Supplementary Material

Serum Metabolomic Profiling Identifies Potential Biomarkers in Arthritis in Older Adults: An exploratory study

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Table S1. Tukey HSD post-hoc analysis displaying P values between EORA vs Control, PMR vs Control and PMR vs EORA

Metabolite	P value EORA vs Control	P value PMR vs Control	P value PMR vs EORA
3-Hydroxybutyrate	**0.004**	0.29	0.18
3-Hydroxyisobutyrate	0.96	0.41	0.50
3-Phenyllactate	0.97	0.33	0.38
Acetate	**0.001**	0.69	**0.01**
Acetoacetate	0.39	0.78	0.80
Acetone	0.96	0.33	0.39
Adenine	0.40	0.98	0.50
Alanine	0.93	0.51	0.25
Arginine	0.38	0.98	0.49
Asparagine	0.78	0.64	0.95
Betaine	0.82	0.69	0.96
Butyrate	0.17	0.67	0.61
Choline	0.12	0.14	1.00
Citrate	0.91	1.00	0.92
Creatine	0.94	0.79	0.93
Creatine Phosphate	0.87	0.13	0.24
Creatinine	0.92	0.96	0.99
Dimethylamine	0.36	0.33	0.99
Formate	0.92	0.77	0.47
Fumarate	0.96	0.95	0.81
Glucose	**0.005**	0.38	0.16
Glutamate	0.25	0.52	0.90
Glutamine	0.93	0.32	0.13
Glycine	0.09	**0.006**	0.39
Glycolate	0.97	1.00	0.97
Hydroxyacetone	0.99	0.57	0.57
Isobutyrate	0.98	0.93	0.98
Isoleucine	0.27	0.96	0.40
Isovalerate	0.87	0.79	0.98
Lactate	**0.006**	0.98	**0.008**
Leucine	0.47	0.60	0.99
Lysine	0.74	0.94	0.91
Malate	0.28	0.93	0.13
Methionine	0.77	0.98	0.62
Methylamine	0.95	0.54	0.30
Methylsuccinate	0.36	0.35	0.99
N,N-Dimethylglycine	0.89	0.87	1.00
N-Acetylaspartate	0.59	0.59	1.00
N-Phenylacetylglycine	0.92	0.80	0.95
O-Acetylcholine	1.00	0.07	**0.04**
O-Phosphocholine	0.29	0.85	0.61
Ornithine	0.69	0.58	0.97
Pantothenate	**0.04**	0.35	0.55
Phenylalanine	0.34	**0.05**	0.47
Proline	0.48	0.39	0.96
Pyruvate	0.75	0.12	0.32
Sarcosine	0.59	0.77	0.97
Serine	0.55	0.25	0.77
SG3PC	0.87	0.09	0.17
Succinate	0.32	1.00	0.27
Taurine	0.86	0.24	0.06

Threonine	0.13	0.06	0.85
Trimethylamine	0.64	0.94	0.84
TMAO	0.80	0.55	0.88
Tryptophan	0.91	0.93	0.69
Tyrosine	0.61	0.33	0.82
Valine	0.35	0.48	0.98
t-Methylhistidine	0.70	0.74	1.00

Tukey's post-hoc analysis after ANOVA adjusted by sex and DM. SG3PC: sn-glycero-3-phosphocholine; TMAO: Trimethylamine N- oxide.

Table S2:Comparison of polar metabolites' concentration between EORA^{neg} and PMR patients.

Metabolite	PMR N=20	EORA^{neg} N=28	P
3-Hydroxybutyrate	24.27±21.86	36.40±28.26	0.12
3-Hydroxyisobutyrate	10.37±3.04	9.41±3.03	0.29
3-Phenyllactate	14.73±5.10	12.62±4.38	0.13
Acetate	25.53±6.35	31.48±8.67	**0.01**
Acetoacetate	4.78±6.08	6.28±12.89	0.63
Acetone	3.03±5.60	1.63±2.44	0.24
Adenine	12.43±3.99	11.60±3.22	0.43
Alanine	118.65±32.44	134.09±36.30	0.14
Arginine	43.36±15.48	49.13±23.20	0.34
Asparagine	16.25±7.31	16.20±5.10	0.98
Betaine	10.90±7.70	10.68±6.08	0.91
Butyrate	8.79±2.78	8.26±2.23	0.47
Choline	10.71±2.58	10.93±2.15	0.75
Citrate	8.99±2.58	9.30±2.23	0.65
Creatine	11.35±3.85	11.81±4.48	0.71
Creatine Phosphate	11.77±6.75	9.85±3.34	0.25
Creatinine	6.27±3.83	6.23±2.74	0.97
Dimethylamine	1.70±.80	1.72±0.95	0.92
Formate	14.35±6.14	12.85±3.81	0.30
Fumarate	2.84±1.70	2.62±1.10	0.59
Glucose	1321.01±357.16	1561.20±643.18	0.14
Glutamate	123.99±35.21	127.46±38.66	0.75
Glutamine	27.05±9.22	22.35±8.02	0.07
Glycine	54.29±22.56	61.40±14.69	0.19
Glycolate	7.63±4.70	7.81±4.14	0.89
Hydroxyacetone	3.28±2.23	3.73±1.76	0.44
Isobutyrate	4.83±4.37	4.75±2.93	0.94
Isoleucine	28.44±5.65	31.14±8.28	0.21
Isovalerate	9.65±2.97	10.02±3.87	0.72
Lactate	710.61±153.32	894.90±256.11	**0.01**
Leucine	44.07±10.89	45.15±18.82	0.82
Lysine	39.89±10.09	41.64±16.29	0.67
Malate	20.71±8.85	16.67±6.06	0.07
Methionine	1.95±1.14	2.24±1.18	0.40
Methylamine	4.00±5.65	2.55±0.91	0.27
Methylsuccinate	4.89±2.09	4.88±2.42	0.99
N,N-Dimethylglycine	1.27±.81	1.25±0.51	0.95
N-Acetylaspartate	4.11±3.37	4.02±1.79	0.90
N-Phenylacetylglycine	9.13±6.03	8.44±5.79	0.69
O-Acetylcholine	2.36±1.59	4.18±2.70	**0.01**
O-Phosphocholine	19.16±6.29	20.81±5.95	0.36
Ornithine	27.60±9.15	27.10±7.03	0.83
Pantothenate	6.07±3.05	7.06±4.07	0.37
Phenylalanine	15.68±4.49	14.27±4.10	0.27
Proline	58.42±38.03	56.74±18.34	0.84
Pyruvate	4.80±2.07	6.04±3.33	0.15
Sarcosine	5.65±1.67	5.33±2.51	0.60
Serine	49.85±13.48	46.41±14.70	0.41
SG3PC	135.18±43.97	113.23±44.72	0.10
Succinate	4.09±1.01	3.68±0.71	0.11
Taurine	33.44±21.42	54.95±33.14	**0.01**

Threonine	27.73±8.48	29.61±7.45	0.42
Trimethylamine	0.69±.46	0.64±0.31	0.69
TMAO	4.91±6.55	4.08±4.93	0.62
Tryptophan	16.25±6.83	17.65±5.38	0.43
Tyrosine	24.10±5.39	23.21±4.90	0.56
Valine	91.27±17.29	92.96±21.40	0.77
t-Methylhistidine	9.92±3.30	9.89±5.74	0.99

Variables expressed in mean ± standard deviation. Student *t*-test was used to compare concentration of metabolites between groups. SG3PC: sn-glycero-3-phosphocholine; TMAO: Trimethylamine N- oxide.

Table S3: Comparison of demographic and clinical characteristics between responders and non-responder in EORA^{neg} patients.

Variable	Responder N=21	Non-Responder N=7	p
Female, n (%)	5(24)	7(100)	**<0.001**
Age, years	76.00±6.86	79.00±7.44	0.34
BMI	27.92±3.08	31.52±7.66	0.27
DM, n (%)	9(43)	6(86)	0.08
HBP, n (%)	15(71)	5(71)	1.00
DL, n (%)	13(62)	6(86)	0.37
Weight loss, n (%)	7(33)	3(43)	0.67
Anorexia, n (%)	9(43)	5(71)	0.39
Shoulder Pain, n (%)	15(71)	6(86)	0.64
Pelvis Pain, n (%)	10(48)	3(43)	1.00
Stiffness, n (%)	20(95)	7(100)	1.00
Fatigue, n (%)	14(67)	6(86)	0.63
Tender joints	7.76±4.55	13.14±7.65	0.12
Swollen joints	11.67±5.64	10.86±7.01	0.76
Pain	69.29±17.91	84.29±12.72	**0.05**
DAS-ESR	6.04±0.96	7.11±0.89	**0.02**
DAS-CRP	5.46±1.00	6.17±0.79	0.10
CRP mg/dL	41.60±56.86	41.48±38.15	1.00
ESR mm/h	46.57±27.14	70.43±19.37	**0.04**
TNF (pg/mL)	14.22±7.00	12.50±5.77	0.62
IL-6 (pg/mL)	17.80±23.27	26.32±45.10	0.52
NSAIDs, mg	557.14±53.45	200.00±261.01	**0.01**
Glucocorticoids, mg	8.95±3.32	9.14±3.02	0.89
Methotrexate, n (%)	6 (28.6)	2 (28.6)	1.00

Continuous variables expressed in mean ± standard deviation; Categorical variables expressed in percentage. A student *t*-test was used to determine the significance of continuous variables while Pearson's Chi-square test was used for categorical variables. Note. BMI: Body mass index; DM: Diabetes mellitus; HBP: High blood pressure; DL: Dyslipidemia; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; TNF: Tumor necrosis factor; IL-6: Interleukin 6; NSAIDs: Non-steroidal anti-inflammatory drugs.

Table S4: Comparison of clinical characteristics between non-diabetic and diabetic in EORA^{neg} patients.

Variable	Non-Diabetic N=13	Diabetic N=15	P
Tender Joints	9.54±4.94	8.73±6.65	0.72
Swollen Joints	11.31±5.19	11.60±6.61	0.90
ESR	54.00±30.02	51.27±25.51	0.80
CRP	56.77±66.53	28.40±32.38	0.18
DAS28ESR	6.36±0.92	6.07±1.08	0.46
DAS28CRP	5.89±0.97	5.42±0.98	0.21
IL-6	24.77±41.78	15.74±11.65	0.46
TNF	13.70±7.81	14.00±5.89	0.92

Variables expressed in mean ± standard deviation. Student *t*-test was used to compare quantitative clinical variables between groups.

Table S5: Polar metabolites' concentrations in EORA^{neg} responders and non-responder at baseline according DAS-ESR based EULAR response criteria.

Metabolite	Responder N=21	Non-Responder N=7	P adj.
3-Hydroxybutyrate	31.40±28.05	51.41±24.91	**0.02**
3-Hydroxyisobutyrate	9.32±2.89	9.66±3.67	0.65
3-Phenyllactate	12.01±4.01	14.44±5.24	0.25
Acetate	29.77±6.94	36.61±11.72	0.09
Acetoacetate	6.95±14.91	4.24±0.67	0.62
Acetone	1.83±2.80	1.01±0.36	0.47
Adenine	10.92±2.57	13.63±4.26	**0.02**
Alanine	132.97±27.99	137.46±57.44	0.75
Arginine	47.20±22.41	54.93±26.39	0.56
Asparagine	15.83±5.42	17.28±4.13	0.72
Betaine	11.16±5.39	9.21±8.12	0.57
Butyrate	8.31±2.24	8.11±2.36	0.96
Choline	10.91±2.28	10.99±1.86	0.73
Citrate	8.90±1.90	10.51±2.82	0.09
Creatine	11.56±4.06	12.59±5.88	0.61
Creatine Phosphate	9.98±3.51	9.46±3.00	0.85
Creatinine	6.34±3.02	5.90±1.79	0.78
Dimethylamine	1.72±0.93	1.73±1.11	0.96
Formate	12.88±3.35	12.77±5.28	0.95
Fumarate	2.51±1.03	2.94±1.33	0.37
Glucose	1371.61±297.06	2129.96±1031.61	**<0.001**
Glutamate	135.29±38.91	104.00±28.65	**0.05**
Glutamine	21.27±8.44	25.27±6.00	0.24
Glycine	63.37±11.32	55.49±22.14	0.21
Glycolate	7.27±3.67	9.43±5.30	0.28
Hydroxyacetone	3.60±1.84	4.11±1.58	0.66
Isobutyrate	4.79±2.97	4.63±3.04	0.98
Isoleucine	30.95±7.57	31.71±10.82	0.78
Isovalerate	10.06±3.56	9.90±5.02	0.96
Lactate	870.05±206.86	969.44±379.32	0.43
Leucine	44.28±17.42	47.77±23.92	0.65
Lysine	43.80±16.11	35.19±16.24	0.24
Malate	15.85±6.26	19.13±4.98	0.25
Methionine	2.13±1.28	2.56±0.82	0.50
Methylamine	2.47±0.89	2.80±1.01	0.45
Methylsuccinate	4.55±1.90	5.87±3.58	0.27
N,N-Dimethylglycine	1.17±0.40	1.51±0.74	0.12
N-Acetylaspartate	3.93±1.51	4.29±2.57	0.82
N-Phenylacetylglycine	7.91±6.38	10.00±3.37	0.54
O-Acetylcholine	4.31±3.08	3.77±0.95	0.56
O-Phosphocholine	20.77±6.46	20.91±4.52	0.96
Ornithine	28.02±7.11	24.33±6.48	0.29
Pantothenate	6.45±3.71	8.87±4.84	0.21
Phenylalanine	14.35±4.19	14.03±4.11	0.87
Proline	58.04±18.15	52.84±19.79	0.58
Pyruvate	5.87±3.44	6.57±3.14	0.70
Sarcosine	5.35±2.40	5.27±3.04	0.88
Serine	44.46±15.70	52.26±9.86	0.29
SG3PC	107.10±39.80	131.60±56.53	0.21
Succinate	3.61±0.64	3.89±0.92	0.29
Taurine	26.77±7.10	80.70±47.64	**0.02**

Threonine	30.55±7.49	26.77±7.10	0.33
Trimethylamine	0.64±0.33	0.64±0.28	0.87
TMAO	2.98±3.54	7.40±7.10	**0.04**
Tryptophan	17.48±3.73	18.17±9.13	0.77
Tyrosine	22.53±4.75	25.23±5.12	0.23
Valine	91.71±22.95	91.71±22.95	0.95
t-Methylhistidine	9.46±4.29	11.19±9.17	0.55

A student *t*-test was used to determine the significance of metabolic concentrations between groups. Variables expressed in mean ± standard deviation. Concentration expressed in μM. The DAS-ESR was considered to calculate the response criteria, according to EULAR. Note. TMAO: Trimethylamine N-Oxide, SG3PC: Sn-Glycero-3-Phosphocholine. P adj:P adjusted by DM and sex.

Table S6: Polar metabolites' concentrations in control, EORA^{neg} responders and non-responders at baseline according DAS-ESR based EULAR response criteria.

Metabolite	Control N=18	Responder N=21	Non-Responder N=7	P adj.
3-Hydroxybutyrate	15.61±7.60**	31.40±28.05	51.41±24.91***	**<0.001**
3-Hydroxyisobutyrate	8.92±4.53	9.32±2.89	9.66±3.67	0.90
3-Phenyllactate	12.38±5.47	12.01±4.01	14.44±5.24	0.54
Acetate	23.63±5.52**	29.77±6.94*	36.61±11.72	**<0.001**
Acetoacetate	2.79±1.80	6.95±14.91	4.24±0.67	0.41
Acetone	1.37±1.09	1.83±2.80	1.01±0.36	0.59
Adenine	12.63±2.72	10.92±2.57	13.63±4.26***	**0.02**
Alanine	130.05±31.56	132.97±27.99	137.46±57.44	0.88
Arginine	41.98±20.04	47.20±22.41	54.93±26.39	0.39
Asparagine	18.03±6.80	15.83±5.42	17.28±4.13	0.73
Betaine	9.11±6.04	11.16±5.39	9.21±8.12	0.69
Butyrate	9.49±2.68	8.31±2.24	8.11±2.36	0.16
Choline	12.19±2.91	10.91±2.28	10.99±1.86	0.14
Citrate	8.96±2.22	8.90±1.90	10.51±2.82	0.20
Creatine	12.21±3.84	11.56±4.06	12.59±5.88	0.81
Creatine Phosphate	9.00±2.68	9.98±3.51	9.46±3.00	0.76
Creatinine	6.54±3.84	6.34±3.02	5.90±1.79	0.89
Dimethylamine	2.18±1.31	1.72±0.93	1.73±1.11	0.46
Formate	13.26±4.06	12.88±3.35	12.77±5.28	0.89
Fumarate	2.71±1.15	2.51±1.03	2.94±1.33	0.62
Glucose	1142.19±273.30**	1371.61±297.06	2129.96±1031.61***	**<0.001**
Glutamate	111.29±36.57	135.29±38.91	104.00±28.65	**0.04**
Glutamine	23.14±6.62	21.27±8.44	25.27±6.00	0.39
Glycine	74.28±22.06**	63.37±11.32	55.49±22.14	**0.03**
Glycolate	7.61±3.47	7.27±3.67	9.43±5.30	0.50
Hydroxyacetone	3.92±1.86	3.60±1.84	4.11±1.58	0.90
Isobutyrate	4.46±2.25	4.79±2.97	4.63±3.04	0.97
Isoleucine	27.86±7.07	30.95±7.57	31.71±10.82	0.38
Isovalerate	10.31±2.60	10.06±3.56	9.90±5.02	0.88
Lactate	697.53±199.29**	870.05±206.86	969.44±379.32	**0.02**
Leucine	39.26±13.96	44.28±17.42	47.77±23.92	0.49
Lysine	38.53±10.28	43.80±16.11	35.19±16.24	0.31
Malate	19.93±5.61	15.85±6.26	19.13±4.98	0.12
Methionine	2.03±1.13	2.13±1.28	2.56±0.82	0.62
Methylamine	2.87±1.32	2.47±0.89	2.80±1.01	0.52
Methylsuccinate	5.94±2.61	4.55±1.90	5.87±3.58	0.20
N,N-Dimethylglycine	1.16±.67	1.17±0.40	1.51±0.74	0.31
N-Acetylaspartate	4.86±1.56	3.93±1.51	4.29±2.57	0.37
N-Phenylacetylglycine	8.06±3.69	7.91±6.38	10.00±3.37	0.72
O-Acetylcholine	4.23±2.65	4.31±3.08	3.77±0.95	0.83
O-Phosphocholine	18.06±6.00	20.77±6.46	20.91±4.52	0.32
Ornithine	24.90±9.84	28.02±7.11	24.33±6.48	0.43
Pantothenate	4.51±2.58**	6.45±3.71	8.87±4.84	**0.03**
Phenylalanine	12.53±3.21	14.35±4.19	14.03±4.11	0.34
Proline	46.99±19.09	58.04±18.15	52.84±19.79	0.23
Pyruvate	6.84±3.69	5.87±3.44	6.57±3.14	0.76
Sarcosine	6.34±3.33	5.35±2.40	5.27±3.04	0.68
Serine	42.07±15.79	44.46±15.70	52.26±9.86	0.33
SG3PC	106.86±39.32	107.10±39.80	131.60±56.53	0.37
Succinate	4.07±1.15	3.61±0.64	3.89±0.92	0.25
Taurine	49.31±33.76	26.77±7.10	80.70±47.64***	**0.04**

Threonine	34.29±10.66	30.55±7.49	26.77±7.10	0.13
Trimethylamine	0.73±.39	0.64±0.33	0.64±0.28	0.60
TMAO	3.21±2.92	2.98±3.54	7.40±7.10***	**0.05**
Tryptophan	16.92±4.78	17.48±3.73	18.17±9.13	0.86
Tyrosine	21.64±5.19	22.53±4.75	25.23±5.12	0.29
Valine	84.84±16.07	91.71±22.95	91.71±22.95	0.43
t-Methylhistidine	8.87±2.26	9.46±4.29	11.19±9.17	0.56

Variables expressed in mean ± standard deviation. ANOVA (Tukey HSD post hoc) was used to determine the significance of metabolites between groups. Concentration expressed in μM. DAS-ESR was considered to calculate the response criteria, according to EULAR. Note. TMAO: Trimethylamine N-Oxide, SG3PC: Sn-Glycero-3-Phosphocholine. P adj: P value of variables adjusted by DM and sex.*P<0.05 control vs responder; **P<0.05 control vs non-responder; *** P<0.05 responder & non-responder.

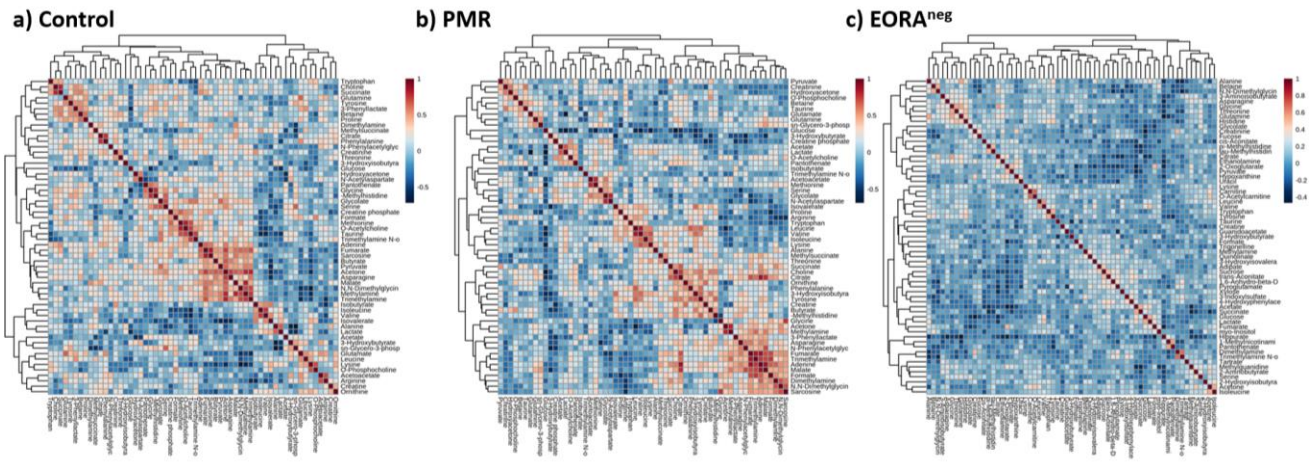

Figure S1: Heatmap and hierarchical cluster analysis indicating associations between polar metabolites identified by ^{1}H-NMR in serum. a) Controls, b) PMR and c) EORA^{neg} samples showing differences in patterns.

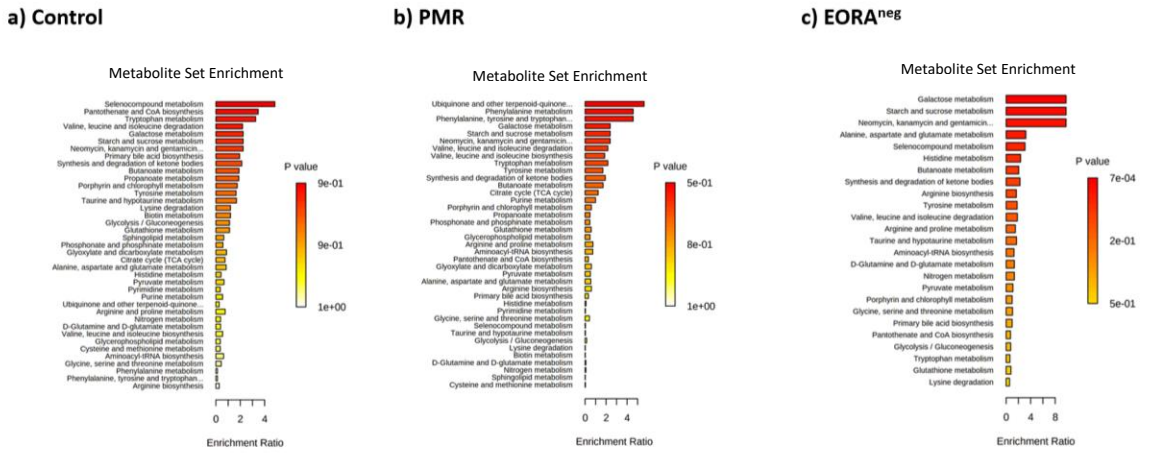

Figure S2: Metabolomic pathway analysis obtained by MetaboAnalyst in the three groups. a) Control b) PMR c) EORA^{neg} samples. The metabolite sets enrichment overview considering the top 25 metabolic sets of enrichment in the 3 groups are very different. While for controls, selenocompound and tryptophan metabolism and pantothenate and CoA biosynthesis are the most enriched pathways, for EORA^{neg}, the galactose metabolism is the predominant pathway. On the other hand, phenylalanine, tyrosine, tryptophan and ubiquinone metabolism were the most enriched sets for PMR.

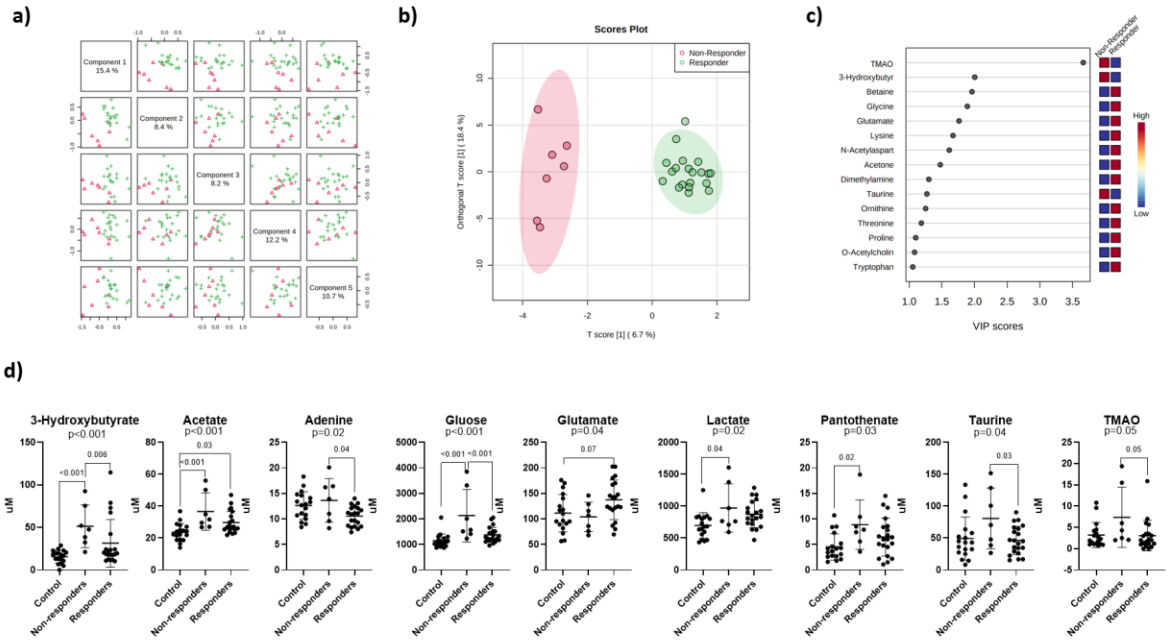

Figure S3. Comparison of metabolomic profile between responders and non-responders according DAS-ESR based EULAR response criteria. a) Component analysis with a total variance of 54.9% b) OPLS-DA showing a clear separation between responders and non-responders c) VIP Scores d) Metabolite concentration in controls, responders, and non-responders according DAS-ESR based EULAR response criteria. P-value below metabolite name shows the overall ANOVA significance while the p-value between groups is from the Tukey HSD post-hoc analysis. TMAO: Trimethylamine N-Oxide.