1: Viability analysis of the control cultures after incubation period (T36)

Microbiome	1	2	3	4	5	6	7	8	9	10
Viability [%]	93.3	92.4	92.5	91.2	92.2	90.8	91.7	92.2	91.0	92.8



Supplementary Figure S1: Heatmap of fractional change in relative abundance induced by Menthacarin within the seven most abundant phyla (expressed in percentage) across 10 microbiomes. The numbers in each cell denote the fractional change (in %) with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
	18.7		28.3	217.8	71	27.8			-20.4	57.2	[Clostridium] innocuum group
-4.6	50.3		7.3					-25.5	88.7	23.2	[Eubacterium] brachy group
		-70	73.1	9.4	-19.1	209.6	-42.9	25	79.2	33.1	[Eubacterium] eligens group
24	104.2	45.6	26.7	56.3	15.7	-6.1	24.9	53.8	75.4	42.1	[Eubacterium] hallii group
-25.5	12.6	56.7	106.5	17.6	18.2	27.2	-14.8	-68	44.7	17.5	[Eubacterium] nodatum group
-20.5	-14.7	-34.2	-44.3	19.9	11.6	-23.3	-8.6	-30.3	28.5	-11.6	[Eubacterium] oxidoreducens group
	-48.5	-23.7			-73.9					-48.7	[Eubacterium] ruminantium group
		-61.5						23.3		-19.1	[Eubacterium] siraeum group
-37	66.4				-6.2		292.8	65.1	-33	58	[Eubacterium] ventriosum group
							-38.3			-38.3	[Eubacterium] xylanophilum group
-62.2	-80.1	-50.4	4.3	186.5	37.7	-35	4.5	93.4	49.5	14.8	[Ruminococcus] gauvreauii group
24.1	-50.7	111.3	37.5	11	6.4	-9.5	10.5	1.3		15.8	[Ruminococcus] gnavus group
29.9	-25.3	83.3	63.5	43.6	8.4	0.1	-7	162	5.9	36.5	[Ruminococcus] torques group
				-9.2						-9.2	28-4
								42.1		42.1	Acetitomaculum
								-25.4	-47.7	-36.6	Acidaminococcus
	-5.9									-5.9	Actinobacillus
68.5	260.1									164.3	Actinomyces
	49.3			11.5	-41.4	30.6	8.6	-31.4		4.5	Adlercreutzia
-17.9	-19.7	25	7.3	39.3	9	49.2	30.5	39.6	-3	15.9	Agathobacter
-49.4	-13.4		-68.8	-71	29.8	-51.2	35.8	26.8	-5.1	-18.5	Akkermansia
-65.6	-41.7	4.4	-20.7	-3.9	27.8	7.9	22.9	-40.2	-55.9	-16.5	Alistipes
		29.6					-27.7			0.9	Allisonella
		-76.1	-54.4							-65.3	Alloprevotella
	90.4									90.4	Anaerofilum
8.7	9.4	-1.1	-4.6	47.3	15.3	5.7	22.6	45.6	-30.2	11.9	Anaerostipes
-40.6	-6.6		113.6	141.4	31.2	118.5		-28.2	-14.8	39.3	Anaerotruncus
		-67.3								-67.3	Anaerovibrio
					-44			-34.5		-39.3	Asaccharobacter
					-89.7		-65.2			-77.4	Bacteroidales bacterium 55.9

Supplementary Figure S2 (1 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
-36.5	-44.3	17.2	-7.9	-7.9	-5.3	-13.5	5.7	-35.1	-27.6	-15.5	Bacteroides
-64.5	-72.1			76.5	1.3	-22.6	2.5	-31.9	39.2	-9	Barnesiella
31	159.2	234	117.4		-1.4	47.6	92.9	159.9	313.9	128.3	Bifidobacterium
211.8	22.6	37.2	35.5	-20.7	-13.9	-41.6	26.8	-67.4	-49.9	14	Bilophila
19.1	46.2	17.4	21.2	45.8	19.8	-15.7	-0.1	23	-33.8	14.3	Blautia
-51.5	12.8	-48.5	-0.4	-18.7	-43.2	21.4	-22	-70.9	338.2	11.7	Butyricococcus
-41.4	21.5		-20.8	-29	-31.6	-23.9	-3.7	-53.1	22.1	-17.8	Butyricimonas
							-47.4			-47.4	CAG-352
		-39.4	67.3	10.9	-41.5	16.2	-12.1	-20.5		-2.7	CAG-56
-13.8	-6.8	69.4	-5	85	30.2	5	1.4	-41.5	70.7	19.5	Candidatus Soleaferrea
43.6	201.4	10.4	-17.2	292.8	63.2	15.2	27.1			79.6	Catenibacterium
	150.8	22.8		68.3	-13.4					57.1	Catenisphaera
								-65.1		-65.1	CHKCI002
	142.4									142.4	Christensenella
-11.5	-19.5	-4.9	28.5	45.7	-1.3	-10.7	18.8	20.2	146.9	21.2	Christensenellaceae R-7 group
					-20.3		102.7			41.2	Citrobacter
					41.9		3.9			22.9	Clostridiales bacterium 42.27
-0.2			-83.7				-65.4			-49.8	Clostridium sensu stricto 1
-20.8	-7.2	-60.2	-17	22.7	-11.5	1.7	-14.8	-5	113.9	0.2	Colidextribacter
16.1	45.4	238.2			-85.1				-15	39.9	Collinsella
								-77.2		-77.2	Coprobacter
19	-14.9	111.6	14	65.4	31.5	1.1	34.2	-13.9		27.6	Coproccoccus
	-88	-55.8	-50.2	24.3	11.1	-64.8	10.6	156.6		-7	Cupriavidus
							-60.3			-60.3	Defluviitaleaceae UCG-011
-38.5	-69.5	-28.8			-0.3	-49.1	-83.9			-45	Desulfovibrio
-20		-28.3	-29.7		-2.5		15.9	32.5		-5.4	Dialister
	-34.3								-54.3	-44.3	Dielma
19.9	-2	86.2	40.1	27.5	4.9	18.9	18.7	3.3	-0.1	21.7	Dorea
	-51								-6.8	-28.9	DTU089
	293.1		-28.1	-19	20.6	43.3	59.3	-50.3		45.6	Eggerthella

Supplementary Figure S2 (2 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
83.5	-28.5		-57.1	-39.4	-3.9	-27.7	60.7	-47.5	214.7	17.2	Eisenbergiella
	-9.4		-3.2	-32.9	-15.4		-68.3			-25.8	Enterobacter
-60.4		154.8	56.7		-49.1		4.3	-51.2	-20.3	5	Enterorhabdus
87.3	172.1			68.3	-22.6		-5.6	364.9	150.5	116.4	Erysipelatoclostridium
-11.4		-29.1								-20.2	Erysipelotrichaceae UCG-002
53.4	56.3		0.2	70.7	-8.6	13	80	81.7		43.3	Erysipelotrichaceae UCG-003
		-0.3								-0.3	Erysipelotrichaceae UCG-006
4.5	54.7	64.6	6.1	0.8	-7.1	20.5	-47.3	-23.2	17.3	9.1	Escherichia
								-28.6		-28.6	Eubacteriaceae bacterium CH
5	54.5	23.1	6.1	-2.7	-19.1	25.5	-21.6	-21.6	18.8	6.8	Faecalibacterium
	120.1				40.1					80.1	Faecalicoccus
	157.8	29	47.9	81.9	-2					62.9	Faecalitalea
-22.2	-15.6	6.9	112.2	7.9	28.5	76.6	21.4	51.1	0.6	26.7	Family XIII AD3011 group
-54.8	-54.2	86.4	3.4	30.3	2.8	-64.9	13.2	22.1		-1.7	Family XIII UCG-001
-83			29.6							-26.7	Fenollaria
	80.3	-12.5	-25.3	-41.9	-44.8	-58.9	-6.9	-44.1	-12.6	-18.5	Flavonifractor
	-3.2	-44.5		-28.8	17		-7.4			-13.4	Fournierella
				79.4	25.5				-26.7	26.1	Frisingicoccus
19.5	43.3	9.2	-50.3	-0.5	1.7	7.1	27.4	-24.9	36	6.9	Fusicatenibacter
		-51								-51	Fusobacterium
								188.4		188.4	Garicola
-38.5		-36.1		-85.2	48.8		24.1	13.5	9.1	-9.2	GCA-900066575
	-85.9									-85.9	GCA-900066755
	-16.7			55.7	3.1	-27	-8.4	-47.7	116.9	10.9	Gordonibacter
					-22.6		-8.3			-15.5	Hafnia-Obesumbacterium
13.3	31	29		40.3	28.6	3	1.5		52.2	24.9	Holdemanella
65	-35.6		-8.6	51.5	-33	73	19.3	192.4	65	43.2	Holdemania
		-14.2		-33.4	60.4	5.3				4.5	Howardella
35.4										35.4	Huakuichenia
-80.1			-17.9			-61.4			-7.5	-41.7	Hungatella

Supplementary Figure S2 (3 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
15.4	5.9	60.5	5.3	21.1	1.5	12.4	38.5	0	-34.2	12.6	Incertae Sedis
		-73.9	3.4	-4.4	88.9	28.7	14.3	1099.2	75	153.9	Intestinibacter
111.5	-42.3	-37.1	109.2	104.6	30.8	-7.5	185.1	72.7	205.3	73.2	Intestinimonas
77.1		-71.5								2.8	Kandleria
47.7	8.6	3	18.9	-23.1	0.2	-11.7	7.8	12.1	33.5	9.7	Lachnoclostridium
		-64.2	49.6	12.9	-13.2	533.7	-20.5	-20.7	57.7	66.9	Lachnospira
						-23.9				-23.9	Lachnospiraceae AC2044 group
-4.3		33.8	-28.1	31.5	-24.9	5.1	-27.4	-16	-4.6	-3.9	Lachnospiraceae FCS020 group
-32.6	-18.7	-11.8	-19.1	-27.5	-13.7	-9.9	4.3	27.4	30	-7.2	Lachnospiraceae NC2004 group
	2.6	8.9	22.5	79.9	-8.3	165.4	-18.4	43.8	-61.2	26.1	Lachnospiraceae ND3007 group
		-6.5								-6.5	Lachnospiraceae NK3A20 group
86.5	47.4	16.8	-35.6	-2.4	-16.8	14.9	20.7	-6	76.3	20.2	Lachnospiraceae NK4A136 group
							-72.8			-72.8	Lachnospiraceae NK4B4 group
38.8	35.4	-5.8		13	43.4	33.1	13.8	8.8		22.6	Lachnospiraceae UCG-001
-31.5	-55.9	5.1	-3.5	15.5	-9.9	57	54.3	37	-49.9	1.8	Lachnospiraceae UCG-004
-33.5	-48.8	16.3	-7.1	2.6	10.4	37.7	100.6	35.9		12.7	Lachnospiraceae UCG-006
27.8	46.2	14.7	9.8	-4.4	2.2	-7.4	6.4	19.3	11.9	12.7	Lachnospiraceae UCG-008
-6.8	16.1	-19.1	-13.6	22.8	46.6	12.7	10.3	-21.9		5.2	Lachnospiraceae UCG-010
							28.5			28.5	Lactacaseibacillus
		139.4						-16.1		61.6	Lactobacillus
	50.8				17.9		15.1			27.9	Lactococcus
6.8		132.5								69.6	Libanicoccus
					13.5		55			34.3	Ligilactobacillus
		-33.1								-33.1	Mailhella
-15.3	-1.4	24.6	-10.7	-3.9	-10.6	26.7	-17.4	0.3	-18.1	-2.6	Marvinbryantia
-53.2		-100								-76.6	Megasphaera
								-50.6		-50.6	Merdibacter
100.9	70.6				151.9	-11.4	-31.4			56.1	Methanobrevibacter
	92.4				84.3					88.4	Methanosphaera
			-65.5							-65.5	Methylophaga

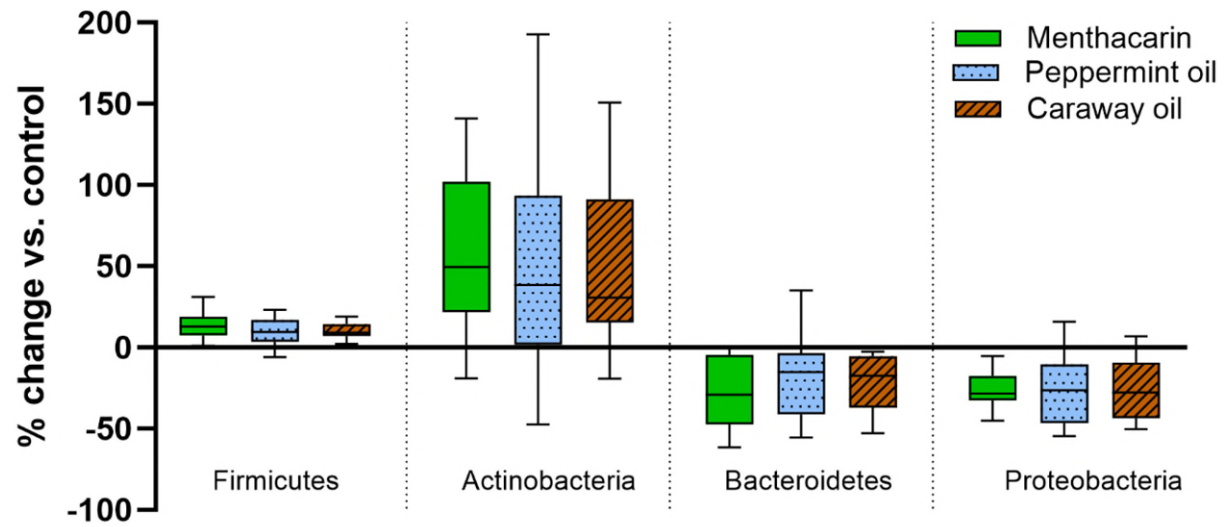
Supplementary Figure S2 (4 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
		-47.3								-47.3	Mitsuokella
		-5.7	7.2	12.1	14.6					7.1	Mogibacterium
-56.1	214		24	140.7	13.6	9.8	22.5	-26.7	35.9	42	Monoglobus
-24.5	54.7	51.1	-40.2	80.3	47.1	-10.9	30.8	41.5	-40	19	Moryella
-23.2	-74.8	-21.2	-32.2	-18.5	19.4		-28.9	60.6	124.2	0.6	Negativibacillus
-42.3	18.9	-27.6	8.8	17	-13	22.7	40.4	25.8		5.6	NK4A214 group
-100	-94.8			-4.8	-30.9	-46.7	-11.4	-92.2	-66.4	-55.9	Odoribacter
2.4		20.6	-91.9							-23	Olsenella
		17	14.1		-31.6		83	-47.1		7.1	Oribacterium
54.1	45.3	7.6	10.7	7.7	1.8	-13.6	-13.3	47	36.5	18.4	Oscillibacter
	-47.8	15.3	-33.9	-55.5	-71.1	-14.6	-57.5	-29	-37.7	-36.9	Oscillospira
				-89.3		-87.2				-88.3	Paeniclostridium
-29.8										-29.8	Paludicola
-81.2	-59.1	104.2	13.9	-62.7	-11.1	-13.6	-19.1	-76.2	-19.8	-22.5	Parabacteroides
						-88.1				-88.1	Paraclostridium
				-89.2	-56.4	92.7	-84.5			-34.3	Paraprevotella
	-82.6	46.6	-41.3	-8.2	-21.2	-26.1	-25.2	135.6	-32	-6	Parasutterella
56.4			76.6					420.1		184.4	Peptoniphilus
			-7		-13.7		220.2	-28.7		42.7	Peptostreptococcus
	107.8	-30.6		18.3	-21.7	-12.4	-16.6	78.1	22.3	18.2	Phascolarctobacterium
-8.4	59	40.2	69.7	18.1	34.8	-10.9	13.7	91.9	-54.8	25.3	Phoceia
-29.2	-73.9	-82.3	-70.9	-35.6	-75.1	47.3	-84.3	-72.2	100.9	-37.5	Prevotella
-84.9	-65.4	-59.4							-100	-77.4	Prevotellaceae NK3B31 group
		-62.1	-88.5							-75.3	Prevotellaceae UCG-003
-84.8			115.1							15.1	Raoultibacter
		-87								-87	Rikenellaceae RC9 gut group
122.9	16	-74.1	-16.4		-1.2	-45.4	-16.4			-2.1	Romboutsia
5.5	-31.9	42.1	-6.6	51	16.6	36.9	17.3	-0.7	59.6	19	Roseburia
-3	-14.9	25.6	-8.2	10.1	14.5	26.4	-0.6	39	32.9	12.2	Ruminococcus
-78.5			31.3						40.7	-2.2	S5-A14a

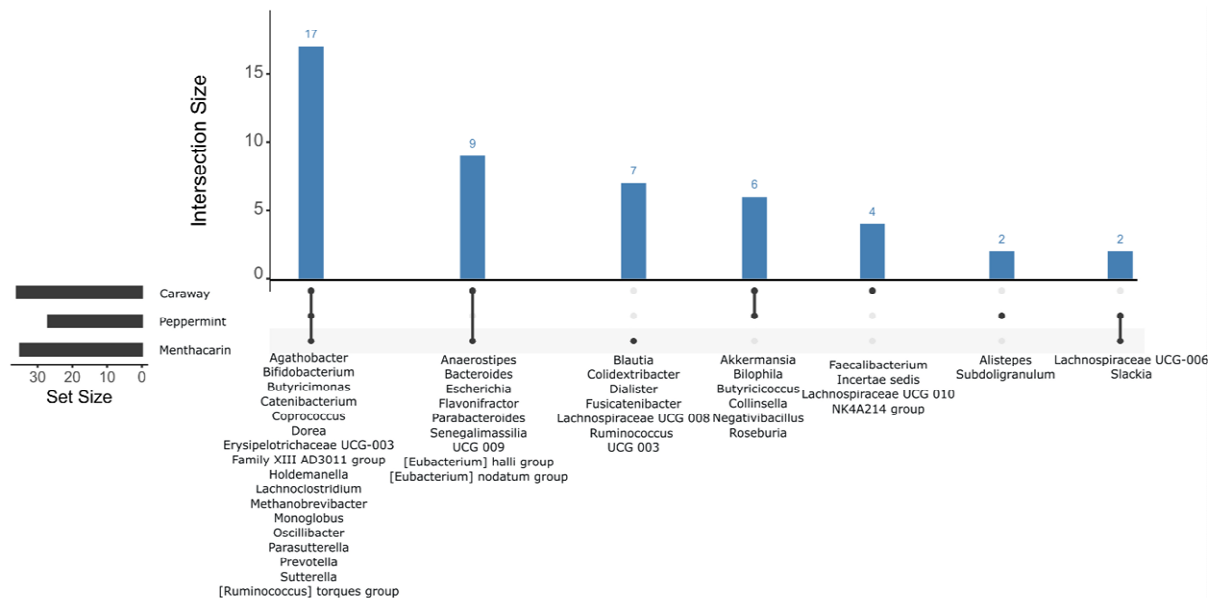
Supplementary Figure S2 (5 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.

microbiome 1	microbiome 2	microbiome 3	microbiome 4	microbiome 5	microbiome 6	microbiome 7	microbiome 8	microbiome 9	microbiome 10	Means	
				49.4	-16.6					16.4	Selenomonadales bacterium M
	110.8			120.3	27.6			-88.5	-75.2	19	Sellimonas
19.3		-4.9	-24.1		-35.4	32.7	-3.6		36.4	2.9	Senegalimassilia
			14.3							14.3	Serratia
								-42.1		-42.1	Shuttleworthia
-69.7		15.4		33.3	-58.6		50.5	54.7	6.1	4.5	Slackia
		69	148.3							108.6	Solobacterium
-31.6			54.9	6.1	10		1.2	53.6		15.7	Streptococcus
26.5	23	0.6	-31.8	-5.5	-22.4	19.3	-21.9	47.8	52.6	8.8	Subdoligranulum
		-33.1								-33.1	Succinivibrio
-60.3	-90.5	-52.3	-19.1	-13.2	-32.1	-26.5	-31.4	-45	-79.1	-44.9	Sutterella
							-67.6			-67.6	Terrisporobacter
	-4.5									-4.5	Turicibacter
			-35.8						-13.1	-24.5	Tuzzerella
	0.1		-18.3	-60.5	-6.9	0.7	6.4		-8.1	-12.4	Tyzzereella
-17.5										-17.5	UBA1819
				-48	-48.7					-48.4	UC5-1-2E3
-30.9	24	79.8	-18.5	28.9	-1.5	8.9	0.8	-1.4	-3.8	8.6	UCG-002
-39	-68.9	-39.4	-9.4	-25.1	0.6	-28.8	-15.4	-4.1	-33.4	-26.3	UCG-003
-18.5	37.3	-14.4	-17.9	2.4	-11.1	0.8	24.7	-27.4		-2.7	UCG-005
								-54.3		-54.3	UCG-008
-70.7	26.6	10.5	18.9	29	4.1	-54.6	-33.2	32.7		-4.1	UCG-009
-59.3							86.1			13.4	Victivallis

Supplementary Figure S2 (6 of 6): Heatmap of fractional change in relative abundance induced by Menthacarin (expressed in percentage) across all identified 173 genera. The numbers in each cell denote the fractional change with mean for each genus across 10 microbiomes shown in the final column.



Supplementary Figure S3: Peppermint and caraway oil induce similar fractional changes in relative abundance of the phyla that are significantly affected by Menthacarin. Data are presented as box-and-whiskers plots with quartiles and min-max values.



Supplementary Figure S4: UpSet plot showing the proportions of genera exhibiting consistent fractional change with treatment of Menthacarin, peppermint oil, and caraway oil in at least 70% of tested microbiomes. Genera present in at least 4 microbiomes were considered.

