

Supplemental Material

Improved method for the quantification of lysophospholipids including enol ether species by liquid chromatography-tandem mass spectrometry

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Supplemental Material Table 1. Autosampler and ESI-MSMS data collection parameters.

Injection type	Sequential	
Fill mode	Partial loop	
Pre-sample airy boundary	2 μ L	
Post-sample air boundary	2 μ L	
Post-injection wash frequency	10 cycles	
Flush time	6 sec	
Wash time	15 sec	
Secondary wash volume	600 μ L	
	Negative Mode	Positive Mode
Capillary Voltage (kV)	2.8	3.0
Extractor (V)	3	3
RF Offset	0.3	0.3
Dwell Time (msec) ¹	40	100
Inter-Scan Delay (msec) ¹	10	10
Inter-Channel Delay (msec) ¹	100	100
Source Temp ($^{\circ}$ C)	130	130
Desolvation Temp ($^{\circ}$ C)	450	450
Desolvation Gas (L/Hr)	1100	1100
Cone Gas (L/Hr)	50	50
Collision Cell Entrance (V)	-1	-1
Collision Cell Exit (V)	1	1

¹Each channel consists of all lysophospholipid species with a given polar head group (i.e. all LPG species constitute 1 channel, all LPI species constitute a second channel etc.). Data collection was carried out by cycling through the set of 89 ion reactions (negative mode). The instrument was switched to positive mode at 27.75 min post injection, and a set of 27 ion reactions were monitored. In each cycle, each ion reaction was monitored for 40 msec (Dwell Time) with a 10 msec period between each reaction monitoring in which no data was collected (Inter-Scan Delay, to allow for instrument parameter changes). After collecting data for the set of ion reactions in each lysophospholipid class, there was a 100 msec period in which no data was collected (Inter-Channel Delay).

Supplemental Material Table 2. Extraction yields, liquid chromatography retention times, and tandem mass spectrometry parameters for lysophospholipid molecular species.

Lysophospholipid ¹	Extr. yield (%) ²	LC ret. time (min)	Limit of quant. (fmole) ³	Parent ion ⁴ (<i>m/z</i>)	Fragment ion ⁴ (<i>m/z</i>)	Cone voltage ⁵ (V)	Collision energy ⁵ (eV)
12:0-LPG		9.8		-427.22	-199.22	-38	26
14:0-LPG	65	9.5	80	-455.25	-227.25	-38	26
16:1-LPG		9.3		-481.26	-253.26	-38	26
16:0-LPG	93	9.2	120	-483.28	-255.28	-38	26
d ₃₁ -16:0-LPG Int. Std.		9.2		-514.44	-286.44	-38	26
18:3-LPG		9.2		-505.25	-277.25	-38	26
18:2-LPG		9.1		-507.27	-279.27	-38	26
18:1-LPG		9		-509.29	-281.29	-38	26
18:0-LPG	106	8.9	200	-511.31	-283.31	-38	26
20:4-LPG		9.1		-531.25	-303.25	-38	26
20:3-LPG		9		-533.27	-305.27	-38	26
20:2-LPG		8.8		-535.29	-307.29	-38	26
20:1-LPG		8.7		-537.31	-309.31	-38	26
20:0-LPG		8.7		-539.33	-311.33	-38	26
22:6-LPG		9		-555.24	-327.24	-38	26
22:5-LPG		8.9		-557.26	-329.26	-38	26
22:4-LPG		8.8		-559.28	-331.28	-38	26
12:0-LPI		13.9		-515.2	-199.22	-45	35
14:0-LPI		13.5		-543.23	-227.25	-45	35
16:1-LPI		13.1		-569.26	-253.26	-45	35
16:0-LPI	55	12.9	65	-571.28	-255.28	-45	35
d ₃₁ -16:0-LPI Int. Std.		12.9		-602.44	-286.44	-45	35
18:3-LPI		13		-593.25	-277.25	-45	35
18:2-LPI		12.8		-595.27	-279.27	-45	35
18:1-LPI	72	12.6	120	-597.29	-281.29	-45	35
18:0-LPI	71	12.4	25	-599.31	-283.31	-45	35
20:4-LPI		12.3		-619.26	-303.25	-45	35
20:3-LPI		12.2		-621.28	-305.27	-45	35
20:2-LPI		11.9		-623.3	-307.29	-45	35
20:1-LPI		11.9		-625.32	-309.31	-45	35
20:0-LPI		11.7		-627.34	-311.33	-45	35
22:6-LPI		12.3		-643.25	-327.24	-45	35
22:5-LPI		12.2		-645.27	-329.26	-45	35
22:4-LPI		12		-647.29	-331.28	-45	35
12:0-LPE		15.6		-396.23	-199.22	-32	23
14:0-LPE	94	15.2	40	-424.26	-227.25	-32	23
16:1-LPE		14.9	60	-450.27	-253.26	-32	23
16:0-LPE	95	14.7	60	-452.29	-255.28	-32	23
d ₃₁ -16:0-LPE Int. Std.		14.7		-483.44	-286.44	-32	23
18:3-LPE		14.6		-474.26	-277.25	-32	23
18:2-LPE		14.4		-476.28	-279.27	-32	23
18:1-LPE	80	14.3		-478.3	-281.29	-32	23
18:0-LPE	85	14	50	-480.32	-283.31	-32	23
20:4-LPE		13.6		-500.27	-303.25	-32	23
20:3-LPE		13.6		-502.29	-305.27	-32	23
20:2-LPE		13.9		-504.31	-307.29	-32	23
20:1-LPE		13.7		-506.33	-309.31	-32	23

20:0-LPE		13.5		-508.35	-311.33	-32	23
22:6-LPE		13.6		-524.26	-327.24	-32	23
22:5-LPE		13.4		-526.28	-329.26	-32	23
22:4-LPE		13.2		-528.3	-331.28	-32	23
12:0-LPS		17.4		-440.2	-153.05	-28	30
14:0-LPS		17.2		-468.23	-153.05	-28	30
16:1-LPS		16.6		-494.26	-153.05	-28	30
16:0-LPS	67	16.3		-496.28	-153.05	-28	30
d ₃₁ -16:0-LPS Int. Std.		16.3		-527.44	-153.05	-28	30
18:3-LPS		16.3		-518.25	-153.05	-28	30
18:2-LPS		16.1		-520.27	-153.05	-28	30
18:1-LPS	77	15.9		-522.29	-153.05	-28	30
18:0-LPS		15.6	180	-524.31	-153.05	-28	30
20:4-LPS	82	15.9		-544.26	-153.05	-28	30
20:3-LPS	83	15.7		-546.28	-153.05	-28	30
20:2-LPS	98	15.7		-548.3	-153.05	-28	30
20:1-LPS		16.2		-550.32	-153.05	-28	30
20:0-LPS		15.6		-552.34	-153.05	-28	30
22:6-LPS		15.8		-568.25	-153.05	-28	30
22:5-LPS		15.6		-570.27	-153.05	-28	30
22:4-LPS		15.4		-572.29	-153.05	-28	30
12:0-LPA		22.8		-353.22	-153.05	-28	20
14:0-LPA	96	22.2	300	-381.25	-153.05	-28	20
16:1-LPA		22		-407.26	-153.05	-28	20
16:0-LPA		21.6		-409.28	-153.05	-28	20
d ₃₁ -16:0-LPA Int. Std.		21.6		-440.44	-153.05	-28	20
18:3-LPA		21.9		-431.25	-153.05	-28	20
18:2-LPA		21.6		-433.27	-153.05	-28	20
18:1-LPA		21.2	250	-435.29	-153.05	-28	20
18:0-LPA		20.9		-437.31	-153.05	-28	20
20:4-LPA		21.2		-457.26	-153.05	-28	20
20:3-LPA		21.2		-459.28	-153.05	-28	20
20:2-LPA		21		-461.3	-153.05	-28	20
20:1-LPA		20.5		-463.32	-153.05	-28	20
20:0-LPA		20.2		-465.34	-153.05	-28	20
22:6-LPA		21.2		-481.25	-153.05	-28	20
22:5-LPA		21.2		-483.27	-153.05	-28	20
22:4-LPA		20.9		-485.29	-153.05	-28	20
12:0-LPC	137	37.5	20	+440.27	+184.15	+35	30
14:0-LPC	109	35.8		+468.30	+184.15	+35	30
16:1-LPC	105	35.1		+494.31	+184.15	+35	30
16:0-LPC	98	34.4	20	+496.33	+184.15	+35	30
d ₃₁ -16:0-LPC Int. Std.		34.4		+527.49	+184.15	+35	30
18:3-LPC		34.8		+518.30	+184.15	+35	30
18:2-LPC		34.4		+520.32	+184.15	+35	30
18:1-LPC	106	33.8		+522.34	+184.15	+35	30
18:0-LPC	102	33	14	+524.36	+184.15	+35	30
20:4-LPC		33		+544.32	+184.15	+35	30
20:3-LPC		33.4		+546.33	+184.15	+35	30
20:2-LPC	87	32.9		+548.35	+184.15	+35	30
20:1-LPC	91	32.4		+550.37	+184.15	+35	30
20:0-LPC	107	31.6		+552.39	+184.15	+35	30

22:6-LPC		33.7		+568.34	+184.15	+35	30
22:5-LPC		332.7		+570.36	+184.15	+35	30
22:4-LPC		31.7		+572.38	+184.15	+35	30
24:0-LPC	89	29.3		+608.45	+184.15		
16:1-enyl-LPE		13.2	1200	-436.21	-196.12	-52	29
18:2-enyl-LPE	89	12.9		-462.22	-196.12	-52	29
18:1-enyl-LPE	84	12.6	1400	-464.24	-196.12	-52	29
16:1-enyl-LPC		32.2		+480.30	+181.12	+45	31
18:2-enyl-LPC	105	32		+506.31	+181.12	+45	31
18:1-enyl-LPC		30.8		+508.33	+181.12	+45	31
16:0-alkyl-LPC	68	34.0		+482.38	+184.15	+37	30
d ₄ -16:0-alkyl-LPC		34.0		+486.41	+184.15	+37	30
18:1-alkyl-LPC		33.4		+508.40	+184.15	+37	30
18:0-alkyl-LPC		33.7		+510.41	+184.15	+37	30

¹The internal standard used for enol ether LPE and LPC species are d₃₁-16:0-LPE and d₃₁-16:0-LPC, respectively (see Main Text). Other internal standards are as indicated.

²Extraction yields were determined only for a subset of lysophospholipids as indicated.

³Limit of quantification is defined as a signal-to-noise ratio of ~10/1, as measured with the Waters QuanLynx software. Values are given only for those lysophospholipid species which were prepared as standardized stock solutions.

⁴*m/z* Values listed were derived from instrument tuning using a set of commercially available lysophospholipid standards (see Main Text for the list). Values for other species were obtained by subtracting or adding the appropriate exact atomic masses. Exact *m/z* values will vary in an instrument-dependent manner.

⁵Cone voltages and collision energies were optimized for each commercially available lysophospholipid (see Main Text for the list). We used the same values across the set of molecular species in each lysophospholipid head group class. Values were derived from instrument tuning and are instrument dependent.

Analysis of isobars.

To look for isobaric species, we considered the masses of a general set of lysophospholipids that contain either a fatty acyl *sn*-1 chain or an ether linked *sn*-1 chain (both enol ether and alkyl ether). Polar head groups included were phosphate, phosphocholine, phosphoethanolamine, phosphoglycerol, phosphoinositol, and phosphoserine. We considered acyl and alkyl *sn*-1 chains 12:0, 14:0, 16:0, d_{31} -16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 22:4, 22:5, and 22:6. As a first step, we ignored isobars due to different positions of the double bonds in the fatty chain. We considered all lysophospholipids that are analyzed in negative mode (LPA, LPE, LPG, LPI, and LPS) as one group, and positive mode analytes (LPC) as a second group. Isobars between these two groups are not considered to cause an issue because of the different charge polarity used to analyze ions in negative mode and in positive mode and because it is highly likely that the LC step will lead to separation of all LPC species from all other lysophospholipid species (the group of LPC species are well separated from the group of LPA species). For each of the above lysophospholipids we also analyzed the ion mass + 1 since natural heavy isotopic substitution results in a satellite peak whose intensity is about 30% of the monoisotopic peak (lacking heavy isotopes). We also considered the ion mass -1, -2, and +2 to account for the isotope distribution for the d_{31} -16:0 internal standard for each head group. Thus, the number of negative mode masses considered is 360 (17 fatty chains x 5 polar head groups = 85 *sn*-1-acyl lysophospholipids plus 16 fatty chains (all but d_{31} -16:0) x 5 polar head groups = 80 *sn*-1-ether lysophospholipids gives 165 species, which gives 330 species after consideration of the ion mass + 1 ions). We also considered 6 lysophospholipids with the *sn*-1 fatty acyl chains 17:0 and 17:1 since these are commonly used commercially available internal standards (17:1-LPG, 17:1-LPI, 19:2-enyl-LPE, 17:1-LPE, 17:1-LPS, 17:0-LPA). As a second independent analysis, we considered 74 positive mode masses (all of negative mode masses plus 24:0). Thus, we considered a total of 440 lysophospholipid masses. Supplemental Material Table 3 list all isobars derived from the set of 360 masses for negative mode lysophospholipids (LPA, LPE, LPG, LPI, and LPS).

Supplemental Material Table 3. Isobar analysis of LPA, LPE, LPG, LPI and LPS species.¹

The table is organized as follows. The first column gives the mass at which isobaric lysophospholipid species exists. For all lysophospholipids except the perdeuterated species we considered its parent ion mass as well as the carbon-13 satellite (M+1). For the d_{31} -internal standards we considered the parent ion mass (d_{31}) and the partially deuterated species (d_{30} and d_{29}) as well as the carbon-13 satellite (M+1) and the additional species M+2. For example, when the [M-1] mass equals the isobaric mass, this lysophospholipid species appears in column 3.

The "e" in e12:0-LPE designates an *sn*-1 ether linked fatty chain, which includes alkyl and enol ethers. We use a single designation for ethers since this table does not make a distinction between double bond regioisomers. For example, e18:1-LPC could have an enol ether or an alkyl chain with the double bond more distant from the ether oxygen.

In the “Resolution of Isobars” column, “chromatography” means that the isobars are likely to be resolved by LC. “Q3” means that they are likely to be resolved by MSMS (vs MS). “calculation” means that the peak area for the putative analyte peak is significantly larger than the area calculated for the isobaric internal standard-derived peak where the latter is obtained by knowing the area of the d_{31} internal standard peak and the relative amounts of partially deuterated internal standard species. “Unresolved” means isobaric resolution cannot be made based on observations made in this study. “?” means no definitive statement about isobaric resolution can be made.

In the “Comments on Resolution” column, “likely” means the resolution of isobar statement is based on experimental trends seen with related lysophospholipid species, “experimental” means that isobaric resolution is based on experimental observations made in this study. Isobaric overlaps that are difficult to resolve are flagged in red, all others are in green.

Analysis of LPC isobars shows that aside from double bond regioisomers, there are no isobars as long as d_{31} -16:0-LPC and d_4 -alkyl-16:0-LPC are used as internal standards. If LPC species with odd carbon number fatty acyl chains are used, isobars between multiple LPC species exist. This is of course true for any particular head group; the isobars listed in Supplemental Table 3 are between two lysophospholipids that have different polar head groups. As already noted, all LPC species elute well after the region of LPA elution, and other head group species elute earlier than LPA species. As described in the Main Text, when one of the double bonds is part of an enol ether, it is possible to distinguish the species from a double bond regioisomer isobar which does not contain an enol ether.

Isobaric Mass [M-2]	Isobars [M-1]	Isobars	Parent Ion Isobars	[M+1] Isobars	[M+2] Isobars	Resolution of Isobars	Comments on Resolution
382			e12:0-LPE	14:0-LPA		Chromatography	Likely
396			12:0-LPE	e16:0-LPA		Chromatography	Likely
410			e14:0-LPE	16:0-LPA		Chromatography	Likely
423			e18:0-LPA, 17:0-LPA			Unresolved	
424			14:0-LPE 14:0-LPE	e18:0-LPA 17:0-LPA e18:0-LPA, 17:0-LPA		Chromatography Chromatography Unresolved	Likely Likely
427			12:0-LPG	e12:0-LPS		Chromatography	Likely
436			e16:1-LPE	18:1-LPA		Chromatography	Likely
437			18:0-LPA	e16:1-LPE		Chromatography	Experimental
438	d31-16:0-LPA d31-16:0-LPA		e16:0-LPE e16:0-LPE	18:0-LPA 18:0-LPA		Chromatography calculation Chromatography	Likely Experimental Likely
439	d31-16:0-LPA			e16:0-LPE		Chromatography	Likely
440			d31-16:0-LPA, 12:0-LPS			Chromatography	Likely
441			e14:0-LPG e14:0-LPG	d31-16:0-LPA 12:0-LPS d31-16:0-LPA, 12:0-LPS		Chromatography Chromatography Chromatography	Likely Likely Likely
442				e14:0-LPG	d31-16:0-LPA	Chromatography	Likely
450			16:1-LPE	e20:1-LPA		Q3/Chromatography	Likely
451			e20:0-LPA	16:1-LPE		Chromatography	Likely
452			16:0-LPE	e20:0-LPA		Chromatography	Likely
455			14:0-LPG	e14:0-LPS		Chromatography	Likely
460			e18:3-LPG	20:3-LPA		Chromatography	Likely
461			20:2-LPA	e18:3-LPE		Chromatography	Likely
462			e18:2-LPE	20:2-LPA		Chromatography	Likely
463			20:1-LPA	e18:2-LPE		Chromatography	Likely
464			e18:1-LPE	20:1-LPA		Chromatography	Likely
465			20:0-LPA	e18:1-LPE		Chromatography	Likely
466			e18:0-LPE	20:0-LPA		Chromatography	Likely
467			e22:6-LPA, e16:1-LPG e22:6-LPA e16:1-LPG	e18:0-LPE e18:0-LPE		Chromatography Chromatography Chromatography	Likely Likely Likely
468			14:0-LPS 14:0-LPS	e16:1-LPG e22:6-LPA e22:6-LPA, e16:1-LPG		Chromatography Chromatography Chromatography	Likely Likely Likely
469			e22:5-LPA, e16:0-LPG e22:5-LPA e16:0-LPG	14:0-LPS 14:0-LPS		Chromatography Chromatography Chromatography	Likely Likely Likely
470				e16:0-LPG, e22:5-LPA		Chromatography	Likely
476			18:2-LPE, e19:2-LPE			Unresolved	Experimental
477				18:2-LPE, e19:2-LPE		Unresolved	Experimental
480			e16:1-LPS, 18:0-LPE			Chromatography	Likely

481		22:6-LPA, 16:1-LPG		Chromatography	Likely
		22:6-LPA	e16:1-LPS	Chromatography	Likely
		22:6-LPA	18:0-LPE	Chromatography	Experimental
		16:1-LPG	e16:1-LPS	Chromatography	Likely
		16:1-LPG	18:0-LPE	Q3/Chromatography	Experimental
	d31-16:0-LPE		Chromatography	Likely	
	d31-16:0-LPE	22:6-LPA		Chromatography	Likely
	d31-16:0-LPE	16:1-LPG		Chromatography	Likely
	d31-16:0-LPE		e16:1-LPS	Chromatography	Likely
	d31-16:0-LPE		18:0-LPE	calculation	Likely
482		e16:0-LPS	22:6-LPA	Chromatography	Likely
		e16:0-LPS	16:1-LPG	Chromatography	Likely
			22:6-LPA, 16:1-LPG	Chromatography	Likely
	d31-16:0-LPE	e16:0-LPS		Chromatography	Likely
	d31-16:0-LPE		22:6-LPA	Q3/Chromatography	Likely
	d31-16:0-LPE		16:1-LPG		Likely
483		22:5-LPA, d31-16:0-LPE		Chromatography	Experimental
		22:5-LPA, 16:0-LPG		Chromatography	Likely
		16:0-LPG, d31-16:0-LPE		Q3/Chromatography	Likely
484			22:5-LPA, d31 16:0-LPE	Chromatography	Likely
			16:0-LPG, d31 16:0-LPE	Q3/Chromatography	Likely
			22:5-LPA, 16:0-LPG	Chromatography	Likely
486		e20:4-LPE	22:4-LPA	Chromatography	Likely
491		e18:3-LPG	e20:2-LPE	Chromatography	Likely
492		e20:1-LPE	e18:3-LPG	Chromatography	Likely
493		e18:2-LPG	e20:1-LPE	Chromatography	Likely
494		16:1-LPS, e20:0-LPE		Chromatography	Likely
		16:1-LPS	e18:2-LPG	Chromatography	Likely
		e20:0-LPE	e18:2-LPG	Chromatography	Likely
495		e18:1-LPG, 17:1-LPG		Unresolved	
		e18:1-LPG	16:1-LPS	Chromatography	Likely
		e18:1-LPG	e20:0-LPE	Chromatography	Likely
		17:1-LPG	16:1-LPS	Chromatography	Likely
		17:1-LPG	e20:0-LPE	Chromatography	Likely
		16:1-LPS, e20:0-LPE	Chromatography	Likely	
496		16:0-LPS	e18:1-LPG	Chromatography	Likely
		16:0-LPS	17:1-LPG	Chromatography	Experimental
			e18:1-LPG, 17:1-LPG	Unresolved	
497		e18:0-LPG	16:0-LPS	Chromatography	Likely
501		e12:0-LPI	20:4-LPE	Chromatography	Likely
502		20:3-LPE	e12:0-LPI	Chromatography	Likely
504		e18:3-LPS, 20:2-LPE		Chromatography	Likely
505		18:3-LPG	e18:3-LPS	Chromatography	Likely
		18:3-LPG	20:2-LPE	Q3/Chromatography	Experimental
			e18:3-LPS, 20:2-LPE	Chromatography	Likely
506		e18:2-LPS, 20:1-LPE		Chromatography	Likely
		e18:2-LPS	18:3-LPG	Chromatography	Likely
		20:1-LPE	18:3-LPG	Q3/Chromatography	Experimental
507		18:2-LPG	e18:2-LPS	Chromatography	Likely
		18:2-LPG	20:1-LPE	Q3/Chromatography	Experimental
			e18:2-LPS, 20:1-LPE	Chromatography	Likely
508		e18:1-LPS, 20:0-LPE		Chromatography	Likely
		e18:1-LPS, 17:1-LPS		Unresolved	
		20:0-LPE, 17:1-LPS		Chromatography	Likely
		e18:1-LPS	18:2-LPG	Chromatography	Likely
		20:0-LPE	18:2-LPG	Q3/Chromatography	Experimental
		17:1-LPS	18:2-LPG	Chromatography	Likely

509		18:1-LPG 18:1-LPG 18:1-LPG	e18:1-LPS 20:0-LPE 17:1-LPS 17:1-LPS, e18:1-LPS 17:1-LPS, 20:0-LPE e18:1-LPS, 20:0-LPE		Q3/Chromatography Chromatography Unresolved Chromatography Chromatography	Likely Experimental Experimental Likely Likely
510		e18:0-LPS, e22:6-LPE e18:0-LPS e22:6-LPE	18:1-LPG 18:1-LPG		Chromatography Chromatography Q3/Chromatography	Likely Likely Likely
511		18:0-LPG 18:0-LPG	e18:0-LPS e22:6-LPE e18:0-LPS, e22:6-LPE		Chromatography Chromatography Chromatography	Likely Likely Likely
512	d31-16:0-LPG d31-16:0-LPG	e22:5-LPE e22:5-LPE	18:0-LPG 18:0-LPG		Chromatography Chromatography calculation	Likely Likely Likely
513	d31-16:0-LPG		e22:5-LPE		Chromatography	Likely
514		d31-16:0-LPG, e22:4-LPE			Chromatography	Likely
515		12:0-LPI 12:0-LPI	d31-16:0-LPG e22:4-LPE d31-16:0-LPG, e22:4-LPE		Q3/Chromatography Q3 Q3/Chromatography	Likely Likely Likely
516			12:0-LPI	d31-16:0-LPG	Q3/Chromatography	Likely
518		18:3-LPS	e20:4-LPG		Chromatography	Likely
519		e20:3-LPG	18:3-LPS		Chromatography	Likely
520		18:2-LPS	e20:3-LPG		Chromatography	Likely
521		e20:2-LPG	18:2-LPS		Chromatography	Likely
522		18:1-LPS	e20:2-LPG		Chromatography	Likely
523		e20:1-LPG	18:1-LPS		Chromatography	Likely
524		18:0-LPS, 22:6-LPE 18:0-LPS 22:6-LPE	e20:1-LPG e20:1-LPG		Chromatography Chromatography Chromatography	Experimental Likely Likely
525	d31-16:0-LPS d31-16:0-LPS d31-16:0-LPS	e20:0-LPG e20:0-LPG e20:0-LPG	18:0-LPS 22:6-LPE 18:0-LPS, 22:6-LPE 18:0-LPS 22:6-LPE		Chromatography Chromatography Chromatography Chromatography calculation Chromatography	Likely Likely Likely Experimental Experimental Experimental
526	d31-16:0-LPS d31-16:0-LPS	22:5-LPE 22:5-LPE	e20:0-LPG e20:0-LPG		Chromatography Chromatography Chromatography	Likely Experimental Likely
527		d31-16:0-LPS	22:5-LPE		Chromatography	Experimental
528		22:4-LPE	d31-16:0-LPS		Chromatography	Experimental
529		e14:0-LPI e14:0-LPI	22:4-LPE 22:4-LPE	d31-16:0-LPS d31-16:0-LPS	Chromatography Chromatography Chromatography	Likely Likely Experimental
530		e20:4-LPS	e14:0-LPI		Chromatography	Likely
531		20:4-LPG	e20:4-LPS		Chromatography	Likely
532		e20:3-LPS	20:4-LPG		Chromatography	Likely
533		20:3-LPG	e20:3-LPS		Chromatography	Likely
534		e20:2-LPS	20:3-LPG		Chromatography	Likely
535		20:2-LPG	e20:2-LPS		Chromatography	Likely
536		e20:1-LPS	20:2-LPG		Chromatography	Likely
537		20:1-LPG	e20:1-LPS		Chromatography	Likely
538		e20:0-LPS	20:1-LPG		Chromatography	Likely
539		20:0-LPG	e20:0-LPS		Chromatography	Likely
543		e22:5-LPG, 14:0-LPI			Chromatography, Q3	Likely

544	20:4-LPS 20:4-LPS	e22:5-LPG 14:0-LPI e22:5-LPG, 14:0-LPI	Chromatography Chromatography Chromatography,Q3	Likely Likely Likely
545		e22:4-LPG 20:4-LPS	Chromatography	Likely
546		20:3-LPS e22:4-LPG	Chromatography	Likely
555	22:6-LPG, e16:1-LPI 22:6-LPG e16:1-LPI	e22:6-LPS e22:6-LPS	Chromatography,Q3 Chromatography Chromatography	Likely Likely Likely
556	e22:5-LPS e22:5-LPS	e16:1-LPI 22:6-LPG e16:1-LPI, 22:6-LPG	Chromatography Chromatography Q3	Likely Likely Likely
557	22:5-LPG, e16:0-LPI 22:5-LPG e16:0-LPI	e22:5-LPS e22:5-LPS	Chromatography,Q3 Chromatography Chromatography	Likely Likely Likely
558	e22:4-LPS e22:4-LPS	e16:0-LPI 22:5-LPG e16:0-LPI, 22:5-LPG	Chromatography Chromatography Q3	Likely Likely Likely
559		22:4-LPG e22:4-LPS	Chromatography	Likely
569		16:1-LPI 22:6-LPS	Chromatography	Experimental
570		22:5-LPS 16:1-LPI	Chromatography	Likely
571		16:0-LPI 22:5-LPS	Chromatography	Experimental
572		22:4-LPS 16:0-LPI	Chromatography	Experimental
583		17:1-LPI, e18:1-LPI	Unresolved	
584		17:1-LPI, e18:1-LPI	Unresolved	

Supplemental Material Table 4. Analysis of lysophospholipids in mouse serum.

Runs 1-4 are independent experiments prepared with 100 μ L of the same batch of mouse serum. Each sample was independently extracted and analyzed by LC/ESI-MSMS as described in the main text. We analyzed for 104 different lysophospholipids as described in the main text. Of these, discernable peaks in the selected ion chromatograms were observed for 80 species, and the levels of these are given in the table on the next page. Listed for each of the 4 runs are the LC retention time, the peak area in the selective ion chromatogram of the analyte and the internal standard, the ratio of peak area of analyte to that of the internal standard, the derived pmoles of analyte, the average pmole of the analyte for the 4 runs and the standard deviation for the 4 runs.

Lysophospholipid	LC Ret. Time (min)	Analyte Area	IS Area	Area Ratio	Pmoles Analytes	Average	Std Dev
16:1-LPG	9.6	596.945	23123.543	0.03	0.041304743	0.04	0.01
	9.6	765.564	30133.988	0.03	0.040648533		
	9.6	606.248	26056.566	0.02	0.037226579		
	10.18	905.967	27666.781	0.03	0.052393056		
16:0-LPG	9.44	17717.629	23123.543	0.77	1.225945626	1.20	0.08
	9.52	23569.637	30133.988	0.78	1.251457962		
	9.52	20431.846	26056.566	0.78	1.25461481		
	9.93	18801.172	27666.781	0.68	1.087292201		
18:2-LPG	9.44	17271.713	23123.543	0.75	1.195091116	1.08	0.08
	9.44	20437.828	30133.988	0.68	1.085170831		
	9.44	17151.859	26056.566	0.66	1.053207641		
	9.85	17196.244	27666.781	0.62	0.994477471		
18:1-LPG	9.27	25804.059	23123.543	1.12	1.785474415	1.70	0.06
	9.27	31694.576	30133.988	1.05	1.682861279		
	9.27	27136.906	26056.566	1.04	1.666338135		
	9.69	28853.877	27666.781	1.04	1.668651051		
18:0-LPG	9.11	7264.343	23123.543	0.31	0.502645672	0.52	0.03
	9.11	9314.28	30133.988	0.31	0.494552795		
	9.11	8973.521	26056.566	0.34	0.551017874		
	9.36	9429.198	27666.781	0.34	0.545300763		
20:4-LPG	9.11	1803.158	23123.543	0.08	0.124766901	0.11	0.01
	9.36	2037.95	30133.988	0.07	0.108207384		
	9.36	1857.881	26056.566	0.07	0.114082938		
	9.85	1838.37	27666.781	0.07	0.106314934		
20:3-LPG	9.11	1017.641	23123.543	0.04	0.070414192	0.08	0.01
	9.36	1539.328	30133.988	0.05	0.081732454		
	8.95	1337.669	26056.566	0.05	0.082139389		
	9.52	1464.645	27666.781	0.05	0.084702011		
20:2-LPG	9.19	1055.71	23123.543	0.05	0.073048321	0.06	0.01
	9.19	1237.691	30133.988	0.04	0.065716678		
	9.19	1106.735	26056.566	0.04	0.067958917		
	9.52	877.706	27666.781	0.03	0.050758691		
20:1-LPG	9.36	917.234	23123.543	0.04	0.063466676	0.05	0.01
	8.78	888.394	30133.988	0.03	0.047170338		
	8.86	869.624	26056.566	0.03	0.053399147		
	9.03	950.755	27666.781	0.03	0.054983194		

16:0-LPI	12.91	16825.674	3175.722	5.30	11.65608413	11.70	0.48
	12.99	19720.25	3927.876	5.02	11.04529522		
	12.99	16410.34	3029.682	5.42	11.91634898		
	13.07	12372.168	2237.335	5.53	12.16571037		
18:2-LPI	12.82	26873.063	3175.722	8.46	18.61647166	18.46	1.16
	12.91	32623.684	3927.876	8.31	18.2724976		
	12.99	27399.543	3029.682	9.04	19.89614573		
	13.07	17358.066	2237.335	7.76	17.06840737		
18:1-LPI	12.58	11422.723	3175.722	3.60	7.913158205	7.69	0.89
	12.66	14428.296	3927.876	3.67	8.081276293		
	12.66	11516.819	3029.682	3.80	8.362924492		
	12.66	6492.835	2237.335	2.90	6.384487348		
18:0-LPI	12.17	138730.938	3175.722	43.68	96.10666916	91.23	6.63
	12.25	165596.141	3927.876	42.16	92.75025744		
	12.33	130214.383	3029.682	42.98	94.55502016		
	12.5	82872.438	2237.335	37.04	81.48952374		
20:4-LPI	12.66	141580.188	3175.722	44.58	98.08050377	93.37	7.92
	12.66	172446.453	3927.876	43.90	96.58711135		
	12.74	133953.453	3029.682	44.21	97.27014142		
	12.82	82909.055	2237.335	37.06	81.5255297		
20:3-LPI	12.58	27225.314	3175.722	8.57	18.8604956	17.38	1.68
	12.58	32215.93	3927.876	8.20	18.04411494		
	12.5	24257.314	3029.682	8.01	17.61441986		
	12.74	15238.328	2237.335	6.81	14.984042		
20:2-LPI	12.33	2365.138	3175.722	0.74	1.63846319	1.68	0.29
	12.33	2710.016	3927.876	0.69	1.517877652		
	12.41	2905.794	3029.682	0.96	2.110038875		
	12.17	1491.392	2237.335	0.67	1.466504748		
20:1-LPI	11.92	629.74	3175.722	0.20	0.43625607	0.44	0.03
	12.17	795.069	3927.876	0.20	0.445317469		
	12.09	540.279	3029.682	0.18	0.392322957		
	12	483.816	2237.335	0.22	0.475742435		
22:6-LPI	12.74	1988.308	3175.722	0.63	1.377412003	1.06	0.21
	12.74	1750.127	3927.876	0.45	0.980244641		
	12.74	1275.872	3029.682	0.42	0.926472943		
	12.91	982.383	2237.335	0.44	0.965989715		
		12.7825					
22:5-LPI	12.66	1492.09	3175.722	0.47	1.033654079	0.88	0.12
	12.58	1350.16	3927.876	0.34	0.756223465		
	12.58	1217.607	3029.682	0.40	0.884163883		
	12.58	847.875	2237.335	0.38	0.833726286		

22:4-LPI	12.25	933.576	3175.722	0.29	0.646740237	0.54	0.11
	12.09	1004.349	3927.876	0.26	0.562535019		
	12.25	523.999	3029.682	0.17	0.380501254		
	12.2	594	2237.335	0.27	0.584087765		
16:1-enyl-LPE	13.09	53683.219	18501.73	2.90	22.0515846	18.40	2.55
	13.17	55483.023	23061.918	2.41	18.28429772		
	13.09	40662.332	18680.604	2.18	16.5430263		
	13.34	26557.752	12062.629	2.20	16.73258087		
18:2-enyl-LPE	12.84	20154.563	18501.73	1.09	8.278938175	6.11	1.49
	13.01	17686.398	23061.918	0.77	5.828510222		
	13.01	12088.612	18680.604	0.65	4.918119949		
	12.84	8607.646	12062.629	0.71	5.423204975		
18:1-enyl-LPE	12.52	27848.055	18501.73	1.51	11.43921233	8.47	2.01
	12.43	24011.322	23061.918	1.04	7.912873821		
	12.52	18248.207	18680.604	0.98	7.424083996		
	12.51	11284.938	12062.629	0.94	7.110019615		
16:1-LPE	14.82	2265.786	18501.73	0.12	0.293912321	0.26	0.04
	14.82	2707.739	23061.918	0.12	0.28178808		
	14.82	1846.819	18680.604	0.10	0.237271		
	14.81	1102.06	12062.629	0.09	0.219267624		
16:0-LPE	14.57	253239.188	18501.73	13.69	32.84957954	32.07	1.49
	14.57	324570.125	23061.918	14.07	33.77725565		
	14.57	241469.047	18680.604	12.93	31.02285733		
	14.57	154027.625	12062.629	12.77	30.64558315		
18:2-LPE	14.49	99344.695	18501.73	5.37	12.88675535	12.63	0.59
	14.49	127728.883	23061.918	5.54	13.29244685		
	14.57	96761.977	18680.604	5.18	12.43154369		
	14.73	59932	12062.629	4.97	11.92416678		
18:1-LPE	14.24	63280.305	18501.73	3.42	8.208569253	7.37	0.75
	14.24	74767.281	23061.918	3.24	7.780856493		
	14.32	52960.699	18680.604	2.84	6.804152457		
	14.4	33580.047	12062.629	2.78	6.681139974		
18:0-LPE	13.83	352539.563	18501.73	19.05	45.7305858	43.57	3.26
	13.91	449193.75	23061.918	19.48	46.74654554		
	13.99	327456.656	18680.604	17.53	42.07015867		
	13.99	199601.813	12062.629	16.55	39.71309664		
20:4-LPE	14.16	56808.68	18501.73	3.07	7.369085594	6.97	0.42

	14.24	68952.43	23061.918	2.99	7.175718516		
	14.32	53887.637	18680.604	2.88	6.923241283		
	14.24	32145.559	12062.629	2.66	6.395731942		
20:3-LPE	14.16	10479.255	18501.73	0.57	1.359343802	1.31	0.16
	14.16	14199.36	23061.918	0.62	1.477694266		
	14.24	10067.767	18680.604	0.54	1.293461432		
	14.32	5483.031	12062.629	0.45	1.090912636		
20:2-LPE	13.91	3912.191	18501.73	0.21	0.507480025	0.53	0.02
	13.99	5185.378	23061.918	0.22	0.53963019		
	13.99	4284.974	18680.604	0.23	0.550514191		
	14.08	2617.201	12062.629	0.22	0.520722506		
20:1-LPE	13.75	8734.34	18501.73	0.47	1.132997617	0.97	0.15
	13.66	10218.946	23061.918	0.44	1.063461868		
	13.75	6549.239	18680.604	0.35	0.841416776		
	13.66	4253.891	12062.629	0.35	0.846360972		
20:0-LPE	13.25	2159.367	18501.73	0.12	0.280107903	0.25	0.03
	13.5	2361.955	23061.918	0.10	0.245803146		
	13.34	1639.391	18680.604	0.09	0.210621584		
	13.42	1410.454	12062.629	0.12	0.280626189		
22:6-LPE	14.16	35443.754	18501.73	1.92	4.597678682	4.30	0.45
	14.24	45813.246	23061.918	1.99	4.767677623		
	14.24	30989.908	18680.604	1.66	3.981444026		
	14.4	19299.932	12062.629	1.60	3.839945405		
22:5-LPE	14.08	3886.364	18501.73	0.21	0.504129808	0.53	0.04
	14.16	5536.838	23061.918	0.24	0.576205813		
	14.32	4128.241	18680.604	0.22	0.53037784		
	14.24	2481.588	12062.629	0.21	0.493740726		
22:4-LPE	13.99	2253.82	18501.73	0.12	0.29236012	0.26	0.04
	13.75	2733.046	23061.918	0.12	0.284421721		
	13.75	2050.746	18680.604	0.11	0.263470624		
	13.99	1061.871	12062.629	0.09	0.211271556		
16:0-LPS	15.98	695.745	2373.985	0.29	0.703369229	0.73	0.08
	15.98	1398.813	5050.69	0.28	0.664691597		
	16.06	1568.396	5470.979	0.29	0.688021358		
	16.48	1411.013	3999.983	0.35	0.846611398		
18:2-LPS	15.98	286.786	2373.985	0.12	0.289928706	0.41	0.14
	16.15	881.571	5050.69	0.17	0.418907199		
	16.15	717.987	5470.979	0.13	0.314965347		
	16.15	1013.23	3999.983	0.25	0.607940584		

18:1-LPS	15.9	1344.99	2373.985	0.57	1.359728895	1.78	0.29
	15.65	4035.037	5050.69	0.80	1.917379368		
	15.82	4601.515	5470.979	0.84	2.018584974		
	15.98	3055.957	3999.983	0.76	1.833581993		
18:0-LPS	14.17	3616.073	2373.985	1.52	3.655699257	3.09	0.45
	15.32	6834.187	5050.69	1.35	3.247486739		
	15.49	6333.017	5470.979	1.16	2.778157401		
	15.57	4460.246	3999.983	1.12	2.676158974		
20:4-LPS	15.74	2597.117	2373.985	1.09	2.625577162	2.97	0.33
	15.82	7178.017	5050.69	1.42	3.410868772		
	15.65	6461.268	5470.979	1.18	2.834418337		
	15.98	5030.203	3999.983	1.26	3.018134627		
20:3-LPS	15.24	667.918	2373.985	0.28	0.675237291	0.84	0.12
	15.41	2022.891	5050.69	0.40	0.961242602		
	15.49	1989.514	5470.979	0.36	0.872756704		
	15.49	1400.653	3999.983	0.35	0.840395372		
22:6-LPS	15.74	1051.759	2373.985	0.44	1.063284562	1.37	0.30
	15.82	3746.568	5050.69	0.74	1.780303919		
	15.9	2945.069	5470.979	0.54	1.291937988		
	15.82	2213.227	3999.983	0.55	1.327941844		
22:5-LPS	15.32	435.617	2373.985	0.18	0.440390651	0.38	0.06
	15.49	682.738	5050.69	0.14	0.324425217		
	15.74	765.128	5470.979	0.14	0.335645083		
	15.98	696.729	3999.983	0.17	0.418039177		
22:4-LPS	12.78	2916.323	2373.985	1.23	2.948281139	1.79	0.78
	12.86	3196.228	5050.69	0.63	1.518791927		
	12.86	3142.564	5470.979	0.57	1.378574767		
	13.27	2192.772	3999.983	0.55	1.315668792		
16:1-LPA	21.91	12428.062	13708.649	0.91	3.988976069	3.36	0.52
	21.91	10762.171	16711.295	0.64	2.833625545		
	22	9942.192	14402.116	0.69	3.037445664		
	22.24	8930.133	11004.484	0.81	3.570597695		
16:0-LPA	21.5	350604.938	13708.649	25.58	112.5320028	100.16	9.38
	21.59	357437.281	16711.295	21.39	94.11143998		
	21.59	334618.281	14402.116	23.23	102.2294527		
	21.75	229559.234	11004.484	20.86	91.78627818		
18:3-LPA	21.75	4129.667	13708.649	0.30	1.325479615	1.31	0.14
	22	4307.937	16711.295	0.26	1.134258165		

	21.83	4246.736	14402.116	0.29	1.297423129		
	22	3695.517	11004.484	0.34	1.477604475		
			21.895				
18:2-LPA	21.59	759249.313	13708.649	55.38	243.6926481	227.86	13.49
	21.59	856066.625	16711.295	51.23	225.3980407		
	21.59	756680.75	14402.116	52.54	231.1740372		
	21.67	528155.75	11004.484	47.99	211.1762169		
18:1-LPA	21.09	154127.547	13708.649	11.24	49.46958718	54.83	4.94
	21.17	207213.094	16711.295	12.40	54.55816641		
	21.17	201046.531	14402.116	13.96	61.42185887		
	21.34	134746.125	11004.484	12.24	53.87648798		
18:0-LPA	20.76	62609.645	13708.649	4.57	20.09552057	24.28	4.08
	20.85	88131.992	16711.295	5.27	23.20471063		
	20.85	97734.492	14402.116	6.79	29.8589294		
	20.93	59905.402	11004.484	5.44	23.95239693		
20:4-LPA	21.17	210261.297	13708.649	15.34	67.48657047	75.15	6.70
	21.17	290805.125	16711.295	17.40	76.56752813		
	21.17	273266.031	14402.116	18.97	83.48568616		
	21.34	182683.172	11004.484	16.60	73.04349362		
20:3-LPA	21.09	53277.234	13708.649	3.89	17.10014091	19.28	2.51
	21.09	72465.219	16711.295	4.34	19.07972803		
	21.17	74747.414	14402.116	5.19	22.83613197		
	21.26	45262.148	11004.484	4.11	18.09748201		
20:2-LPA	21.01	4738.052	13708.649	0.35	1.520750061	1.78	0.18
	21.09	7126.683	16711.295	0.43	1.876419823		
	20.93	6267.542	14402.116	0.44	1.914800908		
	21.09	4483.174	11004.484	0.41	1.792538896		
			21.03			0.40	
20:1-LPA	20.35	1341.266	13708.649	0.10	0.430499782	0.51	0.10
	20.43	1710.788	16711.295	0.10	0.450441884		
	20.52	2157.772	14402.116	0.15	0.659222353		
	20.6	1286.739	11004.484	0.12	0.514485877		
22:6-LPA	21.17	163263.984	13708.649	11.91	52.40206599	57.70	5.95
	21.17	211831.578	16711.295	12.68	55.77419004		
	21.17	216761.891	14402.116	15.05	66.22306892		
	21.34	141101.375	11004.484	12.82	56.41755215		
22:5-LPA	21.09	13919.162	13708.649	1.02	4.467567358	4.97	0.42
	21.17	18392.666	16711.295	1.10	4.842696535		
	21.01	17894.838	14402.116	1.24	5.467063812		
	21.42	12757.006	11004.484	1.16	5.10072316		

22:4-LPA	20.85	1268.932	13708.649	0.09	0.407283081	0.42	0.04
	21.01	1457.795	16711.295	0.09	0.383830098		
	20.68	1476.652	14402.116	0.10	0.451132931		
	21.17	1141.835	11004.484	0.10	0.456547894		
14:0-LPC	35.71	77921.539	7870.779	9.90	5.940063036	7.66	1.46
	35.7	110587.086	9066.454	12.20	7.318434704		
	35.75	142168.109	9013.73	15.77	9.463436935		
	35.7	145978.703	11050.068	13.21	7.926396634		
16:1-LPC	35.01	366384.844	7870.779	46.55	27.93000622	33.51	4.49
	35.06	491318.281	9066.454	54.19	32.51447243		
	35.06	580473.688	9013.73	64.40	38.63929947		
	35.06	643580.25	11050.068	58.24	34.94531889		
16:0-LPC	34.31	28442774	7870.779	3613.72	2168.230667	2156.28	89.09
	34.31	33360640	9066.454	3679.57	2207.741196		
	34.31	33381886	9013.73	3703.45	2222.069177		
	34.31	37332472	11050.068	3378.48	2027.090078		
18:3-LPC	34.91	63133.68	7870.779	8.02	4.812764785	5.66	0.68
	35.01	83140.406	9066.454	9.17	5.502067688		
	34.96	96280.953	9013.73	10.68	6.408952986		
	35.01	109188.359	11050.068	9.88	5.92874319		
18:2-LPC	34.46	13414184	7870.779	1704.30	1022.58117	1013.73	51.59
	34.46	15627053	9066.454	1723.61	1034.167471		
	34.46	15901680	9013.73	1764.16	1058.497204		
	34.51	17305610	11050.068	1566.11	939.6653487		
18:1-LPC	33.72	8137876	7870.779	1033.94	620.3611612	627.63	24.55
	33.67	9789562	9066.454	1079.76	647.8538577		
	33.67	9708864	9013.73	1077.12	646.2716767		
	33.72	10977009	11050.068	993.39	596.0330199		
18:0-LPC	32.97	28269252	7870.779	3591.67	2155.002853	2181.40	94.32
	33.02	33749136	9066.454	3722.42	2233.451093		
	32.92	34187580	9013.73	3792.83	2275.700293		
	33.02	37965076	11050.068	3435.73	2061.439405		
20:4-LPC	33.92	2044180.25	7870.779	259.72	155.8305919	157.27	5.71
	33.96	2437235	9066.454	268.82	161.2913935		
	33.86	2436060.25	9013.73	270.26	162.1566377		
	33.92	2758854	11050.068	249.67	149.8011053		
20:3-LPC	33.72	980375.5	7870.779	124.56	74.73533433	74.28	3.04
	33.72	1153778.875	9066.454	127.26	76.35480476		
	33.72	1144177.25	9013.73	126.94	76.16229352		
	33.76	1286374.125	11050.068	116.41	69.84793894		

20:2-LPC	33.27	318250.094	7870.779	40.43	24.26062991	24.48	0.86
	33.22	375879.844	9066.454	41.46	24.87498491		
	33.17	381346.938	9013.73	42.31	25.38440388		
	33.27	430597.375	11050.068	38.97	23.38070906		
20:1-LPC	32.32	384655.844	7870.779	48.87	29.32282896	29.00	1.49
	32.32	454614.125	9066.454	50.14	30.08546395		
	32.42	447245.844	9013.73	49.62	29.77097233		
	32.47	493699.75	11050.068	44.68	26.80706128		
20:0-LPC	31.53	249262.797	7870.779	31.67	19.00163608	17.91	1.11
	31.53	278261.219	9066.454	30.69	18.41477731		
	31.43	267580.625	9013.73	29.69	17.81153585		
	31.53	302138.813	11050.068	27.34	16.40562645		
22:6-LPC	33.87	3361822.75	7870.779	427.13	256.2762403	244.35	14.19
	33.91	3825779.75	9066.454	421.97	253.1825397		
	33.91	3652674.25	9013.73	405.23	243.140692		
	33.92	4140182.75	11050.068	374.67	224.8049198		
22:5-LPC	33.87	359645.656	7870.779	45.69	27.41626891	26.85	0.94
	33.86	415781.344	9066.454	45.86	27.51558728		
	33.86	405428.938	9013.73	44.98	26.98742505		
	33.87	469200.625	11050.068	42.46	25.47680023		
22:4-LPC	33.17	34575.82	7870.779	4.39	2.635760958	2.48	0.16
	32.97	38880.352	9066.454	4.29	2.573024823		
	32.92	36643.434	9013.73	4.07	2.439174504		
	33.17	42030.207	11050.068	3.80	2.282169141		
24:0-LPC	29.19	63399.984	7870.779	8.06	4.833065495	4.27	0.39
	29.19	64133	9066.454	7.07	4.24419514		
	29.14	60614.059	9013.73	6.72	4.034781983		
	29.19	73252.422	11050.068	6.63	3.9774826		
16:1-enyl-LPC	32.08	52219.355	7870.779	6.63	13.26917069	13.86	0.66
	32.08	64338.27	9066.454	7.10	14.19259834		
	32.07	65946.734	9013.73	7.32	14.63250708		
	32.08	73752.469	11050.068	6.67	13.34878102		
18:2-enyl-LPC	32.92	137894.875	7870.779	17.52	35.03970192	34.81	1.39
	33.02	158065.641	9066.454	17.43	34.86823867		
	32.97	163866.516	9013.73	18.18	36.35931318		
	32.97	182204.922	11050.068	16.49	32.97806348		
18:1-enyl-LPC	30.48	6259.815	7870.779	0.80	1.590646872	1.58	0.01

	30.53	7187.655	9066.454	0.79	1.585549323		
	30.43	7035.433	9013.73	0.78	1.56104809		
	30.58	8654.604	11050.068	0.78	1.566434523		
16:0-alkyl-LPC	33.92	222849.234	6978.524	31.93	57.48043873	53.21	3.60
	33.86	259374.266	9512.854	27.27	49.07819239		
	33.81	277169.375	9159.732	30.26	54.46719129		
	33.92	314538.531	10924.943	28.79	51.82355238		
18:1-alkyl-LPC	33.27	173007.422	6978.524	24.79	44.62453086	41.93	1.95
	33.27	212114.438	9512.854	22.30	40.13579819		
	33.22	213677.922	9159.732	23.33	41.99033985		
	33.32	248703.781	10924.943	22.76	40.97658045		
18:0-alkyl-LPC	33.67	969999	6978.524	139.00	250.1959154	228.41	15.04
	33.67	1172888.375	9512.854	123.30	221.931197		
	33.62	1147448.125	9159.732	125.27	225.4876698		
	33.67	1311073.875	10924.943	120.01	216.0132987		

Supplemental Material Table 5. Changes in lysophospholipase species in HEK293 cells in response to human group X secreted phospholipase A₂ action. See main text for more details.

WT HEK (A) WT HEK 2 (E) Average Standard Deviation				WT HEK (+) 200 ng/ml hGX (B) WT HEK (+) 200 ng/ml hGX (F) Average Standard Deviation				pre-pro-hGX overexpressed (C) pre-pro-hGX overexpressed (D) pre-pro-hGX overexpressed (G) Average Standard Deviation					
(12:0)-LPG	0.23	0.32	0.28	0.06	0.30	0.23	0.26	0.05	0.22	0.32	0.14	0.22	0.09
(14:0)-LPG	7.60	7.66	7.63	0.04	11.50	10.18	10.84	0.93	4.82	4.54	3.20	4.19	0.87
(16:0)-LPG	1.22	1.30	1.31	0.01	1.32	1.34	1.33	0.01	0.90	0.82	0.62	0.78	0.14
(18:0)-LPG	9.44	8.54	8.99	0.64	10.32	10.96	10.64	0.45	7.80	7.12	6.72	7.21	0.55
(18:1)-LPG	49.12	45.32	47.22	2.89	51.68	51.54	51.61	0.13	34.22	30.22	28.40	30.28	3.91
(20:0)-LPG	1.10	1.06	1.08	0.03	1.22	1.24	1.23	0.01	2.48	2.08	1.94	2.17	0.28
(20:3)-LPG	0.04	0.02	0.03	0.00	0.00	0.04	0.02	0.00	0.22	0.12	0.10	0.15	0.08
(20:4)-LPG	0.02	0.18	0.18	0.01	0.14	0.20	0.17	0.04	0.12	0.18	0.39	0.13	0.05
(20:1)-LPG	1.78	1.98	1.88	0.14	2.32	2.24	2.28	0.08	1.08	1.04	0.86	0.99	0.12
(20:2)-LPG	0.88	0.76	0.82	0.08	1.00	0.98	0.99	0.01	1.40	1.08	1.02	1.17	0.20
(20:3)-LPG	0.90	0.92	0.91	0.01	0.96	1.06	0.96	0.01	1.36	1.16	1.16	1.05	0.16
(20:4)-LPG	0.12	0.14	0.13	0.01	0.18	0.18	0.18	0.00	0.26	1.00	0.92	1.06	0.18
(22:4)-LPG	0.12	0.10	0.11	0.01	0.10	0.10	0.05	0.07	0.86	0.64	0.72	0.62	0.18
(22:5)-LPG	0.24	0.22	0.23	0.01	0.28	0.28	0.28	0.00	2.28	1.48	1.88	1.88	0.40
(22:6)-LPG	0.30	0.38	0.34	0.06	0.24	0.30	0.27	0.04	1.68	1.32	1.54	1.51	0.18

Exogenous/WT SD		Overexpression/WT SD	
(12:0)-LPG	0.95	0.28	0.81
(14:0)-LPG	1.42	0.12	0.55
(16:0)-LPG	1.12	0.02	0.60
(18:0)-LPG	1.18	0.10	0.80
(18:1)-LPG	1.09	0.06	0.64
(20:0)-LPG	1.11	0.03	0.21
(20:3)-LPG	0.67	0.99	0.89
(20:4)-LPG	0.89	0.35	0.87
(20:1)-LPG	1.21	0.10	1.53
(20:2)-LPG	1.21	0.13	0.42
(20:3)-LPG	1.12	0.16	0.52
(20:4)-LPG	1.38	0.15	0.15
(22:4)-LPG	0.45	0.65	0.55
(22:5)-LPG	1.13	0.07	0.81
(22:6)-LPG	0.79	0.18	0.45

WT HEK (A) WT HEK 2 (E) Average Standard Deviation				WT HEK (+) 200 ng/ml hGX (B) WT HEK (+) 200 ng/ml hGX (F) Average Standard Deviation				pre-pro-hGX overexpressed (C) pre-pro-hGX overexpressed (D) pre-pro-hGX overexpressed (G) Average Standard Deviation					
(12:0)-LPI	9.90	7.96	8.93	1.37	10.23	8.29	9.26	1.37	2.06	2.04	1.36	1.70	0.48
(14:0)-LPI	0.92	0.93	0.93	0.00	1.02	0.71	0.87	0.22	0.51	0.25	0.13	0.19	0.08
(16:0)-LPI	123.38	106.85	115.12	11.69	110.92	103.78	107.35	5.05	25.86	24.70	22.33	23.51	1.88
(18:0)-LPI	23.25	20.02	21.64	2.29	12.44	15.28	13.86	2.01	12.44	12.44	2.80	3.23	0.60
(18:2)-LPI	2.00	1.48	1.74	0.37	1.10	1.17	1.13	0.05	1.41	1.07	0.65	0.86	0.30
(20:0)-LPI	1.84	1.98	1.87	0.04	0.89	1.28	1.08	0.27	0.00	0.00	0.00	0.00	0.00
(20:1)-LPI	5.67	4.12	4.90	1.10	3.15	3.48	3.32	0.23	0.00	0.53	0.34	0.44	0.13
(20:3)-LPI	3.09	2.24	2.67	0.61	1.50	2.22	1.86	0.51	1.59	2.13	1.80	1.96	0.24
(20:4)-LPI	6.67	4.09	5.38	1.82	3.23	3.77	3.50	0.38	6.57	7.04	6.44	6.74	0.42
(22:4)-LPI													
(22:5)-LPI													
(22:6)-LPI													

Exogenous/WT SD		Overexpression/WT SD	
(12:0)-LPI	1.04	0.22	0.19
(14:0)-LPI	0.94	0.23	0.20
(16:0)-LPI	0.93	0.10	0.20
(18:0)-LPI	0.65	0.14	0.15
(18:2)-LPI	0.65	0.14	0.49
(20:0)-LPI	0.85	0.14	0.50
(20:1)-LPI	0.68	0.16	0.09
(20:3)-LPI	0.70	0.23	0.74
(20:4)-LPI	0.65	0.23	1.25
(22:4)-LPI			
(22:5)-LPI			
(22:6)-LPI			

WT HEK (A) WT HEK 2 (E) Average Standard Deviation				WT HEK (+) 200 ng/ml hGX (B) WT HEK (+) 200 ng/ml hGX (F) Average Standard Deviation				pre-pro-hGX overexpressed (C) pre-pro-hGX overexpressed (D) pre-pro-hGX overexpressed (G) Average Standard Deviation					
(12:0)-LPE	1.36	1.17	1.27	0.13	2.63	2.86	2.74	0.16	1.08	0.87	0.98	0.92	0.08
(14:0)-LPE	40.02	39.65	39.83	0.28	99.74	104.23	101.99	3.18	52.44	45.52	43.77	44.64	1.24
(16:0)-LPE	4.51	3.93	4.22	0.41	4.07	9.79	8.86	1.31	3.40	3.75	3.16	3.45	0.41
(18:0)-LPE	39.93	37.99	38.96	1.38	172.01	169.31	169.31	3.81	146.15	137.27	126.30	131.76	7.76
(18:1)-LPE	40.50	38.64	39.67	1.32	194.81	194.81	192.08	3.57	50.69	44.72	45.02	44.67	0.21
(18:2)-LPE	1.82	2.02	1.92	0.14	3.20	3.31	3.26	0.08	3.25	2.76	2.72	2.74	0.02
(20:0)-LPE	0.79	0.94	0.87	0.10	0.54	4.64	5.09	0.63	3.70	3.96	3.34	3.65	0.44
(20:1)-LPE	3.31	2.90	3.11	0.29	11.42	11.13	11.27	0.21	5.32	4.74	4.92	4.83	0.13
(20:2)-LPE	0.59	0.69	0.64	0.07	0.66	0.87	0.76	0.15	0.87	0.76	0.84	0.85	0.14
(20:3)-LPE	0.59	0.66	0.64	0.07	0.66	0.87	0.76	0.15	0.87	0.76	0.84	0.85	0.14
(20:4)-LPE	0.42	2.16	1.87	0.41	1.48	1.58	1.52	0.00	1.58	1.52	1.52	1.52	0.00
(22:4)-LPE	0.34	0.47	0.41	0.09	0.42	0.49	0.45	0.00	2.28	2.15	2.55	2.35	0.28
(22:5)-LPE	0.25	0.21	0.23	0.03	0.00	0.14	0.07	0.10	0.59	0.77	0.79	0.78	0.01
(22:6)-LPE	0.21	0.22	0.21	0.01	0.16	0.25	0.21	0.06	0.40	0.35	0.35	0.35	0.00

Exogenous/WT SD		Overexpression/WT SD	
(12:0)-LPE	2.17	0.28	0.73
(14:0)-LPE	2.50	0.08	1.12
(16:0)-LPE	2.10	0.37	0.82
(18:0)-LPE	4.35	0.18	3.38
(18:1)-LPE	2.58	0.12	1.13
(18:2)-LPE	1.70	0.13	1.43
(20:0)-LPE	5.87	1.01	4.21
(20:1)-LPE	3.63	0.24	1.56
(20:2)-LPE	1.19	0.27	1.32
(20:3)-LPE	1.19	0.27	1.32
(20:4)-LPE	0.80	0.19	0.80
(22:4)-LPE	1.12	0.29	0.76
(22:5)-LPE	0.30	0.43	0.41
(22:6)-LPE	0.98	0.27	1.66

WT HEK (A) WT HEK 2 (E) Average Standard Deviation				WT HEK (+) 200 ng/ml hGX (B) WT HEK (+) 200 ng/ml hGX (F) Average Standard Deviation				pre-pro-hGX overexpressed (C) pre-pro-hGX overexpressed (D) pre-pro-hGX overexpressed (G) Average Standard Deviation					
(12:0)-LPS	12.84	9.57	11.21	2.31	16.77	16.88	16.73	0.08	1.23	1.38	1.17	1.28	0.15
(14:0)-LPS	6.48	6.84	7.56	1.53	7.44	9.81	8.63	1.68	1.59	2.13	2.27	2.25	0.17
(16:0)-LPS	69.84	78.60	74.22	6.19	82.40	96.45	94.43	2.86	25.11	30.00	29.91	29.96	0.06
(18:0)-LPS	16.00	16.77	17.39	0.87	19.56	18.15	18.88	1.00	5.22	6.16	5.28	5.73	0.64
(18:2)-LPS	1.65	2.91	2.28	0.89	2.76	2.22	2.49	0.38	0.18	0.27	0.18	0.23	0.06
(20:0)-LPS	6.86	6.84	7.56	1.53	7.44	9.81	8.63	1.68	1.59	2.13	2.27	2.25	0.17
(20:1)-LPS	3.24	3.54	3.39	0.21	4.80	2.46	3.63	1.65	1.02	1.38	0.60	0.99	0.55
(20:2)-LPS	0.48	0.75	0.58	0.38	0.76	0.80	0.69	0.13	0.76	0.80	0.84	0.84	0.17
(20:3)-LPS	7.23	14.40	10.82	5.07	9.87	11.16	10.52	0.91	2.55	4.35	4.08	4.22	0.19
(20:4)-LPS	1.68	2.73	2.21	0.74	2.21	1.92	1.76	0.23	1.02	0.72	0.99	0.86	0.15
(22:4)-LPS	0.60	0.83	0.62	0.09	0.08	0.69	0.69	0.00	0.69	0.60	0.33	0.47	0.19
(22:5)-LPS	0.60	0.63	0.62	0.02	0.57	0.42	0.57	0.21	0.38	0.00	0.21	0.11	0.19
(22:6)-LPS	0.60	0.51	0.56	0.06	0.45	0.45	0.45	0.00	0.00	0.00	0.27	0.14	0.19

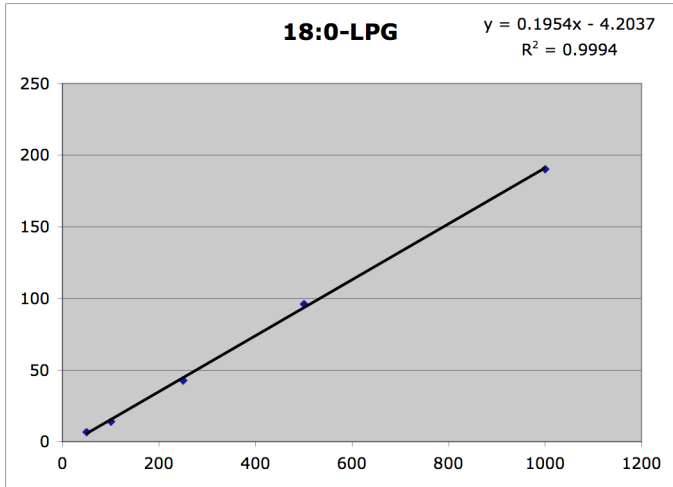
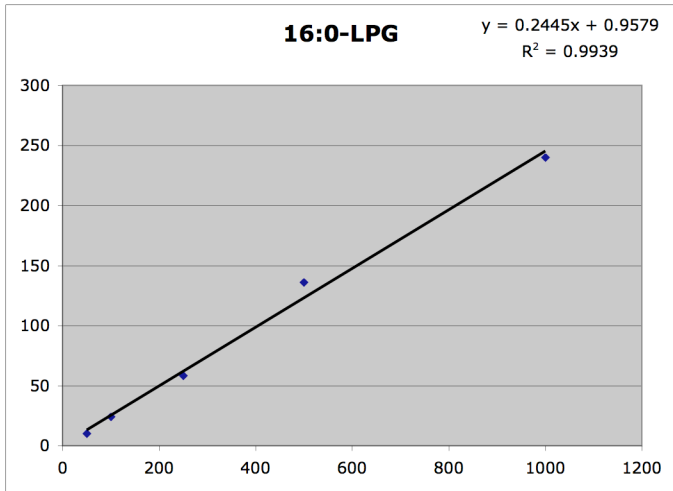
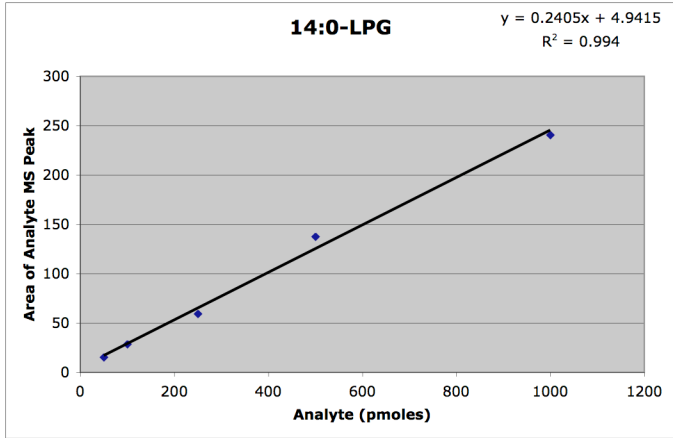
Exogenous/WT SD		Overexpression/WT SD	
(12:0)-LPS	1.49	0.31	0.11
(14:0)-LPS	0.99	0.06	0.74
(16:0)-LPS	1.27	0.11	0.40
(18:0)-LPS	1.08	0.08	0.33
(18:2)-LPS	1.09	0.46	0.10
(20:0)-LPS	2.37	0.32	0.30
(20:1)-LPS	0.97	0.49	0.29
(20:2)-LPS	0.92	0.50	0.16
(20:3)-LPS	1.07	0.46	0.39
(20:4)-LPS	0.80	0.29	0.16
(22:4)-LPS	0.15	0.49	0.15
(22:5)-LPS	0.93	0.35	0.17
(22:6)-LPS	0.81	0.09	0.24

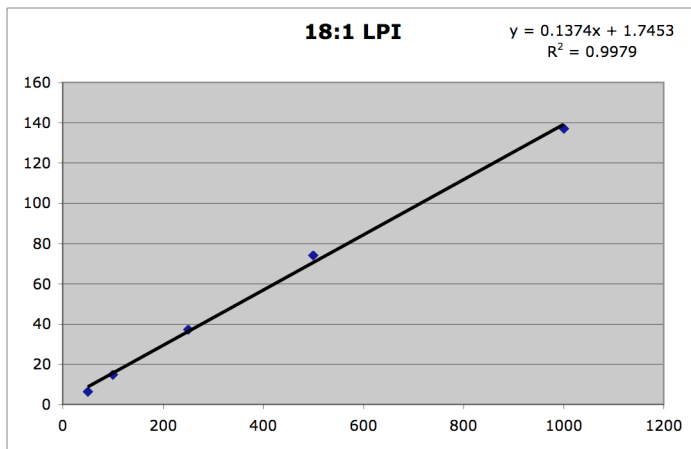
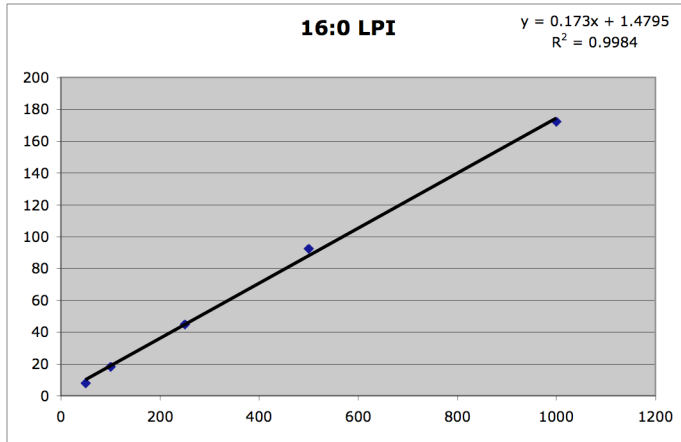
WT HEK (A) WT HEK 2 (E) Average Standard Deviation				WT HEK (+) 200 ng/ml hGX (B) WT HEK (+) 200 ng/ml hGX (F) Average Standard Deviation				pre-pro-hGX overexpressed (C) pre-pro-hGX overexpressed (D) pre-pro-hGX overexpressed (G) Average Standard Deviation					
(12:0)-LPA	4.72	2.58	3.65	1.51	2.54	4.41	3.47	1.32	1.28	1.40	2.59	1.99	0.84
(14:0)-LPA	4.00	4.21	4.10	0.15	3.26	4.25	3.76	0.70	0.00	1.14	1.21	1.17	0.05
(16:0)-LPA	6.29	6.86	6.48	0.21	3.15	6.38	4.75	2.27	3.06	3.17	5.29	4.23	1.50
(18:0)-LPA													
(18:1)-LPA													
(18:2)-LPA													
(20:0)-LPA													
(20:1)-LPA													
(20:2)-LPA													
(20:3)-LPA													
(20:4)-LPA													
(22:4)-LPA													
(22:5)-LPA													
(22:6)-LPA													

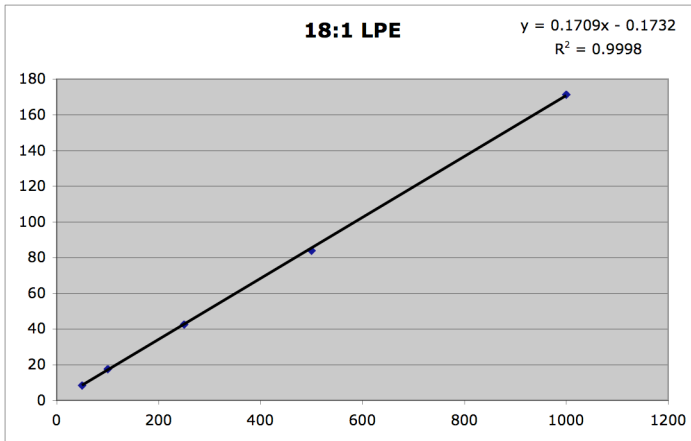
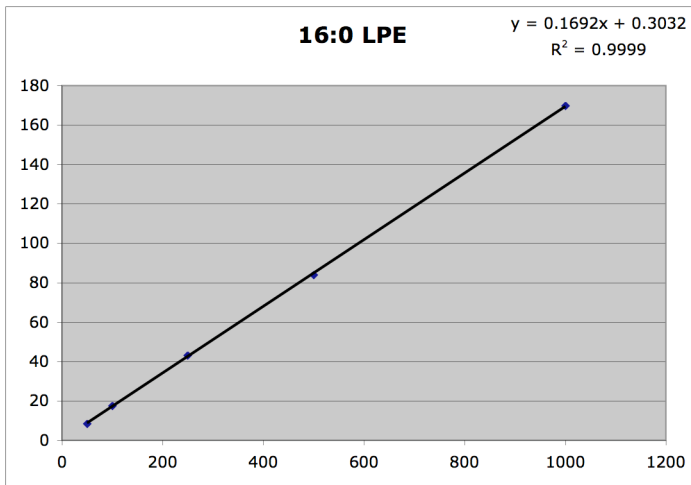
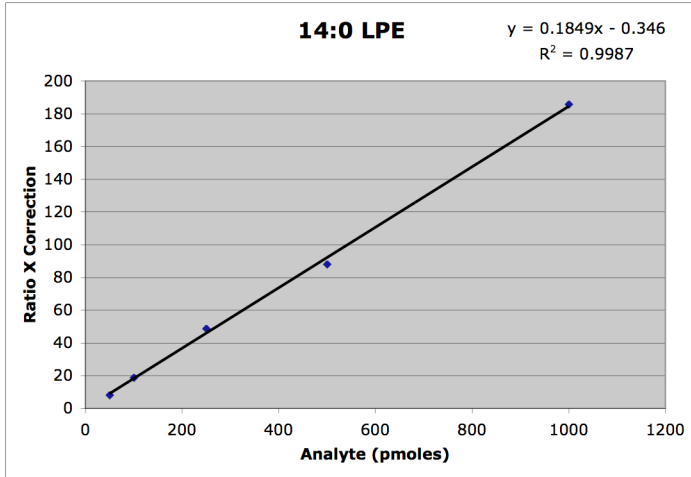
Exogenous/WT SD		Overexpression/WT SD	
(12:0)-LPA	0.95	0.54	0.55
(14:0)-LPA	0.92	0.17	0.29
(16:0)-LPA	0.73	0.35	0.65
(18:0)-LPA			
(18:1)-LPA			
(18:2)-LPA			
(20:0)-LPA			
(20:1)-LPA			
(20:2)-LPA			
(20:3)-LPA			
(20:4)-LPA			
(22:4)-LPA			
(22:5)-LPA			</

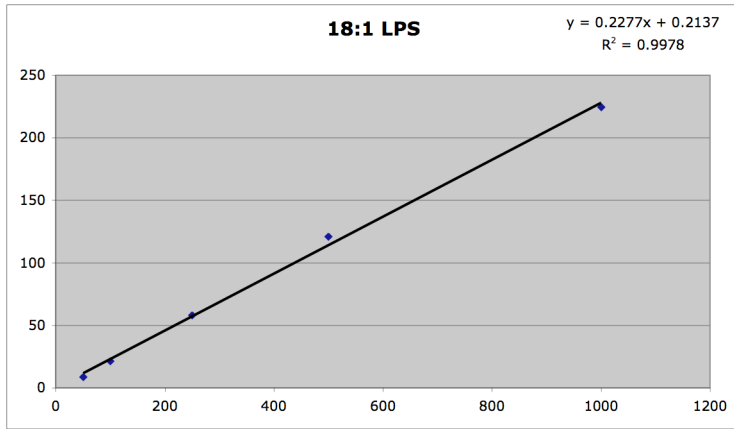
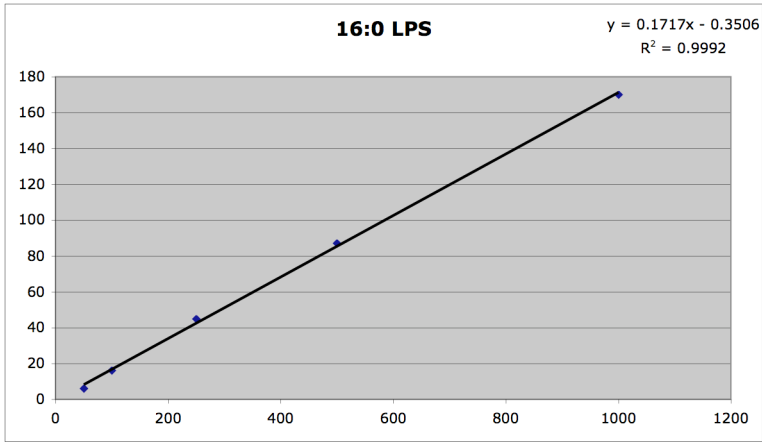
(18:2)-PIaLPC	3.20	3.55	3.36	0.25	6.08	4.30	5.19	1.26	3.50	4.05	3.00	3.53	0.74	(18:2)-PIaLPC	1.54	0.39	1.04	0.23
(18:0)-LvsPAPF	40.37	37.56	38.96	1.99	45.23	44.10	44.66	0.80	7.72	8.93	7.36	8.15	1.11	(18:0)-LvsPAPF	1.15	0.06	0.21	0.03
(18:0)-LvsPAPF	23.81	25.61	24.71	1.27	32.69	34.79	33.74	1.48	19.85	21.06	22.12	21.60	0.73	(18:0)-LvsPAPF	1.37	0.09	0.87	0.05
(18:1)-LvsPAPF	14.83	14.56	14.68	0.19	17.87	15.55	16.71	1.64	5.90	6.50	5.74	6.12	0.54	(18:1)-LvsPAPF	1.14	0.11	0.42	0.04

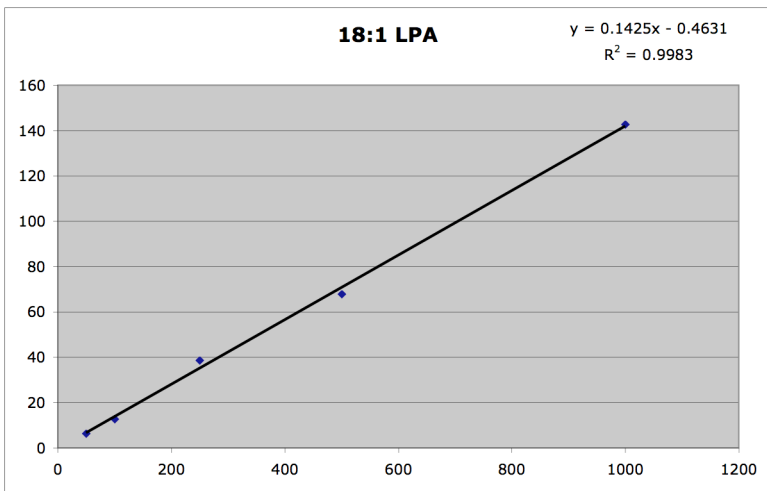
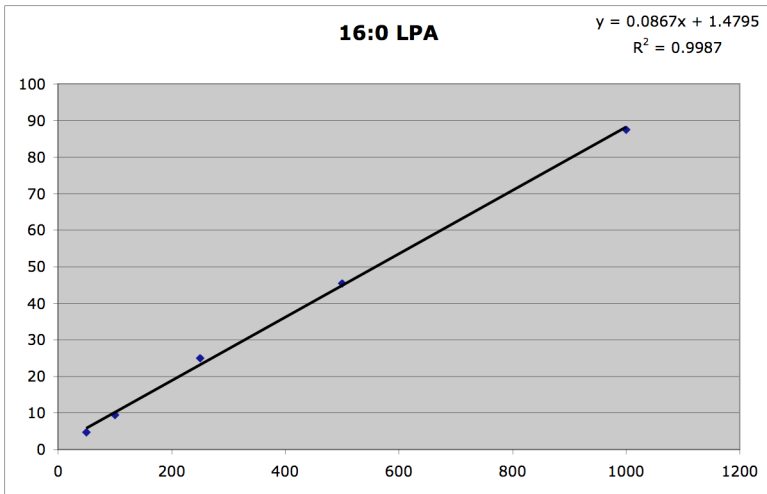
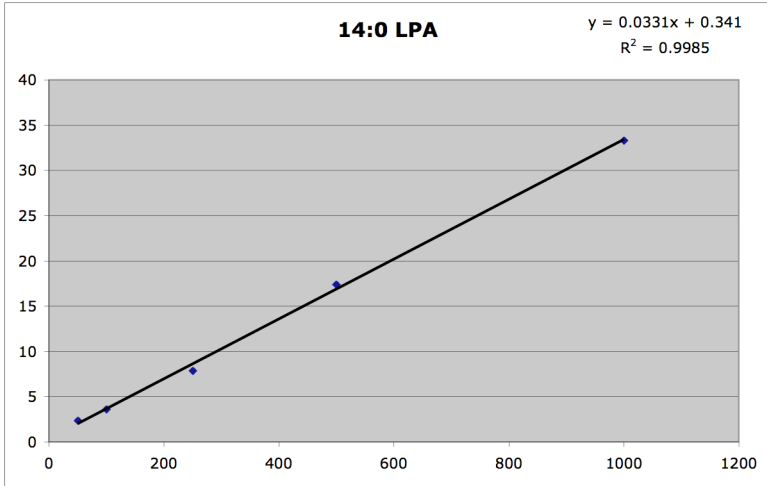
Supplemental Material Fig. 1. Standard curves for commercially available lysophospholipids. The Y-axis gives the area of the peak in the selected ion trace chromatogram (arbitrary units) and the X-axis gives the total pmole analytes injected (based on calibration of lysophospholipid stock solutions using inorganic phosphorous analysis) (note each analyte is present in the stock solution in equi-molar concentration).

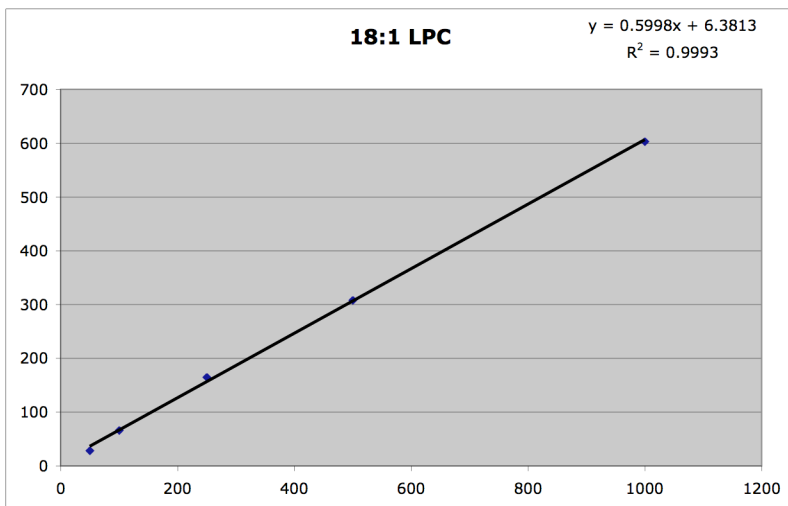
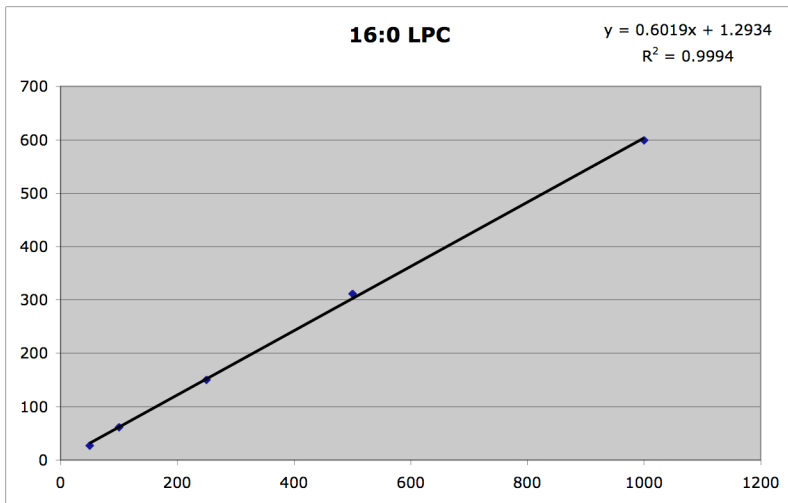
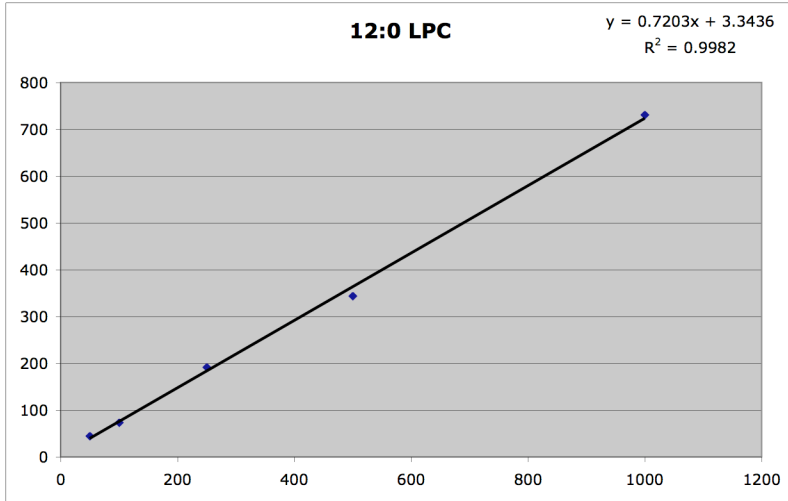


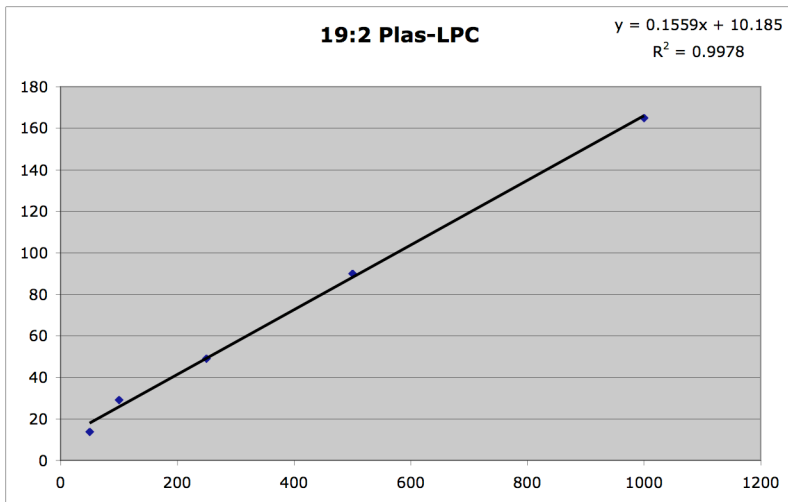
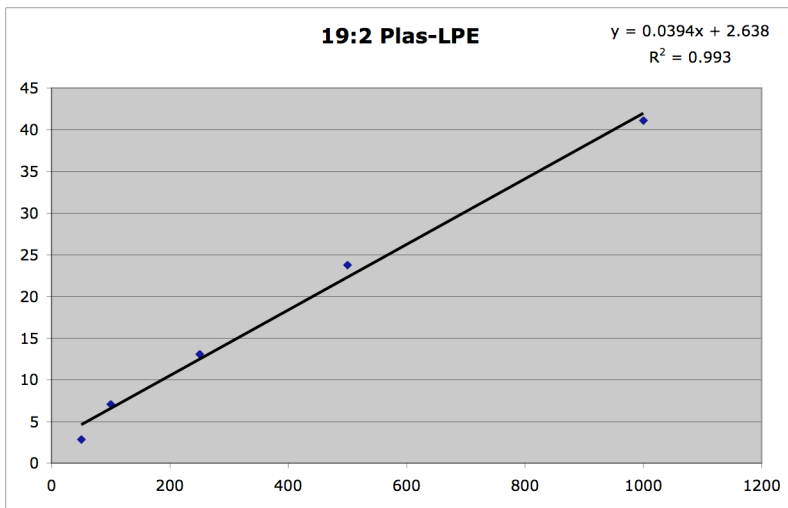
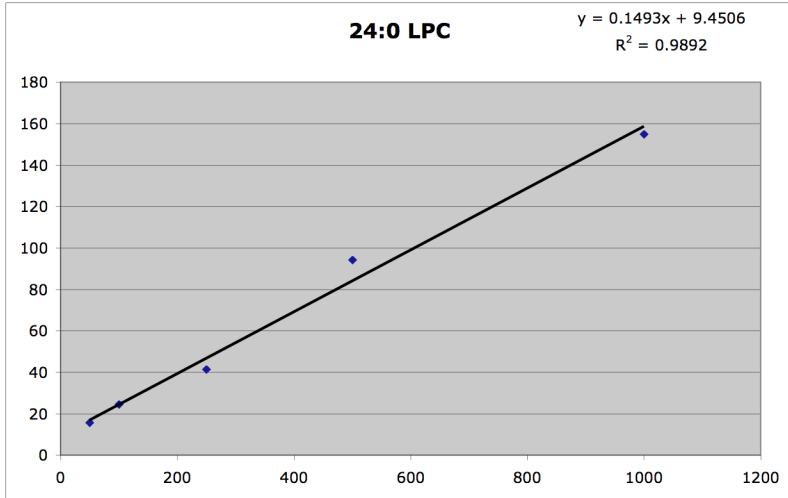


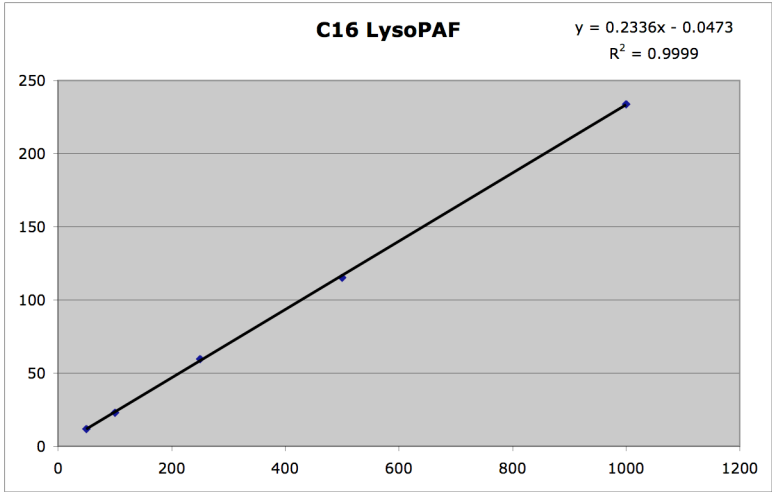




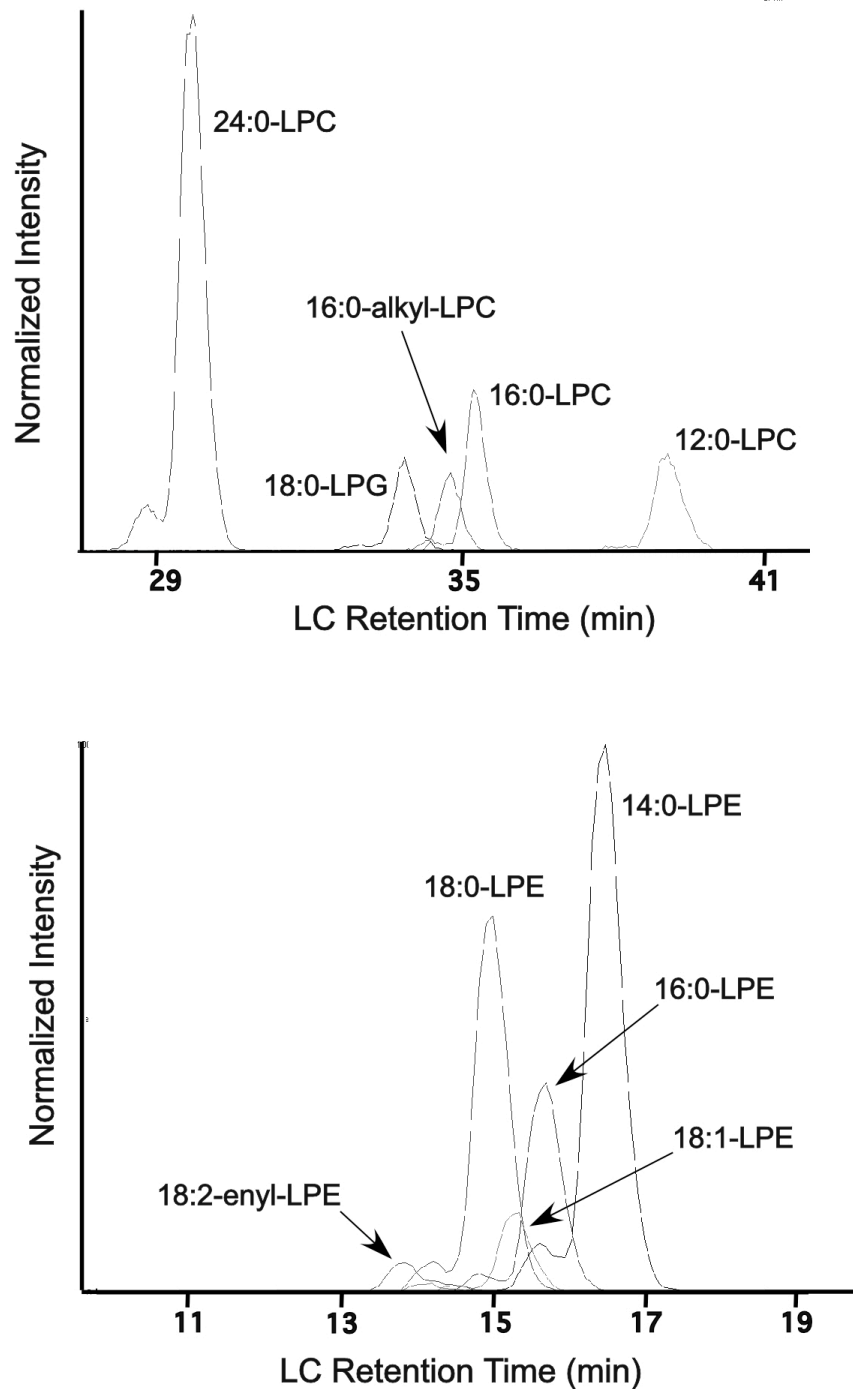






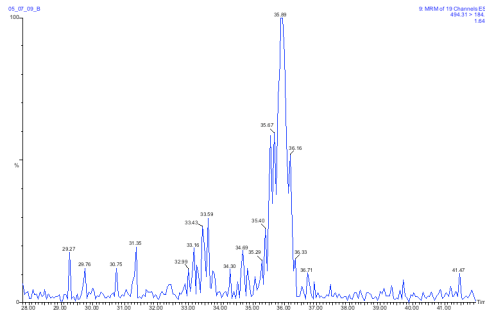


Supplemental Material Fig. 2. LC/ESI-MSMS detection of a standard mixture of lysophospholipids. The top panel shows positive mode detection of 24:0-LPC (1.5 pmole), 18:0-LPC (0.2 pmole), 16:0-LPC (0.4 pmole), 12:0-LPC (0.3 pmole), and 16:0-alkyl-LPC (1.0 pmole). The bottom panel shows negative mode detection of 18:0-LPE (3.8 pmole), 18:1-LPE (0.8 pmole), 16:0-LPE (2.2 pmole), 14:0-LPE (5.8 pmole), and 18:2-enyl-LPE (2.1 pmole). The X-axis is the LC retention time (min), and the Y-axis is the intensity of the ion trace peaks normalized to the largest peak.

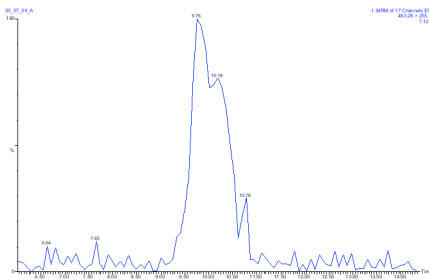


Supplemental Material Fig. 3. LC/ESI-MSMS detection of quantities of lysophospholipids near the limit of quantification.

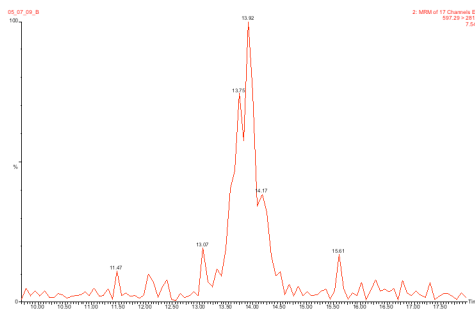
10 fmoles 16:1 LPC



200 fmoles 16:0 LPG

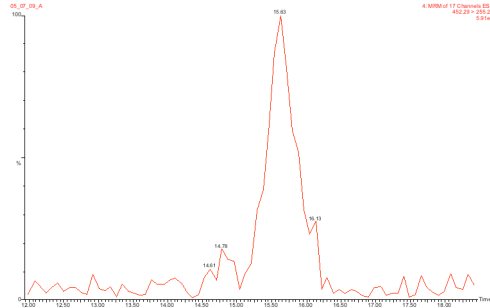


100 fmoles 18:1 LPI

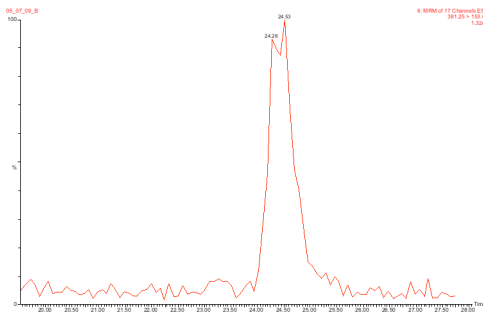


Supplemental Material Fig. 3 continued

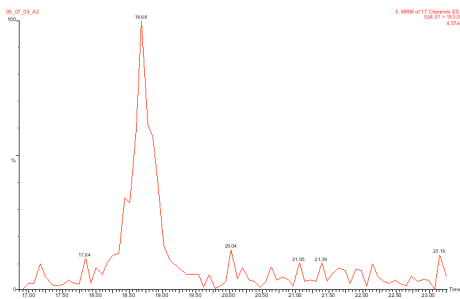
75 fmoles 16:0 LPE



LPA 1 pmole



150 fmoles 18:0 LPS



Supplemental Material Figure 4. Distribution of coefficient of variances for the analysis of lysophospholipids in mouse serum. For each of the 80 lysophospholipid species listed in Supplemental Material Table ***, we calculated the coefficient of variance among the 4 runs ($100 \times (\text{standard deviation}/\text{mean})$). The coefficient of variances are displayed along the X-axis. It can be seen that most of the values are < 20%.

