Supplemental table 1. The average coefficients of variation and intra-class correlations among major tryptophan metabolites in three cohorts.

	HPFS		NHS		NHSII	
Plasma tryptophan metabolites	Mean CV	Mean ICC	Mean CV	Mean ICC	Mean CV	Mean ICC
Tryptophan	9.4	0.79	9.8	0.78	9.8	0.78
IPA	28.3	0.98	41.0	0.94	15.4	1.00
IS	11.3	0.97	15.1	0.95	14.7	0.94
Serotonin	-	-	32.7	0.98	32.4	0.98
Kynurenine	9.9	0.85	14.7	0.88	23.0	0.84
Kynurenic acid	-	-	25.3	0.75	-	-
Quinolinic acid	20.7	0.76	21.0	0.90	20.2	0.93
Xanthurenic acid	27.9	0.70	30.9	0.72	22.5	0.92

Supplemental table 2. Beta coefficients and p values of the 2-way interaction terms between total and individual species rank score and dietary tryptophan on plasma IPA concentrations.

Species rank score	**Beta coefficients**	**P**
Total	0.000564	0.36614
coprobacillus_unclassified	0.001661	0.804105
lactococcus_lactis	-0.00436	0.334466
clostridium_symbiosum	0.003245	0.518947
clostridium_clostridioforme	0.008579	0.516225
flavonifractor_plautii	-0.00075	0.852686
clostridium_nexile	-0.02995	0.104941
ruminococcus_gnavus	0.003207	0.408465
bacteroides_dorei	-0.00032	0.90837
eubacterium_eligens	0.002177	0.394987
eubacterium_sp_3_1_31	-0.01203	0.177632
clostridiales_bacterium_1_7_47faa	0.004688	0.448636
butyrivibrio_crossotus	0.007695	0.740985
lachnospiraceae_bacterium_2_1_58faa	0.005346	0.381801
lachnospiraceae_bacterium_5_1_57faa	0.05884	0.005343
lachnospiraceae_bacterium_1_4_56faa	-0.00593	0.192924
bacteroides_faecis	0.006236	0.302629
faecalibacterium_prausnitzii	0.000582	0.81658

Supplemental table 3. Beta coefficients and p values of the 3-way interaction terms among total species rank score, dietary tryptophan and tryptophan food contributors, and dietary fiber on plasma IPA concentrations

Food contributors	**Beta**	**P**	**Beta**	**P**	**Beta**	**P**	**Beta**	**P**
	Total fiber		Soluble fiber		Insoluble fiber		Pectin	
Tryptophan	1.7E-04	7.8E-04	4.0E-04	3.4E-02	2.6E-04	1.2E-04	1.3E-03	8.2E-04
Vegetables	2.0E-05	2.0E-03	9.0E-05	1.2E-03	3.0E-05	3.8E-03	9.0E-05	4.0E-02
Legume	5.0E-05	3.4E-01	1.8E-04	3.9E-01	6.0E-05	3.8E-01	7.0E-05	8.1E-01
Nuts	2.0E-05	3.5E-02	7.0E-05	2.8E-02	2.0E-05	5.7E-02	9.0E-05	1.0E-01
Fruits	1.0E-05	1.3E-01	3.0E-05	1.7E-01	1.0E-05	1.4E-01	2.0E-05	5.1E-01
Red/processed meat	-2.0E-05	1.1E-02	-8.0E-05	1.3E-02	-3.0E-05	2.0E-02	-6.0E-05	2.9E-01
Poultry	0.0E+00	9.5E-01	2.0E-05	6.7E-01	0.0E+00	9.3E-01	3.0E-05	7.7E-01
Fish	-1.0E-05	6.9E-01	2.0E-05	6.5E-01	-1.0E-05	4.8E-01	-2.0E-05	7.7E-01
Egg	-9.0E-05	1.7E-02	-1.9E-04	9.9E-02	-1.2E-04	1.4E-02	-2.5E-04	1.7E-01
Dairy	2.0E-05	1.3E-02	5.0E-05	8.3E-02	3.0E-05	6.8E-03	1.7E-04	1.5E-03
Whole grain	2.0E-05	1.7E-03	5.0E-05	7.4E-03	3.0E-05	2.0E-03	1.4E-04	1.0E-04

Supplemental table 4. Associations between DNA pathways within IPA-associated species and plasma IPA concentrations

IPA-associated species	Beta	Pathways	Function groups
faecalibacterium_prausnitzii	0.004283	4-deoxy-l-threo-hex-4-enopyranuronate degradation	Energy metabolism
butyrivibrio_crossotus	0.002999	5-aminoimidazole ribonucleotide biosynthesis I	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.002981	5-aminoimidazole ribonucleotide biosynthesis I	Nucleotides biosynthesis
butyrivibrio_crossotus	0.003094	5-aminoimidazole ribonucleotide biosynthesis II	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003153	5-aminoimidazole ribonucleotide biosynthesis II	Nucleotides biosynthesis
bacteroides_faecis	-0.00174	adenine and adenosine salvage III	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003778	adenine and adenosine salvage III	Nucleotides biosynthesis
bacteroides_faecis	-0.0021	adenosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
butyrivibrio_crossotus	0.003363	adenosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.004079	adenosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
ruminococcus_gnavus	-0.00236	adenosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003749	adenosylcobalamin salvage from cobinamide I	Energy metabolism
faecalibacterium_prausnitzii	0.004483	calvin-benson-bassham cycle	Energy metabolism
bacteroides_faecis	-0.00163	cdp-diacylglycerol biosynthesis I	Energy metabolism
butyrivibrio_crossotus	0.002647	cdp-diacylglycerol biosynthesis I	Energy metabolism
faecalibacterium_prausnitzii	0.003877	cdp-diacylglycerol biosynthesis I	Energy metabolism
bacteroides_faecis	-0.00163	cdp-diacylglycerol biosynthesis II	Energy metabolism
butyrivibrio_crossotus	0.002647	cdp-diacylglycerol biosynthesis II	Energy metabolism
faecalibacterium_prausnitzii	0.003877	cdp-diacylglycerol biosynthesis II	Energy metabolism
butyrivibrio_crossotus	0.002801	chorismate biosynthesis from 3-dehydroquinate	Amino acids biosynthesis
butyrivibrio_crossotus	0.002766	chorismate biosynthesis I	Amino acids biosynthesis
bacteroides_faecis	-0.00158	cmp-3-deoxy-d-manno-octulosonate biosynthesis I	Homeostasis
faecalibacterium_prausnitzii	0.003317	coenzyme a biosynthesis I	Coenzyme biosynthesis
bacteroides_dorei	-0.00434	coenzyme a biosynthesis II (mammalian)	Coenzyme biosynthesis
bacteroides_faecis	-0.00161	coenzyme a biosynthesis II (mammalian)	Coenzyme biosynthesis
butyrivibrio_crossotus	0.002631	coenzyme a biosynthesis II (mammalian)	Coenzyme biosynthesis
faecalibacterium_prausnitzii	0.00319	coenzyme a biosynthesis II (mammalian)	Coenzyme biosynthesis
faecalibacterium_prausnitzii	0.004435	d-fructuronate degradation	Energy metabolism
faecalibacterium_prausnitzii	0.004129	d-galactose degradation v (leloir pathway)	Energy metabolism
faecalibacterium_prausnitzii	0.004061	d-galacturonate degradation I	Energy metabolism
faecalibacterium_prausnitzii	0.004139	dtdp-l-rhamnose biosynthesis I	Nucleotides biosynthesis

bacteroides_dorei	-0.00435	folate transformations II	Nucleotides biosynthesis
bacteroides_faecis	-0.00162	folate transformations II	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.004129	galactose degradation I (leloir pathway)	Energy metabolism
faecalibacterium_prausnitzii	0.003715	glutaryl-coa degradation	Homeostasis
bacteroides_faecis	-0.00187	glycolysis Iv (plant cytosol)	Energy metabolism
faecalibacterium_prausnitzii	0.004423	glycolysis Iv (plant cytosol)	Energy metabolism
bacteroides_faecis	-0.00179	gondoate biosynthesis (anaerobic)	Energy metabolism
bacteroides_faecis	-0.00178	guanosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
butyrivibrio_crossotus	0.002682	guanosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003461	guanosine ribonucleotides de novo biosynthesis	Nucleotides biosynthesis
bacteroides_faecis	-0.00173	Inosine-5'-phosphate biosynthesis I	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003585	Inosine-5'-phosphate biosynthesis I	Nucleotides biosynthesis
bacteroides_faecis	-0.00168	Inosine-5'-phosphate biosynthesis II	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.003552	Inosine-5'-phosphate biosynthesis II	Nucleotides biosynthesis
bacteroides_faecis	-0.00168	l-histidine biosynthesis	Amino acids biosynthesis
faecalibacterium_prausnitzii	0.004582	l-isoleucine biosynthesis I (from threonine)	Amino acids biosynthesis
faecalibacterium_prausnitzii	0.004418	l-isoleucine biosynthesis III	Amino acids biosynthesis
bacteroides_dorei	-0.00415	l-lysine biosynthesis III	Amino acids biosynthesis
bacteroides_faecis	-0.00191	l-lysine biosynthesis III	Amino acids biosynthesis
faecalibacterium_prausnitzii	0.00395	l-lysine biosynthesis III	Amino acids biosynthesis
ruminococcus_gnavus	-0.0023	l-lysine biosynthesis III	Amino acids biosynthesis
bacteroides_faecis	-0.00186	l-lysine biosynthesis vi	Amino acids biosynthesis
faecalibacterium_prausnitzii	0.003531	l-lysine biosynthesis vi	Amino acids biosynthesis
ruminococcus_gnavus	-0.00238	l-lysine biosynthesis vi	Amino acids biosynthesis
bacteroides_faecis	-0.00185	l-rhamnose degradation I	Energy metabolism
butyrivibrio_crossotus	0.00307	l-valine biosynthesis	Amino acids biosynthesis
faecalibacterium_prausnitzii	0.004712	l-valine biosynthesis	Amino acids biosynthesis
butyrivibrio_crossotus	0.002974	methylerythritol phosphate pathway I	Homeostasis
faecalibacterium_prausnitzii	0.002713	pantothenate and coenzyme a biosynthesis III	Homeostasis
faecalibacterium_prausnitzii	0.003062	pentose phosphate pathway (non-oxidative branch)	Homeostasis
faecalibacterium_prausnitzii	0.003987	peptidoglycan biosynthesis I (meso-diaminopimelate containing)	Homeostasis
faecalibacterium_prausnitzii	0.00393	peptidoglycan biosynthesis III (mycobacteria)	Homeostasis
faecalibacterium_prausnitzii	0.00332	peptidoglycan maturation (meso-diaminopimelate containing)	Homeostasis

bacteroides_faecis	-0.00183	phosphopantothenate biosynthesis I	Homeostasis
butyrivibrio_crossotus	0.003287	phosphopantothenate biosynthesis I	Homeostasis
butyrivibrio_crossotus	0.002747	preq0 biosynthesis	Nucleotides biosynthesis
butyrivibrio_crossotus	0.00281	purine ribonucleosides degradation	Nucleotides degradation
faecalibacterium_prausnitzii	0.003856	purine ribonucleosides degradation	Nucleotides degradation
ruminococcus_gnavus	-0.00235	purine ribonucleosides degradation	Nucleotides degradation
faecalibacterium_prausnitzii	0.003224	putrescine biosynthesis Iv	Homeostasis
bacteroides_faecis	-0.00158	pyridoxal 5'-phosphate biosynthesis I	Amino acids biosynthesis
butyrivibrio_crossotus	0.003079	pyruvate fermentation to Isobutanol (engineered)	Energy metabolism
faecalibacterium_prausnitzii	0.004793	pyruvate fermentation to Isobutanol (engineered)	Energy metabolism
bacteroides_faecis	-0.00171	queuosine biosynthesis	Nucleotides degradation
butyrivibrio_crossotus	0.002822	queuosine biosynthesis	Nucleotides degradation
butyrivibrio_crossotus	0.002981	s-adenosyl-l-methionine cycle I	Homeostasis
faecalibacterium_prausnitzii	0.004009	s-adenosyl-l-methionine cycle I	Homeostasis
ruminococcus_gnavus	-0.00243	s-adenosyl-l-methionine cycle I	Homeostasis
butyrivibrio_crossotus	0.002849	starch degradation v	Energy metabolism
faecalibacterium_prausnitzii	0.004153	starch degradation v	Energy metabolism
ruminococcus_gnavus	-0.00246	starch degradation v	Energy metabolism
faecalibacterium_prausnitzii	0.004096	sucrose degradation III (sucrose Invertase)	Energy metabolism
faecalibacterium_prausnitzii	0.003991	thiamin formation from pyrithiamine and oxythiamine (yeast)	Energy metabolism
butyrivibrio_crossotus	0.002869	udp-n-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing)	Homeostasis
faecalibacterium_prausnitzii	0.003513	udp-n-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing)	Homeostasis
butyrivibrio_crossotus	0.002935	udp-n-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)	Homeostasis
faecalibacterium_prausnitzii	0.003935	udp-n-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)	Homeostasis
bacteroides_faecis	-0.00169	ump biosynthesis	Nucleotides biosynthesis
butyrivibrio_crossotus	0.002865	ump biosynthesis	Nucleotides biosynthesis
faecalibacterium_prausnitzii	0.00405	ump biosynthesis	Nucleotides biosynthesis
bacteroides_faecis	-0.00178	urate biosynthesis/inosine 5'-phosphate degradation	Nucleotides degradation
faecalibacterium_prausnitzii	0.003952	urate biosynthesis/inosine 5'-phosphate degradation	Nucleotides degradation

Supplemental table 5. Mediation analysis for IPA and kynurenine pathway (KP) metabolites in the association between dietary tryptophan intake and type 2 diabetes risk.

	Proportion of association explained (95% CI) *	P value
IPA	12.2% (4.2%, 30.4%)	0.003
Plasma tryptophan	1.2% (0.3%, 5.0%)	0.08
All KP metabolites	8.2% (2.7%, 22.2%)	0.01
KP metabolites without kynurenic acid	7.8% (2.6%, 21.0%)	0.01
Individual KP metabolite		
-Kynurenine	<1%	-
-Kynurenic acid	1.3% (0.2%, 9.8%)	0.16
-Quinolinic acid	3.8% (0.7%, 19.1%)	0.12
-Xanthurenic acid	5.9% (1.6%, 20.0%)	0.04

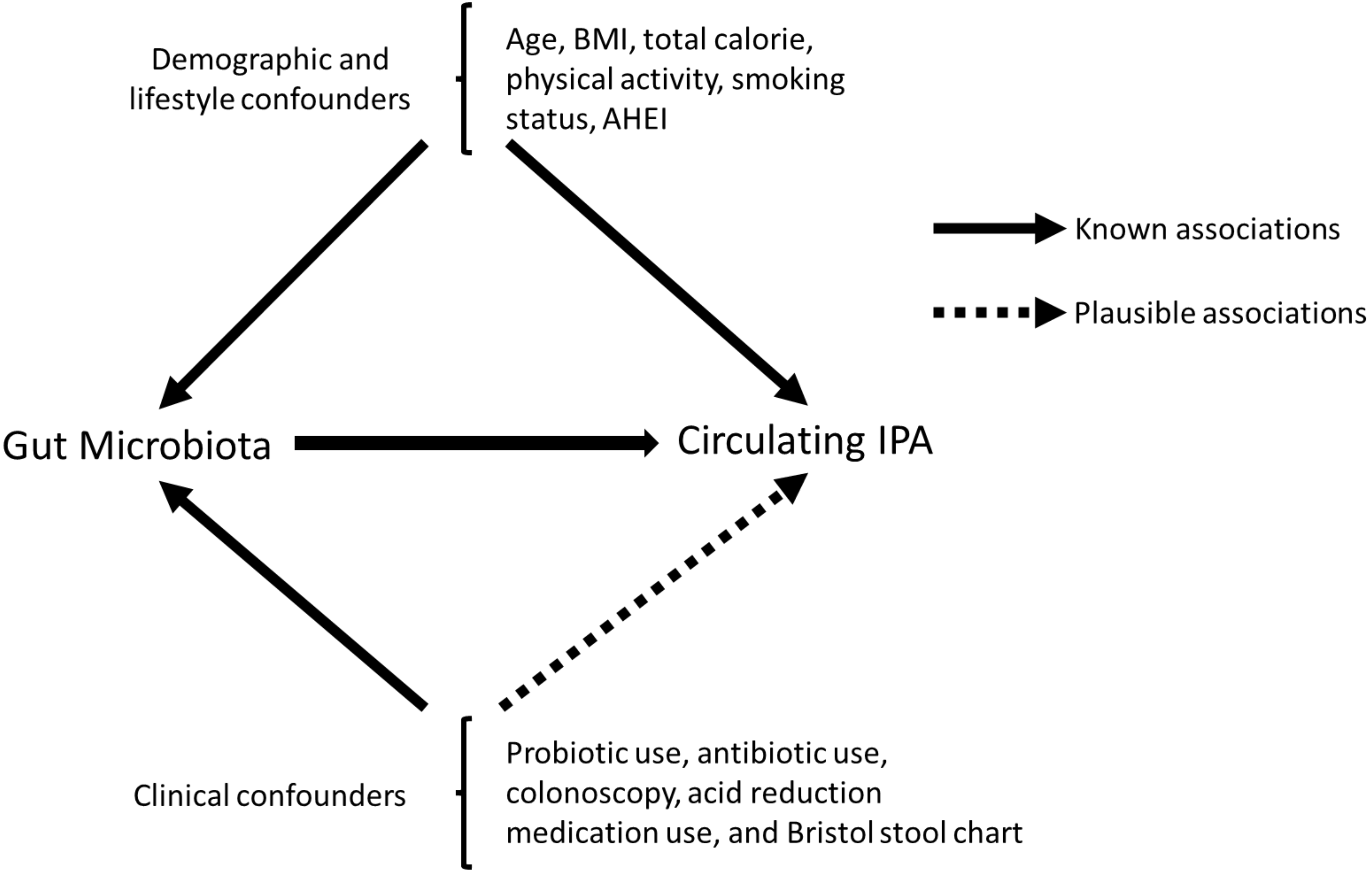
Proportional hazard model adjusted for age, race, cohort origin, physical activity, alcohol intake, AHEI, smoking status, BMI, and total energy intake.

*The mathematical transformation used in calculating the confidence interval of the mediation effects did not allow for non-positive value, and therefore the p values were used to determine statistical significance.

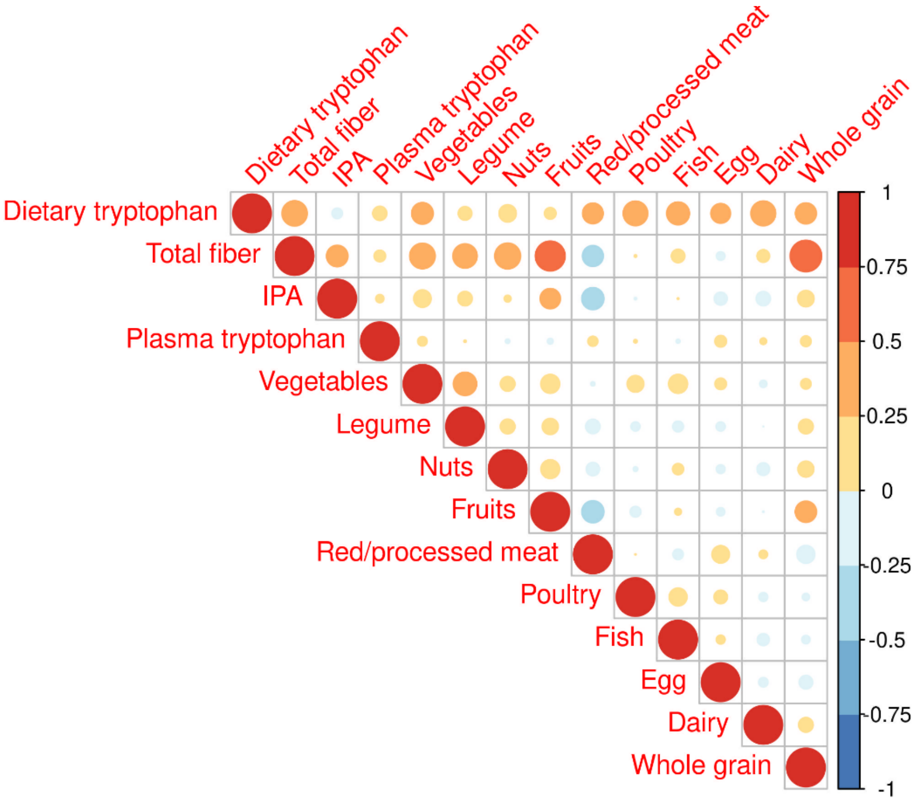
Supplemental table 6. Associations between fiber intake and kynurenine pathway metabolites

	Total fiber		Soluble fiber		Insoluble fiber	
	Beta	P	Beta	P	Beta	P
Kynurenine	-0.004	0.225	-0.018	0.086	-0.004	0.236
Kynurenic acid	-0.005	0.033	-0.023	0.004	-0.005	0.069
Quinolinic acid	-0.005	0.147	-0.014	0.162	-0.007	0.044
Xanthurenic acid	-0.002	0.476	-0.004	0.722	-0.006	0.113

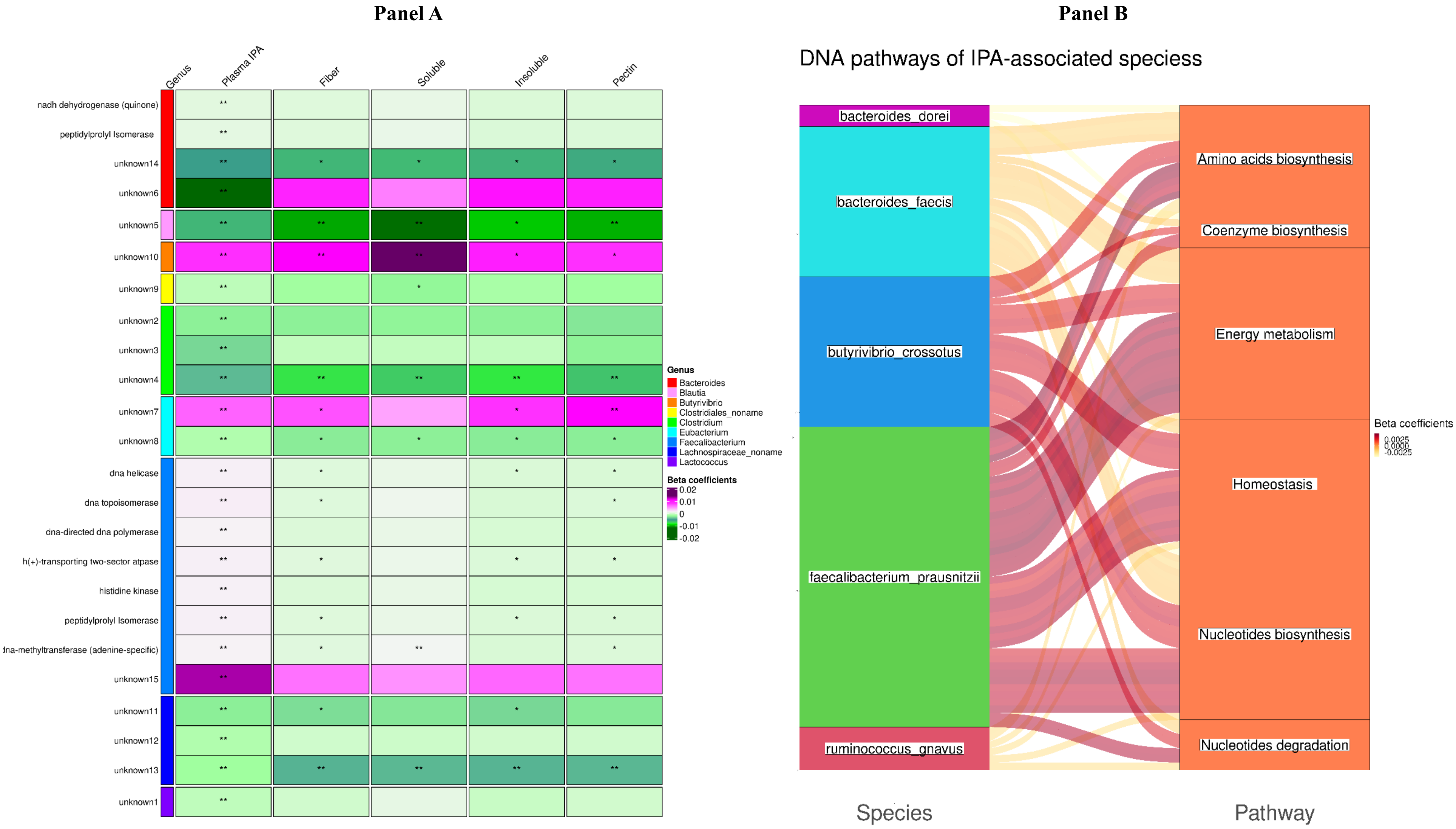
Model adjusted for age, race, cohort origin, physical activity, alcohol intake, AHEI, smoking status, BMI, and total energy intake.



Supplemental figure 1. Conceptual framework of the associations between gut microbiome and circulating IPA level.



Supplemental figure 2. Pearson correlations among dietary tryptophan, fiber, and major tryptophan food contributors.



Supplemental figure 3. Functional analysis of the associations between relative abundance of DNA enzymes and pathways and identified IPA-associated species. **Panel A** shows the DNA enzymes associated with IPA and fiber among IPA-related species. Panel B illustrates the DNA pathways associated with the IPA-related species.