# Multi-omics architecture of obesity and metabolic dysfunction in childhood: identifying biological pathways and prenatal determinants

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## Supplementary Table 1: Omics search strategy used in the present study

| Omics layer | Pubmed Search Strategy, as of 15 March 2024 | Eligibility criteria | No. of records screened by title and abstract | No. of full-text records assessed for eligibility | No. of records included | No. of molecules identified and linked to HELIX dataset | No. of molecules previously examined and found to associate with body mass index in HELIX |
|---|---|---|---|---|---|---|---|
| DNA methylation | (epigenomics [mh] OR "DNA methylation" [tiab] OR methyl* [tiab] OR epigenetic*[tiab] OR cpg [tiab]) AND ("pediatric obesity"[mh] OR (child*[tiab] OR adolescen*[tiab] OR puberty[tiab] OR school*[tiab] OR pediatric*[tiab] OR "early-life"[tiab]) AND (obese[tiab] OR obesity[tiab] OR overweight[tiab] OR bmi[tiab] OR "body mass index"[tiab] OR weight[tiab] OR adiposity[tiab] OR "waist-to-hip ratio"[tiab] OR "fat mass"[tiab] OR "waist circumference"[tiab] OR "ponderal index"[tiab] OR "body composition"[tiab] OR "body constitution"[tiab] OR anthropometry[tiab])) AND systematic [sb] | Reviews including human studies assessing the association between DNA methylation markers and overweight and/or obesity (and/or related anthropometric parameters such as body mass index, weight change, waist circumference, and fat mass) in children. Additional eligibility criteria for individual studies to be considered were as follows: 1) the paper describes an observational study, excluding controlled experiments conducted in a manipulated rather than naturalistic settings (i.e., the administration of drugs); 2) the paper is peer-reviewed; and 3) the paper provides the differentially methylated cytosine-phosphate-guanine (CpG) site across the comparisons. | N=40 | N=10 | N=4 | N= 976 | N=10 (PMID: 33239103) as part of the PACE consortium |
| miRNAs | (micrornas OR microrna [tiab] OR miR [tiab] or miRNA [tiab] OR (("non coding" OR "non-coding") AND RNA)) AND ("pediatric obesity"[mh] OR (child*[tiab] OR adolescen*[tiab] OR puberty[tiab] OR school*[tiab] OR pediatric*[tiab] OR "early-life"[tiab]) AND (obese[tiab] OR obesity[tiab] OR overweight[tiab] OR bmi[tiab] OR "body mass index"[tiab] OR weight[tiab] OR adiposity[tiab] OR "waist-to-hip ratio"[tiab] OR "fat mass"[tiab] OR "waist circumference"[tiab] OR "ponderal index"[tiab] OR "body composition"[tiab] OR "body constitution"[tiab] OR anthropometry[tiab])) AND systematic [sb] | Reviews assessing the association between miRNA expression levels and overweight and/or obesity (and/or related parameters such as body mass index, weight change, waist circumference, and fat mass) in children. Additional eligibility criteria for individual studies to be considered were as follows: 1) the paper describes an observational study, excluding controlled experiments conducted in a manipulated rather than naturalistic settings (i.e., the administration of drugs); 2) the paper is peer-reviewed; and 3) the paper provides the differentially expressed miRNAs across the comparisons. | N=8 | N=5 | N=4 | N=51 | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Transcriptome | (mRNA OR transcriptome OR transcriptomics OR (coding AND RNA)) AND (blood[tiab] OR plasma[tiab] OR serum[tiab]) AND ("pediatric obesity"[mh] OR (child*[tiab] OR adolescen*[tiab] OR puberty[tiab] OR school*[tiab] OR pediatric*[tiab] OR "early-life"[tiab]) AND (obese[tiab] OR obesity[tiab] OR overweight[tiab] OR bmi[tiab] OR "body mass index"[tiab] OR weight[tiab] OR adiposity[tiab] OR "waist-to-hip ratio"[tiab] OR "fat mass"[tiab] OR "waist circumference"[tiab] OR "ponderal index"[tiab] OR "body composition"[tiab] OR "body constitution"[tiab] OR anthropometry[tiab])) Filter: Humans | Studies assessing the association between blood gene expression and overweight and/or obesity (and/or related anthropometric parameters such as body mass index, weight change, waist circumference, and fat mass) in children. Additional eligibility criteria for studies to be considered were as follows: 1) the paper describes an observational study (prospective or cross-sectional), excluding controlled experiments conducted in a manipulated rather than naturalistic settings (i.e., the administration of drugs); 2) the paper is written in English; 3) the paper is peer-reviewed; and 4) the paper provides the differentially expressed genes across the comparisons. | N=215 | N=65 | N=9 | N=219 | - |
| Metabolome | (metabolomics[mh] OR (metabolom*[tiab] OR metabonom*[tiab] OR metabolit*))AND ("pediatric obesity"[mh] OR ((child*[tiab] OR adolescen*[tiab] OR puberty[tiab] OR OR school*[tiab] OR pediatric*[tiab] OR "early-life"[tiab]) AND (obese[tiab] OR obesity[tiab] OR overweight[tiab] OR bmi[tiab] OR "body mass index"[tiab] OR weight[tiab] OR adiposity[tiab] OR "waist-to-hip ratio"[tiab] OR "fat mass"[tiab] OR "waist circumference"[tiab] OR "ponderal index"[tiab] OR "body composition"[tiab] OR "body constitution"[tiab] OR anthropometry[tiab]))) AND systematic [sb] | Reviews assessing the association between metabolome biomarkers and overweight and/or obesity (and/or related anthropometric parameters such as body mass index, weight change, waist circumference, and fat mass) in children. Additional eligibility criteria for individual studies to be considered were as follows: 1) the paper describes an observational study, excluding controlled experiments conducted in a manipulated rather than naturalistic settings (i.e., the administration of drugs); 2) the paper describes a metabolomics approach in the assessment of metabolites (including application of nuclear magnetic resonance spectroscopy or mass spectrometry, coupled to various types of chromatography, to measure at least 10 molecules); 3) the paper is peer-reviewed; and 4) the paper provides the individual metabolites across the comparisons. | N=17 | N=4 | N=3 | N=98 | N=41 (PMID: 30404627) |

**Supplementary Table 2: Sociodemographic characteristics of the identified multi-omics clusters across cohorts**

| | Northern/Western cohort | | | | Southern/Mediterranean cohort | | | |
|---|---|---|---|---|---|---|---|---|
| | Cluster A (N=227) | Cluster B (N=150) | Cluster C (N=180) | P value[b] | Cluster A (N=238) | Cluster B (N=21) | Cluster C (N=47) | P value[b] |
| Study site[a] | | | | 0.998 | | | | 0.969 |
| BiB, Bradford, UK | 62 (27.3%) | 43 (28.7%) | 51 (28.3%) | | - | - | - | |
| EDEN, Poitiers, FR | 31 (13.7%) | 20 (13.3%) | 24 (13.3%) | | - | - | - | |
| KANC, Kaunas city, LT | 56 (24.7%) | 38 (25.3%) | 43 (23.9%) | | - | - | - | |
| MoBa, Oslo, NO | 78 (34.4%) | 49 (32.7%) | 62 (34.4%) | | - | - | - | |
| INMA, Sabadell, SP | - | - | - | | 122 (51.3%) | 11 (52.4%) | 25 (53.2%) | |
| RHEA, Heraklion, GR | - | - | - | | 116 (48.7%) | 10 (47.6%) | 22 (46.8%) | |
| Child sex[a] | | | | 0.216 | | | | 0.641 |
| Male | 126 (55.5%) | 87 (58.0%) | 88 (48.9%) | | 130 (54.6%) | 11 (52.4%) | 29 (61.7%) | |
| Female | 101 (44.5%) | 63 (42.0%) | 92 (51.1%) | | 108 (45.4%) | 10 (47.6%) | 18 (38.3%) | |
| Child race/ethnicity[a] | | | | 0.989 | | | | - |
| white people | 190 (83.7%) | 124 (82.7%) | 149 (82.8%) | | 238 (100.0%) | 21 (100.0%) | 47 (100.0%) | |
| South Asian | 27 (11.9%) | 19 (12.7%) | 24 (13.3%) | | - | - | - | |
| other | 10 (4.4%) | 7 (4.7%) | 7 (3.9%) | | - | - | - | |
| Birth weight, grams[a] | 3421.58 (529.2) | 3493.29 (588.3) | 3402.62 (488.1) | 0.188 | 3291.22 (432.8) | 3153.57 (410.3) | 3178.80 (453.8) | 0.217 |
| Gestational age, weeks[a] | 39.69 (1.88) | 39.71 (1.74) | 39.81 (1.49) | 0.998 | 39.31 (1.61) | 38.89 (1.79) | 38.90 (1.85) | 0.250 |
| Breastfeeding[a] | | | | 0.520 | | | | 0.527 |
| No | 31 (13.7%) | 27 (18.0%) | 28 (15.6%) | | 26 (10.9%) | 4 (19.0%) | 6 (12.8%) | |
| Yes | 196 (86.3%) | 123 (82.0%) | 152 (84.4%) | | 212 (89.1%) | 17 (81.0%) | 41 (87.2%) | |
| Child age at assessment, years[a] | 7.78 (1.53) | 7.75 (1.55) | 7.82 (1.54) | 0.937 | 7.71 (1.23) | 7.94 (1.34) | 7.78 (1.27) | 0.680 |

*BiB* Born in Bradford study, *BMI* body mass index, *EDEN* Etude des Determinants Pre et Postnatals du Developpement et de la Sante de l'Enfant study, *INMA* Infancia y Medio Ambiente study, *MoBa* Norwegian Mother, Father and Child study, *RHEA* Rhea Mother- Child study.

[a] Values are mean (SD) or n (%).

[b] P values for differences across the clusters were derived using kruskall-wallis test for continuous variables and chi-squared test for categorical ones, based on a two-sided alternative hypothesis.

**Supplementary Table 3: Childhood clinical characteristics of the identified multi-omics clusters across cohorts**

| | Northern/Western cohort | | | | Southern/Mediterranean cohort | | | |
|---|---|---|---|---|---|---|---|---|
| | Cluster A (N=227) | Cluster B (N=150) | Cluster C (N=180) | P value[b] | Cluster A (N=238) | Cluster B (N=21) | Cluster C (N=47) | P value[b] |
| BMI, kg/m$^{2}$[a] | 16.1 (1.9) | 16.7 (2.4) | 16.7 (2.4) | 0.017 | 17.0 (2.5) | 17.8 (3.3) | 19.1 (3.5) | < 0.001 |
| Waist circumference, cm[a] | 55.7 (6.4) | 57.8 (6.8) | 57.8 (6.9) | < 0.001 | 59.5 (7.0) | 61.3 (8.7) | 65.2 (10.1) | 0.004 |
| Total fat mass, kg[a] | 5.6 (3.1) | 6.3 (3.6) | 6.6 (4.2) | 0.026 | 6.8 (3.6) | 8.3 (5.5) | 9.7 (5.2) | < 0.001 |
| Triglycerides, mg/dl[a] | 84.2 (37.7) | 82.7 (44.4) | 89.7 (40.6) | 0.151 | 80.3 (35.4) | 90.0 (51.2) | 92.8 (49.3) | 0.281 |
| HDL cholesterol, mg/dl[a] | 60.7 (11.7) | 58.6 (11.1) | 57.8 (12.5) | 0.016 | 62.4 (13.1) | 58.2 (11.0) | 53.7 (12.1) | < 0.001 |
| Insulin, µg/ml[a] | 6.2 (4.6) | 6.7 (6.4) | 7.0 (5.5) | 0.049 | 6.8 (5.0) | 8.8 (5.3) | 8.4 (6.1) | 0.033 |
| Blood pressure, mm Hg[a] | | | | | | | | |
| Systolic | 98.1 (10.9) | 99.1 (11.2) | 100.8 (12.7) | 0.065 | 97.8 (10.6) | 100.5 (7.0) | 103.0 (11.2) | 0.007 |
| Diastolic | 58.0 (11.5) | 59.5 (9.6) | 60.2 (10.7) | 0.007 | 56.2 (8.9) | 58.4 (6.6) | 59.3 (8.2) | 0.013 |
| ALT, U/L[a] | 13.9 (5.1) | 13.7 (4.6) | 14.5 (5.4) | 0.589 | 17.4 (5.9) | 18.8 (6.1) | 21.7 (7.9) | < 0.001 |
| Metabolic syndrome score, SD[a,c] | -0.5 (2.1) | 0.1 (2.2) | 0.5 (2.4) | < 0.001 | -0.5 (2.1) | 0.6 (2.5) | 1.4 (2.7) | < 0.001 |
| Weight status[a, d] | | | | 0.133 | | | | < 0.001 |
| Normal weight | 185 (81.5%) | 111 (74.0%) | 134 (74.4%) | | 155 (65.1%) | 14 (66.7%) | 17 (36.2%) | |
| Overweight/obesity | 42 (18.5%) | 39 (26.0%) | 46 (25.6%) | | 83 (34.9%) | 7 (33.3%) | 30 (63.8%) | |
| Metabolic health[a, e] | | | | 0.009 | | | | 0.013 |
| Healthy | 132 (75.4%) | 90 (74.4%) | 92 (60.9%) | | 155 (70.8%) | 12 (60.0%) | 19 (47.5%) | |
| Unhealthy | 43 (24.6%) | 31 (25.6%) | 59 (39.1%) | | 64 (29.2%) | 8 (40.0%) | 21 (52.5%) | |

[a] Values are mean (SD) or n (%).

[b] P values for differences across the clusters were derived using kruskall-wallis test for continuous variables and chi-squared test for categorical ones, based on a two-sided alternative hypothesis.

[c] The metabolic syndrome score (expressed in SD) was derived using z scores for waist circumference, HDL cholesterol level, triglyceride level, insulin level, and systolic and diastolic blood pressure.

[d] Overweight/obesity was defined according to the World Health Organization criteria.

[e] Metabolically unhealthy status was defined as the presence of at least one of the following risk factors: systolic or diastolic blood pressure≥90$^{th}$ percentile, insulin ≥90$^{th}$ percentile, HDL cholesterol ≤40mg/dl, triglycerides ≥150mg/dl, alanine aminotransferase ≥22.1 U/L for females and ≥25.8 U/L for males.

**Supplementary Table 4: Associations of multi-omics clusters with metabolic health outcomes by cohort and sex**

| | Northern/Western cohort | | | Southern/Mediterranean cohort | | | Pooled population | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Males** | **Females** | **P interaction[c]** | **Males** | **Females** | **P interaction[c]** | **Males** | **Females** | **P interaction[c]** |
| **Fat mass[a]** | | | | | | | | | |
| Cluster A | Ref. | | | Ref. | | | Ref. | | |
| Cluster B | 0.29 (0.01, 0.57) | 0.21 (-0.09, 0.51) | 0.707 | 0.08 (-0.52, 0.68) | 0.77 (0.14, 1.41) | 0.121 | 0.3 (0.05, 0.55) | 0.33 (0.06, 0.60) | 0.775 |
| Cluster C | 0.30 (0.02, 0.58) | 0.30 (0.04, 0.57) | 0.962 | 0.76 (0.37, 1.15) | 0.66 (0.16, 1.15) | 0.745 | 0.43 (0.21, 0.66) | 0.40 (0.17, 0.64) | 0.935 |
| **Metabolic syndrome score[a,b]** | | | | | | | | | |
| Cluster A | Ref. | | | Ref. | | | Ref. | | |
| Cluster B | 0.53 (-0.05, 1.11) | 0.72 (0.01, 1.43) | 0.703 | 1.03 (-0.28, 2.35) | 1.17 (-0.33, 2.67) | 0.891 | 0.66 (0.13, 1.18) | 0.90 (0.26, 1.53) | 0.383 |
| Cluster C | 1.09 (0.51, 1.67) | 0.80 (0.17, 1.44) | 0.497 | 1.65 (0.79, 2.51) | 2.10 (0.91, 3.28) | 0.545 | 1.27 (0.80, 1.75) | 1.11 (0.55, 1.67) | 0.834 |

[a] Effect estimates represent beta coefficients (expressed in SD) and their 95% CIs, derived from generalized linear regression models controlled for study site and age at examination.

[b] The metabolic syndrome score was derived using z scores for waist circumference, HDL cholesterol level, triglyceride level, insulin level, and systolic and diastolic blood pressure.

[c] Values represent P-values for sex*cluster (interaction) estimates.

**Supplementary Table 5: Childhood clinical characteristics of the multi-omics Cluster C and A (reference) across cohorts and stratified by weight status**

| | Children with normal weight[a] | | | | | | Children with overweight/obesity[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Northern/Western cohort | | | Southern/Mediterranean cohort | | | Northern/Western cohort | | | Southern/Mediterranean cohort | | |
| | Cluster A (N=185) | Cluster C (N=134) | P value[c] | Cluster A (N=155) | Cluster C (N=17) | P value[c] | Cluster A (N=42) | Cluster C (N=46) | P value[c] | Cluster A (N=83) | Cluster C (N=30) | P value[c] |
| Waist circumference, cm[b] | 54.1 (4.0) | 55.1 (3.9) | 0.016 | 56.0 (3.8) | 55.8 (2.1) | 0.729 | 62.4 (9.6) | 65.6 (7.9) | 0.125 | 65.9 (7.1) | 70.7 (8.7) | 0.005 |
| Total fat mass, kg[b] | 4.7 (1.7) | 5.0 (2.2) | 0.355 | 5.1 (1.9) | 5.0 (1.4) | 0.871 | 9.6 (4.7) | 11.1 (5.3) | 0.080 | 10.0 (3.9) | 12.3 (4.7) | 0.012 |
| Triglycerides, mg/dl[b] | 82.9 (38.2) | 86.1 (38.5) | 0.479 | 77.8 (35.8) | 81.5 (37.1) | 0.484 | 89.9 (35.5) | 100.2 (44.9) | 0.440 | 85.0 (34.4) | 99.2 (54.6) | 0.451 |
| HDL cholesterol, mg/dl[b] | 61.3 (11.8) | 59.0 (13.0) | 0.033 | 64.0 (13.3) | 57.1 (12.9) | 0.119 | 57.6 (10.6) | 54.4 (10.2) | 0.205 | 59.4 (12.3) | 51.8 (11.3) | 0.007 |
| Insulin, µg/ml[b] | 5.7 (4.2) | 6.5 (4.0) | 0.002 | 6.2 (4.1) | 5.9 (2.9) | 0.986 | 8.2 (5.7) | 8.4 (8.5) | 0.319 | 7.8 (6.2) | 9.8 (7.0) | 0.179 |
| Blood pressure, mm Hg[b] | | | | | | | | | | | | |
| Systolic | 97.9 (11.4) | 100.0 (12.7) | 0.074 | 94.7 (9.9) | 100.1 (11.6) | 0.037 | 99.0 (8.5) | 103.1 (12.7) | 0.197 | 103.5 (9.5) | 104.7 (10.8) | 0.567 |
| Diastolic | 58.1 (12.1) | 59.4 (11.0) | 0.065 | 54.4 (7.2) | 57.4 (6.8) | 0.075 | 57.6 (8.4) | 62.6 (9.8) | 0.012 | 59.5 (10.8) | 60.4 (8.8) | 0.422 |
| ALT, U/L[b] | 13.6 (4.8) | 14.2 (5.2) | 0.434 | 16.2 (4.8) | 18.9 (5.3) | 0.040 | 15.2 (6.1) | 15.3 (5.8) | 0.908 | 19.5 (6.9) | 23.3 (8.9) | 0.048 |
| Metabolic syndrome score, SD[b, d] | -0.9 (1.8) | -0.2 (1.9) | 0.001 | -1.4 (1.5) | -0.6 (1.5) | 0.045 | 1.2 (2.4) | 2.2 (2.6) | 0.063 | 1.1 (2.0) | 2.5 (2.5) | 0.015 |
| Metabolic health[b, e] | | | 0.044 | | | 0.433 | | | 0.040 | | | 0.025 |
| Healthy | 107 (75.4%) | 72 (63.7%) | | 107 (75.9%) | 10 (66.7%) | | 25 (75.8%) | 20 (52.6%) | | 48 (61.5%) | 9 (36.0%) | |
| Unhealthy | 35 (24.6%) | 41 (36.3%) | | 34 (24.1%) | 5 (33.3%) | | 8 (24.2%) | 18 (47.4%) | | 30 (38.5%) | 16 (64.0%) | |

*ALT* alanine aminotransferase, *HDL* high-density lipoprotein.

[a] Weight status was defined according to the World Health Organization criteria.

[b] Values are mean (SD) or n (%).

[c] P values for differences across the clusters were derived using Mann-Whitney U test for continuous variables and chi-squared test for categorical ones, based on a two-sided alternative hypothesis.

[d] The metabolic syndrome score (expressed in SD) was derived using z scores for waist circumference, HDL cholesterol level, triglyceride level, insulin level, and systolic and diastolic blood pressure.

[e] Metabolically unhealthy status was defined as the presence of at least one of the following risk factors: systolic or diastolic blood pressure≥90$^{th}$ percentile, insulin ≥90$^{th}$ percentile, HDL cholesterol ≤40mg/dl, triglycerides ≥150mg/dl, ALT ≥22.1 U/L for females and ≥25.8 U/L for males.

**Supplementary Table 6: Adolescent characteristics of the study population**

| | Northern/Western cohort (N=233) | Southern/Mediterranean cohort (N=171) | Total (N=404) |
|---|---|---|---|
| Study site[a] | | | |
| BiB, Bradford, UK | 88 (37.8%) | - | 88 (21.8%) |
| EDEN, Poitiers, FR | 28 (12.0%) | - | 28 (6.9%) |
| KANC, Kaunas city, LT | 82 (35.2%) | - | 82 (20.3%) |
| MoBa, Oslo, NO | 35 (15.0%) | - | 35 (8.7%) |
| INMA, Sabadell, SP | 0 (0.0%) | 55 (32.2%) | 55 (13.6%) |
| RHEA, Heraklion, GR | 0 (0.0%) | 116 (67.8%) | 116 (28.7%) |
| Child sex[a] | | | |
| Male | 125 (53.6%) | 90 (52.6%) | 215 (53.2%) |
| Female | 108 (46.4%) | 81 (47.4%) | 189 (46.8%) |
| Race/ethnicity[a] | | | |
| white people | 178 (76.4%) | 171 (100.0%) | 349 (86.4%) |
| South Asian | 43 (18.5%) | 0 (0.0%) | 43 (10.6%) |
| other | 12 (5.2%) | 0 (0.0%) | 12 (3.0%) |
| Child age at assessment, years[a] | 14.65 (1.57) | 14.99 (0.96) | 14.80 (1.36) |
| BMI, $kg/m^{2a}$ | 21.42 (4.35) | 22.64 (4.35) | 21.94 (4.39) |
| Waist circumference, cm[a] | 80.93 (12.14) | 78.46 (11.21) | 79.88 (11.80) |
| Total fat mass, kg[a] | 18.01 (12.12) | 22.68 (10.53) | 20.00 (11.69) |
| Weight status[a, b] | | | |
| Normal weight | 163 (70.0%) | 104 (61.2%) | 267 (66.3%) |
| Overweight/obesity | 70 (30.0%) | 66 (38.8%) | 136 (33.7%) |
| Multi-omics clusters[a] | | | |
| Cluster A | 92 (39.5%) | 136 (79.5%) | 228 (56.4%) |
| Cluster B | 58 (24.9%) | 10 (5.8%) | 68 (16.8%) |
| Cluster C | 83 (35.6%) | 25 (14.6%) | 108 (26.7%) |

*BiB* Born in Bradford study, *BMI* body mass index, *EDEN* Etude des Determinants Pre et Postnatals du Developpement et de la Sante de l'Enfant study, *INMA* Infancia y Medio Ambiente study, *MoBa* Norwegian Mother, Father and Child study, *RHEA* Rhea Mother- Child study.

[a] Values are mean (SD) or n (%).

[b] Overweight/obesity was defined according to the World Health Organization criteria.

**Supplementary Table 7: Model performance of XGboost classifier in the S/M cohort**

| | Prediction of Cluster C membership | Prediction of Cluster B membership |
|---|---|---|
| Accuracy | 0.84 | 0.83 |
| Sensitivity | 0.92 | 1 |
| Specificity | 0.83 | 0.81 |
| Gmean | 0.88 | 0.9 |
| Balanced accuracy | 0.88 | 0.91 |
| AUC | 0.94 | 0.96 |

*AUC* area under the receiver operating characteristic curve.

**Supplementary Table 8: List of prenatal factors assessed in the study**

| Group | Exposure | Common abbreviation |
|---|---|---|
| Air Pollution | Nitrogen dioxide | NO2 |
| Air Pollution | Particulate matter with an aerodynamic diameter of less than 10 µm | PM10 |
| Air Pollution | Particulate matter with an aerodynamic diameter of less than 2.5 µm | PM2.5 |
| Built Environment | Building density using a buffer of 300m from home address | |
| Built Environment | Facility density using a buffer of 300m from home address | - |
| Built Environment | Facility richness using a buffer of 300m from home address | |
| Built Environment | Land Use Evenness Index within a buffer of 300m from home address | - |
| Built Environment | Population density (inhabitants per km2) | - |
| Built Environment | Walkability index within a buffer of 300m from home address | - |
| Green Spaces | Average Normalized Difference Vegetation Index (NDVI) within buffer of 100m from home address | NDVI |
| Green Spaces | Presence of a major green space (park or countryside) within a buffer of 300m from home address | |
| Traffic | Total traffic load of roads in a 100-m buffer from home address | - |
| Traffic | Traffic density on nearest road to home address | |
| Metals | Cadmium | Cd |
| Metals | Mercury | Hg |
| Metals | Lead | Pb |
| OCs | 4,4'dichlorodiphenyl dichloroethylene | DDE |
| OCs | Hexachlorobenzene | HCB |
| OCs | polychlorinated biphenyl-138 | PCB 138 |
| OCs | polychlorinated biphenyl-153 | PCB 153 |
| OCs | polychlorinated biphenyl-180 | PCB 180 |
| PFASs | perfluorohexane sulfonate | PFHxS |
| PFASs | perfluorononanoate | PFNA |
| PFASs | perfluorooctanoate | PFOA |
| PFASs | perfluorooctane sulfonate | PFOS |
| Social and lifestyle | Pre-pregnancy BMI | - |
| Social and lifestyle | Gestational weight gain | - |
| Social and lifestyle | Maternal tobacco smoking during pregnancy | - |
| Social and lifestyle | Maternal alcohol consumption during pregnancy | - |
| Social and lifestyle | Maternal educational level during pregnancy | - |
| Social and lifestyle | Maternal age at birth | - |
| Diet | Maternal fish intake during pregnancy | |
| Diet | Maternal dairy intake during pregnancy | |
| Diet | Maternal fruit intake during pregnancy | |
| Diet | Maternal vegetable intake during pregnancy | |
| Diet | Maternal legume intake during pregnancy | |
| Diet | Maternal meat intake during pregnancy | |

**Supplementary Table 9: Selection proportion and effect estimates of the association of prenatal factors with multi-omics clusters as derived LASSO**

| | Northern/Western cohort | | | Southern/Mediterranean cohort | | |
|---|---|---|---|---|---|---|
| | Selection proportion[a] | OR for cluster B[a] | OR for Cluster C[a] | Selection proportion[a] | OR for cluster B[a] | OR for Cluster C[a] |
| Alcohol intake | 0.17 | 0.99 | 0.99 | 0.66 | 0.93 | 0.93 |
| Building density within 300m | 0.87 | 1.02 | 0.91 | 0.36 | 1.04 | 1.08 |
| Cadmium | 0.58 | 1.03 | 1.04 | 0.76 | 1.08 | 1.11 |
| Dairy intake | 0.49 | 0.99 | 0.99 | 0.75 | 0.93 | 0.9 |
| DDE | 0.45 | 0.97 | 1 | 0.29 | 1.04 | 1.07 |
| Facility density within 300m | 0.24 | 1 | 0.96 | 0.17 | 1.07 | 1.04 |
| Facility richness within 300m | 0.38 | 1.01 | 0.96 | 0.31 | 1.03 | 0.96 |
| Fish intake | 0.54 | 1.03 | 1.04 | 0.6 | 0.93 | 1.1 |
| Fruit intake | 0.79 | 0.96 | 1.01 | 0.88 | 0.98 | 0.88 |
| Gestational weight gain | 0.33 | 0.98 | 0.99 | 0.85 | 1.04 | 1.13 |
| HCB | 0.38 | 0.97 | 0.99 | 0.44 | 0.96 | 0.93 |
| Land use index within 300m | 0.59 | 1 | 0.96 | 0.32 | 0.99 | 1.02 |
| Lead | 0.31 | 1 | 0.98 | 0.69 | 0.98 | 1.03 |
| Legume intake | 0.18 | 0.99 | 0.98 | 0.38 | 1.01 | 0.99 |
| Maternal age | 0.39 | 1.03 | 1.03 | 0.19 | 1 | 0.98 |
| Maternal education | 0.56 | 0.95 | 0.99 | 0.69 | 0.92 | 0.9 |
| Meat intake | 0.52 | 1.04 | 1.02 | 0.33 | 1.03 | 1.01 |
| Mercury | 0.23 | 0.99 | 1 | 0.94 | 1.04 | 1.2 |
| N02 | 0.24 | 1.01 | 0.98 | 0 | 0.99 | 0.99 |
| NDVI within 100m | 0.07 | 1.01 | 1.02 | 0.75 | 0.9 | 0.89 |
| PCB-138 | 0.1 | 0.98 | 0.99 | 0.44 | 1.05 | 1.09 |
| PCB-153 | 0.09 | 0.98 | 0.99 | 0.05 | 1 | 0.96 |
| PCB-180 | 0.08 | 0.98 | 0.99 | 0.2 | 0.96 | 0.93 |
| PFHxS | 0.35 | 1.04 | 1.02 | 0.18 | 1.05 | 1.03 |
| PFNA | 0.06 | 1.01 | 1.02 | 0.25 | 0.98 | 0.94 |
| PFOA | 0.92 | 1.05 | 1.12 | 0.51 | 1.06 | 1.09 |
| PFOS | 0.08 | 0.98 | 1 | 0.82 | 1.03 | 1.16 |
| PM10 | 0.1 | 1.04 | 1.02 | 0.06 | 0.99 | 0.99 |
| PM2.5 | 0.8 | 1.07 | 1.09 | 0.13 | 0.98 | 0.98 |
| Population density | 0.13 | 1.02 | 1.01 | 0.41 | 1.06 | 1.1 |
| Pre-pregnancy BMI | 0.99 | 1.16 | 1.15 | 0.62 | 1 | 1.07 |
| Presence of major green space within 300m | 0.86 | 0.93 | 1 | 0.5 | 1.07 | 1 |
| Smoking | 0.57 | 0.96 | 1 | 0.33 | 1.03 | 1.04 |
| Traffic density on nearest road | 0.15 | 1.02 | 1.02 | 0.15 | 1 | 1 |
| Traffic road load within 100m | 0.31 | 1.02 | 1.01 | 0.57 | 0.93 | 1.05 |
| Vegetable intake | 0.58 | 1.04 | 1.01 | 0.29 | 1 | 0.97 |
| Walkability index within 300m | 0.65 | 0.99 | 0.93 | 0.55 | 1.04 | 1.08 |

[a] Selection proportion and effect estimates were derived from LASSO penalized multinomial regression with a stability selection approach using resampling. The reference category was Cluster A. The threshold of selection proportion was 0.89 for the Northern/Western cohort and 0.88 for the Southern/Mediterranean cohort.

**Supplementary Table 10: Distribution of selected prenatal factors by multi-omics cluster membership**

| | Northern/Western region | | | | Southern/Mediterranean region | | | |
|---|---|---|---|---|---|---|---|---|
| | Cluster A (N=227) | Cluster B (N=150) | Cluster C (N=180) | Total (N=557) | Cluster A (N=238) | Cluster B (N=21) | Cluster C (N=47) | Total (N=306) |
| Pre-pregnancy BMI, $kg/m^2$ | | | | | | | | |
| Mean (SD) | 25.01 (4.78) | 26.19 (5.64) | 26.02 (5.45) | 25.66 (5.26) | 24.09 (4.69) | 24.13 (5.20) | 24.73 (4.01) | 24.19 (4.62) |
| Geometric Mean (SD) | 24.59 (1.2) | 25.63 (1.23) | 25.48 (1.23) | 25.16 (1.22) | 23.7 (1.19) | 23.7 (1.21) | 24.45 (1.16) | 23.81 (1.19) |
| $25^{th}$ Percentile | 21.22 | 22.06 | 21.88 | 21.61 | 21.16 | 21.53 | 22.43 | 21.34 |
| Median | 24.37 | 25.37 | 25.27 | 24.78 | 23.01 | 22.73 | 23.75 | 23.11 |
| $75^{th}$ Percentile | 27.56 | 29.29 | 28.95 | 28.62 | 25.71 | 23.95 | 25.71 | 25.6 |
| $90^{th}$ Percentile | 32.09 | 33.61 | 33.51 | 33.25 | 29.23 | 31.68 | 29.22 | 29.38 |
| $95^{th}$ Percentile | 34.27 | 36.7 | 35.92 | 35.3 | 35.02 | 36.43 | 31.39 | 35.25 |
| Maternal PFOA levels, µg/L | | | | | | | | |
| Mean (SD) | 2.17 (1.31) | 2.29 (1.55) | 2.46 (1.69) | 2.29 (1.51) | 2.77 (1.72) | 3.19 (1.33) | 3.43 (4.52) | 2.90 (2.35) |
| Geometric Mean (SD) | 1.81 (1.85) | 1.83 (2.02) | 2 (1.9) | 1.88 (1.91) | 2.26 (2.09) | 2.62 (2.46) | 2.65 (1.92) | 2.33 (2.09) |
| $25^{th}$ Percentile | 1.16 | 1.14 | 1.22 | 1.18 | 1.83 | 2.33 | 2.27 | 1.91 |
| Median | 1.84 | 1.88 | 2.09 | 1.92 | 2.57 | 3.34 | 2.54 | 2.6 |
| $75^{th}$ Percentile | 2.94 | 3.05 | 3.2 | 3.04 | 3.30 | 3.88 | 3.30 | 3.39 |
| $90^{th}$ Percentile | 4.18 | 4.4 | 4.56 | 4.28 | 4.56 | 4.23 | 4.10 | 4.51 |
| $95^{th}$ Percentile | 4.75 | 4.88 | 5.61 | 5.13 | 5.24 | 4.43 | 4.70 | 5.23 |
| Maternal mercury levels, µg/L | | | | | | | | |
| Mean (SD) | 1.66 (1.38) | 1.56 (1.12) | 1.71 (1.31) | 1.65 (1.29) | 3.94 (3.16) | 3.41 (2.13) | 5.19 (5.53) | 4.10 (3.60) |
| Geometric Mean (SD) | 1.23 (2.22) | 1.19 (2.19) | 1.29 (2.18) | 1.24 (2.2) | 2.99 (2.13) | 2.86 (1.86) | 3.69 (2.3) | 3.08 (2.14) |
| $25^{th}$ Percentile | 0.71 | 0.72 | 0.73 | 0.72 | 1.8 | 1.79 | 2.29 | 1.91 |
| Median | 1.27 | 1.36 | 1.45 | 1.36 | 3.06 | 3.07 | 3.74 | 3.16 |
| $75^{th}$ Percentile | 2.12 | 2.09 | 2.16 | 2.15 | 5.07 | 4.25 | 6.68 | 5.18 |
| $90^{th}$ Percentile | 3.52 | 2.98 | 3.29 | 3.39 | 7.65 | 6.68 | 8.65 | 7.71 |
| $95^{th}$ Percentile | 4.04 | 3.59 | 3.92 | 3.95 | 9.41 | 7.34 | 11.32 | 9.41 |

*BMI* body mass index*, PFOA* perfluorooctanoate.

## Supplementary Fig. 1: Clustering of multi-omics features in children from the Northern/Western European cohort

**a** Eigenvalues and Rotation cost values according to a different number of multi-omics clusters. Across the different numbers of clusters (k of 2-8), the best eigengap and rotation cost were for K=3.

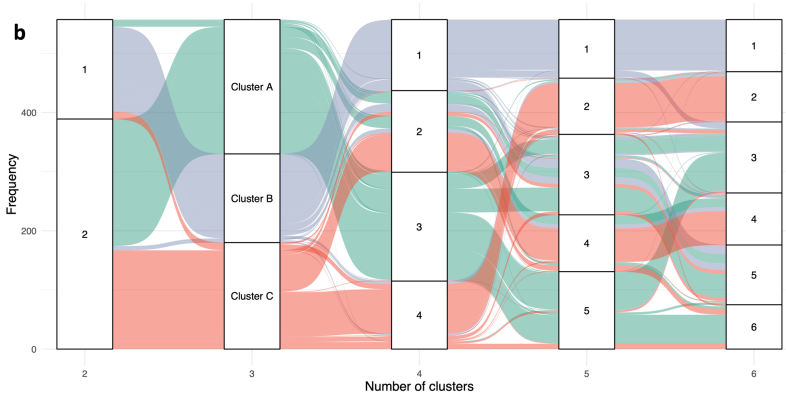
**b** Alluvial plot displaying the flow of participants across different numbers of multi-omics clusters. Line colors represent the three multi-omics clusters (A,B,C), width of the lines represent the number of individuals.

**c** Network representation of the fused similarity network with K=3 clusters. Nodes (circles) with the same colour represent children with a corresponding multi-omics cluster (A, B, C,). Layouts of the networks from the five omics datasets are fixed with the position of the integrated omics similarity network. For network visualization, we present the top 20% edges with the highest similarity.

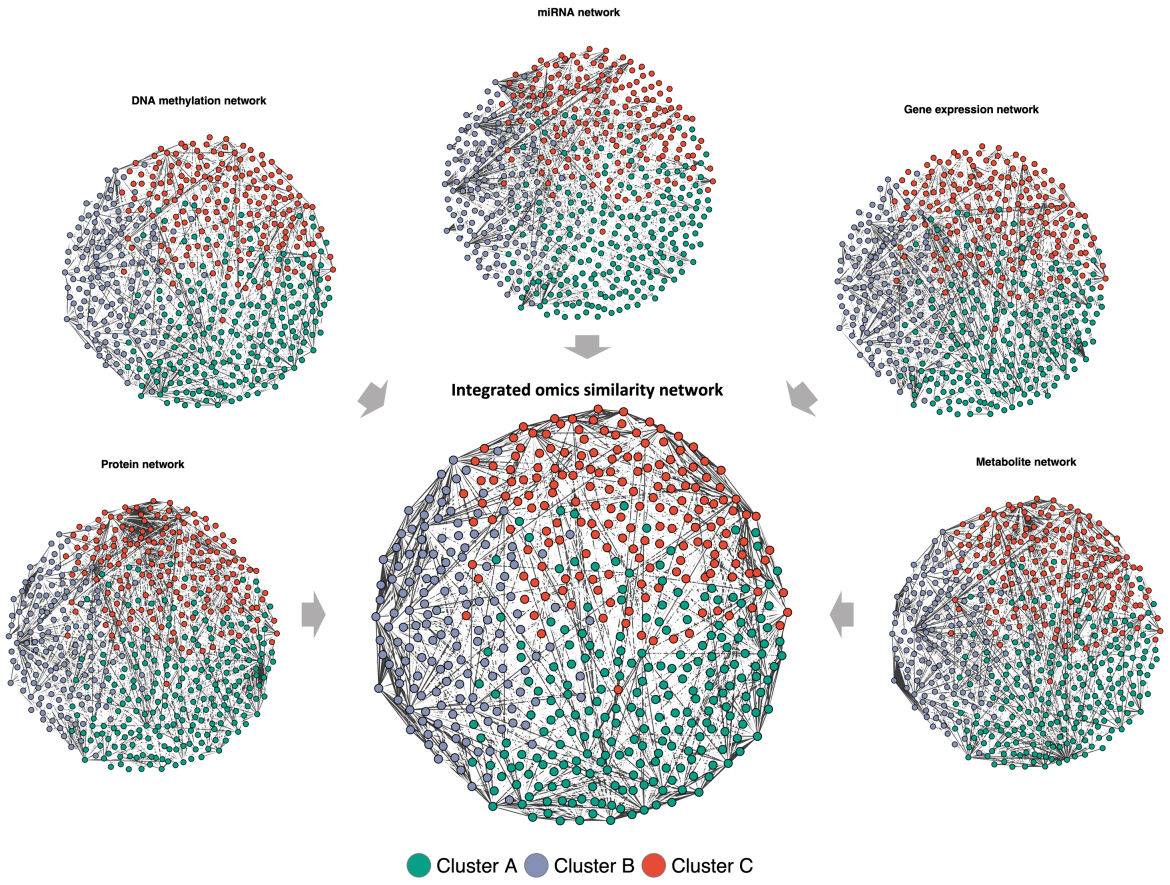
**a**

| Number of clusters | Eigenvalue | Rotation cost value |
|---|---|---|
| 1 | 1.0000 | - |
| 2 | 0.5822 | 501.0087 |
| 3 | 0.5746 | 490.0813 |
| 4 | 0.5652 | 518.0032 |
| 5 | 0.5611 | 493.8436 |
| 6 | 0.5541 | 553.7332 |
| 7 | 0.5535 | 513.9274 |
| 8 | 0.5498 | 529.9453 |

**b**



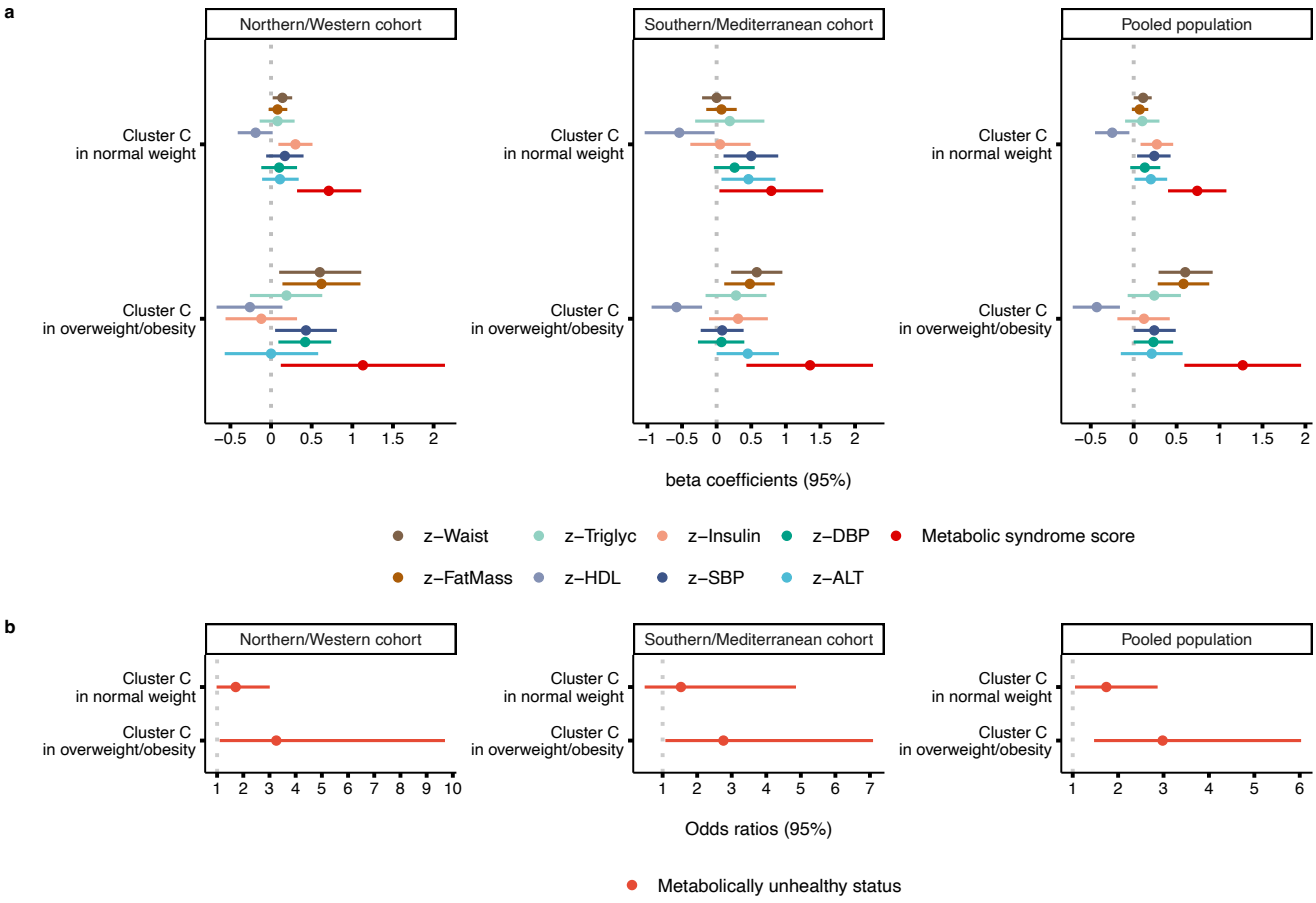

**c**



**Supplementary Fig. 2: Associations of the high-risk Cluster C with metabolic health outcomes in childhood stratified by weight status**

**a** Associations with continuous metabolic health outcomes stratified by weight status defined according to the World Health Organization criteria. Effect estimates and their 95% CIs were derived from generalized linear regression models while controlling for study site, sex, and age at examination. Circles indicate beta coefficients (expressed in SD change) and whiskers indicate 95% CIs. The metabolic syndrome score was derived using z-scores for waist circumference, HDL cholesterol level, triglyceride level, insulin level, and systolic and diastolic blood pressure. Cluster A was the reference category. *ALT* alanine aminotransferase, *BMI* body mass index, *DBP* diastolic blood pressure, *HDL* high-density lipoprotein cholesterol, *SBP* systolic blood pressure.

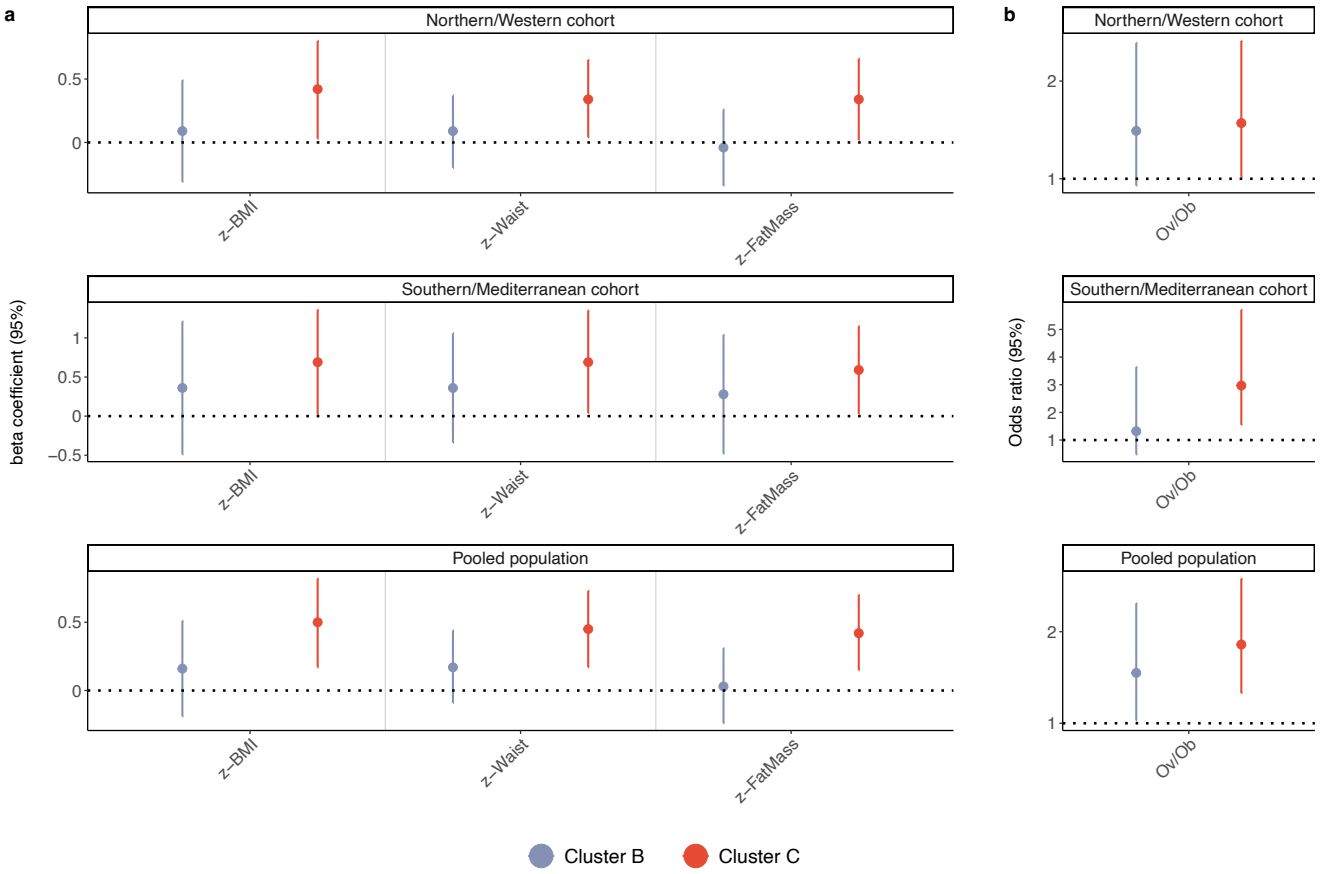
**b** Associations with categorical metabolic health outcomes stratified by weight status defined according to the World Health Organization criteria. Effect estimates represent and their 95% CIs were derived from logistic regression models while controlling for study site, sex, and age at examination. Circles indicate odds ratios and whiskers indicate 95% CIs. Given the asymmetrical nature of the odds ratio scale, odds ratios are not in the centre of the 95% CIs. Metabolically unhealthy status was defined as the presence of at least one of the following risk factors: systolic or diastolic blood pressure≥90$^{th}$ percentile, insulin ≥90$^{th}$ percentile, HDL cholesterol ≤40mg/dl, triglycerides ≥110mg/dl, ALT ≥22.1 U/L for females and ≥25.8 U/L for males. Cluster A was the reference category.



## Supplementary Fig. 3: Associations of the multi-omics clusters with weight-related outcomes in adolescence

**a** Associations with continuous outcomes. Effect estimates and their 95% CIs were derived from generalized linear regression models while controlling for study site, sex, and age at examination. Circles indicate beta coefficients (expressed in SD change) and whiskers 95% CIs. Cluster A was the reference category. *BMI* body mass index.

**b** Associations with the risk of overweight or obesity (Ov/Ob) defined according to the World Health Organization criteria. Effect estimates and their 95% CIs were derived from logistic regression models while controlling for study site, sex, and age at examination. Circles indicate odds ratios and whiskers indicate 95% CIs. Given the asymmetrical nature of the odds ratio scale, odds ratios are not in the centre of the 95% CIs. Cluster A was the reference category.

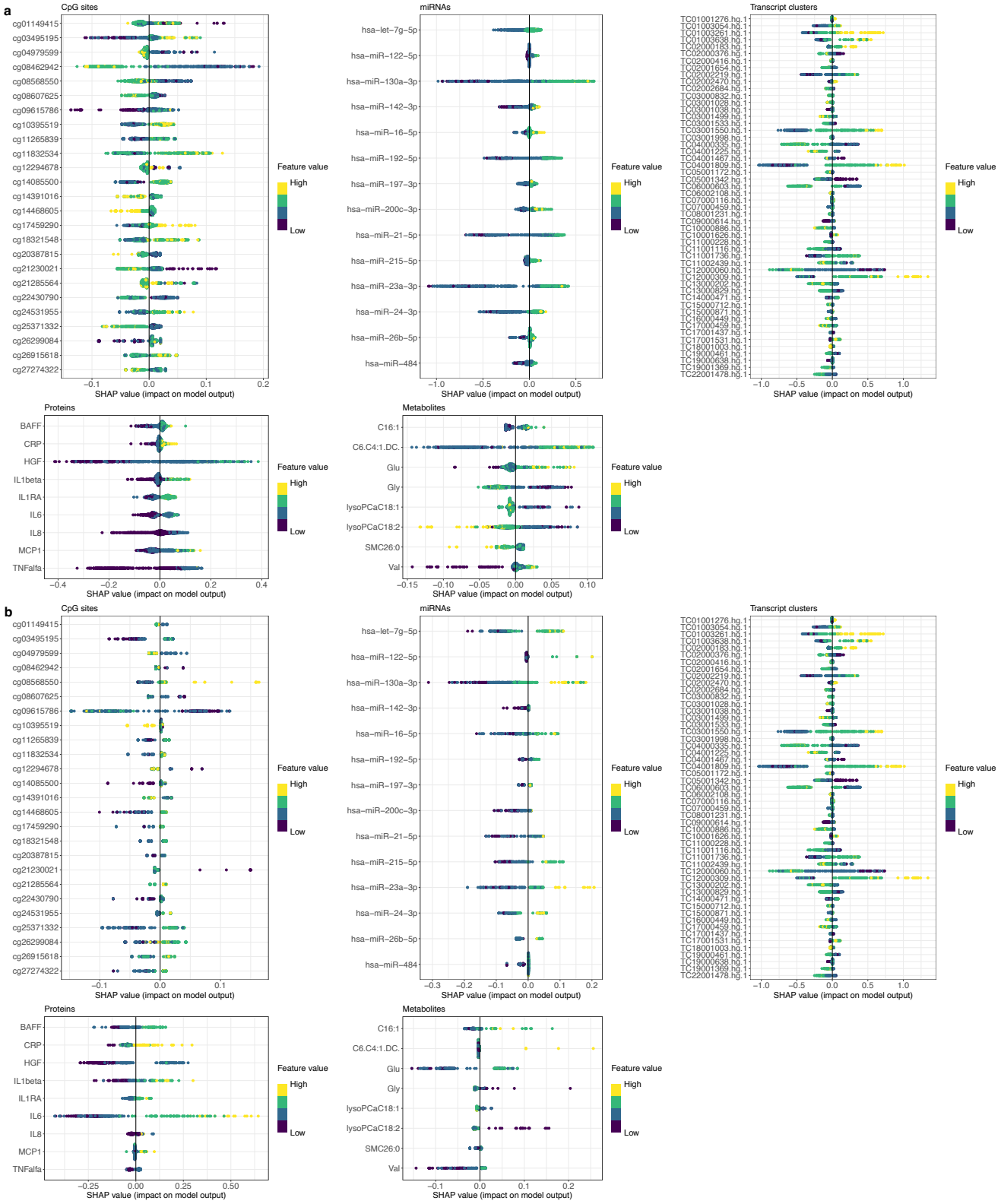


## Supplementary Fig. 4: Local explanations (SHAP values) for the top features contributing to the definition of Cluster C

**a** Local explanations for the Northern/Western cohort.

**b** Same as (**a**) but for the Southern/Mediterranean cohort.

The dot plots represent local (individual) effects for the top features. Each dot represents the contribution (SHAP value) of a feature in the model for each individual. Dots accumulate along each feature to show density. Feature values are shown in a colored range from low to high. SHAP values were calculated based on a classifier comparing membership to Cluster C vs. membership to Cluster A or B.

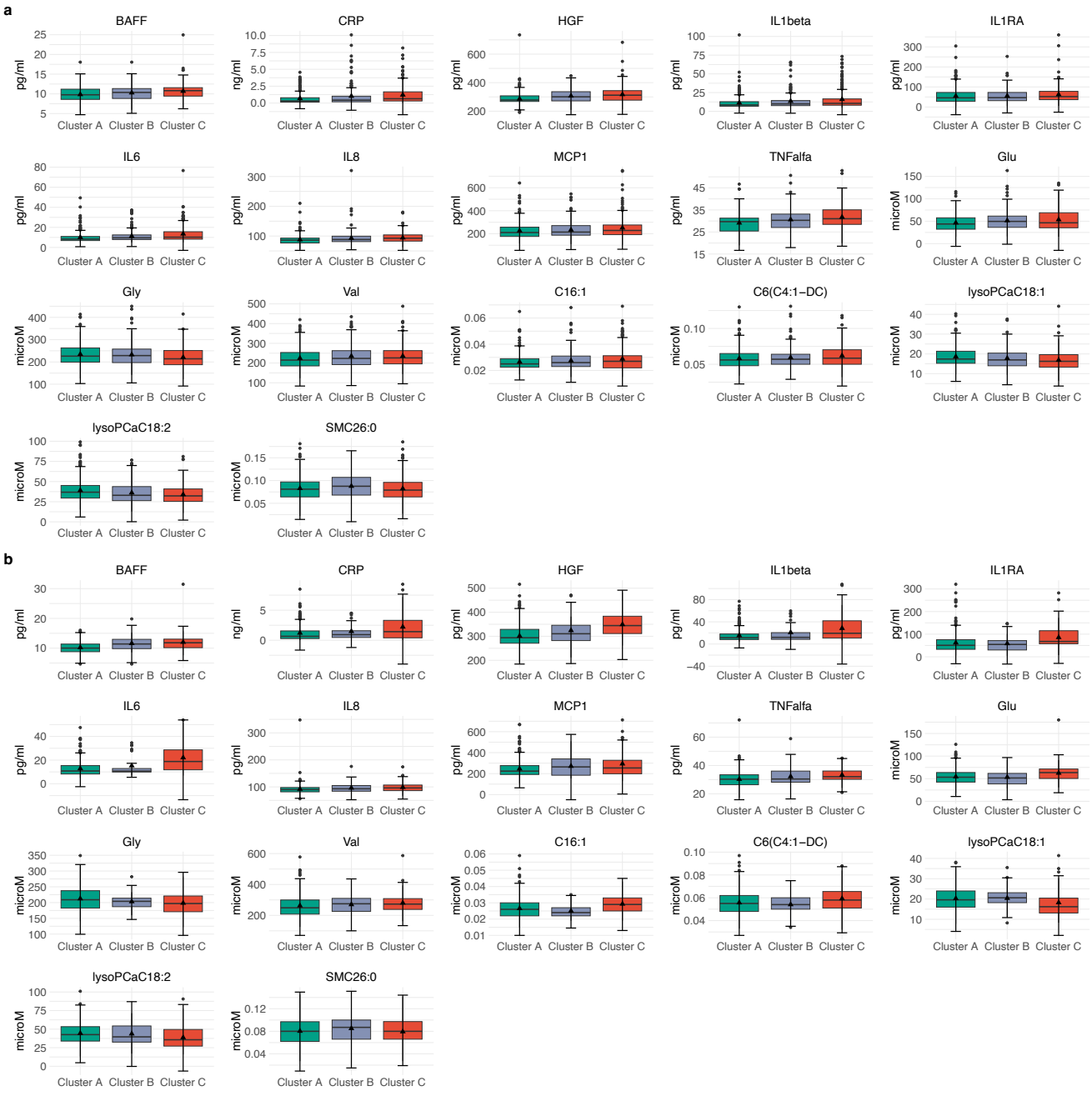


## Supplementary Fig. 5: Distribution of selected cytokines and metabolites across Clusters

**a** Northern/Western cohort.

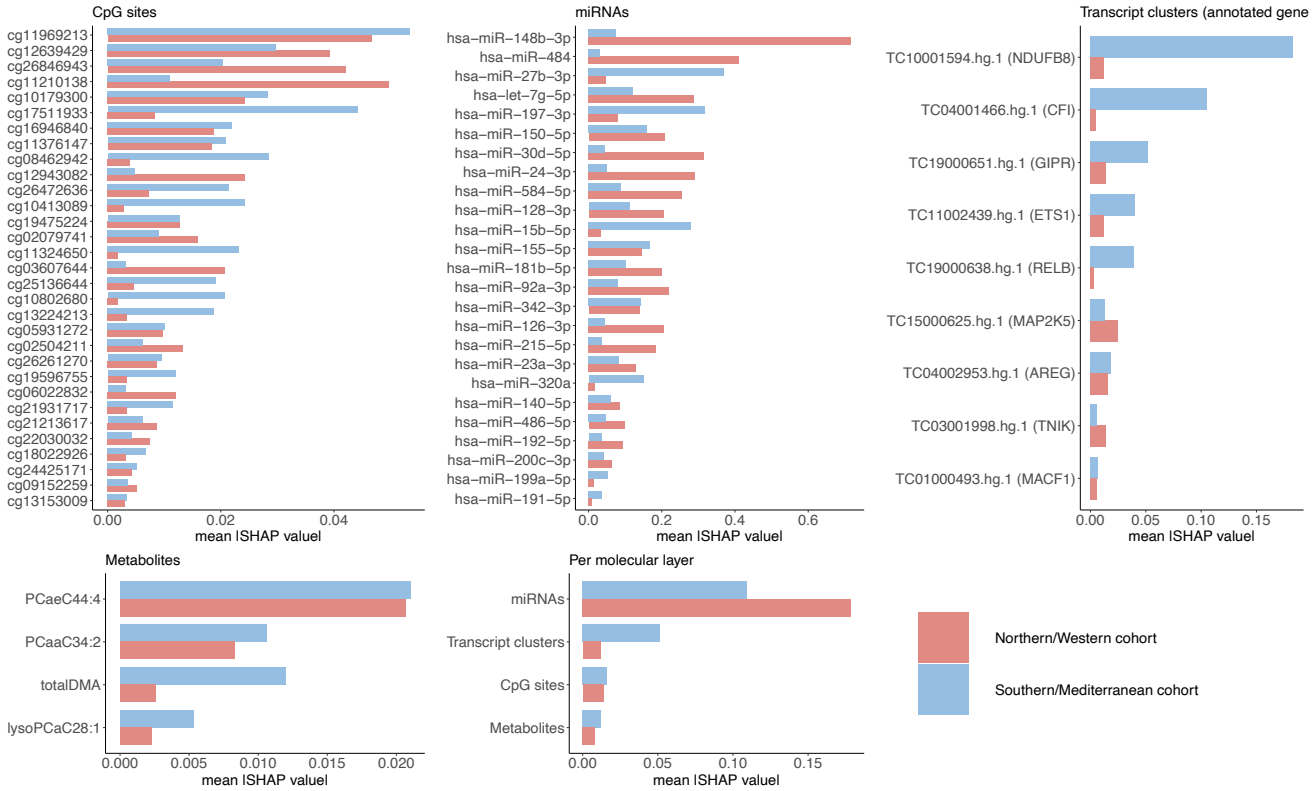
**b** Southern/Mediterranean cohort.

The box-plots represent descriptive statistics for each selected omics biomarker across Clusters. The median value is shown by the black horizontal line within the box, the mean value by the black triangle within the box, the first and third quartiles as the lower and upper border of the box, respectively, and the 1.5 interquartile range by the vertical black lines. Outliers are shown as black circles.



## Supplementary Fig. 6: Molecular drivers of Cluster B

The barplots demonstrate the global importance of the features contributing to the definition of Cluster B. Mean absolute SHAP values were calculated based on a classifier comparing membership to Cluster B vs. membership to Cluster A or C. We present the top features, defined as those with mean SHAP values at concordant direction across cohorts and mean absolute SHAP values ranking within the top quartile in each cohort.

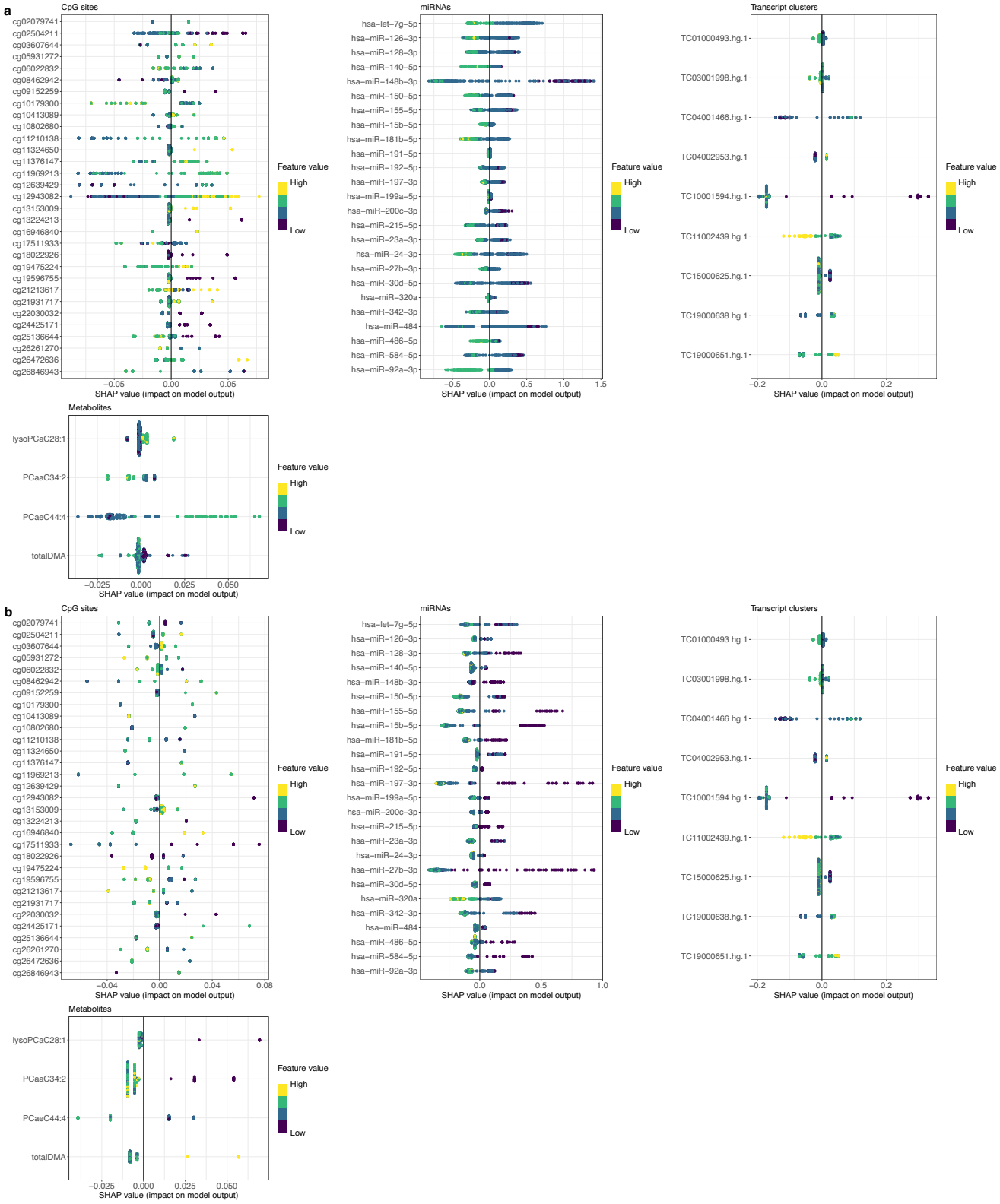


## Supplementary Fig. 7: Local explanations (SHAP values) for the top features contributing to the definition of Cluster B

**a** Local explanations for the Northern/Western cohort.

**b** Same as (**a**) but for the Southern/Mediterranean cohort.

The dot plots represent local (individual) effects for the top features. Each dot represents the contribution (SHAP value) of a feature in the model for each individual. Dots accumulate along each feature to show density. Feature values are shown in a colored range from low to high. SHAP values were calculated based on a classifier comparing membership to Cluster B vs. membership to Cluster A or C.

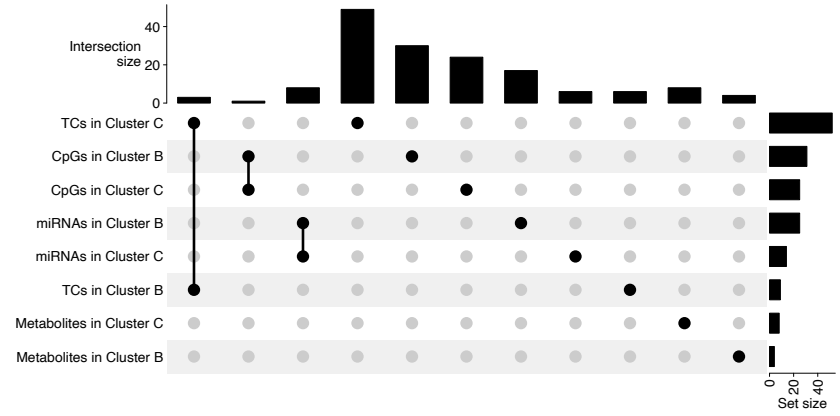


## Supplementary Fig. 8: Intersection of the top features contributing to the definition of Clusters C and B

**a** Upset plot demonstrating the crossover of the top omics features contributing to Cluster C and Cluster B. Vertical stacked bar charts reflect the number of children within each subset. Black dots indicate the sets of subsets; connecting lines indicate relevant intersections related to each stacked bar chart. Horizontal bars indicate the number of features in each subset.

**b** Table demonstrating the common features across Clusters C and B and their direction of association with the clusters.

**a**




**b**

| Common features | Direction of association with Cluster C | Direction of association with Cluster B |
|---|---|---|
| cg08462942 | Downregulation | Upregulation |
| hsa-let-7g-5p | Upregulation | Downregulation |
| hsa-miR-192-5p | Upregulation | Downregulation |
| hsa-miR-197-3p | Upregulation | Downregulation |
| hsa-miR-200c-3p | Upregulation | Downregulation |
| hsa-miR-215-5p | Upregulation | Downregulation |
| hsa-miR-23a-3p | Upregulation | Downregulation |
| hsa-miR-24-3p | Upregulation | Downregulation |
| hsa-miR-484 | Upregulation | Downregulation |
| TC03001998.hg.1 | Downregulation | Downregulation |
| TC11002439.hg.1 | Downregulation | Downregulation |
| TC19000638.hg.1 | Upregulation | Upregulation |

**Supplementary Fig. 9: Tissue-specific gene expression linked to the top features contributing to the definition of Cluster C**

Genes presented were mapped using the top molecular features identified to contribute to the definition of Cluster C. Expression levels are measured in Transcripts Per Million (TPM) and are based on data from the Genotype-Tissue Expression (GTEx) database. The data used were obtained from the GTEx Portal on 10/12/24. Genes with high expression values (TPM ≥80$^{th}$ percentile) in adipose tissue, liver, pancreas, and skeletal muscle are highlighted in bold.

## Supplementary Fig. 10: Correlation among prenatal factors

**a** Correlation matrices in the Northern/Western cohort.

**b** Correlation matrices in the Southern/Mediterranean cohort.

Correlations were estimated as follows: for continuous vs continuous variables – Pearson's correlation; for continuous vs categorical variables – $R^2$ of a lineal model; for categorical vs categorical variables – Cramér's V test. *BMI* body mass index*, Cd* cadmium*, DDE* 4,4'dichlorodiphenyl dichloroethylene*, HCB* hexachlorobenzene*, Hg* mercury*, NDVI* average Normalized Difference Vegetation Index*, NO2* nitrogen dioxide*, Pb* lead*, PCB* polychlorinated biphenyl*, PFHxS* perfluorohexane sulfonate*, PFNA* perfluorononanoate*, PFOA* perfluorooctanoate*, PFOS* perfluorooctane sulfonate*, PM10* particulate matter with an aerodynamic diameter of less than 10 µm*, PM2.5* particulate matter with an aerodynamic diameter of less than 2.5 µm.