

Supplementary information

Supplementary Table 1. Comparison of serum SCFA and cytokines between bipolar depressive and manic groups

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Supplementary figure 1

(A) Distribution of five trials of 5-fold cross-validation error in random forest classification of BD as the number of features increases. The model was constructed using eight SCFAs and six cytokines that were significantly altered in patients with BD from 70% of BD and HC samples (training set). The black curve indicates the average of the five trials (gray lines). The pink line marks the number of features in the optimal set. (B) Receiver operating characteristic analysis was performed to evaluate the diagnostic performance of these six biomarkers, and the areas under the curve of the training set and test set were 97.54% and 98.1%, respectively. (C) The six variables with most weight to discriminate BD and HCs were selected by the cross-validated random forest models. The length of line indicated the importance of the metabolites for classification.

Supplementary Table 1 Comparison of serum SCFA and cytokines between bipolar depressive and manic groups

	BD-M group (n=44)	BD-D group (n=20)	Z	P-value
2-ECA (μg/mL)	0.005(0.001,0.009)	0.009(0.002,0.011)	-0.349	0.727
OA (μg/mL)	0.119(0.096,0.135)	0.137(0.108,0.156)	-1.869	0.062
HPA (μg/mL)	0.021(0.015,0.026)	0.021(0.017,0.027)	-0.029	0.977
ICA (μg/mL)	0.031(0.020,0.046)	0.041(0.030,0.061)	-2.491	0.013
VA (μg/mL)	0.027(0.022,0.035)	0.022(0.020,0.029)	-1.926	0.054
IVA (μg/mL)	0.092(0.073,0.120)	0.087(0.073,0.143)	-0.217	0.828
IBA (μg/mL)	0.037(0.030,0.053)	0.031(0.027,0.040)	-1.883	0.06
CA (μg/mL)	0.145(0.127,0.185)	0.160(0.140,0.200)	-1.246	0.213
BA (μg/mL)	0.078(0.048,0.114)	0.054(0.040,0.087)	-1.724	0.085
AA (μg/mL)	2.235(1.480,3.481)	2.291(1.357,3.494)	-0.29	0.772
PA (μg/mL)	0.189(1.283,0.257)	0.150(0.102,0.176)	-2.433	0.015
TNF-α (pg/mL)	7.34(4.79,11.91)	6.58(3.06,13.55)	-0.54	0.587
IL-6 (pg/mL)	14.54(2.70,38.14)	4.32(1.27,19.03)	-1.67	0.096
IL-8 (pg/mL)	171.92(89.53,412.37)	102.09(10.94,266.44)	-2.02	0.043
IL-10 (pg/mL)	0.50(0.15,0.80)	0.45(0.26,0.78)	-0.05	0.96
CCL2 (pg/mL)	266.51(201.27,356.82)	297.39(192.39,357.05)	-0.08	0.936
IL-1β (pg/mL)	4.74(3.14,10.19)	3.82(2.86,5.24)	-1.12	0.264
IFN-γ (pg/mL)	11.65(9.02,16.72)	11.57(9.95,16.44)	-0.21	0.833
IL-4 (pg/mL)	45.57(27.23,53.65)	46.53(29.68,59.47)	-0.68	0.495
IL-12 (pg/mL)	19.46(13.63,32.14)	19.46(15.96,22.60)	-0.28	0.783

Supplementary Table 2 The linear regression analysis results of cognitive function.

		Single factor linear regression				Multivariate linear regression			
		β	standardized β	R2	p	β	standardized β	p	
TMT	age	-0.111	-0.087	0.008	0.47				
	gender	-5.003	-0.209	0.044	0.08				
	BMI	0.228	0.065	0.004	0.589				
	education	0.671	0.17	0.029	0.156				
	smoking	4.784	0.16	0.026	0.182				
	passive smoking	3.354	0.14	0.019	0.249				
	alcohol	3.695	0.155	0.024	0.198				
	TNF- α	0.223	0.139	0.019	0.247				
	IL-6	-0.167	-0.18	0.032	0.134				
	IL-8	-0.02	-0.309	0.095	0.009	-0.017	-0.271	0.018	R2=0.166
	CCL2	-0.006	-0.133	0.018	0.268				P=0.002
	IL1- β	-0.026	-0.013	<0.001	0.915				FDR=0.004
	IL-4	-0.011	-0.049	0.002	0.684				
	OA	36.537	0.155	0.024	0.196				
	HPA	75.548	0.071	0.005	0.556				
	IVA	44.618	0.307	0.094	0.009	39.051	0.269	0.019	
	IBA	51.559	0.177	0.031	0.139				
	CA	45.545	0.202	0.041	0.091				
	PA	-17.304	-0.098	0.01	0.418				
	BA	-3.463	-0.045	0.002	0.712				
	2-ECA	-0.829	-0.014	<0.001	0.911				
BACS	age	0.073	0.061	0.004	0.614				
	gender	1.875	0.084	0.007	0.488				
	BMI	-0.328	0.1	0.01	0.408				
	education	0.217	0.059	0.003	0.627				
	smoking	0.015	0.001	<0.001	0.996				
	passive smoking	2.215	0.099	0.01	0.415				
	alcohol	1.083	0.048	0.002	0.689				
	TNF- α	0.063	0.042	0.002	0.73				
	IL-6	-0.069	-0.079	0.006	0.513				
	IL-8	-0.022	-0.378	0.143	0.001	-0.022	-0.378	0.001	R2=0.143
	CCL2	-0.015	-0.324	0.105	0.006				P=0.001
	IL1- β	-0.002	-0.001	<0.001	0.992				FDR=0.002
	IL-4	-0.053	-0.253	0.064	0.033				
	OA	3.929	0.018	<0.001	0.883				
	HPA	79.13	0.079	0.006	0.511				
	IVA	25.861	0.19	0.036	0.113				
	IBA	32.082	0.118	0.014	0.329				
	CA	26.792	0.127	0.016	0.293				
	PA	-11.413	-0.069	0.005	0.57				
	BA	5.038	0.069	0.005	0.567				
	2-ECA	5.281	0.096	0.009	0.449				
Fluency	age	-0.107	-0.085	0.007	0.479				
	gender	-4.381	-0.186	0.034	0.121				
	BMI	-0.28	-0.081	0.007	0.501				
	education	1.062	0.273	0.074	0.021	1.012	0.26	0.025	
	smoking	0.272	0.009	<0.001	0.939				
	passive smoking	2.553	0.108	0.012	0.374				
	alcohol	6.259	0.265	0.07	0.025				
	TNF- α	0.205	0.129	0.002	0.284				
	IL-6	-0.108	-0.117	0.014	0.331				
	IL-8	0.002	0.037	0.001	0.759				R2=0.132
	CCL2	0.009	0.198	0.039	0.099				P=0.008
	IL1- β	0.264	0.135	0.018	0.26				FDR=0.011

HVL	IL-4	0.056	0.254	0.065	0.033	0.053	0.24	0.037	R2=0.063 P=0.035 FDR=0.035
	OA	43.437	0.187	0.035	0.119				
	HPA	87.714	0.084	0.007	0.488				
	IVA	10.611	0.074	0.005	0.54				
	IBA	21.364	0.074	0.006	0.537				
	CA	45.118	0.203	0.041	0.09				
	PA	-21.963	-0.126	0.016	0.296				
	BA	-6.679	-0.087	0.008	0.47				
	2-ECA	-5.206	-0.09	0.008	0.477				
	age	-0.002	-0.002	<0.001	0.987				
	gender	2.332	0.114	0.013	0.344				
	BMI	-0.065	-0.022	<0.001	0.858				
	education	0.224	0.066	0.004	0.583				
	smoking	-3.791	-0.148	0.022	0.218				
	passive smoking	-0.325	-0.016	<0.001	0.896				
	alcohol	5.026	0.245	0.06	0.039				
	TNF- α	0.02	0.014	<0.001	0.906				
	IL-6	-0.2	-0.25	0.063	0.035	-0.2	-0.25	0.035	
	IL-8	-0.012	-0.216	0.047	0.071				
	CCL2	-0.005	-0.12	0.014	0.32				
	IL1- β	-0.182	-0.108	0.012	0.371				
	IL-4	-0.026	-0.136	0.019	0.258				
	OA	27.117	0.134	0.018	0.264				
	HPA	-26.225	-0.029	0.001	0.812				
	IVA	12.115	0.097	0.009	0.419				
	IBA	25.649	0.103	0.011	0.393				
	CA	17.594	0.091	0.008	0.45				
	PA	-29.563	-0.195	0.038	0.104				
	BA	-5.528	-0.083	0.007	0.491				
	2-ECA	-4.546	-0.092	0.008	0.469				
	age	-0.344	-0.244	0.059	0.041				
	gender	-0.09	-0.003	<0.001	0.978				
	BMI	-0.302	-0.078	0.006	0.519				
	education	0.785	0.179	0.032	0.135				
	smoking	-5.133	-0.155	0.024	0.198				
	passive smoking	0.292	0.011	<0.001	0.928				
	alcohol	-0.858	-0.032	0.001	0.789				
	TNF- α	0.098	0.055	0.003	0.648				
BVM	IL-6	-0.128	-0.123	0.015	0.306				R2=0.068 P=0.028 FDR=0.032
	IL-8	-0.018	-0.261	0.068	0.028	-0.018	-0.261	0.028	
	CCL2	0.007	-0.125	0.016	0.299				
	IL1- β	0.012	0.005	<0.001	0.965				
	IL-4	-0.027	-0.108	0.012	0.371				
	OA	31.799	0.122	0.015	0.313				
	HPA	81.946	0.069	0.005	0.566				
	IVA	30.636	0.19	0.036	0.113				
	IBA	53.882	0.167	0.028	0.164				
	CA	48.324	0.193	0.037	0.107				
	PA	-16.106	-0.082	0.007	0.497				
	BA	-0.734	-0.009	<0.001	0.944				
	2-ECA	1.645	0.026	0.001	0.837				
	age	-0.113	-0.085	0.007	0.478				
	gender	-5.423	-0.218	0.048	0.068				
	BMI	-0.129	-0.036	0.001	0.769				
	education	1.107	0.27	0.073	0.023				
	smoking	3.585	0.115	0.013	0.338				
	passive smoking	2.694	0.108	0.012	0.374				
	alcohol	7.187	0.289	0.084	0.014				

WMS	TNF- α	0.076	0.046	0.002	0.705				
	IL-6	-0.287	-0.297	0.088	0.012	-0.203	-0.209	0.031	R2=0.414 P<0.001 FDR=0.002
	IL-8	-0.022	-0.332	0.11	0.005				
	CCL2	-0.008	-0.162	0.026	0.178				
	IL1- β	-0.106	-0.052	0.003	0.669				
	IL-4	-0.004	-0.017	<0.001	0.89				
	OA	93.472	0.382	0.146	0.001				
	HPA	416.058	0.376	0.142	0.001				
	IVA	77.07	0.51	0.26	<0.001	54.395	0.36	0.001	
	IBA	136.815	0.453	0.205	<0.001				
	CA	117.924	0.503	0.253	<0.001	78.453	0.334	0.002	
	PA	-17.151	-0.093	0.009	0.44				
	BA	1.252	0.015	<0.001	0.898				
	2-ECA	4.033	0.065	0.004	0.608				
	age	-0.267	-0.196	0.038	0.102				
	gender	-6.177	-0.241	0.058	0.043				
	BMI	0.028	0.007	<0.001	0.951				
	education	0.923	0.218	0.047	0.068				
	smoking	2.697	0.084	0.007	0.486				
	passive smoking	2.414	0.094	0.009	0.439				
NAB	alcohol	6.12	0.238	0.057	0.045				
	TNF- α	0.061	0.035	0.001	0.769				
	IL-6	-0.176	-0.176	0.031	0.142				
	IL-8	-0.024	-0.351	0.123	0.003	-0.02	-0.298	0.01	R2=0.179 P=0.001 FDR=0.002
	CCL2	-0.01	-0.201	0.04	0.094				
	IL1- β	-0.043	-0.02	<0.001	867				
	IL-4	-0.019	-0.079	0.006	0.514				
	OA	68.449	0.271	0.073	0.022				
	HPA	200.143	0.175	0.031	0.144				
	IVA	36.657	0.235	0.055	0.049				
	IBA	75.879	0.243	0.059	0.041				
	CA	74.32	0.307	0.094	0.009	58.554	0.242	0.035	
	PA	-21.608	-0.114	0.013	0.345				
	BA	6.553	0.079	0.006	0.515				
	2-ECA	7.726	0.126	0.016	0.321				

Supplementary Table 3 Mediation analysis results for cognitive function

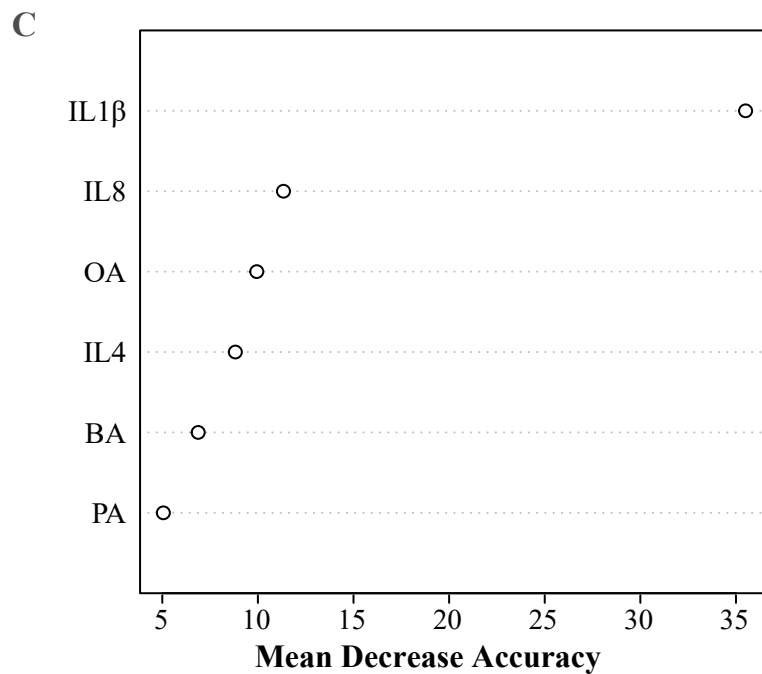
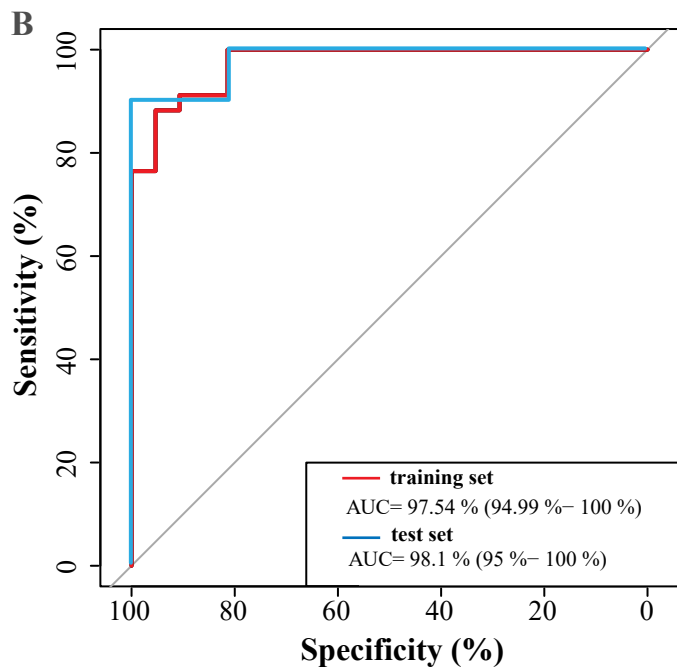
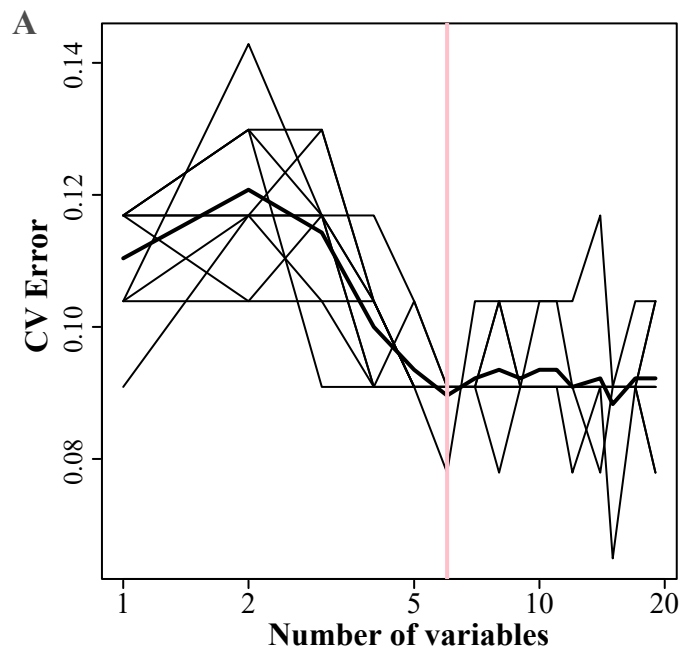
	a	b	c	c'	indirect effect (95% CI)	Proportion Mediated
WMS (Y)						
OA (X)+IL-8 (M)	-869.330*	-0.017*	93.472***	78.788***	14.683 (2.433-28.940)*	15.70%
IVA+IL-6	-16.621	-0.237*	77.070***	73.126***	3.944 (0.563-12.170)*	5.10%
IVA+IL-8	-324.630	-0.017*	77.070**	71.409**	5.661 (1.087-19.560)*	7.30%
IBA+IL-6	-31.119	-0.250*	136.815**	129.162**	7.653 (1.508-30.270)**	5.60%
IBA+IL-8	-607.820	-0.018**	136.815**	125.744*	11.070 (2.254-45.980)**	8.10%
CA+IL-8	-776.550	-0.015*	117.924***	106.000***	11.900 (10.400-24.070)*	10.10%
NAB						
OA+IL-8	-869.330*	-0.021**	68.449***	50.440*	18.009 (2.392-45.890)*	26.30%
IVA+IL-8	-324.630	-0.022**	36.657*	29.493	7.164 (0.069-24.280)*	19.50%
IBA+IL-8	-607.820	-0.022**	75.879*	62.455	13.423 (2.540-59.720)*	17.70%
CA+IL-8	-776.550	-0.020**	74.320**	58.554*	15.766 (0.813-37.780)*	21.20%
TMT						
IVA+IL-8	-342.630	-0.017*	44.618***	39.051***	5.566 (0.483-21.050)*	12.50%

a = beta values for the association between gut microbiota and energy metabolites; b = beta values for the association between energy metabolites and cognition; c = the total effect of gut microbiota on cognition; c' = the effect of gut microbiota on cognition after controlling for the indirect effects; indirect effect = a × b. All regression weights are fully standardized. Bold indicates significant indirect effects. *P < 0.05, **P < 0.001. CI = confidence interval.

Supplementary Table 4 Mediation analysis results for BD

	a	b	c	c'	indirect effect (95% CI)	Proportion Mediated
group (Y)						
PA (X)+IL-6 (M)	52.526	0.004***	2.122***	1.909***	0.213 (0.060-0.789)**	10.00%
PA+IL8	364.28	0.001***	2.122***	1.770***	0.351 (0.061-1.065)**	16.50%
CA+IL8	-781.78	0.001***	-4.241***	-3.493***	-0.748 (-1.284--0.255)*	17.60%
CA+IL1 β	-18.421	0.018**	-4.241***	-3.904***	-0.337 (-1.137--0.011)*	7.90%
IBA+IL-8	-768.39	0.001***	-3.158**	-2.360*	-0.798 (-2.079--0.277)*	25.20%
IVA+IL6	-47.545	0.004***	-1.696**	-1.480*	-0.215 (-1.185--0.021)*	12.70%
IVA+IL8	-452.08	0.001***	-1.696***	-1.231*	-0.464 (-1.247--0.088)*	27.30%
OA+IL8	-904.85	0.001***	-4.316***	-3.504***	-0.811 (-1.342--0.514)***	18.79%
OA+IL1β	-15.966	0.017**	-4.316***	-4.032***	-0.283 (-1.138--0.016)*	6.56%
2-ECA+IL1 β	-2.277	0.019**	-0.314**	-0.269*	-0.045 (-5.598--0.011)*	14.33%
2-ECA+IL8	-70.9	0.001***	-0.314**	-0.223**	-0.091 (-7.396--0.056)*	28.98%
2-ECA+TNFa	-2.060	0.020**	-0.314**	-0.272***	-0.042 (-6.394--0.009)*	13.37%
2-ECA+IL6	-8.162	0.004***	-0.314**	-0.277**	-0.037 (-7.789--0.023)**	11.78%

a = beta values for the association between gut microbiota and energy metabolites; b = beta values for the association between energy metabolites and cognition; c = the total effect of gut microbiota on cognition; c' = the effect of gut microbiota on cognition after controlling for the indirect effects; indirect effect = $a \times b$. All regression weights are fully standardized. Bold indicates significant indirect effects. * $P < 0.05$, ** $P < 0.001$. CI



Dataset	AUC	Accuracy	Sensitivity	Specificity	PPV	F1
Training	0.975	0.916	0.882	0.947	0.937	0.908
Training	0.981	0.888	0.882	0.894	0.882	0.882