Supplementary Materials for

Metabolic Differentiation of Early Lyme Disease from Southern Tick-Associated Rash Illness (STARI)

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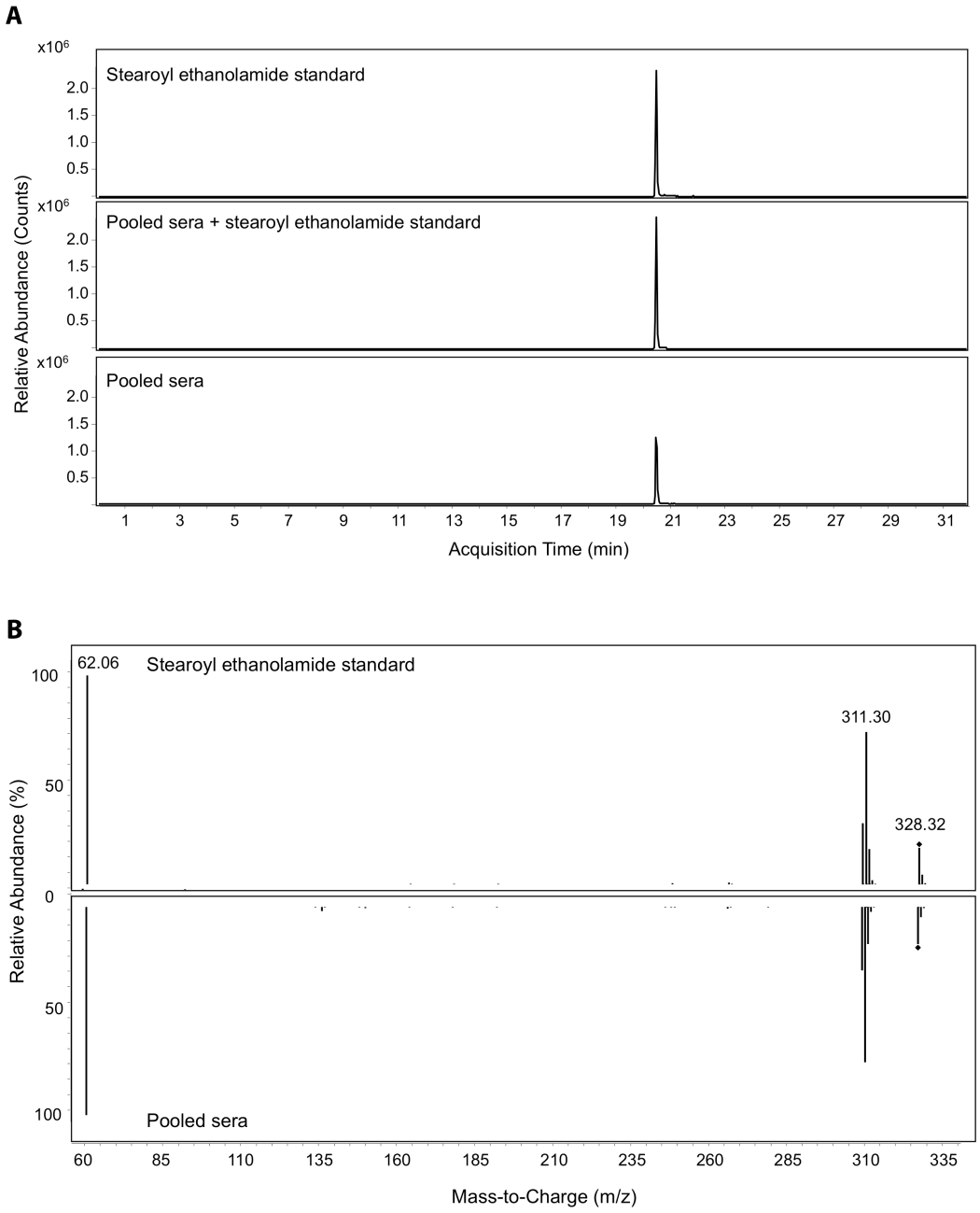

Fig. S1. Level 1 identification of stearoyl ethanolamide. Confirmation of the structural identity of stearoyl ethanolamide was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for stearoyl ethanolamide (**A**) were generated with extracted ion chromatograms for m/z 328.3204. MS/MS spectra for stearoyl ethanolamide were obtained with a collision energy of 20 eV.

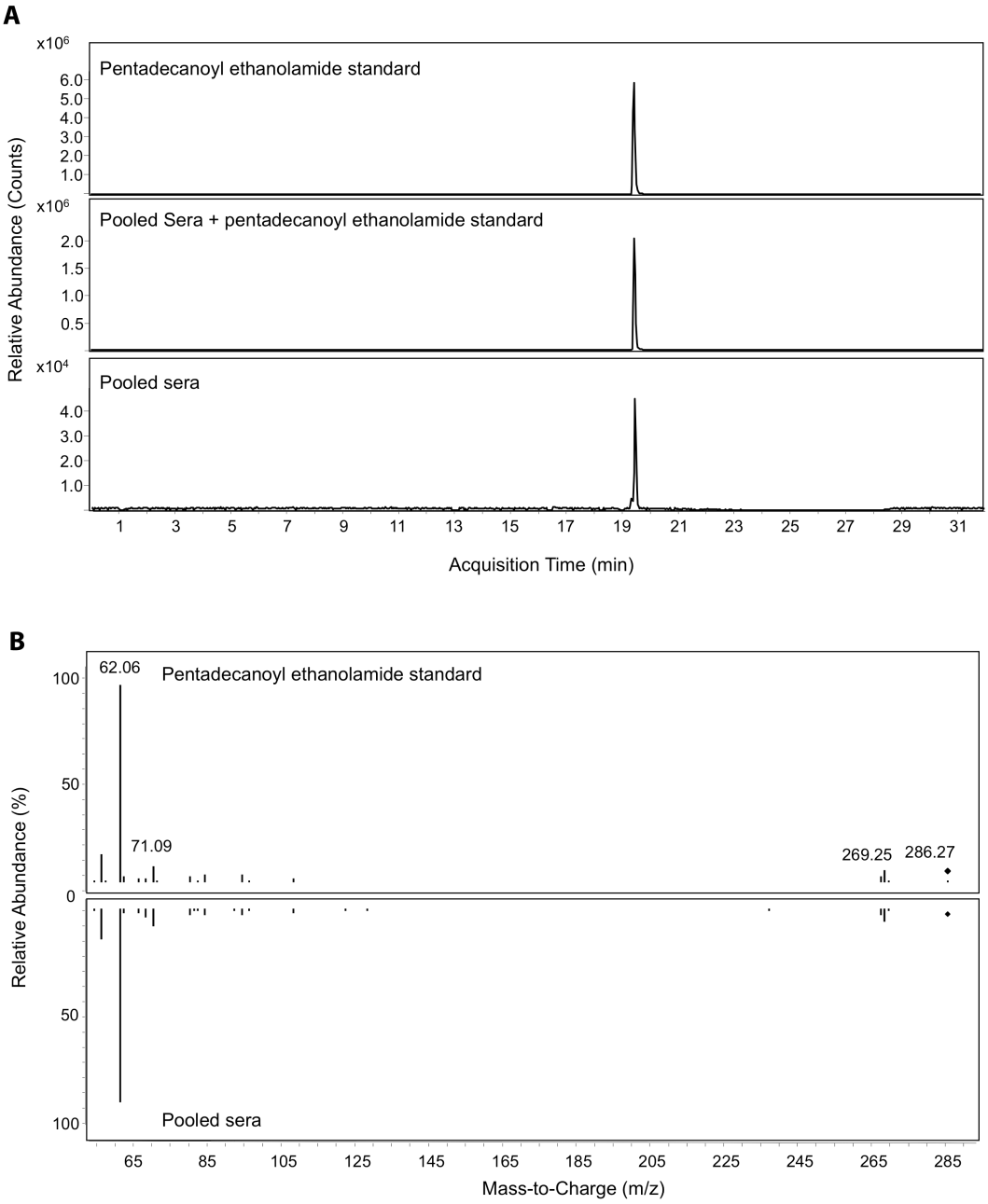

Fig. S2. Level 1 identification of pentadecanoyl ethanolamide. Confirmation of the structural identity of pentadecanoyl ethanolamide was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for pentadecanoyl ethanolamide (**A**) were generated with extracted ion chromatograms for m/z 286.2737. MS/MS spectra for pentadecanoyl ethanolamide were obtained with a collision energy of 20 eV.

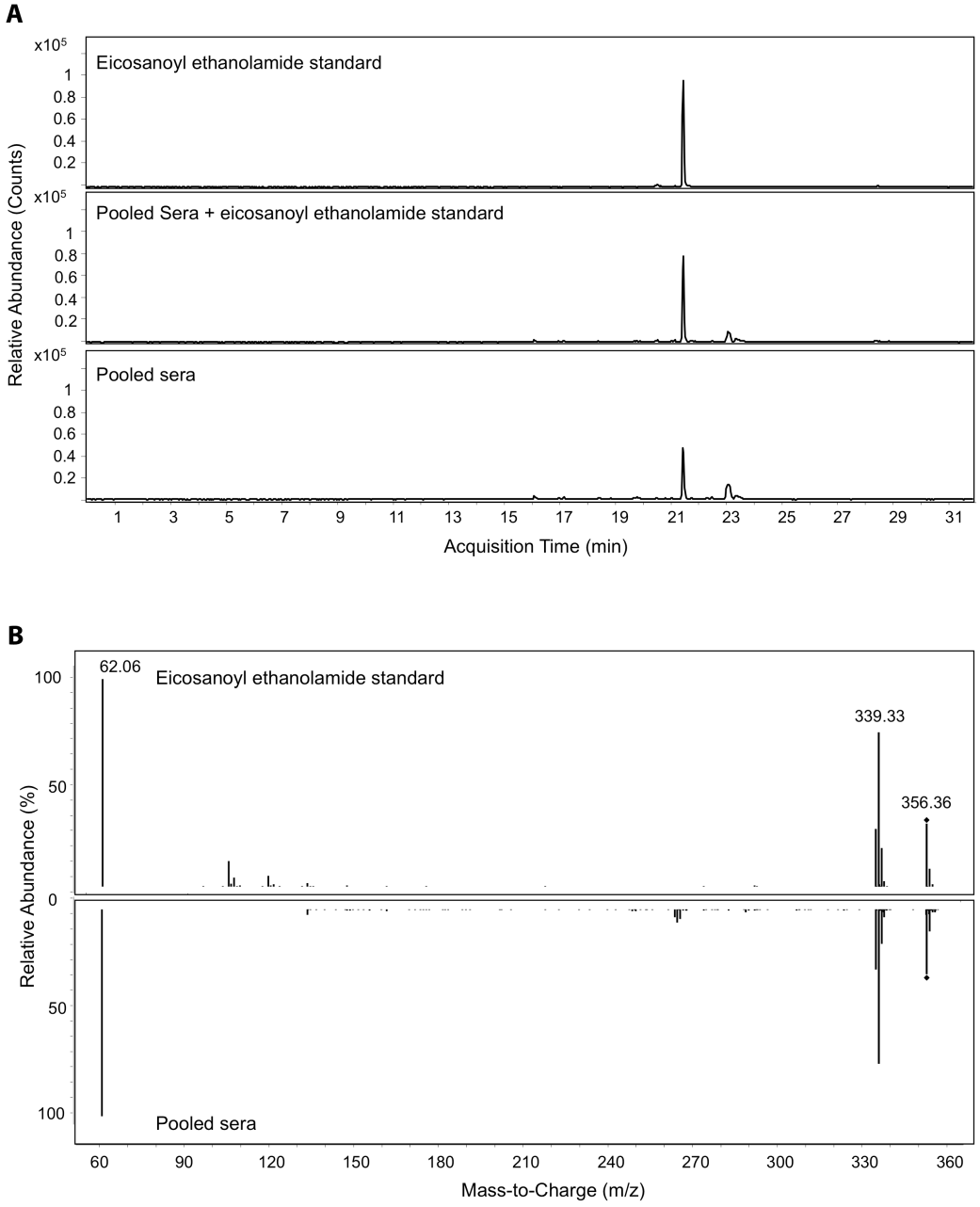

Fig. S3. Level 1 identification of eicosanoyl ethanolamide. Confirmation of the structural identity of eicosanoyl ethanolamide was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for eicosanoyl ethanolamide (**A**) were generated with extracted ion chromatograms for m/z 356.3517. MS/MS spectra for eicosanoyl ethanolamide were obtained with a collision energy of 20 eV.

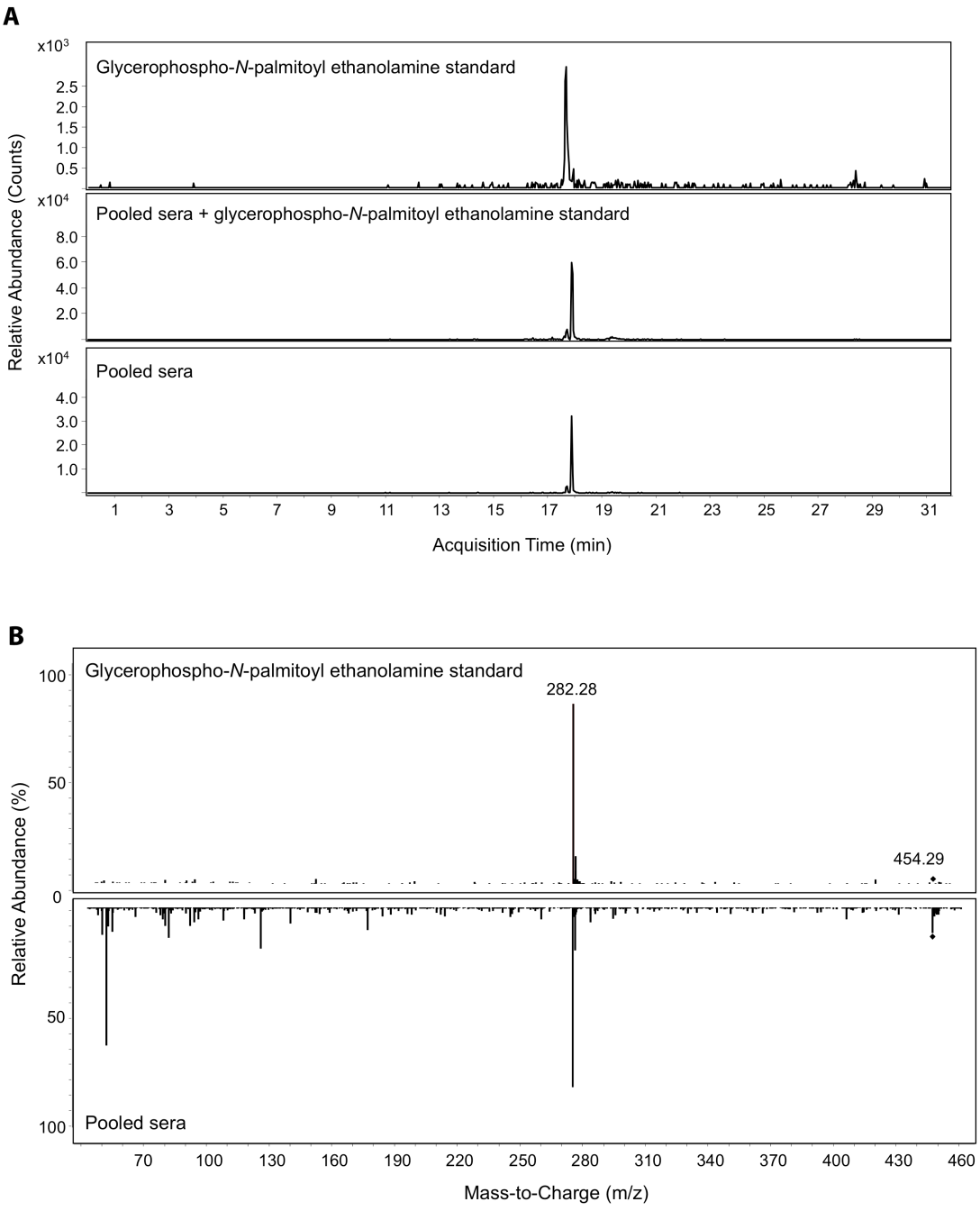

Fig. S4. Level 1 identification of glycerophospho-*N*-palmitoyl ethanolamine. Confirmation of the structural identity of glycerophospho-*N*-palmitoyl ethanolamine was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for glycerophospho-*N*-palmitoyl ethanolamine (**A**) were generated with extracted ion chromatograms for m/z 454.2923. MS/MS spectra for glycerophospho-*N*-palmitoyl ethanolamine were obtained with a collision energy of 20 eV.

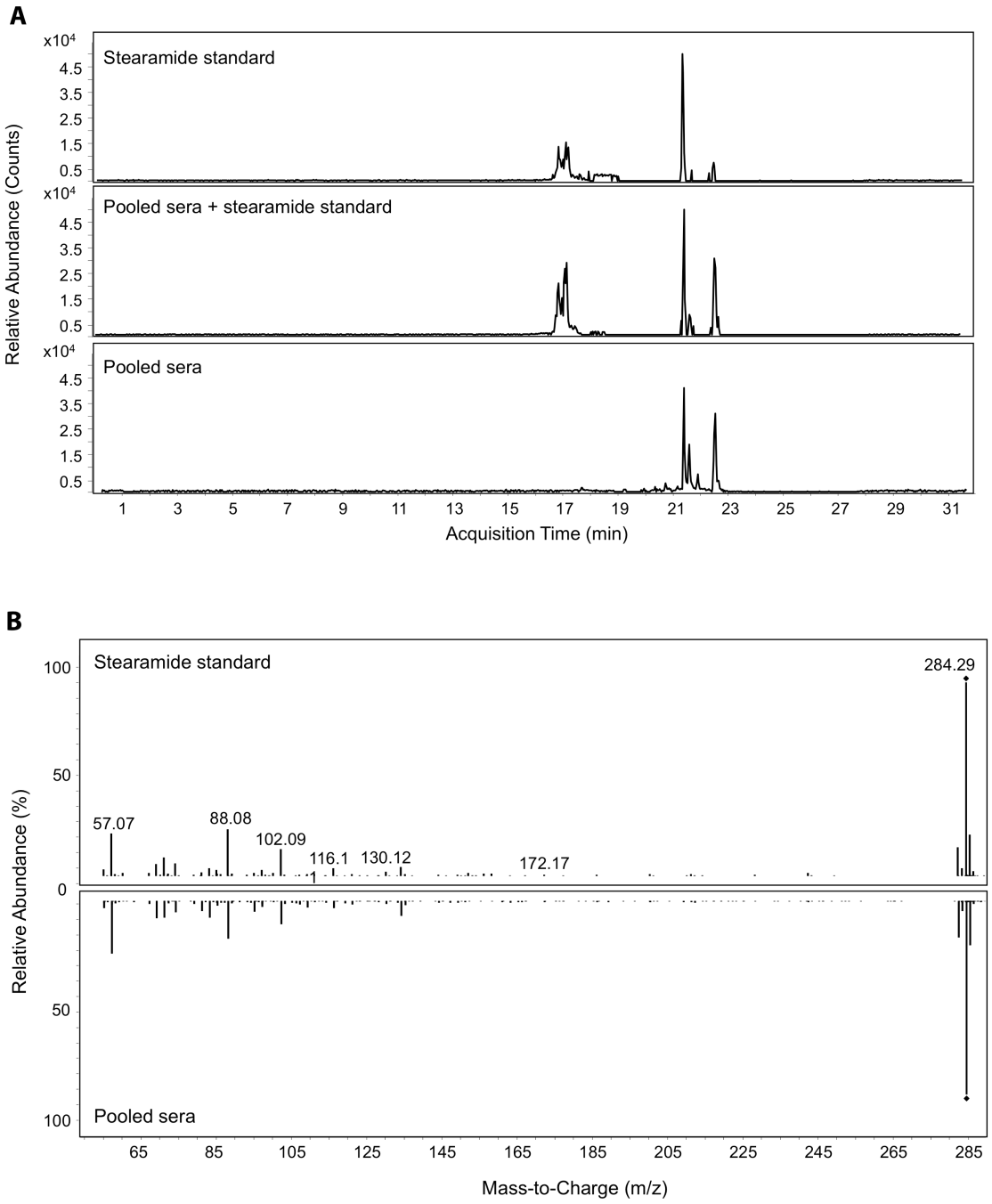

Fig. S5. Level 1 identification of stearamide. Confirmation of the structural identity of stearamide was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for stearamide (**A**) were generated with extracted ion chromatograms for m/z 284.2943. MS/MS spectra for stearamide were obtained with a collision energy of 20 eV.

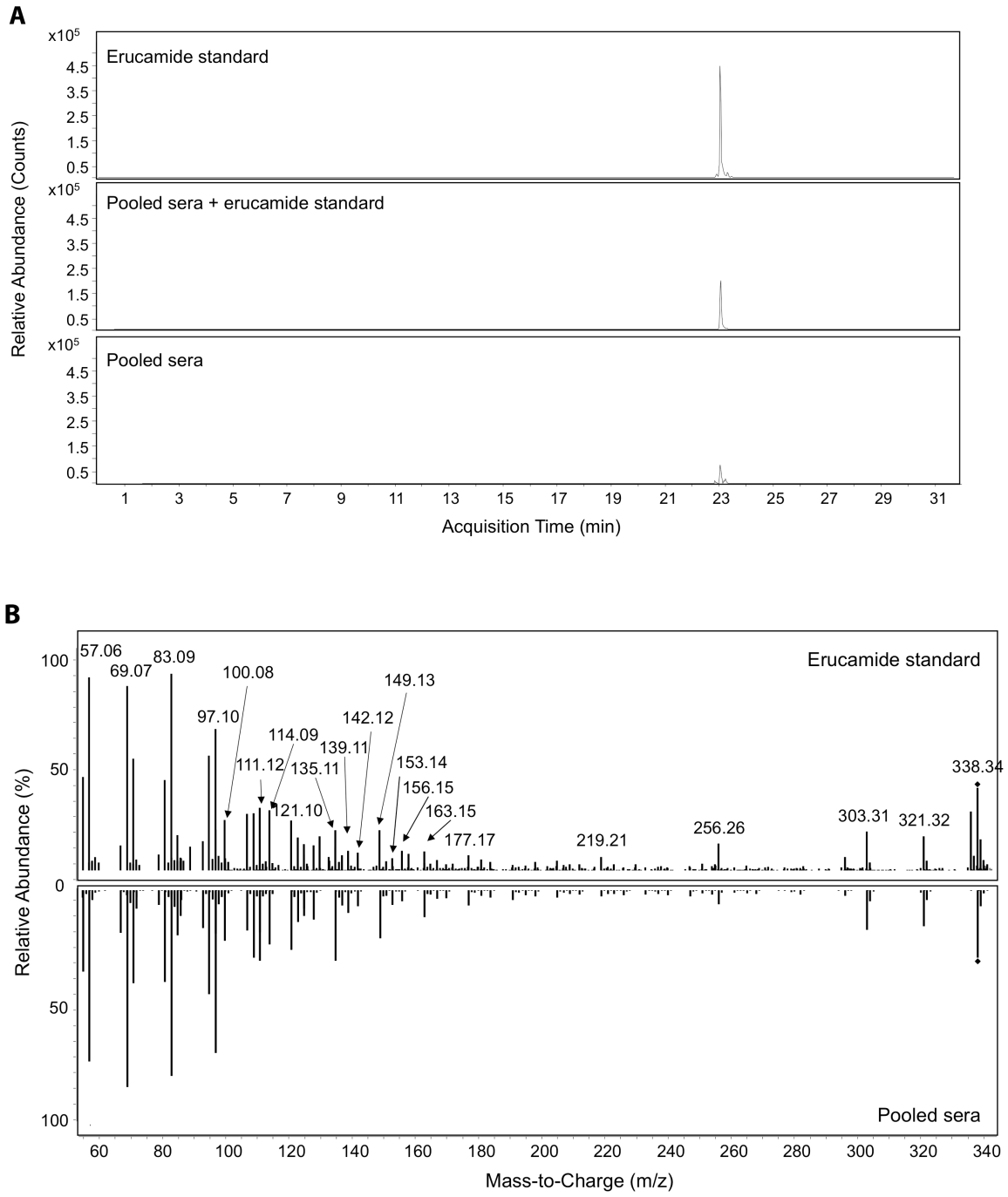

Fig. S6. Level 1 identification of erucamide. Confirmation of the structural identity of erucamide was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for erucamide (**A**) were generated with extracted ion chromatograms for m/z 338.3430. MS/MS spectra for erucamide were obtained with a collision energy of 20 eV.

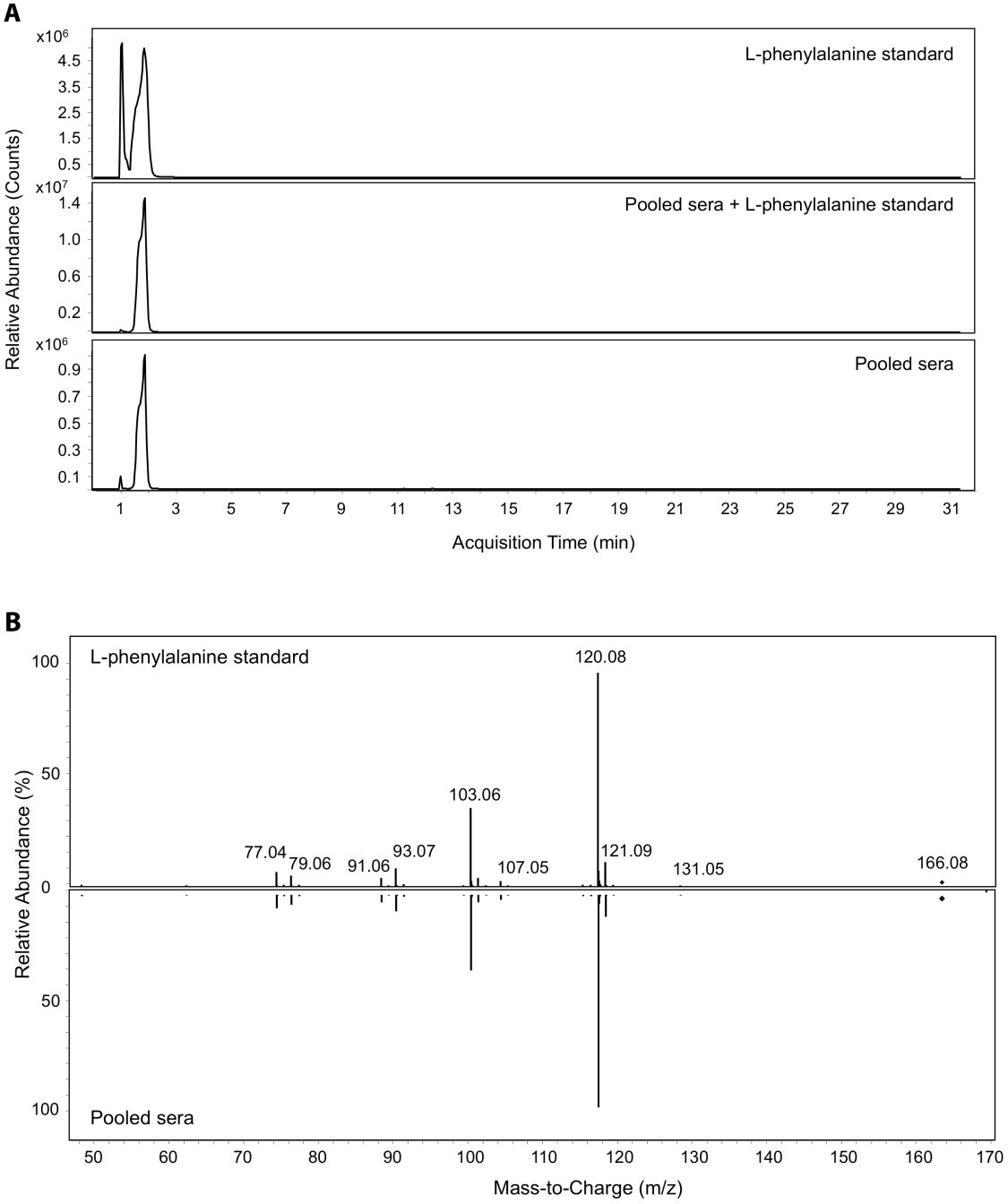

Fig. S7. Level 1 identification of L-phenylalanine. Confirmation of the structural identity of L-phenylalanine was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for L-phenylalanine (**A**) were generated with extracted ion chromatograms for m/z 166.0852. MS/MS spectra for L-phenylalanine were obtained with a collision energy of 20 eV.

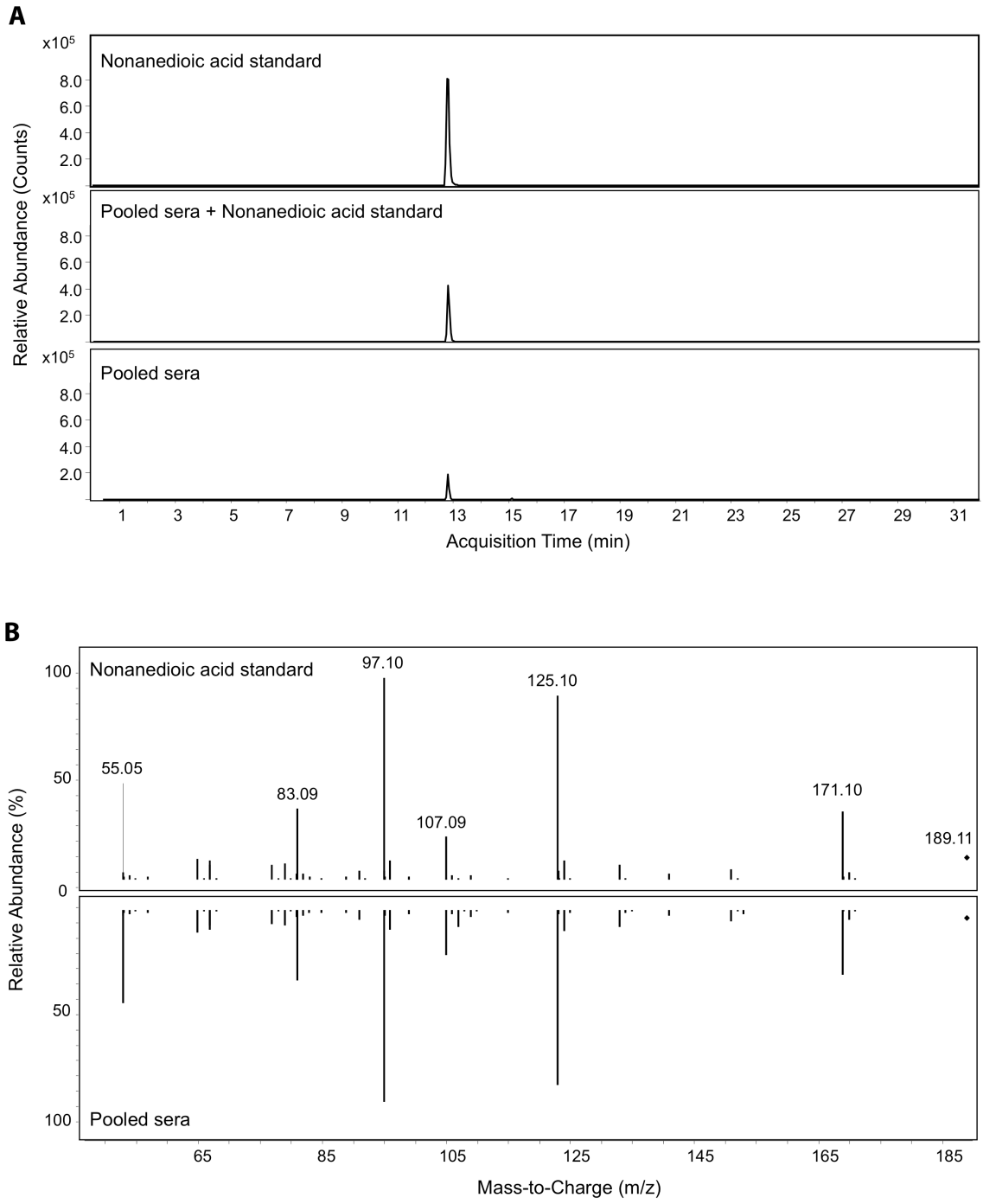

Fig. S8. Level 1 identification of nonanedioic acid. Confirmation of the structural identity of nonanedioic acid was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for nonanedioic acid (**A**) were generated with extracted ion chromatograms for m/z 189.1122. MS/MS spectra for nonanedioic acid were obtained with a collision energy of 10 eV.

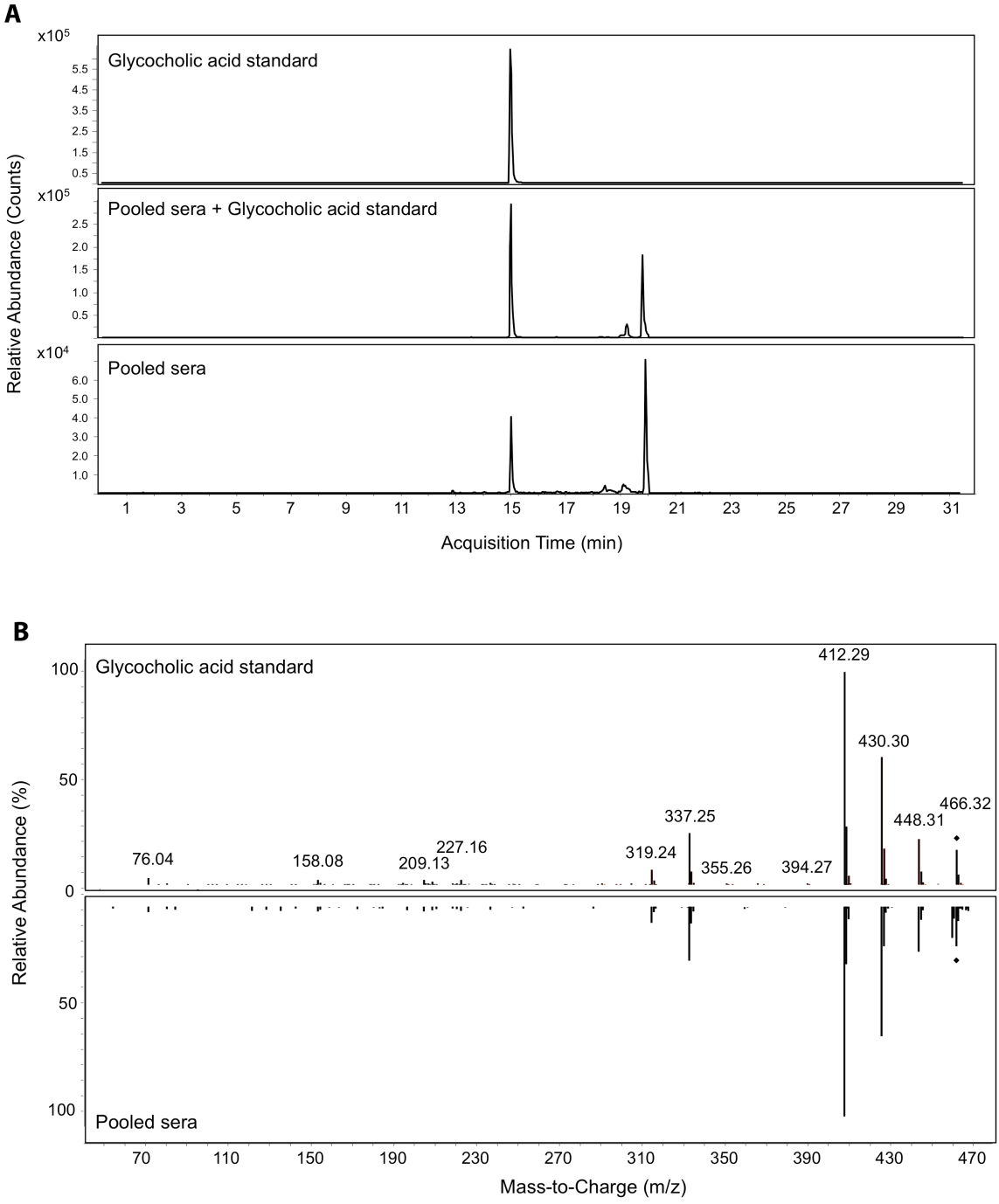

Fig. S9. Level 1 identification of glycocholic acid. Confirmation of the structural identity of glycocholic acid was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for glycocholic acid (**A**) were generated with extracted ion chromatograms for m/z 466.3152. MS/MS spectra for glycocholic acid were obtained with a collision energy of 20 eV.

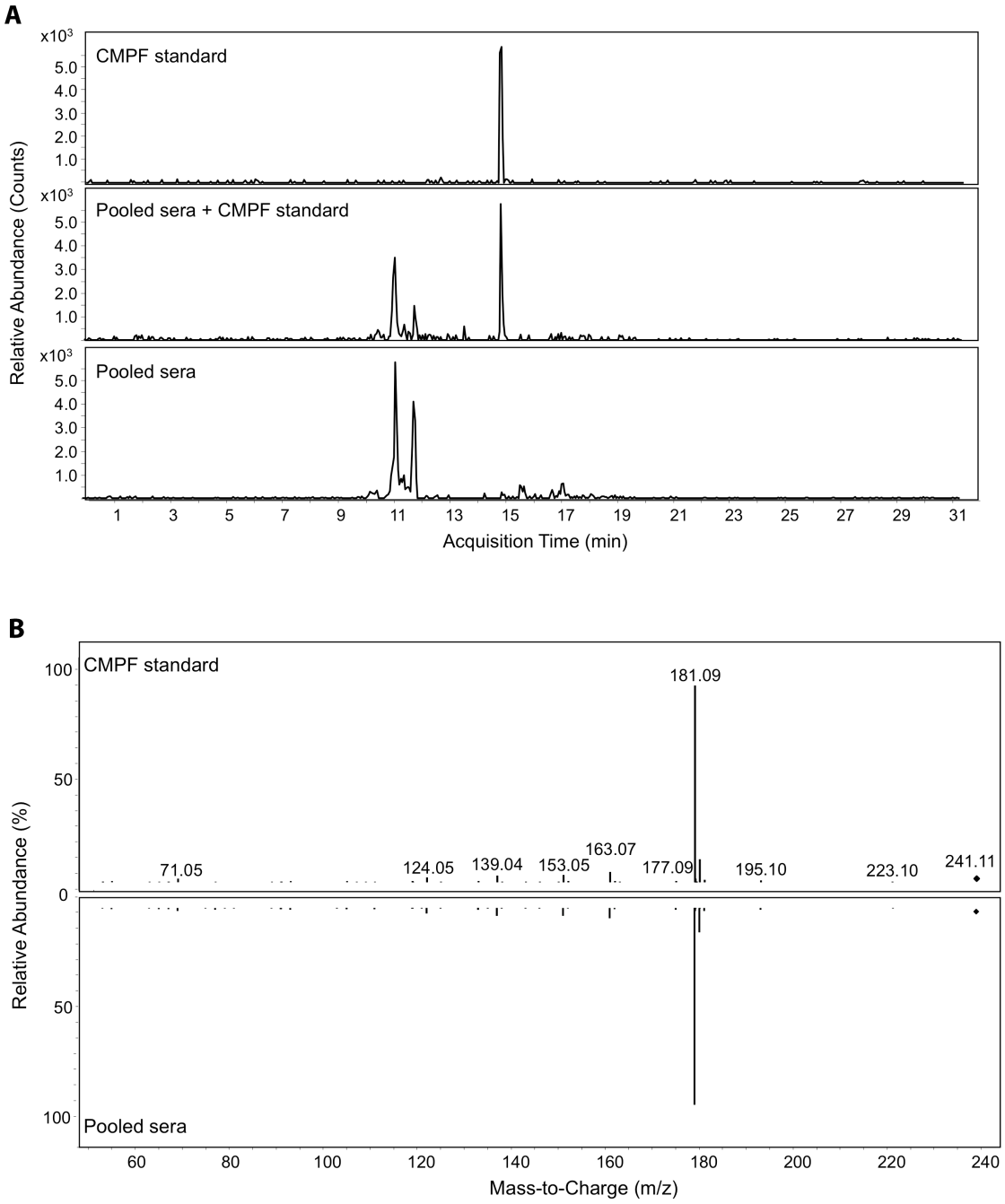

Fig. S10. Level 1 identification of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF). Confirmation of the structural identity of CMPF was achieved by retention-time alignment (**A**) of authentic standard (top panel), authentic standard spiked in pooled patient sera (middle panel), and the targeted metabolite in pooled patient sera; and by comparison of MS/MS spectra (**B**) of the authentic standard (top) and the targeted metabolite in pooled patient sera (bottom). Retention-time alignments for CMPF (**A**) were generated with extracted ion chromatograms for m/z 241.1069. MS/MS spectra for CMPF were obtained with a collision energy of 20 eV.

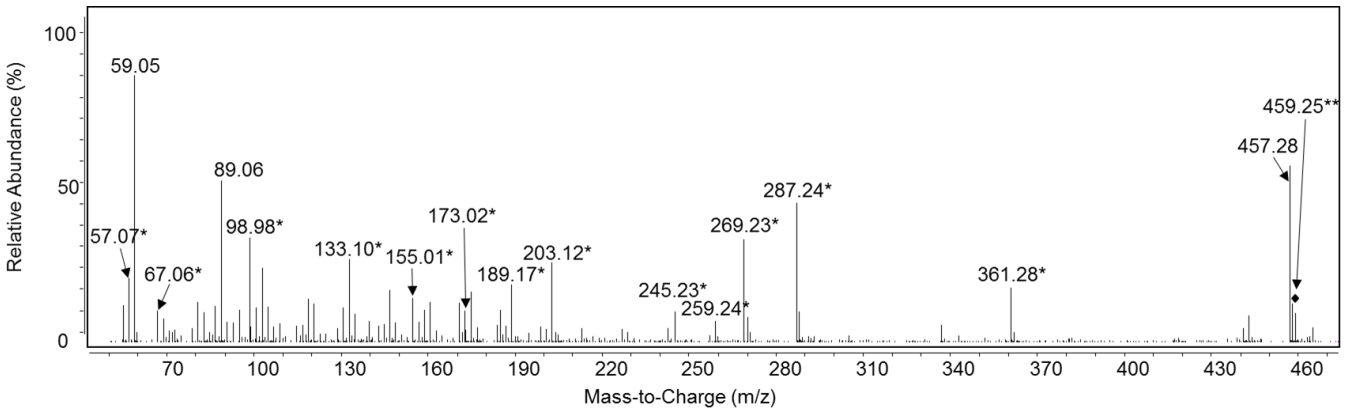

Fig. S11. Level 2 identification of Lyso PA (20:4) by MS/MS spectral matching. The MS/MS fragmentation pattern for m/z 459.2502 (**) in pooled sera at RT 19.02 is shown. A match to the fragmentation of arachidonoyl lysophosphatidic acid (Lyso PA (20:4)) in the Metlin database is indicated by (*). MS/MS spectra for m/z 459.2502 were obtained with a collision energy of 20 eV.

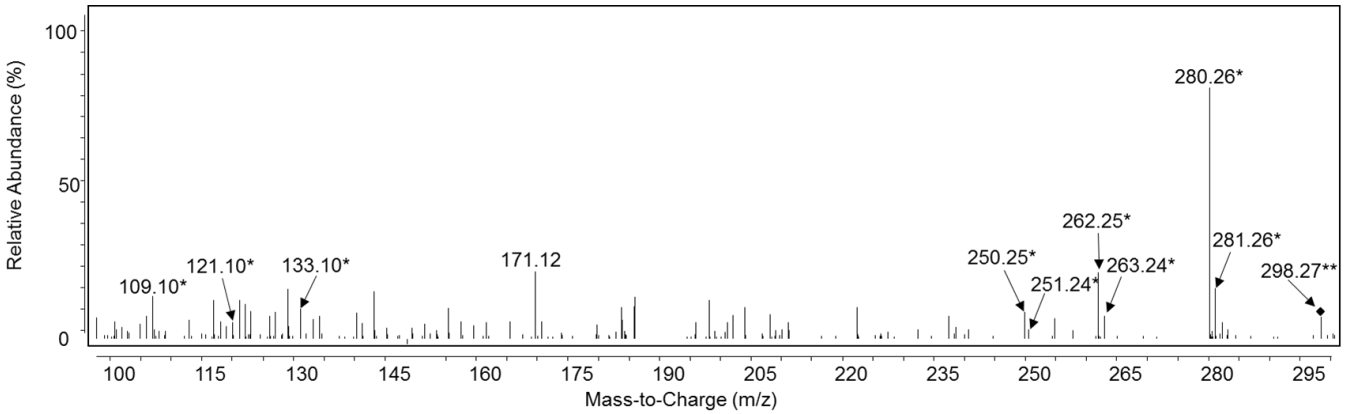

Fig. S12. Level 2 identification of 3-ketosphingosine by MS/MS spectral matching**.** The MS/MS fragmentation pattern for m/z 298.2740 (**) in pooled sera at RT 16.44 is shown. A match to the fragmentation of 3-ketosphingosine in the Metlin database is indicated by (*). MS/MS spectra for m/z 298.2740 were obtained with a collision energy of 20 eV.

Table S1. Serum samples used in this study. NYMC, New York Medical College; CDC, Centers for Disease Control and Prevention; UCF, University of Central Florida

Description of Samples	Sample Numbers	Sample Criteria for Inclusion	Sample Purpose	State Collected	Sample Provider*	Referenc
Early Lyme Disease (n=70)						
Age: 16-81 Male (52), Female (18)	70	At least one EM present on initial visit to the clinic. Samples were collected at initial visit to the clinic and pre-treatment. Positive culture and/or PCR test for *B. burgdorferi*. Patients lived in an endemic area for Lyme disease.	Discovery/Training and Test	NY	NYMC	(*27*)
STARI (n=55)						
STARI Group 1 Age: 4-82 Male (17), Female (16)	33	All patients had a physician-diagnosed erythema migrans-like rash ≥5 cm and a recent history of possible or verified exposure to *Amblyomma americanum* (lone star) ticks before the onset of symptoms. Patients lived in a non-endemic area for Lyme disease with the exception of three patients.± Samples were standard two-tiered negative for Lyme disease.	Discovery/Training and Test	NC, VA, GA, KY, TN, AL, IA and NE	CDC, Fort Collins, CO	(*57*)
STARI Group 2 Age: 8-80 Male (13), Female (9)	22			MO	NYMC	(*20*)
Healthy Donors (n=95)						
Healthy Group 1 Age: 18-unknown Male (8), Female (20)	28	No history of tick-borne disease within the last 12 months and lived in a non-endemic area for Lyme disease. Samples were standard two-tiered negative for Lyme disease.	Discovery/Training and Test	CO	CDC, Fort Collins, CO	-
Healthy Group 2†# Age: 18-74§	30	No history of Lyme disease and lived in an endemic area for Lyme disease. Samples were standard two-tiered negative for Lyme disease.		NY	NYMC	-
Healthy Group 3† Age: 18-60	37	No previous diagnosis with and/or treated for Lyme disease; and could not have lived within the past 10 years in a state with a high incidence of Lyme disease (Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Virginia and Wisconsin). Samples were standard two-tiered negative for Lyme disease.	Verification¥	FL	UCF (*65*)	(*65*)

* Sample handling varied among laboratories that provided samples. ± Two patients were from southwest Iowa and one was from southeast Virginia; both areas are considered to have low risk for Lyme disease and a higher prevalence of *A. americanum* as compared to *I. scapularis*. † The gender of these donors was approximately 50% females and 50% males. # The samples were obtained from the same geographic location as the early Lyme disease samples. § Age ranged from 18-74 for all donors (n=100). Only a subset of 30 donors were used for this study. ¥ Healthy controls from Florida were used to verify that the dysregulation of MFs between EL and STARI were not due to regional differences.

Table S2. 261 MF biosignature list. The experimentally obtained mass of each MF was used to search against the Metlin database and the Human Metabolome Database (HMDB). The predicted chemical structures had to match to the MF mass within 15 ppm. MFs could have matches to multiple chemical structures of within the same classes of chemicals or to structures of a different chemical class. The putative chemical structure data obtained by interrogation against the HMDB were used to evaluate possible metabolic pathways that differed between early Lyme disease and STARI patients (see table S3).

MF #	m/z	Mass	Retention Time	Compound Predicted Formula	Predicted Chemical Structure (based on accurate mass)*	Metabolite Class or Pathway	Level of Identification	# of Alternate Chemical Structures ± 15 ppm	MFs in RF Model	MFs in 2 Way LASSO Model (38 MF)	MFs in 3 Way LASSO Model (82 MF)
CSU/CDC-001	166.0852	165.078	1.86	$C_9H_{11}NO_2$	Phenylalanine	Phenylalanine metabolism	1	> 5	x	x	x
CSU/CDC-002	239.0919	238.0844	11.66	$C_{12}H_{14}O_5$	Trans-2, 3, 4-trimethoxycinnamate	Phenylpropanoid and polyketide metabolism	3	5	x	x	
CSU/CDC-003	886.4296	1770.8438	12.18	-	-	-	4	0	x		x
CSU/CDC-004	181.0859	180.0788	14.7	$C_{10}H_{12}O_3$	5'-(3'-Methoxy-4'-hydroxyphenyl)-gamma-valerolactone	Endogenous metabolite associated with microbiome	3	> 5	x		x
CSU/CDC-005	223.0968	222.0895	14.69	$C_{12}H_{14}O_4$	-	-	4	> 5	x		
CSU/CDC-006	286.1444	285.1371	16.08	$C_{17}H_{19}NO_3$	Piperine	Alkaloid metabolism	1	> 5	x		x
CSU/CDC-007	286.1437	285.1364	16.06	$C_{17}H_{19}NO_3$	-	-	4	> 5	x		
CSU/CDC-008	463.2339	462.2248	16.36	$C_{25}H_{34}O_8$	Ala Lys Met Asn	Peptide	3	> 5	x		x

CSU/CDC-009	242.2844	241.2772	17.1	$C_{16}H_{35}N$	-	-	4	1	x		x
CSU/CDC-010	1112.6727	1111.6663	17.86	-	-	-	4	0	x		
CSU/CDC-011	454.2923	453.2867	18.08	$C_{21}H_{44}NO_7P$	Glycerophospho-*N*-Palmitoyl Ethanolamine	*N*-acyl ethanolamine metabolism	1	> 5	x		
CSU/CDC-012	270.3156	269.3076	18.02	$C_{18}H_{39}N$	-	-	4	1	x	x	x
CSU/CDC-013	284.3314	283.3236	18.13	$C_{19}H_{41}N$	-	-	4	1	x	x	x
CSU/CDC-014	300.6407	599.268	18.27	$C_{33}H_{37}N_5O_6$	Asp Phe Arg Tyr	Peptide	3	> 5	x	x	x
CSU/CDC-015	522.3580	521.3483	18.5	$C_{26}H_{52}NO_7P$	PC(18:1)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-016	363.2192	362.2132	18.58	$C_{21}H_{30}O_5$	4,5α-dihydrocortisone	Sterol metabolism	3	> 5	x		
CSU/CDC-017	590.4237	589.4194	19.24	-	-	-	4	0	x		x
CSU/CDC-018	388.3939	387.3868	19.53	-	-	-	4	0	x		
CSU/CDC-020	256.2632	255.2561	20.08	$C_{16}H_{33}NO$	Palmitic amide	Primary Fatty Acid Amide Metabolism	1	1	x		
CSU/CDC-021	394.3515	376.3171	20.09	-	-	-	4	0	x		
CSU/CDC-022	228.1955	227.1885	20.99	-	-	-	4	0	x		

CSU/CDC-023	284.2943	283.2872	21.15	$C_{18}H_{37}NO$	Stearamide	Primary Fatty Acid Amide Metabolism	1	1	x		
CSU/CDC-024	338.3430	337.3344	22.14	$C_{22}H_{43}NO$	13Z-Docosenamide (Erucamide)	Primary Fatty Acid Amide Metabolism	1	3	x		
CSU/CDC-025	689.5604	688.5504	22.52	$C_{38}H_{77}N_2O_6P$	SM(d18:1-15:0) / SM (d18:1/14:1-OH)	Sphingolipid metabolism	3	> 5	x		
CSU/CDC-026	553.3904	552.3819	23.38	$C_{35}H_{52}O_5$	Furohyperforin	Endogenous metabolite - derived from food	3	3	x		x
CSU/CDC-027	432.2803	431.2727	10.8	$C_{25}H_{37}NO_5$	Ala Ile Lys Thr	Peptide	3	> 5	x		
CSU/CDC-028	389.2174	388.2094	15.47	$C_{19}H_{32}O_8$	Methyl 10,12,13,15-bisepidioxy-16-hydroperoxy-8E-octadecenoate	Fatty acid metabolism	3	> 5	x	x	
CSU/CDC-029	385.2211	384.2147	15.84	$C_{16}H_{28}N_6O_5$	Lys His Thr	Peptides	3	> 5	x		
CSU/CDC-030	399.2364	398.2313	16.23	-	-	-	4	0	x		x
CSU/CDC-031	449.3261	879.6122	17.07	$C_{46}H_{89}NO_{12}S$	C22-OH Sulfatide	Sphingolipid metabolism	3	2	x		
CSU/CDC-032	467.3821	444.2717	17.1	$C_{24}H_{40}O_8$	2-glyceryl-6-keto-PGF1α	Prostaglandin metabolism	3	> 5	x		
CSU/CDC-033	836.5936	835.5845	17.15	$C_{44}H_{85}NO_{11}S$	C20 Sulfatide	Sphingolipid metabolism	3	1	x		
CSU/CDC-034	792.5646	791.5581	17.17	$C_{42}H_{82}NO_{10}P$	PS(36:0)	Glycerophospholipid metabolism	3	> 5	x		

CSU/CDC-035	356.2802	355.2722	17.35	-	-	-	4	0	x		
CSU/CDC-036	806.5798	805.5746	17.71	$C_{43}H_{84}NO_{10}P$	PS(37:0)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-037	762.5582	761.5482	17.79	$C_{41}H_{80}NO_9P$	PS-O(35:1)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-038	718.5308	700.4946	17.88	$C_{39}H_{73}O_8P$	PA(36:2)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-039	734.5079	1449.9753	17.81	-	-	-	4	0	x	x	x
CSU/CDC-040	690.4825	1361.924	17.95	-	-	-	4	0	x		
CSU/CDC-041	426.1798	425.1725	18.03	-	-	-	4	0	x		
CSU/CDC-042	580.4144	1158.8173	18.26	-	-	-	4	0	x		x
CSU/CDC-043	741.5154	1481.0142	18.24	$C_{83}H_{150}O_{17}P_2$	CL(74:6)	Glycerophospholipid metabolism	3	2	x		
CSU/CDC-044	864.6245	863.6166	18.17	$C_{46}H_{89}NO_{11}S$	C22 Sulfatide	Sphingolipid metabolism	3	2	x		
CSU/CDC-045	558.4017	1080.7347	18.28	-	-	-	4	0	x		
CSU/CDC-046	719.5012	1402.9377	18.26	-	-	-	4	0	x		
CSU/CDC-047	536.3897	1053.7382	18.36	-	-	-	4	0	x		

CSU/CDC-048	538.8674	1058.696	18.4	-	-	-	4	0	x		
CSU/CDC-049	653.4619	1270.8593	18.43	-	-	-	4	0	x		
CSU/CDC-050	732.5450	714.5092	18.47	$C_{40}H_{75}O_8P$	PA(37:2)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-051	748.5232	1478.0059	18.58	-	-	-	4	0	x		
CSU/CDC-052	704.4985	1372.925	18.7	-	-	-	4	0	x		x
CSU/CDC-053	682.4841	1328.9008	18.77	-	-	-	4	0	x		
CSU/CDC-054	360.3615	359.3555	18.89	-	-	-	4	0	x		
CSU/CDC-055	441.2412	440.2325	19.09	$C_{20}H_{32}N_4O_7$	Pro Asp Pro Leu	Peptide	3	> 5	x		
CSU/CDC-056	638.4554	1240.847	18.92	-	-	-	4	0	x		
CSU/CDC-057	755.5311	1474.9941	18.94	$C_{83}H_{144}O_{17}P_2$	CL(74:9)	Glycerophospholipid metabolism	3	2	x		
CSU/CDC-058	711.5023	1386.9417	19.09	-	-	-	4	0	x		
CSU/CDC-059	784.5530	1567.0908	19.27	-	-	-	4	0	x		
CSU/CDC-060	645.4660	1271.8896	19.36	-	-	-	4	0	x		

CSU/CDC-061	623.4521	1210.8362	19.55	-	-	-	4	0	x		x
CSU/CDC-062	370.1837	369.1757	19.7	$C_{19}H_{23}N_5O_3$	-	-	4	1	x	x	x
CSU/CDC-063	300.2886	282.2569	19.84	$C_{18}H_{34}O_2$	13Z-octadecenoic acid	Fatty acid metabolism	3	> 5	x		
CSU/CDC-064	309.0981	308.0913	2.06	$C_{15}H_{16}O_7$	-	-	4	3	x		
CSU/CDC-065	561.2965	1120.5778	11.7	$C_{54}H_{88}O_{24}$	Camellioside D	Endogenous metabolite - derived from food	3	5	x		
CSU/CDC-066	811.1942	810.1869	12.07	$C_{42}H_{30}N_6O_{12}$	-	-	4	1	x	x	x
CSU/CDC-067	947.7976	946.7936	14.55	$C_{62}H_{106}O_6$	TAG(59:7)	Triacylglycerol metabolism	3	> 5	x	x	x
CSU/CDC-068	1106.2625	2209.5193	14.53	-	-	-	4	0	x		
CSU/CDC-069	371.2070	370.1997	15.52	$C_{15}H_{26}N_6O_7$	His Ser Lys	Peptide	3	> 5	x		
CSU/CDC-070	389.2178	388.2099	15.52	$C_{19}H_{32}O_8$	-	-	4	> 5	x		x
CSU/CDC-071	443.2649	442.256	15.52	$C_{19}H_{34}N_6O_6$	Pro Gln Ala Lys	Peptide	3	> 5	x		
CSU/CDC-072	410.2033	409.196	17.18	-	-	-	4	3	x	x	x

CSU/CDC-073	850.6093	849.6009	17.63	$C_{48}H_{84}NO_9P$	PS-O(42:6)	Glycerophospholipid metabolism	3	1	x		
CSU/CDC-074	1111.6690	1110.6656	17.89	-	-	-	4	0	x		x
CSU/CDC-075	1487.0005	1485.9987	18.17	-	-	-	4	0	x	x	x
CSU/CDC-076	697.4896	1358.909	18.32	-	-	-	4	0	x		
CSU/CDC-077	439.8234	877.6325	18.71	-	-	-	4	0	x		
CSU/CDC-078	567.8897	566.8818	18.73	-	-	-	4	0	x		
CSU/CDC-079	435.2506	434.243	19	$C_{21}H_{39}O_7P$	Lyso-PA(18:2)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-080	834.6136	833.6057	18.83	$C_{45}H_{88}NO_{10}P$	PS(39:0)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-081	534.8834	533.8771	18.82	-	-	-	4	0	x		
CSU/CDC-082	468.8441	467.8373	19.13	-	-	-	4	0	x		
CSU/CDC-083	482.4040	481.3976	19.99	-	-	-	4	0	x		x
CSU/CDC-084	533.1929	532.1854	20.84	$C_{23}H_{28}N_6O_9$	Asp His Phe Asp	Peptide	3	> 5	x		x
CSU/CDC-085	312.3259	311.319	22.05	-	-	-	4	0	x		

CSU/CDC-086	137.0463	136.0378	1.37	$C_4H_8O_5$	Threonate	Sugar metabolite	3	> 5	x	x	x
CSU/CDC-087	466.3152	465.3085	14.73	$C_{26}H_{43}NO_6$	Glycocholic acid	Bile acid metabolism	1	3	x		x
CSU/CDC-088	228.1955	227.1884	15.22	-	-	-	4	0	x		
CSU/CDC-089	385.2211	384.2143	15.83	$C_{20}H_{32}O_7$	Lys His Thr	Peptide	3	> 5	x		
CSU/CDC-090	403.2338	402.2253	15.84	C16H30N6O6	Lys Gln Gln	Peptide	3	> 5	x		
CSU/CDC-091	683.4728	1347.9062	17.56	-	-	-	4	0	x		x
CSU/CDC-092	675.4753	1348.9377	18.37	-	-	-	4	0	x		
CSU/CDC-093	682.4841	1345.9257	18.76	-	-	-	4	0	x		
CSU/CDC-094	762.5401	1506.0367	19.36	-	-	-	4	0	x		
CSU/CDC-095	227.0897	204.1002	9.68	$C_9H_{16}O_5$	-	-	4	2	x		x
CSU/CDC-177	189.1122	188.1049	12.27	$C_9H_{14}O_4$	Nonanedioic Acid	Fatty acid metabolism	1	> 5	x		
CSU/CDC-097	169.0860	168.0786	9.94	$C_9H_{12}O_3$	2,6-Dimethoxy-4-methylphenol	Endogenous metabolite - derived from food	3	> 5	x		

CSU/CDC-098	183.1016	182.0943	10.89	$C_{10}H_{14}O_3$	-	-	4	> 5	x		x
CSU/CDC-099	476.3055	475.2993	11.09	$C_{26}H_{41}N_3O_5$	-	-	4	5	x		x
CSU/CDC-100	276.1263	275.1196	11.16	$C_{15}H_{17}NO_4$	-	-	4	3	x		
CSU/CDC-101	314.0672	313.06	11.56	$C_{10}H_{12}N_5O_5P$	-	-	4	1	x		
CSU/CDC-102	201.1122	200.1047	11.56	$C_{10}H_{16}O_4$	Decenedioic acid	Fatty acid metabolism	3	> 5	x		
CSU/CDC-103	115.0391	114.0318	11.57	$C_5H_6O_3$	2-Hydroxy-2,4-pentadienoate	Phenylalanine metabolism	3	> 5	x		
CSU/CDC-104	491.1569	490.1504	11.56	$C_{24}H_{26}O_{11}$	-	-	4	> 5	x		
CSU/CDC-105	241.1054	218.1157	11.57	$C_{10}H_{18}O_5$	3-Hydroxy-sebacic acid	Fatty acid metabolism	3	3	x		
CSU/CDC-106	105.0914	104.0841	11.57	-	-		4	0	x		
CSU/CDC-107	811.7965	810.7882	12.07	-	-	-	4	0	x	x	x
CSU/CDC-108	311.1472	328.1391	12.22	$C_{18}H_{20}N_2O_4$	Phe Tyr	Peptide	3	> 5	x		
CSU/CDC-109	271.1543	270.1464	12.24	-	-	-	4	0	x		

CSU/CDC-110	169.0860	168.0787	12.24	$C_9H_{12}O_3$	2,6-Dimethoxy-4-methylphenol	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-111	187.0967	186.0889	12.24	$C_9H_{14}O_4$	-	-	4	4	x		
CSU/CDC-112	215.1283	214.1209	12.32	$C_{11}H_{18}O_4$	alpha-Carboxy-delta-decalactone	Endogenous metabolite - derived from food	3	4	x		x
CSU/CDC-113	475.1635	474.1547	12.25	$C_{25}H_{22}N_4O_6$	His Cys Asp Thr	Peptide	3	> 5	x		
CSU/CDC-114	129.0547	128.0474	12.33	$C_6H_8O_3$	(4E)-2-Oxohexenoic acid	Fatty acid metabolism	3	> 5	x		
CSU/CDC-115	519.1881	518.1813	12.33	$C_{20}H_{30}N_4O_{12}$	Poly-g-D-glutamate	Poly D-glutamate metabolism	3	> 5	x		x
CSU/CDC-116	125.0599	124.0527	13.12	$C_7H_8O_2$	4-Methylcatechol	Catechol metabolism	3	> 5	x		
CSU/CDC-117	247.1550	246.1469	13.13	$C_{12}H_{22}O_5$	3-Hydroxy-dodecanedioic acid	Fatty acid metabolism	3	4	x		
CSU/CDC-118	517.2614	516.2544	13.13	$C_{21}H_{36}N_6O_9$	Gln Glu Gln Ile	Peptide	3	> 5	x		
CSU/CDC-119	301.0739	300.0658	13.14	$C_{16}H_{12}O_6$	Chrysoeriol	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-120	327.1773	304.1885	14.17	$C_{16}H_{24}N_4O_2$	-	-	4	1	x		

CSU/CDC-121	387.2023	386.1935	14.51	$C_{19}H_{30}O_8$	Citroside A	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-122	875.8451	1749.684	14.55	-	-	-	4	0	x		
CSU/CDC-123	737.5118	736.5056	14.52	$C_{42}H_{73}O_8P$	PA(39:5)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-124	1274.3497	1273.3481	14.96	-	-	-	4	0	x		
CSU/CDC-125	1274.2092	1273.2	14.96	-	-	-	4	0	x		
CSU/CDC-126	1486.5728	2971.1328	14.95	-	-	-	4	0	x		
CSU/CDC-127	965.3818	964.3727	15.37	-	-	-	4	0	x		
CSU/CDC-128	1086.1800	2170.3435	15.38	-	-	-	4	0	x		x
CSU/CDC-129	1086.0562	2170.0908	15.38	$C_{97}H_{167}N_5O_{48}$	NeuAcalpha2-3Galbeta1-3GalNAcbeta1-4(9-OAc-NeuAcalpha2-8NeuAcalpha2-3)Galbeta1-4Glcbeta-Cer(d18:1/18:0)	Sphingolipid metabolism	3	1	x		
CSU/CDC-130	1086.4344	2169.8474	15.39	-	-		4	0	x		
CSU/CDC-131	1240.7800	1239.7712	15.38	-	-	-	4	0	x		

CSU/CDC-132	616.1776	615.1699	15.43	-	-	-	4	0	x	x	x
CSU/CDC-133	285.2061	284.1993	15.99	$C_{16}H_{28}O_4$	-	-	4	1	x		x
CSU/CDC-134	357.1363	356.1284	15.98	$C_{20}H_{20}O_6$	Xanthoxylol	Endogenous metabolite - derived from food	3	> 5	x		x
CSU/CDC-135	317.1956	316.1885	16.24	$C_{12}H_{24}N_6O_4$	Arg Ala Ala	Peptide	3	> 5	x		
CSU/CDC-136	299.1853	298.1781	16.24	$C_{16}H_{26}O_5$	Tetranor-PGE1	Prostaglandin metabolism	3	> 5	x		x
CSU/CDC-137	334.2580	333.2514	16.36	-	-	-	4	0	x		x
CSU/CDC-138	317.2317	316.2254	16.63	-	-	-	4	0	x		x
CSU/CDC-139	299.2219	298.2148	16.64	$C_{17}H_{30}O_4$	8E-Heptadecenedioic acid	Fatty acid metabolism	3	2	x		
CSU/CDC-140	748.5408	747.5317	17.23	$C_{40}H_{78}NO_9P$	PS-O(34:1)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-141	331.2471	330.2403	17.26	$C_{18}H_{34}O_5$	11,12,13-trihydroxy-9-octadecenoic acid	Fatty acid metabolism	3	> 5	x		x
CSU/CDC-142	712.4935	1422.9749	17.82	$C_{79}H_{140}O_{17}P_2$	CL(70:7)	Glycerophospholipid metabolism	3	1	x		
CSU/CDC-143	674.5013	673.4957	17.99	$C_{37}H_{72}NO_7P$	PE-P(32:1)	Glycerophospholipid metabolism	3	> 5	x		

CSU/CDC-144	583.3480	582.3379	18.04	$C_{27}H_{46}N_6O_8$	Leu Lys Glu Pro Pro	Peptide	3	1	x		x
CSU/CDC-145	677.9537	676.9478	18.36	-	-	-	4	0	x		
CSU/CDC-146	531.3522	530.3457	18.4	$C_{35}H_{46}O_4$	-	-	4	2	x		
CSU/CDC-147	585.2733	584.2649	18.39	$C_{33}H_{36}N_4O_6$	15,16-Dihydrobiliverdin	Bilirubin breakdown products – Porphyrin metabolism	3	> 5	x		
CSU/CDC-148	513.3431	512.3352	18.4	-	-	-	4	0	x		
CSU/CDC-149	611.9156	610.9073	18.59	-	-	-	4	0	x		
CSU/CDC-150	549.0538	531.0181	18.38	-	-	-	4	0	x		
CSU/CDC-151	755.5311	1509.0457	18.93	-	-	-	4	0	x		
CSU/CDC-152	713.4492	712.4391	19.35	$C_{38}H_{65}O_{10}P$	PG(32:5)	Glycerophospholipid metabolism	3	4	x	x	x
CSU/CDC-153	599.4146	598.4079	19.59	$C_{40}H_{54}O_4$	Isomytiloxanthin	Isoflavinoid	3	> 5	x		
CSU/CDC-154	762.5029	761.4919	19.66	$C_{43}H_{72}NO_8P$	PE(38:7)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-155	502.3376	484.3039	19.87	$C_{27}H_{40}N_4O_4$	Gln Leu Pro Lys	Peptide	3	> 5	x	x	x

CSU/CDC-156	741.4805	740.4698	19.96	$C_{40}H_{69}O_{10}P$	PG(34:5)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-157	648.4672	647.4609	19.98	$C_{34}H_{66}NO_8P$	PE(29:1)	Glycerophospholipid metabolism	3	> 5	x		x
CSU/CDC-158	415.3045	414.2978	20.19	-	-	-	4	0	x	x	x
CSU/CDC-159	516.3532	498.3199	20.27	$C_{23}H_{42}N_6O_6$	Ala Leu Ala Pro Lys	Peptide	3	1	x		
CSU/CDC-160	769.5099	768.5018	20.53	$C_{42}H_{73}O_{10}P$	PG(36:5)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-161	862.5881	861.5818	20.86	-	-	-	4	0	x		
CSU/CDC-162	837.5358	836.5274	21.11	$C_{53}H_{72}O_8$	Amitenone	Endogenous metabolite - derived from food	3	2	x		
CSU/CDC-163	558.3995	540.367	21.44	$C_{26}H_{48}N_6O_6$	Leu Ala Pro Lys Ile	Peptide	3	2	x		
CSU/CDC-164	366.3729	365.3655	22.79	-	-	-	4	0	x	x	x
CSU/CDC-165	445.2880	854.5087	12.48	$C_{45}H_{74}O_{15}$	(3b,21b)-12-Oleanene-3,21,28-triol 28-[arabinosyl-(1->3)-arabinosyl-(1->3)-arabinoside]	Endogenous metabolite - derived from food	3	1	x		x
CSU/CDC-166	333.1446	332.1373	12.89	$C_{12}H_{20}N_4O_7$	Glu Gln Gly	Peptide	3	> 5	x	x	x

CSU/CDC-167	1105.9305	2209.8462	14.53	-	-	-	4	0	x		
CSU/CDC-168	329.1049	328.0976	14.61	$C_{18}H_{16}O_6$	2-Oxo-3-phenylpropanoic acid	Phenylalanine metabolism	3	> 5	x		
CSU/CDC-169	1241.2053	1240.2	15.38	-	-	-	4	0	x		
CSU/CDC-170	1088.6731	1087.6676	17.85	-	-	-	4	0	x		
CSU/CDC-171	667.4391	666.4323	20.35	$C_{37}H_{63}O_8P$	PA(24:5)	Glycerophospholipid metabolism	3	> 5	x		
CSU/CDC-172	133.0497	132.0423	11.57	$C_5H_8O_4$	2-Acetolactic acid	Pantothenate and CoA Biosynthesis Pathway	3	> 5	x		
CSU/CDC-173	259.1540	258.1469	11.75	-	-	-	4	0	x		
CSU/CDC-174	311.1472	288.1574	12.23	$C_{10}H_{20}N_6O_4$	Asn Arg	Dipeptide	3	> 5	x		
CSU/CDC-175	147.0652	146.0579	12.33	$C_6H_{10}O_4$	α-Ketopantoic acid	Pantothenate and CoA Biosynthesis Pathway	3	> 5	x		
CSU/CDC-176	169.0860	168.0788	12.29	$C_9H_{12}O_3$	Epoxyoxophorone	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-096	187.0965	186.0894	9.93	$C_9H_{14}O_4$	5-Butyltetrahydro-2-oxo-3-furancarboxylic acid	Endogenous metabolite - derived from food	3	> 5	x		

CSU/CDC-178	139.1116	138.1044	12.95	$C_9H_{14}O_4$	3,6-Nonadienal	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-179	515.2811	514.2745	13.14	$C_{26}H_{42}O_{10}$	Cofaryloside	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-180	283.1522	282.1444	13.93	$C_{25}H_{42}N_2O_7S$	Epidihydrophaseic acid	Endogenous metabolite - derived from food	3	> 5	x		
CSU/CDC-181	1486.7386	2971.4668	14.97	-	-	-	4	0	x		x
CSU/CDC-182	285.2065	284.1991	16.02	$C_{16}H_{28}O_4$	-	-	4	1	x	x	
CSU/CDC-183	668.4686	1317.8969	18.04	$C_{16}H_{28}O_4$	Omphalotin A	Endogenous metabolite - derived from food	3	1	x		x
CSU/CDC-184	454.2924	436.2587	18.1	$C_{21}H_{41}O_7P$	Lyso-PA(18:1)	Glycerophospholipid metabolism	3	> 5	x		x
CSU/CDC-185	706.9750	705.9684	18.7	-	-	-	4	0	x		
CSU/CDC-186	607.9324	606.9246	19.01	-	-	-	4	0	x		x
CSU/CDC-187	834.5575	833.5502	20.32	-	-	-	4	0	x		
CSU/CDC-188	521.4202	503.3858	21.06	-	-	-	4	0	x		x

CSU/CDC-189	683.4727	1364.9294	17.54	-	-	-	4	0	x		
CSU/CDC-190	728.9890	1455.9633	18.63	-	-	-	4	1	x		
CSU/CDC-191	726.5104	1451.0035	18.64	$C_{81}H_{14}4O_{17}P_2$	CL(72:7)	Glycerophospholipid metabolism	3	2	x		
CSU/CDC-192	633.9280	632.9206	18.47	-	-	-	4	0	x		
CSU/CDC-193	176.0746	175.0667	2.31	-	-	-	4	0	x		x
CSU/CDC-194	596.9082	1191.8033	19.1	-	-	-	4	0	x		x
CSU/CDC-195	209.0784	208.0713	9.92	$C_{17}H_{24}O_3$	Benzylsuccinate	Phenylpropanoic acid metabolism	3	> 5	x		
CSU/CDC-196	792.5483	1566.055	18.46	-	-	-	4	0	x		
CSU/CDC-197	618.9221	1218.8083	19.02	-	-	-	4	0	x		
CSU/CDC-198	549.0543	531.0189	18.37	-	-	-	4	0	x		
CSU/CDC-199	553.7262	552.7188	18.74	-	-	-	4	0	x		
CSU/CDC-200	756.0320	755.0266	18.95	-	-	-	4	0	x		
CSU/CDC-201	639.6307	638.6205	19.58	-	-	-	4	0	x		

CSU/CDC-202	753.4414	730.4513	19.37	$C_{42}H_{67}O_8P$	PA(39:8)	Glycerophospholipid metabolism	3	2	x		
CSU/CDC-203	532.5606	531.5555	18.38	-	-	-	4	0	x		x
CSU/CDC-204	279.1693	278.1629	11.05	$C_{15}H_{22}N_2O_3$	Phe Leu	Dipeptide	3	> 5	x	x	
CSU/CDC-205	241.1069	240.0996	14.7	$C_{12}H_{16}O_5$	3-Carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF)	Fatty acid metabolism	1	> 5	x	x	x
CSU/CDC-206	337.1667	336.1599	20.67	$C_{12}H_{24}N_4O_7$	_	_	4	2	x		x
CSU/CDC-207	328.3204	327.3148	20.72	$C_{20}H_{41}NO_2$	Stearoyl ethanolamide	*N*-acyl ethanolamine metabolism	1	5	x		
CSU/CDC-208	514.3718	1009.7122	18.42	$C_{56}H_{99}NO_{14}$	3-O-acetyl-sphingosine-2,3,4,6-tetra-O-acetyl-GalCer(d18:1/h22:0)	Sphingolipid metabolism	3	1	x		
CSU/CDC-209	630.4594	1241.8737	19.95	-	-	-	4	0	x		
CSU/CDC-210	415.1634	207.0784	12.2	$C_8H_9N_5O_2$	6-Amino-9H-purine-9-propanoic acid	Endogenous metabolite - derived from food	3	2	x		x
CSU/CDC-211	464.1916	463.1849	13.05	$C_{16}H_{29}N_7O_7S$	Arg Asp Cys Ala	Peptide	3	> 5	x	x	x
CSU/CDC-212	1249.2045	1248.1993	15.31	-	-	-	4	0	x	x	x
CSU/CDC-213	1248.9178	1247.9141	15.3	-	-	-	4	0	x	x	x

CSU/CDC-214	244.2270	243.22	17.17	$C_{14}H_{29}NO_2$	Lauroyl ethanolamide	*N*-acyl ethanolamine metabolism	3	3	x		
CSU/CDC-215	463.3426	924.6699	18.08	-	-	-	4	0	x		
CSU/CDC-216	468.3892	450.3553	19.17	$C_{31}H_{46}O_2$	-	-	4	1	x		
CSU/CDC-217	438.3787	420.3453	19.93	-	-	-	4	0	x		
CSU/CDC-218	364.3407	346.3068	20.72	-	-	-	4	0	x		x
CSU/CDC-219	158.1539	157.1466	15.36	-	-	-	4	0	x	x	x
CSU/CDC-220	792.0006	790.995	12.04	-	-	-	4	0	x		
CSU/CDC-221	792.2025	791.1947	12.04	-	-	-	4	0	x		
CSU/CDC-222	989.5004	1976.9858	12.03	-	-	-	4	0	x		x
CSU/CDC-223	791.6016	790.594	12.04	-	-	-	4	0	x		
CSU/CDC-224	819.6064	1635.8239	12.06	-	-	-	4	0	x		x
CSU/CDC-225	1115.5593	2228.1028	14.95	-	-	-	4	0	x		
CSU/CDC-226	1486.9176	2970.7976	14.96	-	-	-	4	0	x		

CSU/CDC-227	529.3381	528.3296	16.89	$C_{24}H_{44}N_6O_7$	Gln Val Leu Leu Gly	Peptide	3	5	x	x	x
CSU/CDC-228	430.3161	412.2845	20.23	$C_{23}H_{40}O_6$	-	-	4	1	x		
CSU/CDC-229	282.2776	264.2456	20.56	$C_{18}H_{32}O$	-	-	4	> 5	x	x	x
CSU/CDC-230	297.2793	296.2734	20.66	$C_{19}H_{36}O_2$	Methyl oleate	Oleic acid ester	1	> 5	x		
CSU/CDC-231	714.3655	1426.718	11.73	-	-	-	4	0	x		
CSU/CDC-232	714.5306	1427.0479	11.76	-	-	-	4	0	x		
CSU/CDC-233	989.7499	1977.4865	12.03	-	-	-	4	0	x		
CSU/CDC-234	221.0744	220.0672	13.7	$C_7H_{12}N_2O_6$	L-beta-aspartyl-L-serine	Peptide	3	> 5	x		
CSU/CDC-235	190.1260	189.1187	14.12	$C_9H_{19}NOS$	8-Methylthiooctanaldoxime	2-oxocarboxylic acid metabolism	3	2	x	x	x
CSU/CDC-019	300.2892	299.2821	19.66	$C_{18}H_{37}NO_2$	Palmitoyl ethanolamide	*N*-acyl ethanolamine metabolism	1	> 5	x		
CSU/CDC-236	313.2734	312.2663	18.91	$C_{19}H_{36}O_3$	2-oxo-nonadecanoic acid	Fatty acid metabolism	3	5	x		
CSU/CDC-237	286.2737	285.2666	19.08	$C_{17}H_{35}NO_2$	Pentadecanoyl ethanolamide	*N*-acyl ethanolamine metabolism	1	4	x		x
CSU/CDC-238	382.3675	381.3603	20.23	$C_{24}H_{47}NO_2$	Erucicoyl ethanolamide	*N*-acyl ethanolamine metabolism	3	1	x	x	x

CSU/CDC-239	337.2712	314.282	20.66	$C_{19}H_{38}O_3$	2-Hydroxy-nonadecanoic acid	Fatty acid metabolism	3	2	x		
CSU/CDC-240	441.3687	440.3614	21.26	$C_{30}H_{48}O_2$	4,4-Dimethyl-14a-formyl-5a-cholesta-8,24-dien-3b-ol	Sterol metabolism	3	> 5	x		
CSU/CDC-241	425.3735	424.3666	21.5	$C_{30}H_{48}O$	Butyrospermone	Sterol metabolism	3	> 5	x		
CSU/CDC-242	356.3517	355.3448	21.67	$C_{22}H_{45}NO_2$	Eicosanoyl ethanolamide	*N*-acyl ethanolamine metabolism	1	2	x		
CSU/CDC-243	393.2970	370.3082	22.46	$C_{22}H_{42}O_4$	-	-	4	3	x		
CSU/CDC-244	477.2968	476.2898	22.79	$C_{31}H_{40}O_4$	Lys Lys Thr Thr	Peptide	3	> 5	x	x	x
CSU/CDC-245	614.4833	613.4772	19.78	-	-	-	4	0	x		x
CSU/CDC-246	167.9935	166.9861	13.2	$C_7H_5NS_2$	-	-	4	1	x		
CSU/CDC-247	714.6967	1427.3824	11.76	-	-	-	4	0	x	x	
CSU/CDC-248	459.3968	458.3904	19.08	-	-	-	4	0	x	x	x
CSU/CDC-249	677.6170	676.6095	20.71	$C_{47}H_{80}O_2$	Cholesterol ester (20:2)	Sterol metabolism	3	> 5	x		
CSU/CDC-250	298.2740	297.2668	16.44	$C_{18}H_{35}NO_2$	3-Ketospingosine	Sphingolipid metabolism	2	> 5	x		x

CSU/CDC-251	460.2695	459.2627	16.87	$C_{26}H_{37}NO_6$	-	-	4	> 5	x		
CSU/CDC-252	1003.7020	1002.696	18.46	-	-	-	4	0	x		x
CSU/CDC-253	342.2635	341.2565	15.62	$C_{19}H_{35}NO_4$	-	-	4	2	x	x	x
CSU/CDC-254	529.3827	1022.6938	17.86	-	-	-	4	0	x	x	x
CSU/CDC-255	630.4765	612.4417	18.11	-	-	-	4	0	x		
CSU/CDC-256	514.3734	1026.7281	18.41	-	-	-	4	0	x		
CSU/CDC-257	667.4754	1315.916	19.28	-	-	-	4	0	x		
CSU/CDC-258	459.2502	458.2429	19.02	$C_{23}H_{39}O_7P$	Lyso PA(20:4)	Glycerohospholipid metabolism	2	> 5	x	x	x
CSU/CDC-259	516.8549	1031.6945	18.43	-	-	-	4	0	x		
CSU/CDC-260	740.5242	1479.0334	19.4	$C_{83}H_{148}O_{17}P_2$	CL(74:7)	Glycerohospholipid metabolism	3	2	x		
CSU/CDC-261	1104.0614	2206.1096	15.2	-	-	-	4	0	x		

* CSU/CDC-230 was positively identified as methyl oleate, a product that was likely an artifact of fatty acid methylation that occurred during sample processing

Table S3. MetaboAnalyst results. Total, the total number of compounds in the pathway; Hits, the actual number of compounds in the pathway matched from the 261 MF biosignature list; Raw p, the original p value calculated from the enrichment analysis; Impact, the pathway impact value calculated from pathway topology analysis. The MetaboAnalyst results were used to target specific MFs in the early Lyme disease-STARI biosignature for structural identification.

Pathway Hit*	Total	Expected	Hits	Raw p	-log(p)	Impact
Glycerophospholipid metabolism	39	1.2638	4†	0.035545	3.337	0.33235
Sphingolipid metabolism	25	0.81014	3±	0.045107	3.0987	0.15499
Valine, leucine and isoleucine biosynthesis	27	0.87495	2	0.21724	1.5268	0.17117
Phenylalanine metabolism	45	1.4582	1	0.77605	0.25353	0.11906
alpha-Linolenic acid metabolism	29	0.93976	2	0.24148	1.421	0
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	14	0.45368	1	0.37027	0.99353	0.0439
Linoleic acid metabolism	15	0.48608	1	0.39079	0.93957	0
Riboflavin metabolism	21	0.68052	1	0.50079	0.69157	0
Phenylalanine, tyrosine and tryptophan biosynthesis	27	0.87495	1	0.59113	0.52572	0.00062
Pantothenate and CoA biosynthesis	27	0.87495	1	0.59113	0.52572	0.02002
Steroid hormone biosynthesis	99	3.2081	3	0.63116	0.4602	0.0382
Glycerolipid metabolism	32	1.037	1	0.65393	0.42476	0.01247
Ubiquinone and other terpenoid-quinone bios+A14:I29ynthesis	36	1.1666	1	0.69723	0.36064	0
Nitrogen metabolism	39	1.2638	1	0.72615	0.32	0
Butanoate metabolism	40	1.2962	1	0.73517	0.30766	0.04772
Ascorbate and aldarate metabolism	45	1.4582	1	0.77605	0.25353	0.00802
Drug metabolism - cytochrome P450	86	2.7869	2	0.77721	0.25205	0.0176
Primary bile acid biosynthesis	47	1.5231	1	0.7906	0.23496	0.00846

Lysine degradation	47	1.5231	1	0.7906	0.23496	0.06505
Fatty acid biosynthesis	49	1.5879	1	0.80422	0.21788	0
Fatty acid metabolism	50	1.6203	1	0.81069	0.20986	0
Starch and sucrose metabolism	50	1.6203	1	0.81069	0.20986	0.01265
Pentose and glucuronate interconversions	53	1.7175	1	0.82888	0.18768	0.009
Arachidonic acid metabolism	62	2.0091	1	0.87371	0.135	0
Aminoacyl-tRNA biosynthesis	75	2.4304	1	0.91874	0.084752	0
Purine metabolism	92	2.9813	1	0.95452	0.046547	0.00791
Porphyrin and chlorophyll metabolism	104	3.3702	1	0.96989	0.030577	0.01101

[*] The Holm adjust, the adjusted p value by the Holm-Bonferroni method and FDR, the p value adjusted using False Discovery Rate for the assigned pathway were 1 for all hits. This is due to small number of metabolites assigned in each pathway relative to the number of known metabolites in each pathway and the number of experimentally determined potential metabolites used in the analysis. [†]The four hits in the glycerophospholipid metabolism pathway were phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine and lysophosphotidylcholine. [±]The three hits in the sphingolipid metabolism pathway were in sphingosine, dehydrosphinganine and sulfatide.

Table S4. Regression coefficients (β) of the LASSO two-way statistical model. The regression coefficient for each of the 38 MFs (CSU/CDC-#) used in the LASSO two-way classification model are provided. The regression coefficients were generated with data from the Training-Set samples, and applied in the classification of the Test-Set samples as shown in table S5.

MF Id	Coefficient
Intercept	-0.5089
CSU/CDC-001	-0.3032
CSU/CDC-002	-0.0359
CSU/CDC-012	-0.31
CSU/CDC-013	-0.2256
CSU/CDC-014	0.05737
CSU/CDC-028	0.21447
CSU/CDC-039	0.29641
CSU/CDC-062	0.0152
CSU/CDC-066	-0.0559
CSU/CDC-067	0.63951
CSU/CDC-072	-0.1451
CSU/CDC-075	0.10409
CSU/CDC-086	0.71497
CSU/CDC-107	-0.2586
CSU/CDC-132	0.88577
CSU/CDC-152	-0.6125
CSU/CDC-155	-0.0083
CSU/CDC-158	-0.027
CSU/CDC-164	0.22005
CSU/CDC-166	-0.2033
CSU/CDC-182	-0.1077
CSU/CDC-204	-0.163
CSU/CDC-205	-0.8688
CSU/CDC-211	0.43327
CSU/CDC-212	-0.3513
CSU/CDC-213	-0.422

CSU/CDC-219	1.01872
CSU/CDC-227	0.43588
CSU/CDC-229	0.11674
CSU/CDC-235	0.3664
CSU/CDC-019	0.52461
CSU/CDC-238	0.7812
CSU/CDC-244	-0.7325
CSU/CDC-247	0.00621
CSU/CDC-248	0.38858
CSU/CDC-253	0.10575
CSU/CDC-254	0.27792
CSU/CDC-258	-0.5593

Table S5. LASSO and RF two-way model classification probability scores. The LASSO and RF probability scores are provided for each patient sample tested in duplicate. These are probability scores for the Test-Set samples. A probability score of ≥ 0.5 classified the samples as early Lyme disease (EL), and a probability score of < 0.5 resulted in the sample being classified as STARI.

Coded Sample ID	LASSO Probability Score	LASSO Classification	RF Probability Score	RF Classification	Sample ID	Patient Type
Valb1618	0.9979	EL	0.8980	EL	EDL134-022315	EL
Valb1591	0.9995	EL	0.8980	EL	EDL134-120214	EL
Valb1454	0.9900	EL	0.6320	EL	EDL135-022315	EL
Valb0820	0.5264	EL	0.8660	EL	EDL135-120214	EL
Valb0989	0.9820	EL	0.8620	EL	EDL136-022315	EL
Valb0546	0.8814	EL	0.8960	EL	EDL136-120214	EL
Valb1573	0.9875	EL	0.5840	EL	EDL137-022315	EL
Valb1299	0.7198	EL	0.4380	STARI	EDL137-120214	EL
Valb0477	0.9247	EL	0.7780	EL	EDL138-022315	EL
Valb0160	0.9868	EL	0.9160	EL	EDL138-120214	EL
Valb0813	0.7300	EL	0.4880	STARI	EDL139-022315	EL
Valb0443	0.8307	EL	0.7680	EL	EDL139-120214	EL
Valb1412	0.9287	EL	0.7200	EL	EDL140-022315	EL

Valb0886	0.9045	EL	0.8140	EL	EDL140-120214	EL
Valb0827	0.9846	EL	0.9040	EL	EDL71-022315	EL
Valb0186	0.9609	EL	0.9180	EL	EDL71-120214	EL
Valb1337	0.9417	EL	0.8200	EL	EDL73-022315	EL
Valb0714	0.9836	EL	0.9000	EL	EDL73-120214	EL
Valb1510	0.9773	EL	0.7720	EL	EDL74-022315	EL
Valb0642	0.9986	EL	0.8520	EL	EDL74-120214	EL
Valb1586	0.9995	EL	0.9020	EL	EDL75-022315	EL
Valb1402	1.0000	EL	0.9160	EL	EDL75-120214	EL
Valb0593	0.9595	EL	0.8020	EL	EDL76-022315	EL
Valb0608	0.6940	EL	0.7980	EL	EDL76-120214	EL
Valb0808	0.9205	EL	0.8720	EL	EDL77-022315	EL
Valb0750	0.9998	EL	0.7240	EL	EDL77-120214	EL
Valb0907	0.9459	EL	0.6720	EL	EDL78-022315	EL
Valb0585	0.9891	EL	0.9180	EL	EDL78-120214	EL
Valb1638	0.9832	EL	0.6000	EL	EDL79-022315	EL
Valb1640	0.9906	EL	0.8500	EL	EDL79-120214	EL
Valb1430	0.9812	EL	0.7580	EL	ELL06-022315	EL
Valb1155	0.9995	EL	0.8080	EL	ELL06-120214	EL

Valb1553	0.9783	EL	0.7780	EL	ELL07-022315	EL
Valb1562	0.9999	EL	0.7920	EL	ELL07-120214	EL
Valb1445	0.8085	EL	0.7160	EL	ELL08-022315	EL
Valb1188	0.9983	EL	0.7860	EL	ELL08-120214	EL
Valb1613	0.9993	EL	0.8640	EL	ELL09-022315	EL
Valb1514	1.0000	EL	0.8820	EL	ELL09-120214	EL
Valb1479	0.3775	STARI	0.6320	EL	ELL10-022315	EL
Valb0933	0.9095	EL	0.8380	EL	ELL10-120214	EL
Valb0923	0.7083	EL	0.8120	EL	ELL16-022315	EL
Valb0338	0.7215	EL	0.8320	EL	ELL16-120214	EL
Valb0783	0.7849	EL	0.8880	EL	ELL17-022315	EL
Valb0261	0.9862	EL	0.9120	EL	ELL17-120214	EL
Valb1264	0.9418	EL	0.8240	EL	ELL18-022315	EL
Valb0545	0.9738	EL	0.8480	EL	ELL18-120214	EL
Valb1427	0.9704	EL	0.8480	EL	ELL61-022315	EL
Valb1071	0.9664	EL	0.7620	EL	ELL61-120214	EL
Valb1211	0.7950	EL	0.7360	EL	ELL62-022315	EL
Valb1217	0.7831	EL	0.8360	EL	ELL62-120214	EL
Valb1414	0.9892	EL	0.9100	EL	ELL63-022315	EL

Valb1104	0.9699	EL	0.8600	EL	ELL63-120214	EL
Valb0736	0.9469	EL	0.9300	EL	ELL64-022315	EL
Valb0384	0.9780	EL	0.9040	EL	ELL64-120214	EL
Valb0672	0.9415	EL	0.7680	EL	ELL65-022315	EL
Valb0300	0.9927	EL	0.8920	EL	ELL65-120214	EL
Valb1018	0.9093	EL	0.8320	EL	ELL66-022315	EL
Valb0458	0.8905	EL	0.8480	EL	ELL66-120214	EL
Valb1356	0.9174	EL	0.8100	EL	ELL67-022315	EL
Valb0492	0.9747	EL	0.7260	EL	ELL67-120214	EL
Valb1561	0.0313	STARI	0.4860	STARI	M06A-022315	STARI
Valb1328	0.8608	EL	0.6060	EL	M06A-120214	STARI
Valb0329	0.1613	STARI	0.2680	STARI	M09A-022315	STARI
Valb0070	0.2476	STARI	0.4080	STARI	M09A-120214	STARI
Valb1052	0.0242	STARI	0.4060	STARI	M13A-022315	STARI
Valb0809	0.8461	EL	0.8340	EL	M13A-120214B	STARI
Valb1256	0.0157	STARI	0.2900	STARI	M16A-022315	STARI
Valb1100	0.3798	STARI	0.4120	STARI	M16A-120214	STARI
Valb1236	0.2314	STARI	0.6800	EL	M19A-022315	STARI
Valb0580	0.5508	EL	0.6140	EL	M19A-120214	STARI

Valb1525	0.7045	EL	0.4720	STARI	M22A-022315	STARI
Valb0534	0.0496	STARI	0.4580	STARI	M22A-120214	STARI
Valb0556	0.1448	STARI	0.3400	STARI	M26A-022315	STARI
Valb0116	0.4234	STARI	0.2860	STARI	M26A-120214	STARI
Valb0461	0.0037	STARI	0.2360	STARI	M27A-022315	STARI
Valb0266	0.1015	STARI	0.2080	STARI	M27A-120214	STARI
Valb0447	0.0316	STARI	0.1220	STARI	S03-022315	STARI
Valb0026	0.0060	STARI	0.1420	STARI	S03-120214	STARI
Valb1114	0.0010	STARI	0.1760	STARI	S09-022315	STARI
Valb0464	0.0254	STARI	0.2120	STARI	S09-120214	STARI
Valb1292	0.0004	STARI	0.1280	STARI	S17-022315	STARI
Valb0754	0.0005	STARI	0.1020	STARI	S17-120214	STARI
Valb0434	0.0257	STARI	0.2520	STARI	S21-022315	STARI
Valb0044	0.0559	STARI	0.4300	STARI	S21-120214	STARI
Valb0873	0.0173	STARI	0.1840	STARI	S33-022315	STARI
Valb0352	0.0012	STARI	0.2200	STARI	S33-120214	STARI
Valb1141	0.0001	STARI	0.1120	STARI	S39-022315	STARI
Valb0480	0.0002	STARI	0.1160	STARI	S39-120214	STARI
Valb0618	0.0158	STARI	0.3220	STARI	S43-022315	STARI

Valb0660	0.1493	STARI	0.3020	STARI	S43-120214	STARI
Valb0223	0.0007	STARI	0.0960	STARI	S47-022315	STARI
Valb0054	0.0095	STARI	0.0940	STARI	S47-120214	STARI
Valb0335	0.0093	STARI	0.0660	STARI	S53-022315	STARI
Valb0197	0.0183	STARI	0.0360	STARI	S53-120214	STARI
Valb0409	0.2080	STARI	0.2080	STARI	S55-022315	STARI
Valb0060	0.0332	STARI	0.1280	STARI	S55-120214	STARI
Valb0437	0.0004	STARI	0.0980	STARI	S65-022315	STARI
Valb0093	0.0003	STARI	0.1500	STARI	S65-120214	STARI

Table S6. Regression coefficients (β) of the LASSO three-way statistical model. The regression coefficient for each of the 82 MFs (CSU/CDC-#) used in the LASSO three-way classification model are provided. The regression coefficients were generated with data from the Training-Set samples, and applied in the classification of the Test-Set samples as shown in table S7.

MF Id	Early Lyme Disease	Healthy Controls	STARI
Intercept	0.5755	-0.4927	-0.0828
CSU/CDC-001	0.37556	0	0
CSU/CDC-003	0	0.4377	0
CSU/CDC-004	0	0.00298	0
CSU/CDC-006	0.0704	0	0
CSU/CDC-008	-0.1193	0	0
CSU/CDC-009	0.22921	0	0
CSU/CDC-012	0.15307	0	-0.2457
CSU/CDC-013	0	0	-0.1007
CSU/CDC-014	0	0	0.72128
CSU/CDC-017	0.11117	0	0
CSU/CDC-026	0	-0.0633	0.05925
CSU/CDC-030	0	0.05795	0
CSU/CDC-039	0	-0.6065	0.06517
CSU/CDC-042	-0.4151	0.02856	0
CSU/CDC-052	0	0.05484	0
CSU/CDC-061	0	0.08714	0
CSU/CDC-062	0	0	0.60672
CSU/CDC-066	0	0	-0.3676
CSU/CDC-067	-1.1528	0	0
CSU/CDC-070	-0.5929	0.5531	0
CSU/CDC-072	0	0	-0.0857
CSU/CDC-074	0.01711	0	0
CSU/CDC-075	0	0	0.18553
CSU/CDC-083	0	-0.0872	0
CSU/CDC-084	0	-0.2013	0.21541
CSU/CDC-086	-1.1622	0	0.06776
CSU/CDC-087	0	0.03553	0
CSU/CDC-091	0	-0.6683	0

CSU/CDC-095	0	0	-0.0694
CSU/CDC-098	0	0.05396	0
CSU/CDC-099	0	-0.0398	0
CSU/CDC-107	0.36836	0	-0.1847
CSU/CDC-112	0	1.11724	0
CSU/CDC-115	0	0.12435	0
CSU/CDC-128	0	0.4206	-0.1927
CSU/CDC-132	0	0	1.0998
CSU/CDC-133	0.35613	-0.1349	0
CSU/CDC-134	0	-0.1009	0
CSU/CDC-136	0	-1.2108	0
CSU/CDC-137	0	-0.2512	0
CSU/CDC-138	-0.0183	0	0
CSU/CDC-141	0	0	0.2233
CSU/CDC-144	0	-0.1318	0
CSU/CDC-152	0.70277	0	0
CSU/CDC-155	0.27512	0	0
CSU/CDC-157	0	0	0.0505
CSU/CDC-158	0	1.89865	0
CSU/CDC-164	-0.2964	0	0
CSU/CDC-165	0	-0.4008	0
CSU/CDC-166	0.14382	0	0
CSU/CDC-181	0	1.3044	0
CSU/CDC-183	0	-0.7613	0.01014
CSU/CDC-184	0.35021	0	0
CSU/CDC-186	0	0.40861	0
CSU/CDC-188	0	0.5533	0
CSU/CDC-193	0	-1.2355	0
CSU/CDC-194	0	0.57412	0
CSU/CDC-203	-0.0308	0	0
CSU/CDC-205	0.50193	0	-0.3139
CSU/CDC-206	0	-0.0668	0
CSU/CDC-210	0	0	-0.218
CSU/CDC-211	-0.7208	0	0.20891
CSU/CDC-212	0	0	-0.0139
CSU/CDC-213	0	0	-0.2463

CSU/CDC-218	0	0.00722	0
CSU/CDC-219	-1.0252	0	0
CSU/CDC-222	0	-0.4632	0
CSU/CDC-224	0	-0.516	0
CSU/CDC-227	-0.4157	0	0.59261
CSU/CDC-229	0	0	0.86651
CSU/CDC-235	-0.9905	0	0
CSU/CDC-019	0	-0.0326	0.52245
CSU/CDC-237	0	0.62355	0
CSU/CDC-238	0	0	0.96539
CSU/CDC-244	1.5845	0	0
CSU/CDC-245	0	-1.3521	0
CSU/CDC-248	-0.0904	0	0.06017
CSU/CDC-250	0	0	-0.0882
CSU/CDC-252	0	-0.0646	0
CSU/CDC-253	0	0	0.16563
CSU/CDC-254	-0.1985	0	0
CSU/CDC-258	0	0	-0.7011

Table S7. LASSO and RF three-way model classification probability scores. The LASSO and RF probability scores are provided for each patient sample tested in duplicate. These are probability scores for the Test-Set samples. Both the LASSO and RF classifiers provided a probability score for a sample being early Lyme disease patient (EL), healthy control (HC) and STARI. The sample was classified based on the highest probability score for membership in one of the three groups (EL, HC, or STARI).

Coded Sample ID	LASSO Probability Score for EL	LASSO Probability Score for HC	LASSO Probability Score for STARI	LASSO Classification	RF Probability Score for EL	RF Probability Score for HC	RF Probability Score for STARI	RF Classification	Sample ID	Patient Type
Valb1618	0.9998	0.0000	0.0002	EL	0.8420	0.0560	0.1020	EL	EDL134-022315	EL
Valb1591	1.0000	0.0000	0.0000	EL	0.8600	0.0320	0.1080	EL	EDL134-120214	EL
Valb1454	0.9978	0.0003	0.0019	EL	0.5140	0.0840	0.4020	EL	EDL135-022315	EL
Valb0820	0.9798	0.0010	0.0192	EL	0.6560	0.1140	0.2300	EL	EDL135-120214	EL
Valb0989	0.9765	0.0190	0.0045	EL	0.3620	0.5660	0.0720	HC	EDL136-022315	EL
Valb0546	0.9184	0.0198	0.0618	EL	0.5760	0.3360	0.0880	EL	EDL136-120214	EL
Valb1573	0.6006	0.3980	0.0015	EL	0.4640	0.1620	0.3740	EL	EDL137-022315	EL
Valb1299	0.0350	0.0012	0.9639	STARI	0.4640	0.1380	0.3980	EL	EDL137-120214	EL
Valb0477	0.9823	0.0001	0.0175	EL	0.5760	0.2480	0.1760	EL	EDL138-022315	EL

Valb0160	0.9570	0.0284	0.0146	EL	0.5800	0.3560	0.0640	EL	EDL138-120214	EL
Valb0813	0.7815	0.1288	0.0897	EL	0.3380	0.3340	0.3280	EL	EDL139-022315	EL
Valb0443	0.1403	0.8550	0.0047	HC	0.5140	0.3480	0.1380	EL	EDL139-120214	EL
Valb1412	0.9258	0.0010	0.0732	EL	0.5260	0.1860	0.2880	EL	EDL140-022315	EL
Valb0886	0.6301	0.1495	0.2204	EL	0.4060	0.4380	0.1560	HC	EDL140-120214	EL
Valb0827	0.9395	0.0600	0.0005	EL	0.5600	0.3240	0.1160	EL	EDL71-022315	EL
Valb0186	0.9623	0.0341	0.0036	EL	0.5460	0.3980	0.0560	EL	EDL71-120214	EL
Valb1337	0.9873	0.0000	0.0127	EL	0.6840	0.0480	0.2680	EL	EDL73-022315	EL
Valb0714	0.9991	0.0000	0.0009	EL	0.7480	0.0740	0.1780	EL	EDL73-120214	EL
Valb1510	0.9795	0.0000	0.0205	EL	0.6700	0.1140	0.2160	EL	EDL74-022315	EL
Valb0642	0.9990	0.0002	0.0008	EL	0.7280	0.1080	0.1640	EL	EDL74-120214	EL
Valb1586	1.0000	0.0000	0.0000	EL	0.8180	0.0920	0.0900	EL	EDL75-022315	EL

Valb1402	1.0000	0.0000	0.0000	EL	0.8460	0.0640	0.0900	EL	EDL75-120214	EL
Valb0593	0.9699	0.0155	0.0146	EL	0.5380	0.3180	0.1440	EL	EDL76-022315	EL
Valb0608	0.2554	0.4250	0.3197	HC	0.4000	0.4320	0.1680	HC	EDL76-120214	EL
Valb0808	0.9747	0.0135	0.0118	EL	0.5080	0.3480	0.1440	EL	EDL77-022315	EL
Valb0750	1.0000	0.0000	0.0000	EL	0.5600	0.2140	0.2260	EL	EDL77-120214	EL
Valb0907	0.9570	0.0006	0.0424	EL	0.5640	0.1900	0.2460	EL	EDL78-022315	EL
Valb0585	0.8967	0.0837	0.0196	EL	0.5760	0.3440	0.0800	EL	EDL78-120214	EL
Valb1638	0.9978	0.0000	0.0022	EL	0.5880	0.0940	0.3180	EL	EDL79-022315	EL
Valb1640	0.9891	0.0000	0.0109	EL	0.8180	0.0700	0.1120	EL	EDL79-120214	EL
Valb1430	0.9960	0.0000	0.0040	EL	0.6740	0.0980	0.2280	EL	ELL06-022315	EL
Valb1155	0.9921	0.0073	0.0006	EL	0.7140	0.1020	0.1840	EL	ELL06-120214	EL
Valb1553	0.9522	0.0308	0.0170	EL	0.4940	0.3240	0.1820	EL	ELL07-022315	EL

Valb1562	0.9989	0.0011	0.0000	EL	0.6360	0.1900	0.1740	EL	ELL07-120214	EL
Valb1445	0.8847	0.0032	0.1122	EL	0.6300	0.1880	0.1820	EL	ELL08-022315	EL
Valb1188	0.9871	0.0124	0.0005	EL	0.6260	0.1600	0.2140	EL	ELL08-120214	EL
Valb1613	1.0000	0.0000	0.0000	EL	0.8320	0.0740	0.0940	EL	ELL09-022315	EL
Valb1514	1.0000	0.0000	0.0000	EL	0.7780	0.1120	0.1100	EL	ELL09-120214	EL
Valb1479	0.2786	0.1610	0.5604	STARI	0.5340	0.2020	0.2640	EL	ELL10-022315	EL
Valb0933	0.5295	0.3586	0.1119	EL	0.6060	0.2880	0.1060	EL	ELL10-120214	EL
Valb0923	0.6352	0.1147	0.2501	EL	0.5600	0.2900	0.1500	EL	ELL16-022315	EL
Valb0338	0.4277	0.0788	0.4935	STARI	0.4760	0.4300	0.0940	EL	ELL16-120214	EL
Valb0783	0.8276	0.0090	0.1634	EL	0.5720	0.3660	0.0620	EL	ELL17-022315	EL
Valb0261	0.9899	0.0038	0.0064	EL	0.6060	0.3060	0.0880	EL	ELL17-120214	EL
Valb1264	0.7738	0.0116	0.2146	EL	0.5880	0.2880	0.1240	EL	ELL18-022315	EL

Valb0545	0.1309	0.8465	0.0225	HC	0.5000	0.3480	0.1520	EL	ELL18-120214	EL
Valb1427	0.9965	0.0022	0.0012	EL	0.5460	0.3180	0.1360	EL	ELL61-022315	EL
Valb1071	0.9949	0.0040	0.0011	EL	0.5240	0.3060	0.1700	EL	ELL61-120214	EL
Valb1211	0.6844	0.3003	0.0153	EL	0.4780	0.3280	0.1940	EL	ELL62-022315	EL
Valb1217	0.0136	0.9855	0.0009	HC	0.4560	0.4140	0.1300	EL	ELL62-120214	EL
Valb1414	0.9456	0.0523	0.0020	EL	0.6260	0.2680	0.1060	EL	ELL63-022315	EL
Valb1104	0.4263	0.5711	0.0026	HC	0.4460	0.4700	0.0840	HC	ELL63-120214	EL
Valb0736	0.8514	0.1341	0.0145	EL	0.4700	0.4880	0.0420	HC	ELL64-022315	EL
Valb0384	0.7501	0.2400	0.0100	EL	0.4000	0.5680	0.0320	HC	ELL64-120214	EL
Valb0672	0.9502	0.0479	0.0019	EL	0.4200	0.4660	0.1140	HC	ELL65-022315	EL
Valb0300	0.9441	0.0527	0.0032	EL	0.5220	0.4020	0.0760	EL	ELL65-120214	EL
Valb1018	0.2340	0.7645	0.0015	HC	0.3360	0.6140	0.0500	HC	ELL66-022315	EL

Valb0458	0.5250	0.4676	0.0074	EL	0.2980	0.6620	0.0400	HC	ELL66-120214	EL
Valb1356	0.6663	0.3313	0.0024	EL	0.6480	0.1860	0.1660	EL	ELL67-022315	EL
Valb0492	0.7816	0.2169	0.0015	EL	0.5200	0.3160	0.1640	EL	ELL67-120214	EL
Valb0408	0.0012	0.9984	0.0004	HC	0.0840	0.8860	0.0300	HC	HCN07-022315	HC
Valb0311	0.0039	0.9653	0.0308	HC	0.0720	0.8880	0.0400	HC	HCN07-120214	HC
Valb0440	0.0006	0.9993	0.0001	HC	0.1480	0.8140	0.0380	HC	HCN08-022315	HC
Valb0123	0.0189	0.9758	0.0053	HC	0.1960	0.7700	0.0340	HC	HCN08-120214	HC
Valb0327	0.0029	0.9970	0.0001	HC	0.1180	0.8600	0.0220	HC	HCN09-022315	HC
Valb0112	0.0000	0.9995	0.0005	HC	0.0540	0.9260	0.0200	HC	HCN09-120214	HC
Valb1108	0.0042	0.9957	0.0001	HC	0.3780	0.5120	0.1100	HC	HCN16-022315	HC
Valb0269	0.0724	0.9238	0.0039	HC	0.0700	0.9120	0.0180	HC	HCN16-120214	HC
Valb0411	0.0243	0.9710	0.0047	HC	0.2760	0.6700	0.0540	HC	HCN17-022315	HC

Valb0029	0.0491	0.9435	0.0074	HC	0.0620	0.9220	0.0160	HC	HCN17-120214	HC
Valb0860	0.1211	0.8540	0.0250	HC	0.3560	0.4300	0.2140	HC	HCN18-022315	HC
Valb0302	0.0198	0.9792	0.0010	HC	0.0240	0.9720	0.0040	HC	HCN18-120214	HC
Valb0709	0.0060	0.9930	0.0010	HC	0.2980	0.5740	0.1280	HC	HCN19-022315	HC
Valb0178	0.0024	0.9940	0.0036	HC	0.0480	0.9260	0.0260	HC	HCN19-120214	HC
Valb0962	0.0978	0.8543	0.0479	HC	0.3700	0.4420	0.1880	HC	HCN25-022315	HC
Valb0418	0.6988	0.1304	0.1708	EL	0.2500	0.5540	0.1960	HC	HCN25-120214	HC
Valb0632	0.0014	0.9982	0.0005	HC	0.1080	0.8440	0.0480	HC	HCN28-022315	HC
Valb0124	0.0226	0.9655	0.0119	HC	0.0800	0.8780	0.0420	HC	HCN28-120214	HC
Valb0690	0.9013	0.0929	0.0058	EL	0.5920	0.3340	0.0740	EL	HCN29-022315	HC
Valb0066	0.0876	0.8866	0.0257	HC	0.1260	0.8560	0.0180	HC	HCN29-120214	HC
Valb1466	0.0038	0.9957	0.0005	HC	0.1860	0.7800	0.0340	HC	HCW13-022315	HC

Valb0777	0.2406	0.7540	0.0054	HC	0.1320	0.8560	0.0120	HC	HCW13 -120214	HC
Valb1405	0.0021	0.9959	0.0019	HC	0.2540	0.5900	0.1560	HC	HCW21 -022315	HC
Valb0802	0.2993	0.6973	0.0034	HC	0.1660	0.8180	0.0160	HC	HCW21 -120214	HC
Valb1254	0.5258	0.4539	0.0203	EL	0.4020	0.3720	0.2260	EL	HCW25 -022315	HC
Valb0697	0.0064	0.9906	0.0031	HC	0.4060	0.4180	0.1760	HC	HCW25 -120214	HC
Valb1138	0.0005	0.9988	0.0007	HC	0.1720	0.7260	0.1020	HC	HCW26 -022315	HC
Valb0520	0.0041	0.9956	0.0004	HC	0.1580	0.7940	0.0480	HC	HCW26 -120214	HC
Valb1119	0.0001	0.9998	0.0001	HC	0.2120	0.7240	0.0640	HC	HCW28 -022315	HC
Valb0572	0.1165	0.8831	0.0004	HC	0.1180	0.8600	0.0220	HC	HCW28 -120214	HC
Valb0943	0.0616	0.9320	0.0064	HC	0.2260	0.5440	0.2300	HC	HCW29 -022315	HC
Valb0419	0.3990	0.5992	0.0018	HC	0.2480	0.6840	0.0680	HC	HCW29 -120214	HC
Valb1282	0.0191	0.6025	0.3783	HC	0.2980	0.4380	0.2640	HC	HCW34 -022315	HC

Valb0719	0.0209	0.9768	0.0024	HC	0.0980	0.8980	0.0040	HC	HCW34-120214	HC
Valb1535	0.0056	0.9885	0.0059	HC	0.2160	0.5380	0.2460	HC	HCW37-022315	HC
Valb1091	0.0163	0.9766	0.0071	HC	0.2120	0.7280	0.0600	HC	HCW37-120214	HC
Valb1509	0.1004	0.8845	0.0151	HC	0.3080	0.5860	0.1060	HC	HCW44-022315	HC
Valb0944	0.0532	0.9143	0.0325	HC	0.2300	0.7280	0.0420	HC	HCW44-120214	HC
Valb1349	0.0037	0.9898	0.0066	HC	0.3080	0.6100	0.0820	HC	HCW46-022315	HC
Valb0801	0.0039	0.9822	0.0139	HC	0.2640	0.6500	0.0860	HC	HCW46-120214	HC
Valb1561	0.0005	0.0000	0.9995	STARI	0.0044	0.1788	0.8168	STARI	M06A-022315	STARI
Valb1328	0.6469	0.0097	0.3434	EL	0.5180	0.0960	0.3860	EL	M06A-120214	STARI
Valb0329	0.2186	0.0048	0.7767	STARI	0.2140	0.0740	0.7120	STARI	M09A-022315	STARI
Valb0070	0.0212	0.0066	0.9722	STARI	0.2480	0.0980	0.6540	STARI	M09A-120214	STARI
Valb1052	0.0298	0.0061	0.9640	STARI	0.3840	0.1920	0.4240	STARI	M13A-022315	STARI

Valb0809	0.9494	0.0020	0.0486	EL	0.6200	0.1560	0.2240	EL	M13A-120214 B	STARI
Valb1256	0.0016	0.0002	0.9982	STARI	0.2340	0.1440	0.6220	STARI	M16A-022315	STARI
Valb1100	0.0232	0.0055	0.9713	STARI	0.2400	0.0820	0.6780	STARI	M16A-120214	STARI
Valb1236	0.1166	0.0227	0.8607	STARI	0.4740	0.2340	0.2920	EL	M19A-022315	STARI
Valb0580	0.1942	0.1003	0.7055	STARI	0.4080	0.1800	0.4120	STARI	M19A-120214	STARI
Valb1525	0.9962	0.0000	0.0038	EL	0.3660	0.1700	0.4640	STARI	M22A-022315	STARI
Valb0534	0.1791	0.0000	0.8208	STARI	0.3520	0.1880	0.4600	STARI	M22A-120214	STARI
Valb0556	0.3684	0.1161	0.5155	STARI	0.3120	0.0300	0.6580	STARI	M26A-022315	STARI
Valb0116	0.4121	0.0005	0.5874	STARI	0.1900	0.0560	0.7540	STARI	M26A-120214	STARI
Valb0461	0.0048	0.0293	0.9659	STARI	0.2000	0.0860	0.7140	STARI	M27A-022315	STARI
Valb0266	0.0169	0.0001	0.9830	STARI	0.1300	0.0560	0.8140	STARI	M27A-120214	STARI
Valb0447	0.0016	0.1106	0.8877	STARI	0.1280	0.0780	0.7940	STARI	S03-022315	STARI

Valb0026	0.0005	0.0004	0.9992	STARI	0.1320	0.0640	0.8040	STARI	S03-120214	STARI
Valb1114	0.0013	0.0004	0.9982	STARI	0.1800	0.2660	0.5540	STARI	S09-022315	STARI
Valb0464	0.1404	0.0000	0.8596	STARI	0.1320	0.2000	0.6680	STARI	S09-120214	STARI
Valb1292	0.0002	0.0000	0.9997	STARI	0.1360	0.1980	0.6660	STARI	S17-022315	STARI
Valb0754	0.0001	0.0000	0.9999	STARI	0.0980	0.1480	0.7540	STARI	S17-120214	STARI
Valb0434	0.0209	0.0896	0.8896	STARI	0.1780	0.2000	0.6220	STARI	S21-022315	STARI
Valb0044	0.0148	0.0203	0.9649	STARI	0.2560	0.1920	0.5520	STARI	S21-120214	STARI
Valb0873	0.0079	0.0169	0.9753	STARI	0.1340	0.2480	0.6180	STARI	S33-022315	STARI
Valb0352	0.0003	0.0087	0.9910	STARI	0.1280	0.2180	0.6540	STARI	S33-120214	STARI
Valb1141	0.0000	0.0169	0.9831	STARI	0.1060	0.1100	0.7840	STARI	S39-022315	STARI
Valb0480	0.0000	0.0002	0.9998	STARI	0.0540	0.0500	0.8960	STARI	S39-120214	STARI
Valb0618	0.0015	0.0010	0.9975	STARI	0.2640	0.2060	0.5300	STARI	S43-022315	STARI

Valb0660	0.0018	0.0008	0.9973	STARI	0.2700	0.1400	0.5900	STARI	S43-120214	STARI
Valb0223	0.0002	0.0340	0.9658	STARI	0.1080	0.3080	0.5840	STARI	S47-022315	STARI
Valb0054	0.0023	0.0168	0.9808	STARI	0.0640	0.0740	0.8620	STARI	S47-120214	STARI
Valb0335	0.0085	0.0023	0.9893	STARI	0.0660	0.0440	0.8900	STARI	S53-022315	STARI
Valb0197	0.0050	0.0001	0.9949	STARI	0.0320	0.0320	0.9360	STARI	S53-120214	STARI
Valb0409	0.0714	0.0715	0.8571	STARI	0.1680	0.1420	0.6900	STARI	S55-022315	STARI
Valb0060	0.0119	0.0059	0.9821	STARI	0.1020	0.1180	0.7800	STARI	S55-120214	STARI
Valb0437	0.0001	0.0078	0.9921	STARI	0.0800	0.1060	0.8140	STARI	S65-022315	STARI
Valb0093	0.0000	0.0001	0.9999	STARI	0.1060	0.0720	0.8220	STARI	S65-120214	STARI

Table S8. ANOVA results on Lasso and Random Forest scores with sample source as the explanatory variable. Shown are the simultaneous 95% confidence intervals using Tukey's method for the difference in mean LASSO score between groups and the Random Forest difference in mean log probability between groups. CI, confidence interval; RF, Random Forest.

Linear hypothesis*	Simultaneous 95% CI (Tukey)±			
	LASSO Healthy Control scores	**LASSO STARI scores**	**Healthy Control classification probabilities from RF**	**STARI classification probabilities from RF**
MO - CO == 0	-10.3 to -7.1	3.3 to 5.6	-3.5 to –2.8	3.3 to 4.5
NC - CO == 0	-9.0 to -5.6	4.0 to 6.4	-3.3 to -2.6	3.4 to 4.6
NY - CO == 0	-2.8 to 0.1	-0.8 to 1.3	-0.4 to 0.2	0.4 to 1.4
OST - CO == 0	-10.9 to -7.4	4.4 to 6.9	-3.2 to -2.5	3.4 to 4.6
NC - MO == 0	-0.4 to 3.2	-0.5 to 2.1	-0.2 to 0.6	-0.6 to 0.7
NY - MO == 0	5.8 to 8.9	-5.3 to -3.1	2.7 to 3.4	-3.6 to -2.4
OST - MO == 0	-2.3 to 1.4	-0.1 to 2.5	-0.2 to 0.6	-0.6 to 0.7
NY - NC == 0	4.4 to 7.6	-6.1 to -3.8	2.5 to 3.2	-3.7 to -2.5
OST - NC == 0	-3.7 to 0.1	-1.0 to 1.8	-0.4 to 0.4	-0.7 to 0.7
OST - NY == 0	-9.5 to -6.1	4.2 to 6.6	-3.2 to -2.5	2.4 to 3.7

* The hypothesis is that the compared groups are the same (0). ± If the 95% CI includes 0 than the groups are defined as the same.

Table S9. Grouping indicated by the ANOVA results. CO samples represented one control group, NY samples represented another control group, and MO, NC, and OST samples comprised a third (STARI) group. CO, Colorado; NY, New York; MO, Missouri; NC, North Carolina; OST, other states from which STARI samples were collected; RF, Random Forest.

Sample Source	LASSO Healthy Control scores	LASSO STARI scores	Healthy Control classification probabilities from RF	STARI classification probabilities from RF
	Group[†]			
CO	A	A	A	A
MO	B	B	B	B
NY	A	A	A	C[*]
NC	B	B	B	B
OST	B	B	B	B

[†] Groups within each analysis were assigned a letter: A, B or C. [*]The NY healthy control samples differed from both the healthy controls from CO and from the STARI samples from MO, NC and OST when STARI classification probabilities from RF were evaluated.