METABOLOMICS

Metabolomics of Osteoporosis: The Vietnam Osteoporosis Study

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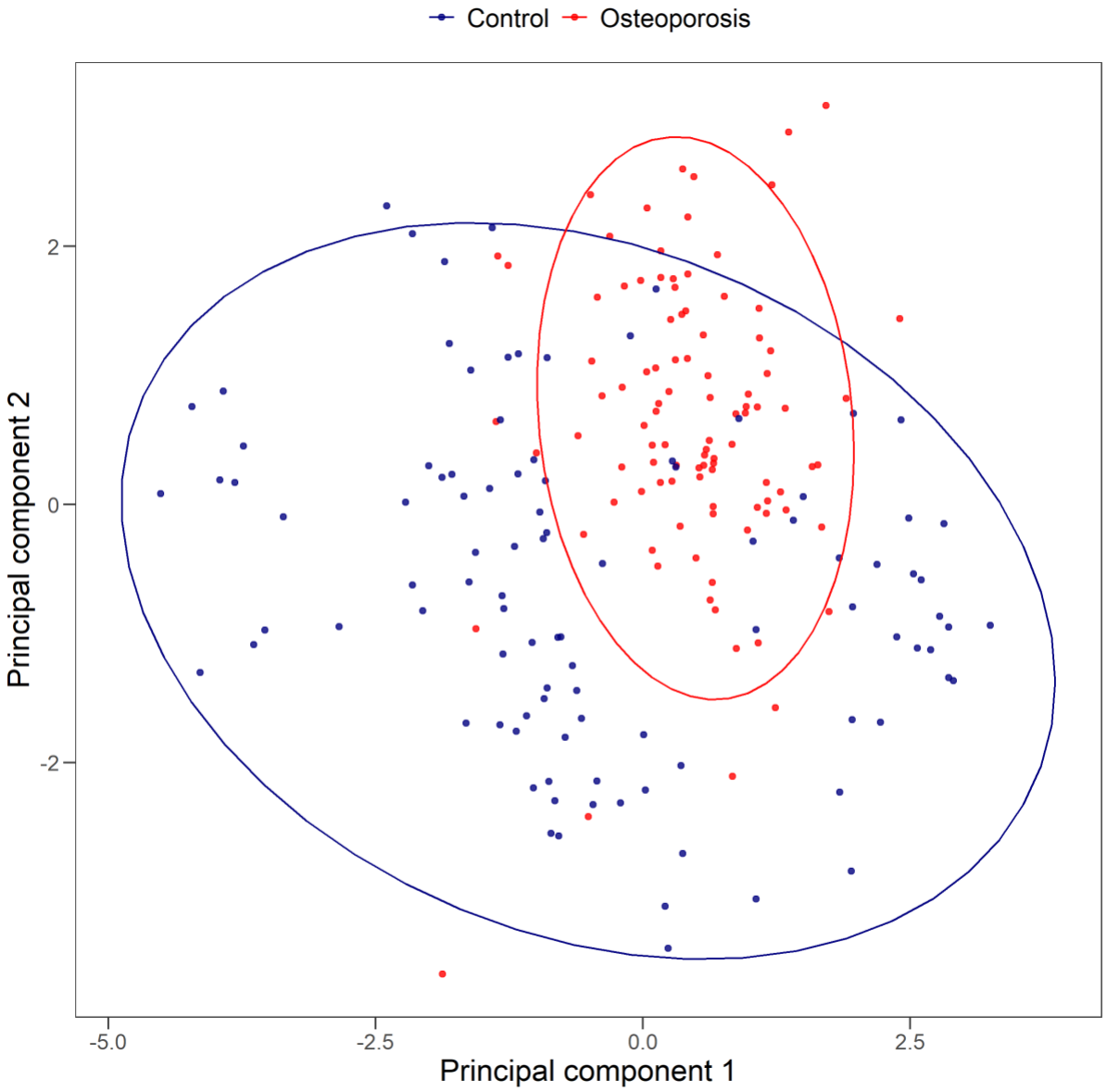
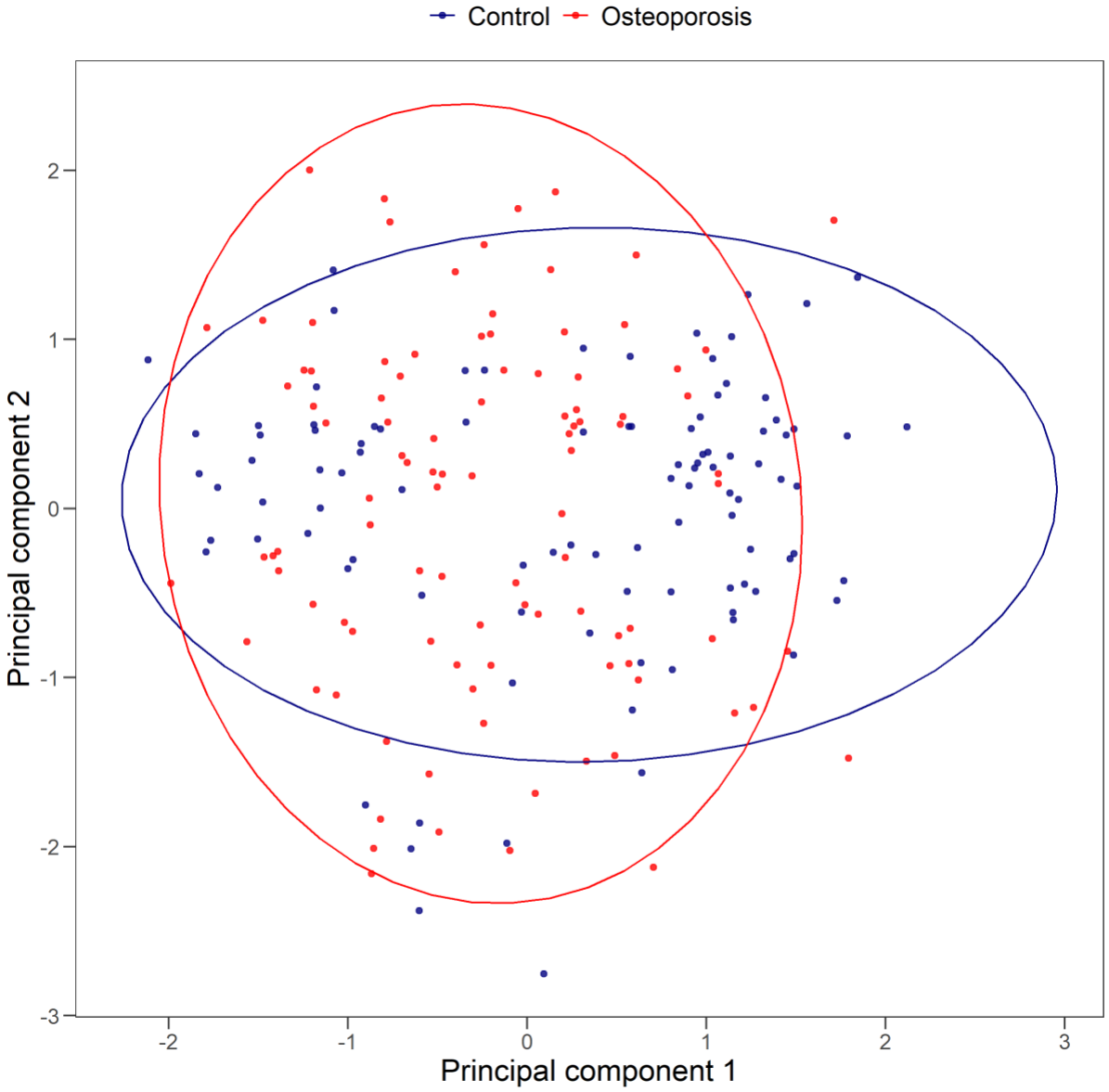
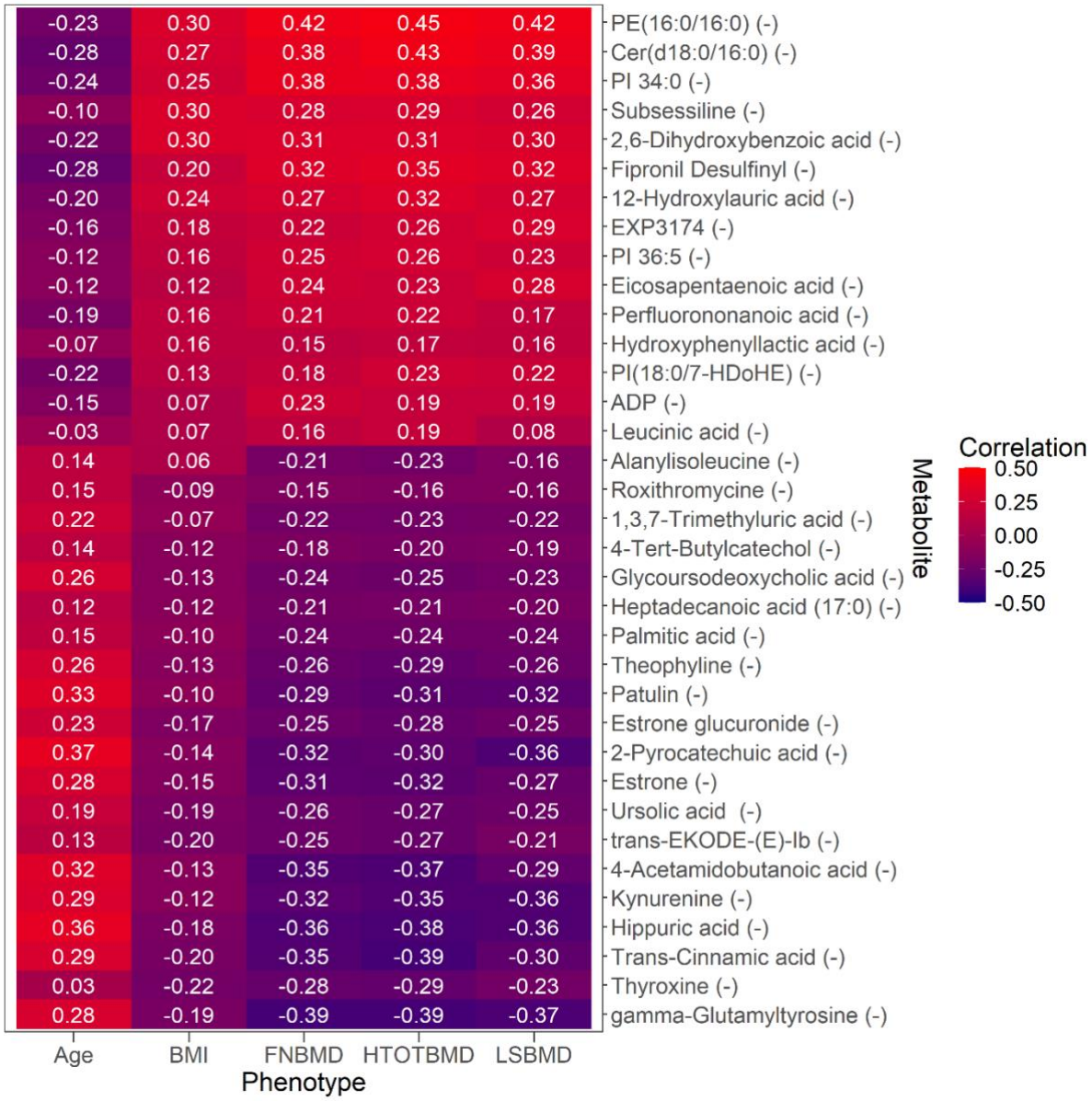
Supplementary Figure 1. Principal component analysis score plots of negative-mode metabolomic data before and after batch correction. Quality-control samples were used to assess batch-related structure.



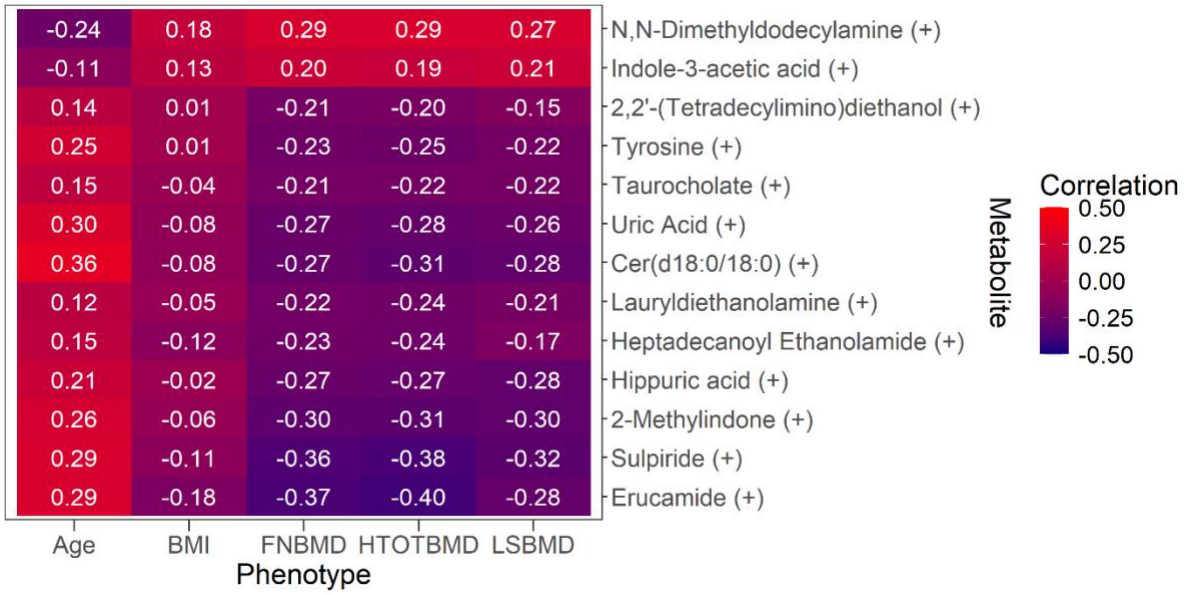
Supplementary Figure 2. Principal component analysis score plots of positive-mode metabolomic data before and after batch correction. Quality-control samples were used to assess batch-related structure.



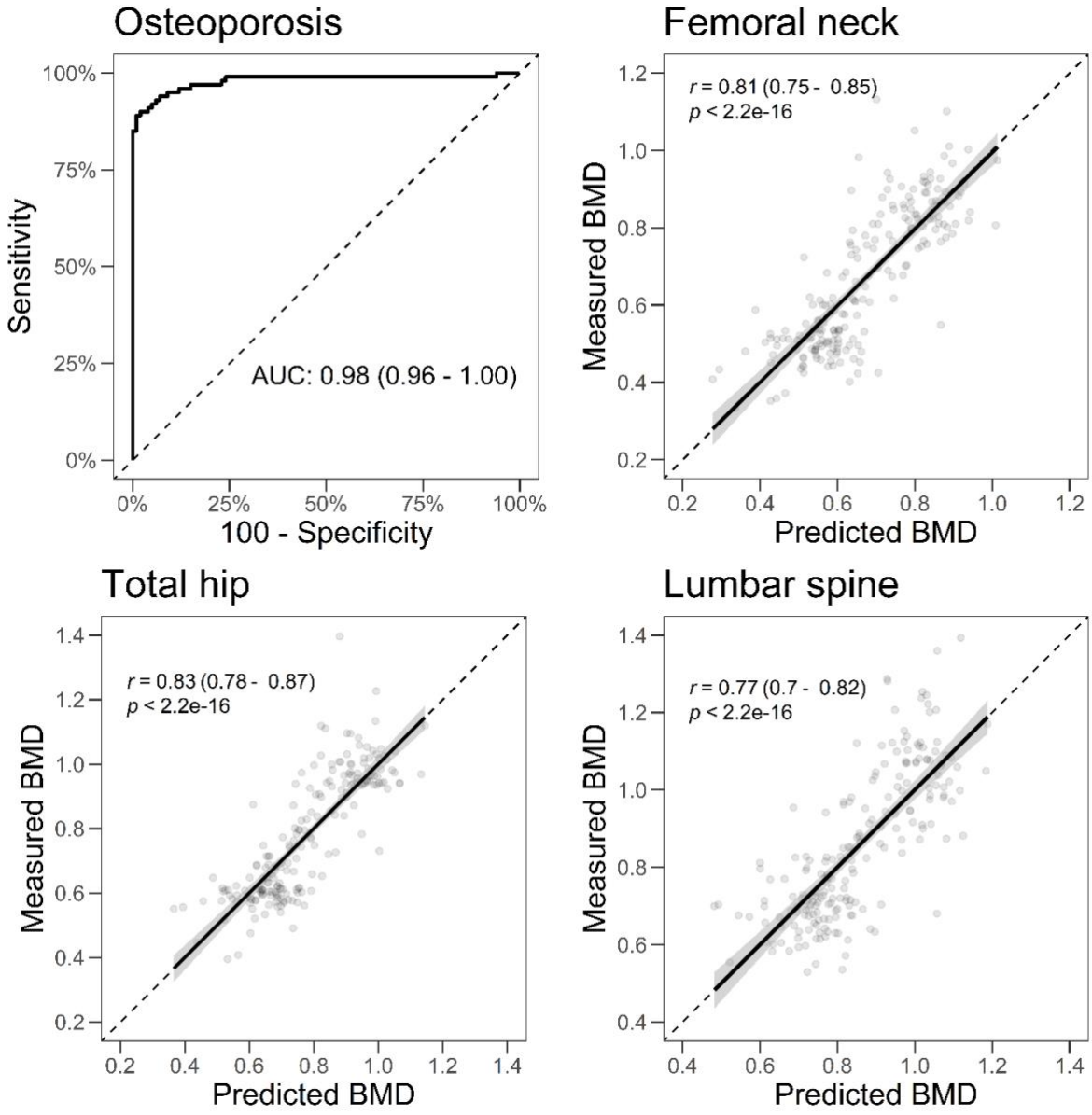
Supplementary figure 3. Correlation heatmap of the 35 prioritized negative-mode features with age, BMI, femoral neck BMD, total hip BMD, and lumbar spine BMD.



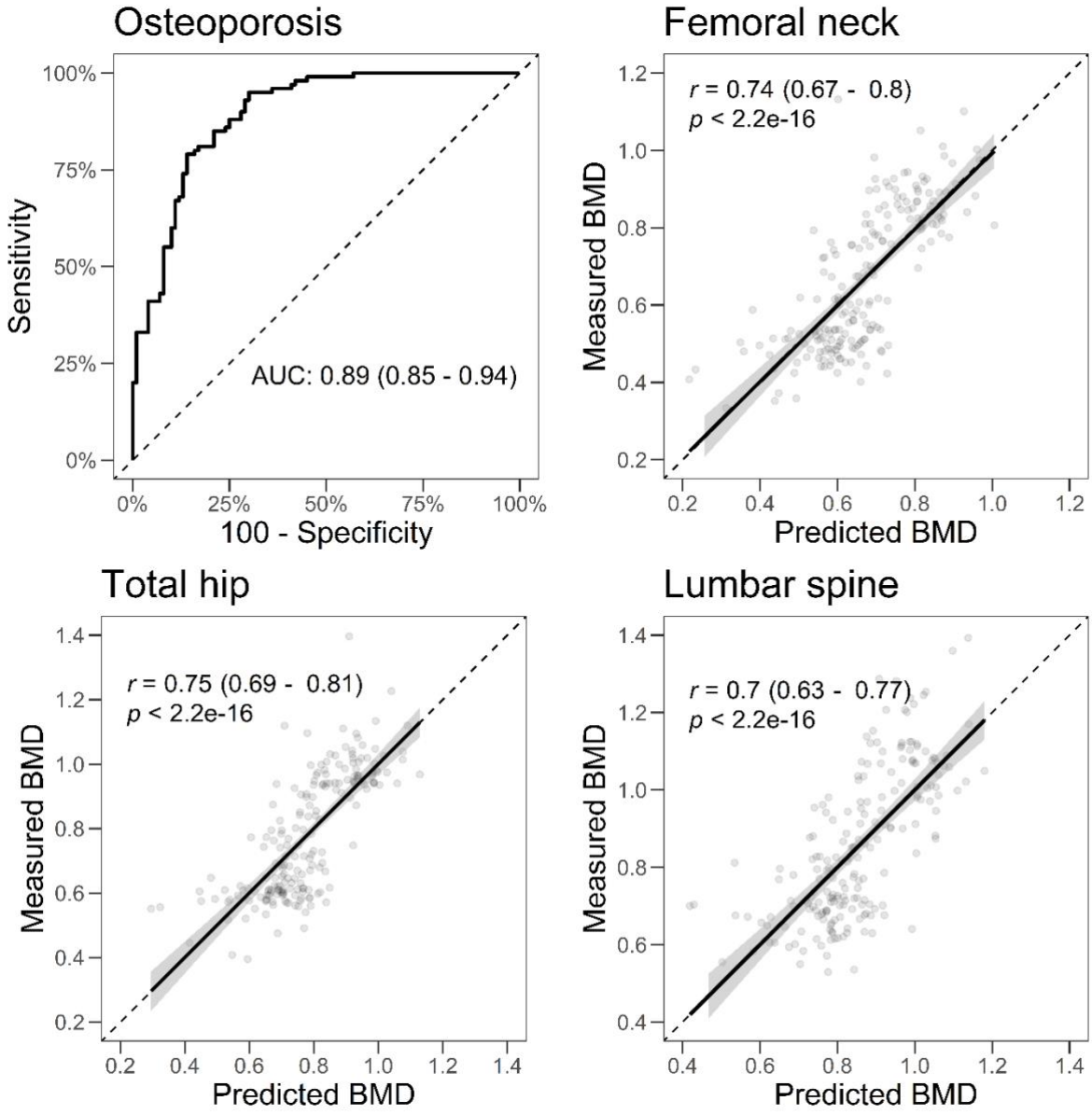
Supplementary figure 4. Correlation heatmap of the 13 prioritized positive-mode features with age, BMI, femoral neck BMD, total hip BMD, and lumbar spine BMD.



Supplementary figure 5. Internal evaluation of the negative-mode MRS for osteoporosis classification and BMD estimation. Osteoporosis classification was assessed using AUC, and BMD estimation was assessed using correlations between predicted and observed BMD.



Supplementary figure 6. Internal evaluation of the positive-mode MRS for osteoporosis classification and BMD estimation. Osteoporosis classification was assessed using AUC, and BMD estimation was assessed using correlations between predicted and observed BMD.



Supplementary table 1. Pathway enrichment analysis in both modes. The analysis includes the total number of metabolites (Total), the ratio of significantly enriched metabolites (Ratio), as well as the raw p-value, Holm-corrected p-value, and false discovery rate (FDR) for each pathway

Analysis	Total	Ratio	Raw p	Holm p	FDR
Negative					
Pathways based					
Biosynthesis of unsaturated fatty acids	36	11	1.37E-02	1	1
Steroid hormone biosynthesis	87	4	7.12E-02	1	1
Arginine and proline metabolism	36	5	1.73E-01	1	1
Fatty acid elongation	38	5	1.82E-01	1	1
Fatty acid degradation	39	5	1.86E-01	1	1
Tryptophan metabolism	41	5	1.95E-01	1	1
Tyrosine metabolism	42	5	1.99E-01	1	1
Fatty acid biosynthesis	47	4	2.21E-01	1	1
Purine metabolism	70	3	3.12E-01	1	1
Chemical structures					
Benzene and substituted derivatives	3050	13	4.23E-05	1.41E-02	1.41E-02
Imidazopyrimidines	198	78	3.13E-04	1.04E-01	5.23E-02
Carboxylic acids and derivatives	3740	8	1.33E-03	4.43E-01	1.48E-01
Steroids and steroid derivatives	2040	11	2.32E-03	7.68E-01	1.94E-01
Fatty Acyls	4680	7	3.01E-03	9.92E-01	2.01E-01
Pyrans	53	145	6.87E-03	1	3.83E-01
Phenylpropanoic acids	78	99	1.01E-02	1	4.82E-01
Hydroxy acids and derivatives	116	66	1.50E-02	1	6.26E-01
Purine nucleotides	134	57	1.73E-02	1	6.42E-01
Cinnamic acids and derivatives	300	26	3.83E-02	1	1
Sphingolipids	629	12	7.87E-02	1	1
Organooxygen compounds	3160	2	3.39E-01	1	1
Prenol lipids	3830	2	3.95E-01	1	1
Glycerophospholipids	40000	0	9.97E-01	1	1
Positive					
Pathways based					
Phenylalanine metabolism	8	38	2.58E-02	1	9.94E-01

Taurine and hypotaurine metabolism	8	38	2.58E-02	1	9.94E-01
Ubiquinone and other terpenoid-quinone biosynthesis	18	17	5.73E-02	1	1
Ether lipid metabolism	20	15	6.36E-02	1	1
Tryptophan metabolism	41	7	1.27E-01	1	1
Tyrosine metabolism	42	7	1.30E-01	1	1
Primary bile acid biosynthesis	46	7	1.41E-01	1	1
Purine metabolism	70	4	2.08E-01	1	1
Chemical structures					
Benzene and substituted derivatives	3050	19	4.32E-03	1	1
Imidazopyrimidines	198	150	6.66E-03	1	1
Indoles and derivatives	559	53	1.87E-02	1	1
Sphingolipids	629	47	2.10E-02	1	1
Steroids and steroid derivatives	2040	15	6.67E-02	1	1
Carboxylic acids and derivatives	3740	8	1.20E-01	1	1

Supplementary table 2. Internal discrimination and BMD estimation metrics for models incorporating the MRS or individual metabolomic features, adjusted for age and BMI. Osteoporosis classification was summarised using AUC, and BMD estimation was summarised using Pearson correlation coefficients

Metabolites adjusted for age and body mass index	Osteoporosis*	FNBMD**	HTOTBMD**	LSBMD**
Naïve model of only age and body mass index	0.90 (0.85 - 0.94)	0.74 (0.67 - 0.80)	0.75 (0.68 - 0.80)	0.70 (0.62 - 0.76)
Negative mode				
MRS (-)	0.98 (0.96 - 1.00)	0.81 (0.75 - 0.85)	0.83 (0.78 - 0.87)	0.77 (0.70 - 0.82)
PE(16:0/16:0) (-)	0.93 (0.88 - 0.96)	0.76 (0.62 - 0.90)	0.78 (0.64 - 0.91)	0.72 (0.58 - 0.86)
Palmitic acid (-)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.58 - 0.85)
Theophyline (-)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.62 - 0.89)	0.70 (0.56 - 0.84)
Roxithromycine (-)	0.90 (0.86 - 0.95)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Alanylisoleucine (-)	0.91 (0.87 - 0.95)	0.75 (0.62 - 0.89)	0.77 (0.63 - 0.91)	0.71 (0.57 - 0.85)
Eicosapentaenoic acid (-)	0.91 (0.87 - 0.95)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.72 (0.58 - 0.86)
2,6-Dihydroxybenzoic acid (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
2-Pyrocatechuic acid (-)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.71 (0.57 - 0.85)
PI 34:0 (-)	0.91 (0.87 - 0.95)	0.76 (0.62 - 0.90)	0.77 (0.63 - 0.91)	0.71 (0.57 - 0.85)
trans-EKODE-(E)-Ib (-)	0.90 (0.85 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Ursolic acid (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.62 - 0.89)	0.70 (0.57 - 0.84)
1,3,7-Trimethyluric acid (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Estrone glucuronide (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
PI 36:5 (-)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.84)
ADP (-)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Fipronil Desulfinyl (-)	0.90 (0.85 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
PI(18:0/7-HDoHE) (-)	0.89 (0.85 - 0.93)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Subsessiline (-)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Glycoursodeoxycholic acid (-)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
12-Hydroxylauric acid (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Estrone (-)	0.91 (0.87 - 0.95)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Hippuric acid (-)	0.90 (0.85 - 0.94)	0.74 (0.61 - 0.88)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
Perfluorononanoic acid (-)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Leucinic acid (-)	0.92 (0.88 - 0.95)	0.75 (0.61 - 0.89)	0.76 (0.63 - 0.90)	0.70 (0.56 - 0.84)
EXP3174 (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
Cer(d18:0/16:0) (-)	0.91 (0.86 - 0.95)	0.75 (0.61 - 0.89)	0.77 (0.63 - 0.91)	0.71 (0.58 - 0.85)
4-Tert-Butylcatechol (-)	0.91 (0.86 - 0.95)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Kynurenine (-)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.72 (0.58 - 0.86)

Patulin (-)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
Thyroxine (-)	0.92 (0.88 - 0.96)	0.76 (0.62 - 0.90)	0.77 (0.63 - 0.91)	0.71 (0.58 - 0.85)
4-Acetamidobutanoic acid (-)	0.91 (0.86 - 0.95)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Trans-Cinnamic acid (-)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.77 (0.63 - 0.91)	0.70 (0.57 - 0.84)
Heptadecanoic acid (17:0) (-)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.89)	0.71 (0.57 - 0.85)
Hydroxyphenyllactic acid (-)	0.91 (0.86 - 0.95)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
gamma-Glutamyltyrosine (-)	0.92 (0.87 - 0.95)	0.76 (0.62 - 0.90)	0.77 (0.63 - 0.91)	0.72 (0.58 - 0.86)
Positive mode				
MRS (+)	0.89 (0.85 - 0.94)	0.74 (0.67 - 0.80)	0.75 (0.69 - 0.81)	0.70 (0.63 - 0.77)
2-Methylindone (+)	0.90 (0.85 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
Lauryldiethanolamine (+)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
N,N-Dimethyldodecylamine (+)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.88)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Uric Acid (+)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Indole-3-acetic acid (+)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
2,2'-(Tetradecylimino)diethanol (+)	0.90 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Sulpiride (+)	0.92 (0.88 - 0.95)	0.76 (0.62 - 0.90)	0.77 (0.63 - 0.91)	0.71 (0.57 - 0.85)
Erucamide (+)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Tyrosine (+)	0.90 (0.86 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)
Hippuric acid (+)	0.91 (0.86 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.72 (0.58 - 0.86)
Heptadecanoyl Ethanolamide (+)	0.91 (0.86 - 0.95)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.70 (0.56 - 0.84)
Taurocholate (+)	0.90 (0.85 - 0.94)	0.75 (0.61 - 0.89)	0.76 (0.62 - 0.90)	0.71 (0.57 - 0.85)
Cer(d18:0/18:0) (+)	0.90 (0.85 - 0.94)	0.74 (0.60 - 0.88)	0.75 (0.61 - 0.89)	0.70 (0.56 - 0.84)

Notes. * Area Under Curve; ** Pearson's correlation coefficient; MRS, metabolomic risk score.

Supplementary table 3. Feature weights from sPLS-DA used to derive the negative- and positive-mode MRSs. Weights are shown for each selected feature by component

Metabolites	Component 1	Component 2
Negative mode		
PE(16:0/16:0) (-)	-0.44673	0
Palmitic acid (-)	0	0
Theophyline (-)	0.006456	0
Roxithromycine (-)	0	0.393126
Alanylisoleucine (-)	0	0.45774
Eicosapentaenoic acid (-)	-0.0681	-0.24546
2,6-Dihydroxybenzoic acid (-)	-0.2305	0
2-Pyrocatechuic acid (-)	0.09549	0
PI 34:0 (-)	-0.31099	0
trans-EKODE-(E)-Ib (-)	0.054413	0
Ursolic acid (-)	0.065603	0
1,3,7-Trimethyluric acid (-)	0.04769	0
Estrone glucuronide (-)	0.111942	0
PI 36:5 (-)	-0.06153	0
ADP (-)	0	-0.11017
Fipronil Desulfinyl (-)	-0.16935	0
PI(18:0/7-HDoHE) (-)	0	0.164231
Subsessiline (-)	-0.15487	0
Glycoursodeoxycholic acid (-)	0.05759	0
12-Hydroxylauric acid (-)	-0.18055	0
Estrone (-)	0.245597	0
Hippuric acid (-)	0.249393	-0.24309
Perfluorononanoic acid (-)	-0.01645	0
Leucinic acid (-)	-0.00947	-0.16933
EXP3174 (-)	-0.12877	0
Cer(d18:0/16:0) (-)	-0.3202	0
4-Tert-Butylcatechol (-)	0.019393	0
Kynurenine (-)	0.227439	0
Patulin (-)	0.148575	0
Thyroxine (-)	0.139255	0.470147
4-Acetamidobutanoic acid (-)	0.168139	0
Trans-Cinnamic acid (-)	0.233319	0
Heptadecanoic acid (17:0) (-)	0.002087	0.277236
Hydroxyphenyllactic acid (-)	-0.04591	-0.38846
gamma-Glutamyltyrosine (-)	0.31934	0
Positive mode		
2-Methylindone (+)	0.21531	0
Lauryldiethanolamine (+)	0	-0.46064
N,N-Dimethyldodecylamine (+)	-0.54494	-0.03081
Uric Acid (+)	0	-0.22157
Indole-3-acetic acid (+)	-0.26393	-0.08052
2,2'-(Tetradecylimino)diethanol (+)	0	-0.50487
Sulpiride (+)	0.552514	0.112503
Erucamide (+)	0.423364	-0.19351
Tyrosine (+)	0.098258	0
Hippuric acid (+)	0.189413	0
Heptadecanoyl Ethanolamide (+)	0.163875	-0.57852
Taurocholate (+)	0.031491	-0.19397
Cer(d18:0/18:0) (+)	0.170699	0.232339

Supplementary table 4. Classification of prioritised metabolomic features. Prioritised metabolomic features classified as endogenous or exogenous based on database annotation, shown by metabolite category and ionization mode

Category	Negative Mode	Positive Mode	Count
Endogenous			**34**
Amino Acid Metabolites	*Kynurenine, Leucinic Acid, Hydroxyphenyllactic Acid, 4-Acetamidobutanoic Acid | Tyrosine, Indole-3-Acetic Acid, 2-Methylindone*	*Tyrosine, Indole-3-Acetic Acid, 2-Methylindone*	7
Peptides	*gamma-Glutamyltyrosine, Alanylisoleucine*		2
Lipids (Sphingolipids)	*Cer(d18:0/16:0)*	*Cer(d18:0/18:0)*	2
Lipids (Phospholipids)	*PE(16:0/16:0), PI 36:5, PI 34:0, PI(18:0/7-HDoHE)*		4
Lipids (Fatty Acids)	*12-Hydroxylauric Acid, Eicosapentaenoic Acid, Palmitic Acid, Heptadecanoic Acid, trans-EKODE-(E)-Ib*		5
Lipids (Endocannabinoid)		*Heptadecanoyl Ethanolamide*	1
Steroid Hormones	*Estrone, Estrone Glucuronide*		2
Hormones	*Thyroxine*		1
Bile Acids	*Glycoursodeoxycholic Acid*	*Taurocholate*	2
Nucleotides	*ADP*		1
Purine Metabolites	*1,3,7-Trimethyluric Acid*	*Uric acid*	2
Microbial Metabolites	*Hippuric Acid, 2,6-Dihydroxybenzoic Acid, 2-Pyrocatechuic Acid, Trans-Cinnamic Acid*	*Hippuric Acid*	5
Exogenous			**14**
Drugs	*Theophyline, Roxithromycine, EXP3174*	*Sulpiride*	4
Environmental Xenobiotics	*Fipronil Desulfinyl, Perfluorononanoic Acid, 4-Tert-Butylcatechol*	*N,N-Dimethyldodecylamine, Erucamide, Lauryldiethanolamine, 2,2'-(Tetradecylimino)diethanol*	7
Mycotoxins	*Patulin*		1
Plant-Derived Xenobiotics	*Subsessiline, Ursolic Acid*		2