Electronic Supplementary Information

Red cabbage microgreens modulation of gut microbiota is associated with attenuation of diet-induced obesity risk factors in a mouse model

Yanbei Wu [a], Quynhchi Pham [b], Yali Wang [a], Haiqiu Huang [b], Xiaojing Jiang [b], Robert W. Li [c], Liangli Yu [d], Yaguang Luo [e], Jing Wang [a], Thomas T.Y. Wang [b*]

[a] China-Canada Joint Lab of Food Nutrition and Health (Beijing), Beijing Technology &Business University, Beijing 100084, PR China

[b] Diet Genomics and Immunology Laboratory, BHNRC, ARS, USDA, Beltsville, MD 20705, USA

[c] Animal Parasitic Diseases Laboratory, BARC, ARS, USDA, Beltsville, MD 20705, USA

[d] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742, USA

[e] Food Quality Laboratory, BARC, ARS, USDA, Beltsville, MD 20705, USA

Table S1. Primer sequence used for microbial analysis by real-time PCR.

Bacteria	Direction	Sequence (5′–3′)
Bacteroidetes	Forward	GGARCATGTGGTTTAATTCGATGAT
	Reverse	AGCTGACGACAACCATGCAG
Firmicutes	Forward	GGAGYATGTGGTTTAATTCGAAGCA
	Reverse	AGCTGACGACAACCATGCAC
Prevotella	Forward	TCCTACGGGAGGCAGCAGT
	Reverse	CAATCGGAGTTCTTCGTG
Enterobacteriaceae	Forward	CATTGACGTTACCCGCAGAAGAAGC
	Reverse	CTCTACGAGACTCAAGCTTGC
Ruminococcus	Forward	GGCGGCCTACTGGGCTTT
	Reverse	CCA GGT GGA TAA CTT ATT GTG TTAA
Bifidobacteria	Forward	TCGCGTCYGGTGTGAAAG
	Reverse	CCACATCCAGCRTCCAC
Lactobacillus	Forward	GAGGCAGCAGTAGGGAATCTTC
	Reverse	GGCCAGTTACTACCTCTATCCTTCTTC
Akkermansia muciniphila	Forward	CAGCACGTGAAGGTGGGGAC
	Reverse	CCT TGCGGTTGGCTTCAGAT

Table S2. Relative abundance (%) of the bacterial taxa at phylum level in cecal samples of mice grouped by diet (LF: low-fat diet, HF: high-fat diet, LFMG: low-fat diet supplemented with red cabbage microgreen powder, HFMG: high-fat diet supplemented with red cabbage microgreen powder).

Phylum	Diet			
	LF	HF	LFMG	HFMG
Bacteroidetes	60.00±2.45	53.47±6.21	55.72±5.21	47.89±4.1
Firmicutes	34.41±2.19	39.19±5.92	37.69±5.55	44.87±4.21
Proteobacteria	3.18±0.61	4.77±0.26	3.15±0.53	4.28±0.96
Deferribacteres	2.17±0.78	2.38±1.24	2.91±0.76	2.83±0.83
Verrucomicrobia	0.16±0.17	0.085±0.164	0.10±0.17	0.02±0.04
TM7	0.08±0.04	0.09±0.11	0.09±0.03	0.09±0.07
Actinobacteria	0.01±0.00	0.01±0.00	0.02±0.01	0.02±0.01

Table S3. Correlation of obesity-related risk factors with differentially abundant microbial features (with the relative abundance greater than 1%) identified in the caecal content of mice fed low- or high-fat diets with or without red cabbage microgreen. [a,b,c]

Features at genus level	Weight gain	LDL	HDL	VLDL	Hepatic Cholesterol Ester	Hepatic Triglycerides	Fecal bile acid
Unclassified *S24-7*	-0.43*	-0.42*	NC	NC	NC	NC	NC
Unclassified *Clostridiales*	NC	NC	NC	NC	-0.43*	NC	NC
Unclassified *Ruminococcaceae*	0.43*	NC	NC	NC	NC	NC	0.49*
AF12	0.52**	0.38*	NC	NC	NC	NC	0.59***
Bilophila	0.49*	NC	NC	NC	NC	NC	0.45*

[a:] NC = no correlation (p>0.05), *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

[b:] Plus/minus symbol indicates the positive/negative correlation between factors and bacteria.

[c:] The value represents Pearson correlation coefficient.

Table S4. Topological properties of the global network inferred using a Random-Matrix theory (RMT) based network pipeline under various experimental conditions.

Network Indexes	LF	HF	LFMG	HFMG
Total nodes	247	245	302	257
Total links	416	483	643	471
Total modules	23	23	29	29
Modularity (M)	0.819	0.72	0.778	0.77
R square of power-law	0.71	0.80	0.786	0.91
Average degree (avgK)	3.368	3.94	4.258	3.67
Average clustering coefficient (avgCC)	0.304	0.32	0.412	0.22
Average path distance (GD)	8.217	6.97	8.1	6.55
Geodesic efficiency (E)	0.166	0.20	0.184	0.20
Harmonic geodesic distance (HD)	6.039	4.92	5.431	4.91
Maximal degree	13	16	17	18
Nodes with max degree	OTU 262625	OTU 1136443 OTU 1684221 OTU 329790 OTU 317633	OTU 716006	OTU 263705 OTU 462832
Centralization of degree (CD)	0.039	0.05	0.043	0.06
Maximal betweenness	6815.31	4996.26	6003.48	4128.33
Nodes with max betweenness	OTU 262677	OTU 275366	OTU 434339	OTU 227886
Centralization of betweenness (CB)	0.204	0.15	0.121	0.11
Maximal stress centrality	48167	33304	277722	40607
Nodes with max stress centrality	262677	418501	434339	341252
Centralization of stress centrality (CS)	1.451	1.04	5.625	1.17
Maximal eigenvector centrality	0.355	0.31	0.318	0.32
Nodes with max eigenvector centrality	OTU 353012	OTU 317633	OTU 716006	OTU 263705
Centralization of eigenvector centrality (CE)	0.335	0.28	0.299	0.31
Density (D)	0.014	0.02	0.014	0.01
Reciprocity	1.00	1.00	1.00	1.00
Transitivity (Trans)	0.359	0.35	0.461	0.37
Connectedness (Con)	0.78	0.63	0.535	0.61
Efficiency	0.987	0.98	0.979	0.98
Hierarchy	0.00	0.00	0.00	0.00
Lubness	1.00	1.00	1.00	1.00
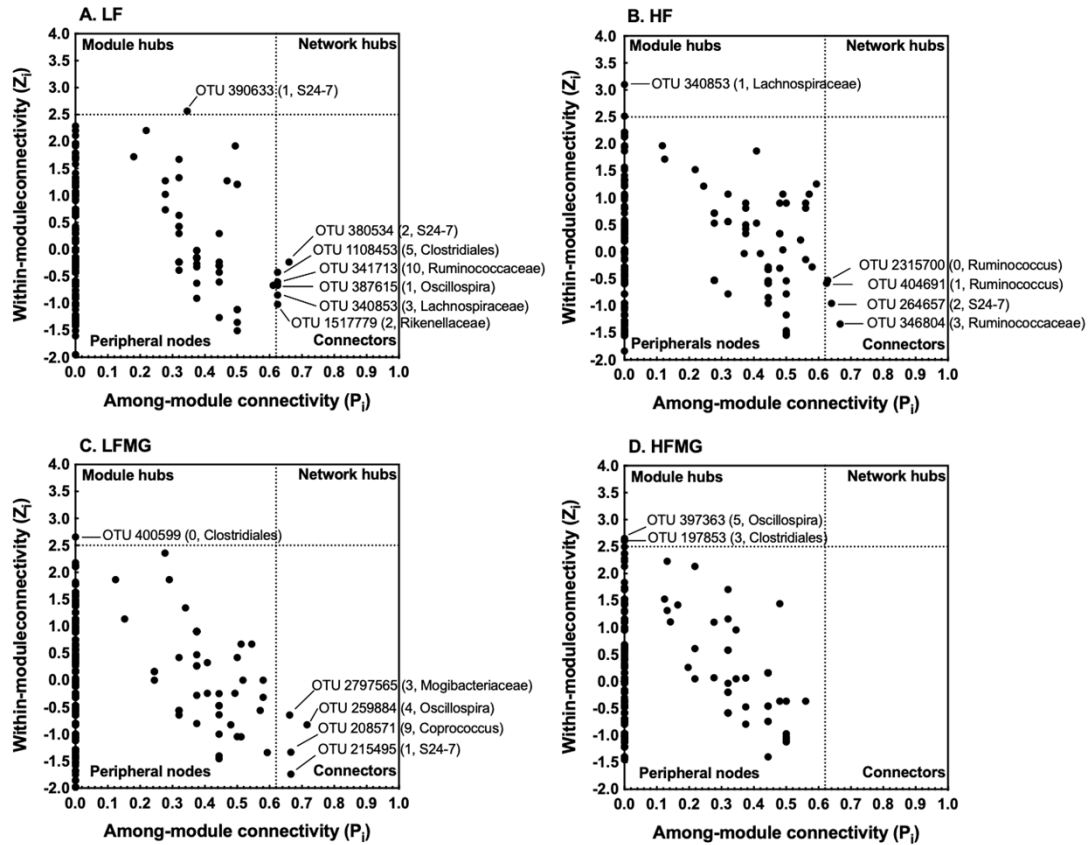
Fig. S1. Z-P plot showing the distribution of OTUs based on their topological roles in the network. Each dot represents an OTU under different diet groups (HF: a high-fat diet, HFMG: a high-fat diet supplemented with RCMG). The topological role of each OTU was determined according to the scatter plot of within-module connectivity (Zi) and among-module connectivity (Pi). The module hubs and connectors are labeled with OTU numbers. In parentheses are the module number and phylogenetic associations.