

Supplementary Materials

Causal roles of immune cells and plasma metabolites in esophageal cancer: A Mendelian randomization study with nutritional insights

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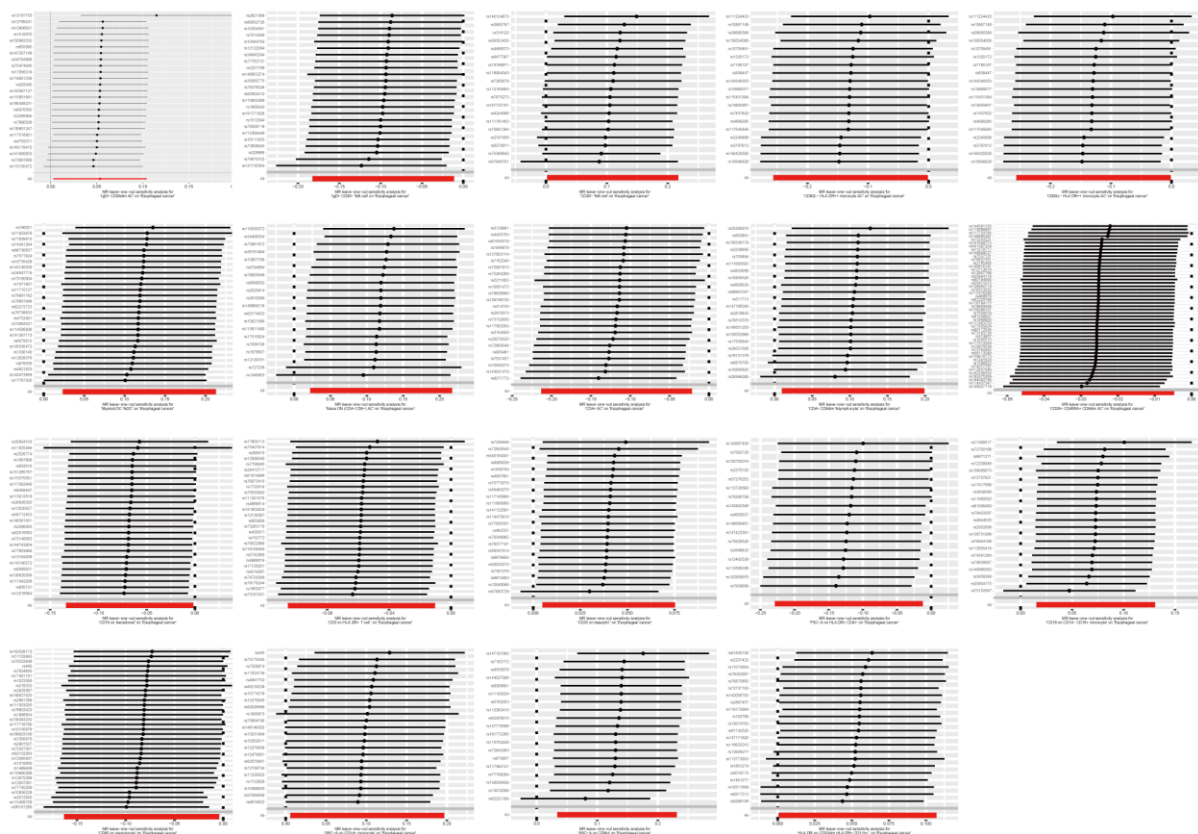
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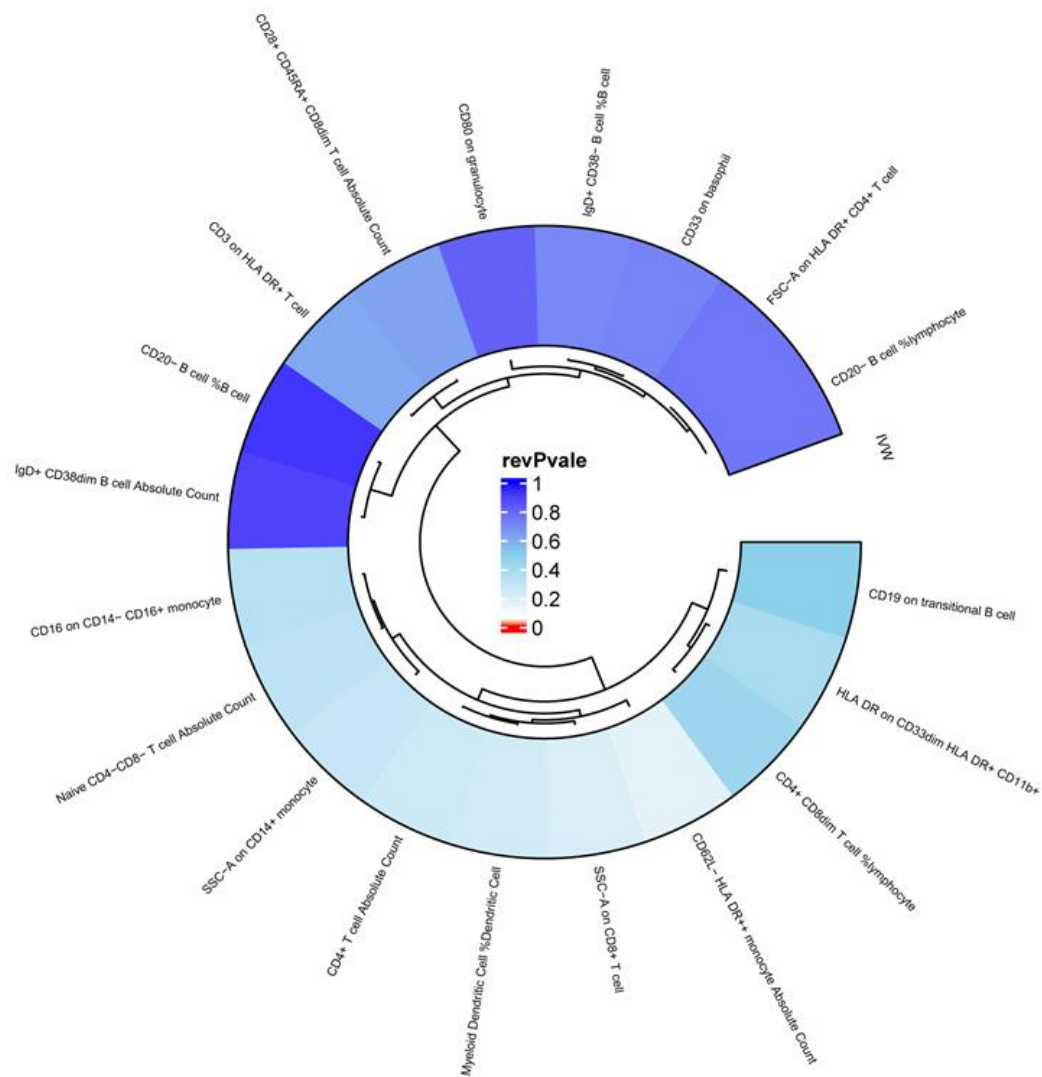
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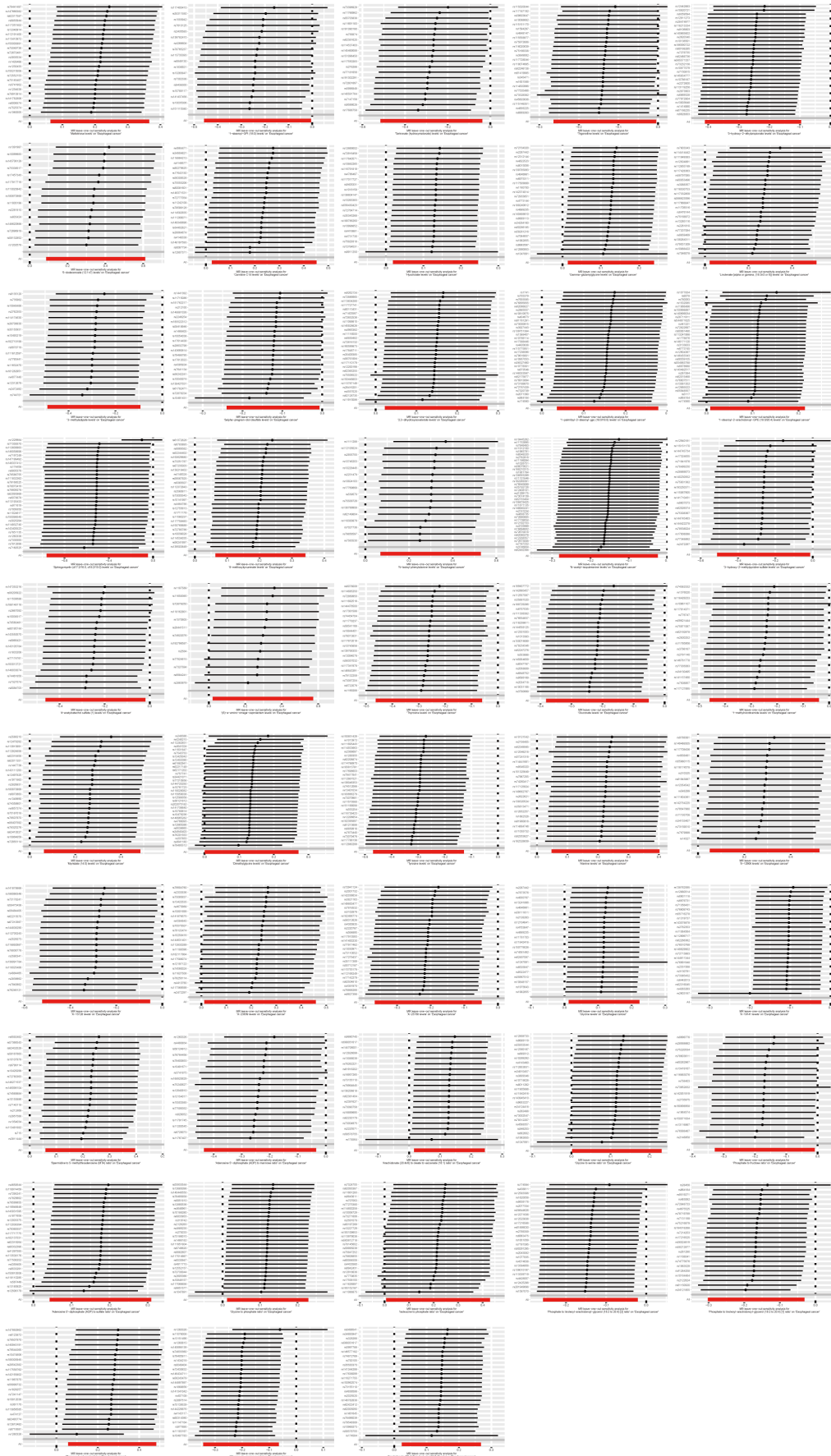
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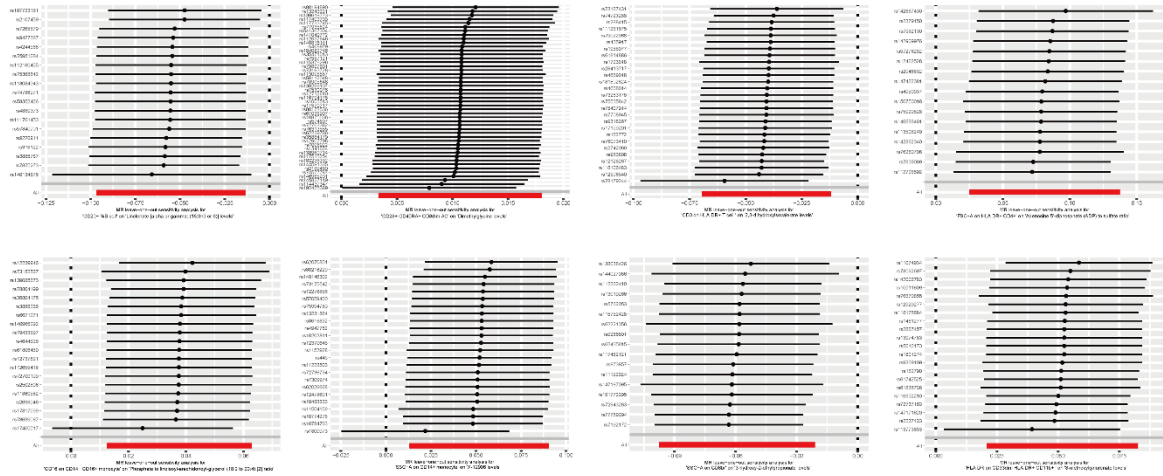
Supplementary Figure 1. The leave-one-out sensitivity analysis of the MR analysis of immune cells to esophageal cancer



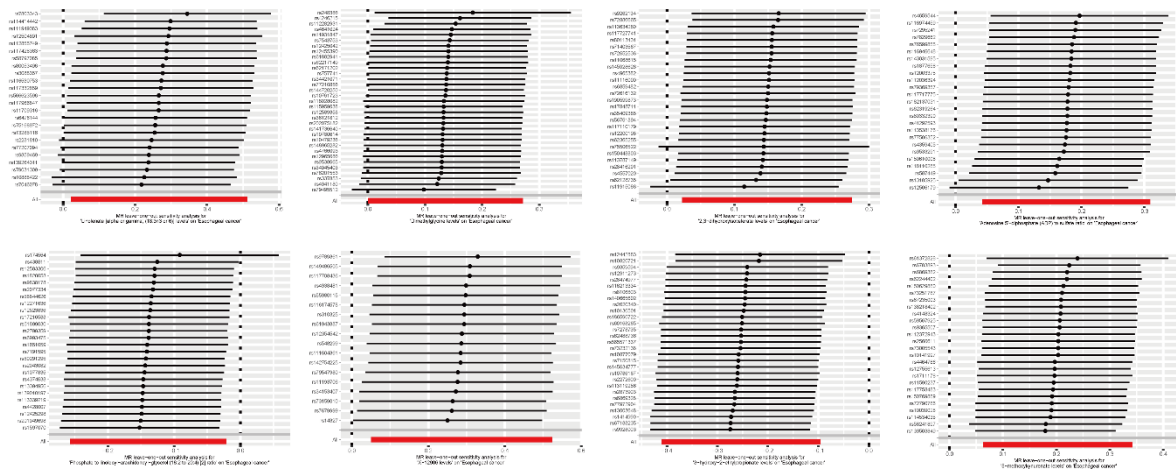
Supplementary Figure 2. Ring heatmap depicting reverse Mendelian randomization analysis of immune cells



Supplementary Figure 3. The leave-one-out sensitivity analysis of the MR analysis of plasma metabolites to esophageal cancer



Supplementary Figure 4. The leave-one-out sensitivity analysis of the MR analysis of immune cells to plasma metabolites



Supplementary Figure 5. The leave-one-out sensitivity analysis of the MR analysis of plasma metabolites highly correlated with immune cells to esophageal cancer

Supplementary Table 1. Checklist for reviewing MR investigations of the study

Checklist item	Satisfied (Yes/No)	Notes / Study details
1. Primary hypothesis	Yes	Investigate causal effect of immune cells on esophageal cancer mediated through plasma metabolites.
2. Data sources	Yes	Immune cells GWAS: GCST90001391–GCST90002121, 3,757 Sardinian European individuals, 731 immune traits. Metabolite GWAS: GCST90199621–GCST90201020, 8,096 European individuals, 1,091 metabolites + 309 ratios, measured by UPLC-MS/MS. Esophageal cancer GWAS: GCST90018841, 998 cases, 475,308 controls, European ancestry.
3. Selection of genetic variants (IVs)	Yes	p-value threshold: Immune cells & metabolites $P < 1 \times 10^{-6}$; Esophageal cancer $P < 5 \times 10^{-8}$. F-statistics: SNPs with $F < 10$ excluded. LD pruning: $r^2 < 0.001$, 1000 Genomes Phase 3 EUR panel. MAF: Immune cell SNPs 0.0001–0.5; Metabolite SNPs 0.00012–0.5. Pleiotropy: Partially; known pleiotropic SNPs not specifically removed, assessed via MR-Egger intercept, Cochran Q, leave-one-out.
4. Variant harmonization	No	This study did not perform variant harmonization because the effect allele orientations of the instrumental variables were already highly consistent across the datasets (primarily European-ancestry GWAS). We also ensured the reliability of the instrumental variables through stringent F-statistic filtering and LD pruning.
5. Primary analysis	Yes	Method: IVW as main analysis. Supplementary methods: MR-Egger, Weighted median, Simple mode, Weighted mode. Multiple testing: FDR < 0.05 .
6 & 7. Supplementary / sensitivity analyses	Yes	Heterogeneity (Cochran Q), horizontal pleiotropy (MR-Egger intercept), leave-one-out analyses performed.
8. Extensions / additional analyses	Yes	Two-step MR mediation analysis performed. Colocalization or subgroup analyses not conducted.
9. Data presentation	Yes	Forest plots, F-statistics, R^2 reported
10. Interpretation	Yes	Causal estimates (β or OR) reported with 95% CI; discussed limitations including small outcome GWAS and potential weak instrument bias.

Supplementary Table 2. Detailed information of the GWAS datasets used in this study

Esophageal cancer	GCST90018841	https://opengwas.io/datasets/ebi-a-GCST90018841	998 cases, 475,308 controls	European
Immune cell traits	GCST90001391–GCST90002121	https://doi.org/10.1038/s41588-020-0684-4	3,757 Sardinian individuals	European
Blood metabolites	GCST90199621–GCST90201020	https://doi.org/10.1038/s41588-022-01270-1	8,299 unrelated European-ancestry participants from CLSA Lyon cohort; after removing 203 relatives, 8,096 included	European

Supplementary Table 3. Detailed information of the GWAS datasets used in this study

SNP	effect_allele. exposure	other_allele. exposure	eaf. exposure	exposure	R2	F	MAF
rs72757888	T	C	0.010	IgD+ %B cell	0.006	23.8	0.010
rs56209800	T	A	0.055	IgD+ %B cell	0.005	20.0	0.055
rs7597061	G	T	0.100	IgD+ %B cell	0.005	19.6	0.100
rs138919897	G	A	0.002	IgD+ %B cell	0.006	21.9	0.002
rs4691917	G	A	0.996	IgD+ %B cell	0.007	24.9	0.004
rs17405603	T	A	0.284	IgD+ %B cell	0.008	31.3	0.284
rs72952600	G	C	0.014	IgD+ %B cell	0.006	22.0	0.014
rs9265870	G	A	0.472	IgD+ %B cell	0.006	22.7	0.472
rs12204136	C	T	0.052	IgD+ %B cell	0.005	19.6	0.052
rs10226722	C	T	0.601	IgD+ %B cell	0.006	21.4	0.399
rs2450220	T	C	0.816	IgD+ %B cell	0.006	23.0	0.184
rs140191306	T	C	0.018	IgD+ %B cell	0.006	22.3	0.018
rs11068290	T	C	0.023	IgD+ %B cell	0.006	23.2	0.023
rs11616703	T	G	0.017	IgD+ %B cell	0.005	19.8	0.017
rs62057183	T	C	0.251	IgD+ %B cell	0.005	19.6	0.251
rs73426855	C	T	0.147	IgD+ %B cell	0.007	25.0	0.147
rs148496020	A	T	0.019	IgD+ %B cell	0.006	21.0	0.019
rs1919856	G	A	0.108	IgD+ CD38br AC	0.005	19.8	0.108
rs116285595	T	C	0.007	IgD+ CD38br AC	0.006	21.1	0.007
rs116104104	T	C	0.000	IgD+ CD38br AC	0.006	20.7	0.000
rs12036556	T	C	0.027	IgD+ CD38br AC	0.006	22.1	0.027
rs1294253	C	T	0.725	IgD+ CD38br AC	0.007	27.0	0.275
rs146959444	C	T	0.002	IgD+ CD38br AC	0.006	23.5	0.002
rs113965271	C	G	0.029	IgD+ CD38br AC	0.006	20.5	0.029
rs733555	T	G	0.264	IgD+ CD38br AC	0.007	26.4	0.264
rs78188887	G	A	0.001	IgD+ CD38br AC	0.006	22.9	0.001
rs140380950	C	T	0.037	IgD+ CD38br AC	0.009	32.1	0.037
rs374727947	G	T	0.010	IgD+ CD38br AC	0.006	21.8	0.010
rs794185	C	T	0.336	IgD+ CD38br AC	0.005	19.7	0.336
rs76112278	A	G	0.019	IgD+ CD38br AC	0.008	28.3	0.019
rs7667070	C	T	0.036	IgD+ CD38br AC	0.006	22.3	0.036
rs80330977	A	G	0.081	IgD+ CD38br AC	0.009	33.9	0.081
rs78658073	A	G	0.001	IgD+ CD38br AC	0.006	21.1	0.001
rs76660971	A	G	0.230	IgD+ CD38br AC	0.005	20.0	0.230
rs117772462	A	T	0.016	IgD+ CD38br AC	0.005	19.8	0.016
rs10265656	G	A	0.938	IgD+ CD38br AC	0.006	20.5	0.063
rs143397127	C	T	0.016	IgD+ CD38br AC	0.006	23.3	0.016
rs1695681	A	C	0.531	IgD+ CD38br AC	0.007	27.1	0.469
rs16868356	C	T	0.020	IgD+ CD38br AC	0.006	23.6	0.020
rs2395904	G	T	0.266	IgD+ CD38br AC	0.006	23.0	0.266
rs10821937	T	C	0.646	IgD+ CD38br AC	0.005	19.8	0.354
rs61878035	A	G	0.189	IgD+ CD38br AC	0.006	22.4	0.189
rs11068290	T	C	0.023	IgD+ CD38br AC	0.006	20.7	0.023
rs118090735	G	T	0.001	IgD+ CD38br AC	0.005	19.9	0.001
rs11159480	T	G	0.817	IgD+ CD38br AC	0.006	22.3	0.183
rs7169431	G	A	0.922	IgD+ CD38br AC	0.006	20.7	0.078
rs76608654	C	T	0.049	IgD+ CD38br AC	0.006	21.1	0.049
rs12939565	T	A	0.427	IgD+ CD38br AC	0.007	26.0	0.427
rs75766272	T	C	0.020	IgD+ CD38dim %B cell	0.005	20.2	0.020
rs7595353	C	T	0.305	IgD+ CD38dim %B cell	0.006	20.5	0.305

SNP: single nucleotide polymorphism; effect_allele.exposure: Allele associated with the reported effect; other_allele.exposure: Alternative allele of the SNP; eaf.exposure: Frequency of the effect allele; R2: Proportion of variance in the exposure explained by the SNP; F: F-statistic indicating instrument strength; MAF: minor allele frequency.

Due to the substantial size of Table S3 (18,623 rows), only the first 50 rows are presented here for reference. The full table is available upon request from the corresponding author or the first author.

Supplementary Table 4. SNP information for metabolites

exposure	SNP	effect_allele. exposure	other_allele. exposure	eaf. exposure	R2	F	MAF
Carnitine levels	rs7537155	A	G	0.199	0.003	21.9	0.199
Carnitine levels	rs78562549	T	G	0.007	0.002	20.0	0.007
Carnitine levels	rs4254516	C	G	0.456	0.003	21.2	0.456
Carnitine levels	rs12692101	T	A	0.444	0.003	26.0	0.444
Carnitine levels	rs13026148	A	G	0.378	0.002	19.8	0.378
Carnitine levels	rs73025372	G	A	0.052	0.003	20.7	0.052
Carnitine levels	rs115168226	G	A	0.006	0.003	21.2	0.006
Carnitine levels	rs10006723	T	G	0.053	0.003	24.5	0.053
Carnitine levels	rs1511808	T	C	0.616	0.002	19.6	0.384
Carnitine levels	rs7705826	G	T	0.834	0.011	94.5	0.166
Carnitine levels	rs149235996	G	A	0.012	0.008	66.0	0.012
Carnitine levels	rs72836546	T	C	0.235	0.003	25.2	0.235
Carnitine levels	rs112819917	A	G	0.008	0.003	26.3	0.008
Carnitine levels	rs13247874	T	C	0.190	0.003	28.0	0.190
Carnitine levels	rs78428648	A	G	0.038	0.002	20.0	0.038
Carnitine levels	rs79717472	G	A	0.028	0.003	22.3	0.028
Carnitine levels	rs76187828	G	A	0.093	0.002	19.8	0.093
Carnitine levels	rs10900223	C	T	0.250	0.004	35.8	0.250
Carnitine levels	rs10826332	A	T	0.077	0.003	24.6	0.077
Carnitine levels	rs1171617	T	G	0.770	0.069	607.0	0.230
Carnitine levels	rs138190991	A	T	0.030	0.003	22.3	0.030
Carnitine levels	rs55775057	A	G	0.150	0.002	19.8	0.150
Carnitine levels	rs2512510	A	G	0.217	0.003	21.8	0.217
Carnitine levels	rs76638161	T	C	0.032	0.002	19.6	0.032
Carnitine levels	rs2625939	T	C	0.228	0.003	21.9	0.228
Carnitine levels	rs1765260	A	G	0.153	0.003	22.3	0.153
Carnitine levels	rs11871125	T	C	0.395	0.003	21.2	0.395
Carnitine levels	rs73006619	C	A	0.047	0.003	20.8	0.047
Benzoate levels	rs72638543	T	C	0.042	0.003	21.1	0.042
Benzoate levels	rs9943251	A	G	0.315	0.003	22.1	0.315
Benzoate levels	rs149187032	A	G	0.012	0.003	20.1	0.012
Benzoate levels	rs186382517	C	T	0.001	0.003	23.9	0.001
Benzoate levels	rs141054369	T	C	0.008	0.003	20.1	0.008
Benzoate levels	rs62192967	T	A	0.054	0.003	20.3	0.054
Benzoate levels	rs113989462	G	C	0.016	0.003	20.6	0.016
Benzoate levels	rs9863931	T	C	0.133	0.003	20.4	0.133
Benzoate levels	rs149023824	G	A	0.003	0.003	19.7	0.003
Benzoate levels	rs11749139	G	T	0.038	0.003	20.0	0.038
Benzoate levels	rs73801273	T	C	0.026	0.003	22.6	0.026
Benzoate levels	rs117191976	T	G	0.004	0.003	21.2	0.004
Benzoate levels	rs6468762	A	G	0.014	0.003	21.7	0.014
Benzoate levels	rs11166865	A	C	0.701	0.003	20.6	0.299
Benzoate levels	rs181702052	C	G	0.003	0.003	24.8	0.003
Benzoate levels	rs533126002	C	T	0.004	0.003	20.6	0.004
Benzoate levels	rs2749463	C	G	0.845	0.003	22.4	0.155
Benzoate levels	rs11625375	T	C	0.966	0.003	20.4	0.034
Benzoate levels	rs11070230	G	A	0.972	0.003	21.1	0.028
Benzoate levels	rs62209511	T	C	0.039	0.003	20.9	0.039
Maltotriose levels	rs7525374	G	A	0.526	0.004	20.0	0.474
Maltotriose levels	rs72670481	T	C	0.044	0.007	32.2	0.044

SNP: single nucleotide polymorphism; effect_allele.exposure: Allele associated with the reported effect; other_allele.exposure: Alternative allele of the SNP; eaf.exposure: Frequency of the effect allele; R2: Proportion of variance in the exposure explained by the SNP; F: F-statistic indicating instrument strength; MAF: minor allele frequency.

Due to the substantial size of Table S4 (34,844 rows), only the first 50 rows are presented here for reference. The full table is available upon request from the corresponding author or the first author.

Supplementary Table 5. MR results of immune cells on esophageal cancer

Outcome	Exposure	Method	nsnp	β	SE	p	OR	or lci95	or uci95	FDR
EC	IgD+ CD38dim B cell Absolute Count	IVW	25	0.0536	0.0258	0.0382	1.06	1.00	1.11	0.0481
EC	IgD+ CD38- B cell %B cell	IVW	24	-0.0969	0.0437	0.0267	0.908	0.833	0.989	0.0481
EC	CD20- B cell %B cell	IVW	19	0.110	0.0550	0.0462	1.12	1.00	1.24	0.0481
EC	CD20- B cell %lymphocyte	IVW	19	0.0747	0.0235	0.0015	1.078	1.029	1.128	0.0293
EC	CD62L- HLA DR++ monocyte Absolute Count	IVW	19	-0.129	0.0652	0.0481	0.879	0.774	0.999	0.0481
EC	Myeloid Dendritic Cell %Dendritic Cell	IVW	28	0.118	0.0483	0.0145	1.13	1.02	1.24	0.0427
EC	Naive CD4-CD8- T cell Absolute Count	IVW	20	0.120	0.0498	0.0162	1.13	1.02	1.24	0.0427
EC	CD4+ T cell Absolute Count	IVW	24	-0.117	0.0488	0.0162	0.889	0.808	0.979	0.0427
EC	CD4+ CD8dim T cell %lymphocyte	IVW	22	0.104	0.0483	0.0306	1.11	1.01	1.22	0.0481
EC	CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	49	-0.0254	0.0105	0.0159	0.975	0.955	0.995	0.0427
EC	CD19 on transitional B cell	IVW	26	-0.0677	0.0337	0.0442	0.935	0.875	0.998	0.0481
EC	CD3 on HLA DR+ T cell	IVW	28	-0.0579	0.0243	0.0171	0.944	0.900	0.990	0.0427
EC	CD33 on basophil	IVW	23	0.0401	0.0180	0.0256	1.04	1.00	1.08	0.0481
EC	FSC-A on HLA DR+ CD4+ T cell	IVW	17	-0.121	0.0553	0.0293	0.886	0.795	0.988	0.0481
EC	CD16 on CD14- CD16+ monocyte	IVW	22	0.0729	0.0294	0.0132	1.08	1.02	1.14	0.0427
EC	CD80 on granulocyte	IVW	33	-0.0837	0.0407	0.0395	0.920	0.849	0.996	0.0481
EC	SSC-A on CD14+ monocyte	IVW	23	0.101	0.0488	0.0379	1.11	1.01	1.22	0.0481
EC	SSC-A on CD8+ T cell	IVW	19	0.133	0.0504	0.0084	1.14	1.03	1.26	0.0427
EC	HLA DR on CD33dim HLA DR+ CD11b+	IVW	22	0.0539	0.0272	0.0475	1.06	1.00	1.11	0.0481

EC: esophageal cancer; IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio; FDR: false discovery rate.

Supplementary Table 6. Heterogeneity test for MR of immune cells on esophageal cancer

Outcome	Exposure	Method	Q	Q df	Q p
EC	IgD+ CD38dim B cell Absolute Count	MR Egger	29.6	23	0.160
EC	IgD+ CD38dim B cell Absolute Count	IVW	29.7	24	0.196
EC	IgD+ CD38- B cell %B cell	MR Egger	18.7	22	0.666
EC	IgD+ CD38- B cell %B cell	IVW	19.6	23	0.664
EC	CD20- B cell %B cell	MR Egger	25.9	17	0.076
EC	CD20- B cell %B cell	IVW	26.8	18	0.083
EC	CD20- B cell %lymphocyte	MR Egger	16.3	17	0.504
EC	CD20- B cell %lymphocyte	IVW	17.0	18	0.520
EC	CD62L- HLA DR++ monocyte Absolute Count	MR Egger	16.4	17	0.496
EC	CD62L- HLA DR++ monocyte Absolute Count	IVW	16.7	18	0.541
EC	Myeloid Dendritic Cell %Dendritic Cell	MR Egger	17.5	26	0.894
EC	Myeloid Dendritic Cell %Dendritic Cell	IVW	18.9	27	0.875
EC	Naive CD4-CD8- T cell Absolute Count	MR Egger	21.9	18	0.235
EC	Naive CD4-CD8- T cell Absolute Count	IVW	22.0	19	0.284
EC	CD4+ T cell Absolute Count	MR Egger	21.1	22	0.514
EC	CD4+ T cell Absolute Count	IVW	21.2	23	0.567
EC	CD4+ CD8dim T cell %lymphocyte	MR Egger	14.5	20	0.807
EC	CD4+ CD8dim T cell %lymphocyte	IVW	14.5	21	0.849
EC	CD28+ CD45RA+ CD8dim T cell Absolute Count	MR Egger	40.8	47	0.727
EC	CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	41.0	48	0.752
EC	CD19 on transitional	MR Egger	17.1	24	0.846
EC	CD19 on transitional	IVW	17.1	25	0.878
EC	CD3 on HLA DR+ T cell	MR Egger	20.8	26	0.751
EC	CD3 on HLA DR+ T cell	IVW	21.4	27	0.766
EC	CD33 on basophil	MR Egger	21.3	21	0.441
EC	CD33 on basophil	IVW	21.5	22	0.491
EC	FSC-A on HLA DR+ CD4+ T cell	MR Egger	9.1	15	0.870
EC	FSC-A on HLA DR+ CD4+ T cell	IVW	9.6	16	0.885
EC	CD16 on CD14- CD16+ monocyte	MR Egger	18.0	20	0.587
EC	CD16 on CD14- CD16+ monocyte	IVW	18.1	21	0.645
EC	CD80 on granulocyte	MR Egger	36.8	31	0.220
EC	CD80 on granulocyte	IVW	37.3	32	0.239
EC	SSC-A on CD14+ monocyte	MR Egger	25.2	21	0.240
EC	SSC-A on CD14+ monocyte	IVW	25.3	22	0.283
EC	SSC-A on CD8+ T cell	MR Egger	19.6	17	0.297
EC	SSC-A on CD8+ T cell	IVW	19.6	18	0.354
EC	HLA DR on CD33dim HLA DR+ CD11b+	MR Egger	25.5	20	0.184
EC	HLA DR on CD33dim HLA DR+ CD11b+	IVW	25.8	21	0.213

EC: esophageal cancer; IVW: inverse variance weighted; Q: Cochran's Q statistic; Q_df: degrees of freedom associated with the Q statistic; Q_p: p-value for the heterogeneity test based on the Q statistic.

Supplementary Table 7. Pleiotropy test for MR of immune cells on esophageal cancer

Outcome	Exposure	egger intercept	SE	<i>p</i>
EC	IgD+ CD38dim B cell Absolute Count	0.001	0.015	0.938
EC	IgD+ CD38- B cell %B cell	-0.015	0.015	0.335
EC	CD20- B cell %B cell	0.018	0.024	0.461
EC	CD20- B cell %lymphocyte	0.012	0.014	0.394
EC	CD62L- HLA DR++ monocyte Absolute Count	0.017	0.028	0.564
EC	Myeloid Dendritic Cell %Dendritic Cell	-0.022	0.019	0.246
EC	Naive CD4-CD8- T cell Absolute Count	0.006	0.021	0.792
EC	CD4+ T cell Absolute Count	0.006	0.016	0.727
EC	CD4+ CD8dim T cell %lymphocyte	-0.002	0.021	0.928
EC	CD28+ CD45RA+ CD8dim T cell Absolute Count	0.005	0.011	0.631
EC	CD19 on transitional B cell	0.003	0.014	0.828
EC	CD3 on HLA DR+ T cell	0.010	0.013	0.443
EC	CD33 on basophil	0.006	0.015	0.673
EC	FSC-A on HLA DR+ CD4+ T cell	-0.015	0.021	0.489
EC	CD16 on CD14- CD16+ monocyte	-0.004	0.017	0.819
EC	CD80 on granulocyte	-0.012	0.017	0.508
EC	SSC-A on CD14+ monocyte	0.007	0.020	0.742
EC	SSC-A on CD8+ T cell	-0.004	0.018	0.820
EC	HLA DR on CD33dim HLA DR+ CD11b+	0.010	0.018	0.603

EC: esophageal cancer; SE: standard error.

Supplementary Table 8. The for the results of reverse MR analysis of immune cells

Outcome	Exposure	Method	nsnp	β	SE	p	OR	or lci95	or uci95	rev-p
EC	IgD+ CD38dim B cell Absolute Count	IVW	25	0.054	0.026	0.038	1.06	1.00	1.11	0.893
EC	IgD+ CD38- B cell %B cell	IVW	24	-0.097	0.044	0.027	0.908	0.833	0.989	0.703
EC	CD20- B cell %B cell	IVW	19	0.110	0.055	0.046	1.12	1.00	1.24	0.927
EC	CD20- B cell %lymphocyte	IVW	19	0.075	0.023	0.001	1.08	1.03	1.13	0.756
EC	CD62L- HLA DR++ monocyte Absolute Count	IVW	19	-0.129	0.065	0.048	0.879	0.774	0.999	0.160
EC	Myeloid Dendritic Cell %Dendritic Cell	IVW	28	0.118	0.048	0.014	1.13	1.02	1.24	0.229
EC	Naive CD4-CD8- T cell Absolute Count	IVW	20	0.120	0.050	0.016	1.13	1.02	1.24	0.306
EC	CD4+ T cell Absolute Count	IVW	24	-0.117	0.049	0.016	0.889	0.808	0.979	0.243
EC	CD4+ CD8dim T cell %lymphocyte	IVW	22	0.104	0.048	0.031	1.11	1.01	1.22	0.425
EC	CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	49	-0.025	0.011	0.016	0.975	0.955	0.995	0.625
EC	CD19 on transitional B cell	IVW	26	-0.068	0.034	0.044	0.935	0.875	0.998	0.480
EC	CD3 on HLA DR+ T cell	IVW	28	-0.058	0.024	0.017	0.944	0.900	0.990	0.608
EC	CD33 on basophil	IVW	23	0.040	0.018	0.026	1.04	1.00	1.08	0.716
EC	FSC-A on HLA DR+ CD4+ T cell	IVW	17	-0.121	0.055	0.029	0.886	0.795	0.988	0.756
EC	CD16 on CD14- CD16+ monocyte	IVW	22	0.073	0.029	0.013	1.08	1.02	1.14	0.327
EC	CD80 on granulocyte	IVW	33	-0.084	0.041	0.039	0.920	0.849	0.996	0.814
EC	SSC-A on CD14+ monocyte	IVW	23	0.101	0.049	0.038	1.11	1.01	1.22	0.274
EC	SSC-A on CD8+ T cell	IVW	19	0.133	0.050	0.008	1.14	1.03	1.26	0.203
EC	HLA DR on CD33dim HLA DR+ CD11b+	IVW	22	0.054	0.027	0.048	1.06	1.00	1.11	0.378

EC: esophageal cancer; IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio.

Supplementary Table 9. MR results of plasma metabolites on esophageal cancer

Outcome	Exposure	Method	nsnp	β	SE	p	OR	or lei95	or uci95	FDR
EC	Maltotriose levels	IVW	24	0.215	0.077	0.0053	1.24	1.07	1.44	0.0346
EC	1-stearoyl-GPI (18:0) levels	IVW	18	-0.231	0.116	0.0456	0.794	0.633	0.995	0.0475
EC	Tartronate (hydroxymalonate) levels	IVW	20	-0.277	0.122	0.0234	0.758	0.597	0.963	0.0387
EC	Trigonelline levels	IVW	25	-0.134	0.066	0.0428	0.874	0.768	0.996	0.0464
EC	3-hydroxy-2-ethylpropionate levels	IVW	29	-0.254	0.080	0.0016	0.776	0.663	0.908	0.0346
EC	5-dodecenoate (12:1n7) levels	IVW	15	0.349	0.134	0.0090	1.42	1.09	1.84	0.0346
EC	Carnitine C14 levels	IVW	22	0.238	0.109	0.0292	1.27	1.02	1.57	0.0421
EC	Hyocholate levels	IVW	21	0.132	0.058	0.0228	1.14	1.02	1.28	0.0387
EC	Gamma-glutamylglycine levels	IVW	25	0.126	0.057	0.0277	1.13	1.01	1.27	0.0421
EC	Linolenate [α or γ ; (18:3n3 or 6)] levels	IVW	24	0.267	0.125	0.0331	1.31	1.02	1.67	0.0428
EC	3-methyladipate levels	IVW	18	-0.292	0.101	0.0039	0.747	0.612	0.911	0.0346
EC	5 α -pregnan-diol disulfate levels	IVW	23	-0.123	0.059	0.0380	0.884	0.787	0.993	0.0437
EC	2,3-dihydroxyisovalerate levels	IVW	27	0.149	0.064	0.0208	1.16	1.02	1.32	0.0379
EC	1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels	IVW	32	0.157	0.062	0.0109	1.17	1.04	1.32	0.0346
EC	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	IVW	32	0.111	0.053	0.0345	1.12	1.01	1.24	0.0428
EC	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	IVW	29	-0.504	0.234	0.0308	0.604	0.382	0.955	0.0423
EC	8-methoxykynurenate levels	IVW	26	0.203	0.071	0.0043	1.22	1.07	1.41	0.0346
EC	N-lactoyl phenylalanine levels	IVW	16	0.299	0.117	0.0108	1.35	1.07	1.70	0.0346
EC	N-acetyl-isoptureanine levels	IVW	38	-0.153	0.066	0.0197	0.858	0.754	0.976	0.0379
EC	3-hydroxy-2-methylpyridine sulfate levels	IVW	21	-0.263	0.127	0.0385	0.769	0.599	0.986	0.0437
EC	4-acetylcatechol sulfate (1) levels	IVW	18	-0.241	0.113	0.0334	0.786	0.629	0.981	0.0428
EC	(S)- α -amino- ω -caprolactam levels	IVW	13	0.297	0.141	0.0347	1.35	1.02	1.77	0.0428
EC	Thyroxine levels	IVW	22	-0.262	0.103	0.0109	0.770	0.629	0.942	0.0346
EC	Gluconate levels	IVW	24	-0.216	0.084	0.0103	0.805	0.683	0.950	0.0346
EC	1-methylnicotinamide levels	IVW	19	-0.249	0.107	0.0201	0.779	0.631	0.962	0.0379
EC	Myristate (14:0) levels	IVW	23	0.274	0.108	0.0111	1.32	1.06	1.63	0.0346
EC	Dimethylglycine levels	IVW	33	0.136	0.069	0.0496	1.15	1.00	1.31	0.0496
EC	Tyrosine levels	IVW	29	-0.299	0.087	0.0006	0.742	0.625	0.880	0.0292
EC	Alanine levels	IVW	22	0.225	0.090	0.0128	1.25	1.05	1.50	0.0346
EC	X-12906 levels	IVW	18	0.287	0.121	0.0175	1.33	1.05	1.69	0.0379
EC	X-13728 levels	IVW	19	-0.288	0.120	0.0165	0.750	0.593	0.949	0.0379
EC	X-23639 levels	IVW	23	0.235	0.114	0.0391	1.27	1.01	1.58	0.0437
EC	X-23780 levels	IVW	27	-0.207	0.081	0.0106	0.813	0.693	0.953	0.0346
EC	Glycine levels	IVW	22	0.118	0.054	0.0285	1.13	1.01	1.25	0.0421
EC	X-19141 levels	IVW	25	-0.106	0.042	0.0123	0.899	0.828	0.977	0.0346

EC: esophageal cancer; IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio; FDR: false discovery rate.

Supplementary Table 9. MR results of plasma metabolites on esophageal cancer (cont.)

Outcome	Exposure	Method	nsnp	β	SE	p	OR	or lci95	or uci95	FDR
EC	Spermidine to 5-methylthioadenosine (MTA) ratio	IVW	19	0.228	0.086	0.0081	1.26	1.06	1.486	0.0346
EC	Adenosine 5'-diphosphate (ADP) to mannose ratio	IVW	18	-0.235	0.102	0.0213	0.791	0.648	0.966	0.0379
EC	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	IVW	20	0.130	0.058	0.0257	1.14	1.02	1.28	0.0412
EC	Glycine to serine ratio	IVW	25	0.116	0.058	0.0470	1.12	1.00	1.26	0.0480
EC	Phosphate to fructose ratio	IVW	17	-0.169	0.082	0.0386	0.845	0.720	0.991	0.0437
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	IVW	25	0.176	0.068	0.0097	1.19	1.04	1.36	0.0346
EC	Glycine to phosphate ratio	IVW	28	0.120	0.055	0.0298	1.13	1.01	1.26	0.0421
EC	Isoleucine to phosphate ratio	IVW	28	0.218	0.108	0.0435	1.24	1.01	1.54	0.0464
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	26	-0.139	0.060	0.0210	0.870	0.773	0.979	0.0379
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	IVW	23	-0.167	0.069	0.0162	0.847	0.739	0.970	0.0379
EC	Mannose to glycerol ratio	IVW	22	0.270	0.109	0.0130	1.31	1.06	1.62	0.0346
EC	Glucose-to-mannose ratio	IVW	25	-0.204	0.071	0.0042	0.815	0.709	0.937	0.0346
EC	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	IVW	25	0.145	0.062	0.0198	1.16	1.02	1.31	0.0379

EC: esophageal cancer; IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio; FDR: false discovery rate.

Supplementary Table 10. Heterogeneity test for MR of plasma metabolites on esophageal cancer

Outcome	Exposure	Method	Q	Q df	Q p
EC	Maltotriose levels	MR Egger	19.5	22	0.613
EC	Maltotriose levels	IVW	20.5	23	0.615
EC	1-stearoyl-GPI (18:0) levels	MR Egger	17.9	16	0.332
EC	1-stearoyl-GPI (18:0) levels	IVW	18.4	17	0.365
EC	Tartronate (hydroxymalonnate) levels	MR Egger	21.4	18	0.260
EC	Tartronate (hydroxymalonnate) levels	IVW	21.4	19	0.316
EC	Trigonelline levels	MR Egger	31.5	23	0.112
EC	Trigonelline levels	IVW	31.9	24	0.130
EC	3-hydroxy-2-ethylpropionate levels	MR Egger	21.9	27	0.745
EC	3-hydroxy-2-ethylpropionate levels	IVW	22.4	28	0.760
EC	5-dodecenoate (12:1n7) levels	MR Egger	13.6	13	0.406
EC	5-dodecenoate (12:1n7) levels	IVW	14.0	14	0.446
EC	Carnitine C14 levels	MR Egger	18.5	20	0.552
EC	Carnitine C14 levels	IVW	18.6	21	0.613
EC	Hyocholate levels	MR Egger	7.5	19	0.991
EC	Hyocholate levels	IVW	7.5	20	0.995
EC	Gamma-glutamylglycine levels	MR Egger	21.1	23	0.576
EC	Gamma-glutamylglycine levels	IVW	21.1	24	0.634
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	MR Egger	27.9	22	0.180
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	IVW	28.0	23	0.216
EC	3-methyladipate levels	MR Egger	6.8	16	0.977
EC	3-methyladipate levels	IVW	6.9	17	0.985
EC	5alpha-pregnan-diol disulfate levels	MR Egger	23.5	21	0.320
EC	5alpha-pregnan-diol disulfate levels	IVW	23.9	22	0.351
EC	2,3-dihydroxyisovalerate levels	MR Egger	20.1	25	0.743
EC	2,3-dihydroxyisovalerate levels	IVW	20.1	26	0.787
EC	1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels	MR Egger	25.3	30	0.711
EC	1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels	IVW	26.9	31	0.676
EC	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	MR Egger	43.0	30	0.059
EC	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	IVW	43.4	31	0.069
EC	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	MR Egger	184.5	27	0.000
EC	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	IVW	194.9	28	0.000
EC	8-methoxykynurenate levels	MR Egger	28.5	24	0.240
EC	8-methoxykynurenate levels	IVW	28.6	25	0.281
EC	N-lactoyl phenylalanine levels	MR Egger	11.9	14	0.617
EC	N-lactoyl phenylalanine levels	IVW	12.4	15	0.648
EC	N-acetyl-isoputrescine levels	MR Egger	50.3	36	0.058
EC	N-acetyl-isoputrescine levels	IVW	50.5	37	0.069
EC	3-hydroxy-2-methylpyridine sulfate levels	MR Egger	21.8	19	0.296
EC	3-hydroxy-2-methylpyridine sulfate levels	IVW	21.8	20	0.353
EC	4-acetylcatechol sulfate (1) levels	MR Egger	14.6	16	0.551
EC	4-acetylcatechol sulfate (1) levels	IVW	14.9	17	0.601
EC	(S)-a-amino-omega-caprolactam levels	MR Egger	9.0	11	0.620
EC	(S)-a-amino-omega-caprolactam levels	IVW	9.7	12	0.642
EC	Thyroxine levels	MR Egger	14.0	20	0.832
EC	Thyroxine levels	IVW	14.0	21	0.869
EC	Gluconate levels	MR Egger	17.9	22	0.714
EC	Gluconate levels	IVW	22.1	23	0.515
EC	1-methylnicotinamide levels	MR Egger	19.6	17	0.297
EC	1-methylnicotinamide levels	IVW	20.2	18	0.322
EC	Myristate (14:0) levels	MR Egger	21.4	21	0.437
EC	Myristate (14:0) levels	IVW	21.6	22	0.483
EC	Dimethylglycine levels	MR Egger	38.5	31	0.167
EC	Dimethylglycine levels	IVW	38.5	32	0.199
EC	Tyrosine levels	MR Egger	25.0	27	0.575
EC	Tyrosine levels	IVW	25.3	28	0.610
EC	Alanine levels	MR Egger	9.3	20	0.979
EC	Alanine levels	IVW	10.3	21	0.975
EC	X-12906 levels	MR Egger	6.8	16	0.977
EC	X-12906 levels	IVW	7.2	17	0.981

EC: esophageal cancer; IVW: inverse variance weighted; Q: Cochran's Q statistic; Q_df: degrees of freedom associated with the Q statistic; Q_p: p-value for the heterogeneity test based on the Q statistic.

Supplementary Table 10. Heterogeneity test for MR of plasma metabolites on esophageal cancer (cont.)

Outcome	Exposure	Method	Q	Q df	Q p
EC	X-12906 levels	MR Egger	6.8	16	0.977
EC	X-12906 levels	IVW	7.2	17	0.981
EC	X-13728 levels	MR Egger	20.2	17	0.266
EC	X-13728 levels	IVW	20.2	18	0.324
EC	X-23639 levels	MR Egger	31.8	21	0.061
EC	X-23639 levels	IVW	32.0	22	0.077
EC	X-23780 levels	MR Egger	24.9	25	0.466
EC	X-23780 levels	IVW	25.0	26	0.521
EC	Glycine levels	MR Egger	11.1	20	0.944
EC	Glycine levels	IVW	11.2	21	0.960
EC	X-19141 levels	MR Egger	18.7	23	0.718
EC	X-19141 levels	IVW	19.5	24	0.723
EC	Spermidine to 5-methylthioadenosine (MTA) ratio	MR Egger	21.1	17	0.224
EC	Spermidine to 5-methylthioadenosine (MTA) ratio	IVW	22.0	18	0.231
EC	Adenosine 5'-diphosphate (ADP) to mannose ratio	MR Egger	34.8	16	0.004
EC	Adenosine 5'-diphosphate (ADP) to mannose ratio	IVW	35.3	17	0.006
EC	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	MR Egger	16.8	18	0.534
EC	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	IVW	16.9	19	0.597
EC	Glycine to serine ratio	MR Egger	19.3	23	0.685
EC	Glycine to serine ratio	IVW	19.4	24	0.731
EC	Phosphate to fructose ratio	MR Egger	17.2	15	0.309
EC	Phosphate to fructose ratio	IVW	17.2	16	0.374
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	MR Egger	15.3	23	0.883
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	IVW	15.6	24	0.900
EC	Glycine to phosphate ratio	MR Egger	21.7	26	0.705
EC	Glycine to phosphate ratio	IVW	21.7	27	0.752
EC	Isoleucine to phosphate ratio	MR Egger	36.0	26	0.092
EC	Isoleucine to phosphate ratio	IVW	39.9	27	0.052
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	MR Egger	19.5	24	0.727
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	19.7	25	0.765
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	MR Egger	25.5	21	0.225
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	IVW	26.1	22	0.247
EC	Mannose to glycerol ratio	MR Egger	24.6	20	0.218
EC	Mannose to glycerol ratio	IVW	25.4	21	0.229
EC	Glucose-to-mannose ratio	MR Egger	25.1	23	0.347
EC	Glucose-to-mannose ratio	IVW	26.2	24	0.344
EC	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	MR Egger	27.4	23	0.238
EC	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	IVW	27.5	24	0.283

EC: esophageal cancer; IVW: inverse variance weighted; Q: Cochran's Q statistic; Q_df: degrees of freedom associated with the Q statistic; Q_p: p-value for the heterogeneity test based on the Q statistic.

Supplementary Table 11. Pleiotropy test for MR of plasma metabolites on esophageal cancer

Outcome	Exposure	egger intercept	SE	<i>p</i>
EC	Maltotriose levels	0.024	0.024	0.344
EC	1-stearoyl-GPI (18:0) levels	-0.023	0.033	0.502
EC	Tartronate (hydroxymalonate) levels	-0.001	0.030	0.978
EC	Trigonelline levels	0.011	0.019	0.583
EC	3-hydroxy-2-ethylpropionate levels	0.017	0.022	0.449
EC	5-dodecenoate (12:1n7) levels	0.025	0.037	0.504
EC	Carnitine C14 levels	-0.006	0.033	0.851
EC	Hyocholate levels	0.000	0.016	0.994
EC	Gamma-glutamylglycine levels	0.000	0.013	0.997
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	-0.010	0.030	0.750
EC	3-methyladipate levels	0.007	0.026	0.793
EC	5alpha-pregnan-diol disulfate levels	-0.019	0.029	0.523
EC	2,3-dihydroxyisovalerate levels	0.003	0.017	0.866
EC	1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels	-0.020	0.016	0.213
EC	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	0.009	0.018	0.601
EC	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	0.066	0.053	0.227
EC	8-methoxykynurenate levels	-0.006	0.019	0.755
EC	N-lactoyl phenylalanine levels	-0.021	0.029	0.477
EC	N-acetyl-isoputrescine levels	-0.008	0.019	0.693
EC	3-hydroxy-2-methylpyridine sulfate levels	-0.001	0.043	0.974
EC	4-acetylcatechol sulfate (1) levels	0.016	0.030	0.602
EC	(S)-a-amino-omega-caprolactam levels	0.025	0.031	0.427
EC	Thyroxine levels	0.008	0.038	0.833
EC	Gluconate levels	0.035	0.017	0.052
EC	1-methylnicotinamide levels	-0.027	0.037	0.470
EC	Myristate (14:0) levels	0.016	0.031	0.617
EC	Dimethylglycine levels	0.001	0.017	0.972
EC	Tyrosine levels	-0.013	0.022	0.565
EC	Alanine levels	-0.023	0.023	0.337
EC	X-12906 levels	0.020	0.030	0.518
EC	X-13728 levels	0.001	0.043	0.986
EC	X-23639 levels	-0.013	0.036	0.719
EC	X-23780 levels	-0.002	0.021	0.912
EC	Glycine levels	0.003	0.012	0.822
EC	X-19141 levels	-0.014	0.015	0.376
EC	Spermidine to 5-methylthioadenosine (MTA) ratio	0.017	0.019	0.386
EC	Adenosine 5'-diphosphate (ADP) to mannose ratio	0.015	0.033	0.645
EC	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	0.004	0.019	0.835
EC	Glycine to serine ratio	-0.004	0.014	0.754
EC	Phosphate to fructose ratio	-0.002	0.023	0.926
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	-0.015	0.026	0.568
EC	Glycine to phosphate ratio	0.001	0.013	0.909
EC	Isoleucine to phosphate ratio	-0.049	0.029	0.105
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	0.008	0.017	0.666
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	0.014	0.020	0.496
EC	Mannose to glycerol ratio	0.025	0.030	0.414
EC	Glucose-to-mannose ratio	0.021	0.021	0.322
EC	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	0.004	0.017	0.838

EC: esophageal cancer; SE: standard error.

Supplementary Table 12. MR results of immune cells on plasma metabolites

Outcome	Exposure	Method	nsnp	β_1	SE	p	OR	or_lci95	or_uci95	FDR
Linolenate [alpha or gamma; (18:3n3 or 6)] levels	CD20- B cell %B cell	IVW	19	-0.0552	0.0213	0.0095	0.946	0.908	0.987	0.0473
Dimethylglycine levels	CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	47	0.0107	0.0038	0.0045	1.01	1.00	1.02	0.0112
2,3-dihydroxyisovalerate levels	CD3 on HLA DR+ T cell	IVW	27	-0.0407	0.0147	0.0056	0.960	0.933	0.988	0.0278
Adenosine 5'-diphosphate (ADP) to sulfate ratio	FSC-A on HLA DR+ CD4+ T cell	IVW	17	0.0813	0.0287	0.0045	1.08	1.03	1.15	0.0227
Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	CD16 on CD14- CD16+ monocyte	IVW	20	0.0377	0.0129	0.0034	1.04	1.01	1.06	0.0170
X-12906 levels	SSC-A on CD14+ monocyte	IVW	24	0.0517	0.0197	0.0089	1.05	1.01	1.09	0.0148
3-hydroxy-2-ethylpropionate levels	SSC-A on CD8+ T cell	IVW	17	-0.0593	0.0196	0.0024	0.942	0.907	0.979	0.0122
8-methoxykynurenate levels	HLA DR on CD33dim HLA DR+ CD11b+	IVW	21	0.0517	0.0157	0.0010	1.05	1.02	1.09	0.0051

IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β_1 : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio; FDR: false discovery rate.

Supplementary Table 13. MR results of immune cell-related metabolites on esophageal cancer

Outcome	Exposure	Method	nsnp	β_2	SE	p	OR	or_lci95	or_uci95	FDR
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	IVW	24	0.267	0.125	0.0331	1.31	1.02	1.67	0.0379
EC	Dimethylglycine levels	IVW	33	0.136	0.0691	0.0496	1.15	1.00	1.31	0.0496
EC	2,3-dihydroxyisovalerate levels	IVW	27	0.149	0.0643	0.0208	1.16	1.02	1.32	0.0280
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	IVW	25	0.176	0.0680	0.0097	1.19	1.04	1.36	0.0259
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	26	-0.139	0.0603	0.0210	0.870	0.773	0.979	0.0280
EC	X-12906 levels	IVW	18	0.287	0.12	0.0175	1.33	1.05	1.69	0.0280
EC	3-hydroxy-2-ethylpropionate levels	IVW	29	-0.254	0.0802	0.0016	0.776	0.663	0.908	0.0125
EC	8-methoxykynurenate levels	IVW	26	0.203	0.0710	0.0043	1.22	1.07	1.41	0.0174

EC: esophageal cancer; IVW: inverse variance weighted; nsnp: number of SNPs used as instrumental variables; β_2 : Estimated causal effect of exposure on outcome; SE: standard error; OR: odds ratio; FDR: false discovery rate

Supplementary Table 14. Heterogeneity test for MR of immune cells on plasma metabolites

Outcome	Exposure	Method	Q	Q df	Q p
Linolenate [alpha or gamma; (18:3n3 or 6)] levels	CD20- B cell %B cell	MR Egger	10.7	17	0.873
Linolenate [alpha or gamma; (18:3n3 or 6)] levels	CD20- B cell %B cell	IVW	10.7	18	0.905
Dimethylglycine levels	CD28+ CD45RA+ CD8dim T cell Absolute Count	MR Egger	44.7	45	0.483
Dimethylglycine levels	CD28+ CD45RA+ CD8dim T cell Absolute Count	IVW	46.9	46	0.435
2,3-dihydroxyisovalerate levels	CD3 on HLA DR+ T cell	MR Egger	30.7	25	0.198
2,3-dihydroxyisovalerate levels	CD3 on HLA DR+ T cell	IVW	31.5	26	0.210
Adenosine 5'-diphosphate (ADP) to sulfate ratio	FSC-A on HLA DR+ CD4+ T cell	MR Egger	10.2	15	0.810
Adenosine 5'-diphosphate (ADP) to sulfate ratio	FSC-A on HLA DR+ CD4+ T cell	IVW	11.1	16	0.806
Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	CD16 on CD14- CD16+ monocyte	MR Egger	11.8	18	0.858
Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	CD16 on CD14- CD16+ monocyte	IVW	12.1	19	0.883
X-12906 levels	SSC-A on CD14+ monocyte	MR Egger	24.7	22	0.312
X-12906 levels	SSC-A on CD14+ monocyte	IVW	25.9	23	0.307
3-hydroxy-2-ethylpropionate levels	SSC-A on CD8+ T cell	MR Egger	17.9	15	0.271
3-hydroxy-2-ethylpropionate levels	SSC-A on CD8+ T cell	IVW	17.9	16	0.328
8-methoxykynurenate levels	HLA DR on CD33dim HLA DR+ CD11b+	MR Egger	31.6	19	0.034
8-methoxykynurenate levels	HLA DR on CD33dim HLA DR+ CD11b+	IVW	32.1	20	0.042

IVW: inverse variance weighted; Q: Cochran's Q statistic; Q_df: degrees of freedom associated with the Q statistic; Q_p: p-value for the heterogeneity test based on the Q statistic

Supplementary Table 15. Pleiotropy test for MR of immune cells on plasma metabolites

Outcome	Exposure	egger intercept	SE	p
Linolenate [alpha or gamma; (18:3n3 or 6)] levels	CD20- B cell %B cell	0.0021	0.0083	0.803
Dimethylglycine levels	CD28+ CD45RA+ CD8dim T cell Absolute Count	-0.0076	0.0052	0.148
2,3-dihydroxyisovalerate levels	CD3 on HLA DR+ T cell	-0.0054	0.0067	0.435
Adenosine 5'-diphosphate (ADP) to sulfate ratio	FSC-A on HLA DR+ CD4+ T cell	-0.0116	0.0122	0.357
Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	CD16 on CD14- CD16+ monocyte	-0.0041	0.0080	0.612
X-12906 levels	SSC-A on CD14+ monocyte	-0.0081	0.0079	0.319
3-hydroxy-2-ethylpropionate levels	SSC-A on CD8+ T cell	0.0025	0.0096	0.801
8-methoxykynurenate levels	HLA DR on CD33dim HLA DR+ CD11b+	-0.0055	0.0101	0.593

SE: standard error

Supplementary Table 16. Heterogeneity test for MR of immune cell–related metabolites on esophageal cancer

Outcome	Exposure	method	Q	Q_df	Q_p
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	MR Egger	27.9	22	0.180
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	IVW	28.0	23	0.216
EC	Dimethylglycine levels	MR Egger	38.5	31	0.167
EC	Dimethylglycine levels	IVW	38.5	32	0.199
EC	2,3-dihydroxyisovalerate levels	MR Egger	20.1	25	0.743
EC	2,3-dihydroxyisovalerate levels	IVW	20.1	26	0.787
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	MR Egger	15.3	23	0.883
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	IVW	15.6	24	0.900
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	MR Egger	19.5	24	0.727
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	19.7	25	0.765
EC	X-12906 levels	MR Egger	6.8	16	0.977
EC	X-12906 levels	IVW	7.2	17	0.981
EC	3-hydroxy-2-ethylpropionate levels	MR Egger	21.9	27	0.745
EC	3-hydroxy-2-ethylpropionate levels	IVW	22.4	28	0.760
EC	8-methoxykynurenate levels	MR Egger	28.5	24	0.240
EC	8-methoxykynurenate levels	IVW	28.6	25	0.281

EC: esophageal cancer; IVW: inverse variance weighted; Q: Cochran's Q statistic; Q_df: degrees of freedom associated with the Q statistic; Q_p: p-value for the heterogeneity test based on the Q statistic

Supplementary Table 17. Pleiotropy test for MR of immune cell–related metabolites on esophageal cancer

Outcome	Exposure	egger intercept	SE	p
EC	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	-0.0098	0.0304	0.750
EC	Dimethylglycine levels	0.0006	0.0165	0.972
EC	2,3-dihydroxyisovalerate levels	0.0028	0.0165	0.866
EC	Adenosine 5'-diphosphate (ADP) to sulfate ratio	-0.0149	0.0258	0.568
EC	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	0.0076	0.0174	0.666
EC	X-12906 levels	0.0199	0.0301	0.518
EC	3-hydroxy-2-ethylpropionate levels	0.0172	0.0223	0.449
EC	8-methoxykynurenate levels	-0.0059	0.0187	0.755

EC: esophageal cancer; SE: standard error

Supplementary Table 18. Exploratory analysis of metabolite-mediated pathways linking immune cells and esophageal cancer

Exposure	Mediator	Outcome	OR	or_lci95	or_uci95	proportion	p
CD3 on HLA DR+ T cell	2,3-dihydroxyisovalerate levels	EC	-0.0061	-0.0105	-0.0016	10.50%	0.0075
SSC-A on CD14+ monocyte	X-12906 levels	EC	0.0148	0.0036	0.0261	14.70%	0.01
SSC-A on CD8+ T cell	3-hydroxy-2-ethylpropionate levels	EC	0.0151	0.0051	0.025	11.30%	0.0032
HLA DR on CD33dim HLA DR+ CD11b+	8-methoxykynurenate levels	EC	0.0105	0.004	0.0169	19.50%	0.0014

EC: esophageal cancer; OR: odds ratio

Supplementary Table 19. Potential dietary or nutritional interventions based on metabolites associated with esophageal cancer risk

Superpathway	Metabolite / Ratio	Association with EC	Endogenous / Exogenous	Dietary Source (HMDB)
Lipid	Maltotriose levels	↑ Risk	Exogenous	Primarily derived from dietary starch, which is hydrolyzed by salivary and pancreatic α -amylase in the mouth and small intestine (HMDB0001262)
Lipid	1-stearoyl-GPI (18:0) levels	↓ Protective	Endogenous	synthesized endogenously from cellular membrane lipids (HMDB0061704)
Lipid	5-dodecenoate (12:1n7) levels	↑ Risk	Exogenous	Medium-chain fatty acid, found in Milk and milk product (HMDB0000529)
Lipid	Carnitine C14 levels	↑ Risk	Exogenous	Formed from long-chain fatty acids and carnitine, usually dietary (HMDB0005066)
Lipid	Hyocholate levels	↑ Risk	Endogenous	Bile acid synthesized in the liver (HMDB0000760)
Lipid	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	↑ Risk	Exogenous	Found in seed oils such as flaxseed and hempseed (HMDB0001388, HMDB0003073)
Lipid	3-methyladipate levels	↓ Protective	Endogenous	Metabolite of plant acids, potentially related to plant-based diets (HMDB0000555)
Lipid	5alpha-pregnan-diol disulfate levels	↓ Protective	Endogenous	Cholesterol metabolite, related to hormone levels (HMDB0094650)
Lipid	1-palmitoyl-2-stearoyl-GPC (16:0/18:0) levels	↑ Risk	Both	Likely from animal fats (HMDB0007970)
Lipid	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	↑ Risk	Both	Likely derived from foods rich in stearic acid and arachidonic acid (HMDB0009003)
Lipid	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	↓ Protective	Both	Likely from sphingolipids, which are mainly found in animal-based foods such as egg yolk, meat, and fish (HMDB0012095)
Lipid	Myristate (14:0) levels	↑ Risk	Both	Likely from animal fats (HMDB0000806)
Amino acid	3-hydroxy-2-ethylpropionate levels	↓ Protective	Endogenous	Related to amino acid metabolism (HMDB0029169)

HMDB: Human Metabolome Database

Supplementary Table 19. Potential dietary or nutritional interventions based on metabolites associated with esophageal cancer risk (cont.)

Superpathway	Metabolite / Ratio	Association with EC	Endogenous / Exogenous	Dietary Source (HMDB)
Amino acid	8-methoxykynurenate levels	↑ Risk	Endogenous	Related to tryptophan metabolism (HMDB0060426)
Amino acid	N-lactoyl phenylalanine levels	↑ Risk	Endogenous	Related to lactate and phenylalanine metabolism (HMDB0062175)
Amino acid	N-acetyl-isoptreanine levels	↓ Protective	Endogenous	Related to gamma amino acid metabolism (HMDB0094713)
Amino acid	Thyroxine levels	↓ Protective	Both	Thyroid hormone, with iodine supplied by foods such as kelp, nori, and fish (HMDB0000248)
Amino acid	Dimethylglycine levels	↑ Risk	Endogenous	Related to choline metabolism (HMDB0000092)
Amino acid	Tyrosine levels	↓ Protective	Both	Synthesized from phenylalanine in the liver; found in protein-rich foods (HMDB0029117)
Amino acid	Alanine levels	↑ Risk	Both	Synthesized in the liver and muscle; found in protein-rich foods (HMDB0001310, HMDB0000161)
Amino acid	Glycine levels	↑ Risk	Both	Produced via the serine pathway; found in protein-rich foods (HMDB0000123)
Xenobiotics	Tartronate (hydroxymalonate) levels	↓ Protective	Exogenous	Possibly from plant-based foods (HMDB0035227)
Xenobiotics	2,3-dihydroxyisovalerate levels	↑ Risk	Both	Valine metabolism; found in meat, fish, eggs, legumes, nuts, and dairy products (HMDB0035227)
Xenobiotics	3-hydroxy-2-methylpyridine sulfate levels	↓ Protective	Both	Produced via the vitamin B6 catabolic pathway; vitamin B6-rich foods include meat, fish, poultry, potatoes, bananas, and nuts (HMDB0341247)
Xenobiotics	4-acetylcatechol sulfate (1) levels	↓ Protective	Both	Derived from flavonoid metabolism; flavonoid-rich foods include citrus fruits, other fruits and vegetables, and green tea (HMDB0240459)
Xenobiotics	(S)-a-amino-omega-caprolactam levels	↑ Risk	Both	Generated from lysine metabolism; lysine-rich foods include meat, dairy products, and legumes (HMDB0062769)
Xenobiotics	Gluconate levels	↓ Protective	Both	A product of glucose metabolism; foods rich in glucuronate or its precursors include grapes, oranges, and lemons (HMDB0000625)
Cofactors & Vitamins	Trigonelline levels	↓ Protective	Exogenous	Mainly derived from coffee beans and legumes (HMDB0000875)
Cofactors & Vitamins	1-methylnicotinamide levels	↓ Protective	Endogenous	An intermediate of nicotinamide metabolism (HMDB0000699)
Peptide	Gamma-glutamylglycine levels	↑ Risk	Endogenous	Produced from protein digestion and hydrolysis; precursor amino acids (e.g., glutamate and glycine) are widely present in foods such as meat, dairy products, and legumes (HMDB0011667)
Unknown	X-12906 levels	↑ Risk	Unknown	Unknown
Unknown	X-13728 levels	↓ Protective	Unknown	Unknown
Unknown	X-23639 levels	↑ Risk	Unknown	Unknown
Unknown	X-23780 levels	↓ Protective	Unknown	Unknown
Unknown	X-19141 levels	↓ Protective	Unknown	Unknown

HMDB: Human Metabolome Database

Supplementary Table 19. Potential dietary or nutritional interventions based on metabolites associated with esophageal cancer risk (cont.)

Superpathway	Metabolite / Ratio	Association with EC	Endogenous / Exogenous	Dietary Source (HMDB)
Metabolite ratio	Spermidine to 5-methylthioadenosine (MTA) ratio	↑ Risk	Both	The increase in the ratio mainly depends on dietary spermidine, while MTA is primarily controlled by endogenous metabolism (HMDB0001257, HMDB0001173)
Metabolite ratio	Adenosine 5'-diphosphate (ADP) to mannose ratio	↓ Protective	Both	Reflects energy metabolism status vs. sugar metabolism status (HMDB0001341, HMDB0000169)
Metabolite ratio	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	↑ Risk	Both	ω-6 polyunsaturated fatty acids / monounsaturated fatty acids (HMDB0001043, HMDB0304034, HMDB0003231)
Metabolite ratio	Glycine to serine ratio	↑ Risk	Both	Can be modulated by adjusting dietary protein and foods rich in glycine or serine (e.g., legumes, meat, eggs) (HMDB0000123, HMDB0000187)
Metabolite ratio	Phosphate to fructose ratio	↓ Protective	Both	Can be modulated by adjusting high-fructose intake and consumption of phosphate-rich foods (HMDB0000660)
Metabolite ratio	Adenosine 5'-diphosphate (ADP) to sulfate ratio	↑ Risk	Both	Energy metabolism / energy expenditure, influenced by foods high in sulfur (HMDB0001341, HMDB0251240)
Metabolite ratio	Glycine to phosphate ratio	↑ Risk	Both	High-protein foods rich in glycine have an impact (HMDB0000123)
Metabolite ratio	Isoleucine to phosphate ratio	↑ Risk	Both	Isoleucine: branched-chain amino acid, a product of protein catabolism (HMDB0000172). High-protein foods such as meat, fish, eggs
Metabolite ratio	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	↓ Protective	Both	Phosphate: product of ATP hydrolysis and phosphorylation metabolism (HMDB0000172). Foods high in phosphate may influence phosphate levels.
Metabolite ratio	Phosphate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	↓ Protective	Both	Phosphate supply vs. signaling lipid levels (HMDB0245187). Intake of phosphate-rich foods and foods providing membrane lipids may affect this balance.
Metabolite ratio	Mannose to glycerol ratio	↑ Risk	Both	Mannose: glucose isomerization, fruits, vegetables (HMDB0000169); Glycerol: product of triglyceride hydrolysis fats and oils (HMDB0000131)
Metabolite ratio	Glucose-to-mannose ratio	↓ Protective	Both	Glucose: from gluconeogenesis and glycogen breakdown, carbohydrate-rich foods (HMDB0000122); Mannose: derived from glucose isomerization, fruits, vegetables (HMDB0000169).
Metabolite ratio	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	↑ Risk	Both	Arachidonate: derived from linoleate via Δ6-desaturase elongation, animal fats, red meat, egg (HMDB0001043); Linoleate: essential fatty acid, vegetable oils, nuts (HMDB0000673)

HMDB: Human Metabolome Database