**SUPPORTING ONLINE MATERIAL**

## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure 1. Reproducibility of Positive Ion Mode (MALDI-TOF & MALDI-FITCR) Phospholipid Imaging Data.** Each column represents a different day 8 implantation site each from a different mouse. Each row represents a unique potassiated phospholipid ($[M+K]^+$) and how it localizes in each of the 8 different implantation sites. SM, sphingomyelin; PC, phosphatidylcholine; MALDI, Matrix-Assisted Laser Desorption/Ionization; TOF, Time-of-flight; FTICR, Fourier Transform Ion Cyclotron Resonance.

**Supplementary Figure 2. Reproducibility of Negative Ion Mode (MALDI-TOF & MALDI-FITCR) Phospholipid Imaging Data.** Each row represents a different day 8 implantation site each from a different mouse. Each column represents a unique phospholipid ($[M-H]^-$) and how it localizes in each of the 4 different implantation sites. PE, phosphatidylethanolamine; PEp, phosphatidylethanolamine plasmalogen; PI, phosphatidylinositol; LPI, lysophosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; MALDI, Matrix-Assisted Laser Desorption/Ionization; TOF, Time-of-flight; FTICR, Fourier Transform Ion Cyclotron Resonance.

**Supplementary Figure 3. Reproducibility of MALDI-FTICR IMS analysis of embryo implantation in the mouse uterus on day 6 of pregnancy. (a)** This picture shows a mouse uterus on day 6 of pregnancy and an enlargement of the implantation site which was sectioned. **(b)** The optical images of the 9 serial center sections (***left***) with corresponding 150 μm lateral resolution MALDI-FTICR images (***right***). Phospholipid identities ($[M+K]^+$) are listed above their respective columns. Each column is set to the same relative intensity scale. SM, Sphingomyelin; PC, phosphatidylcholine; MALDI, Matrix-

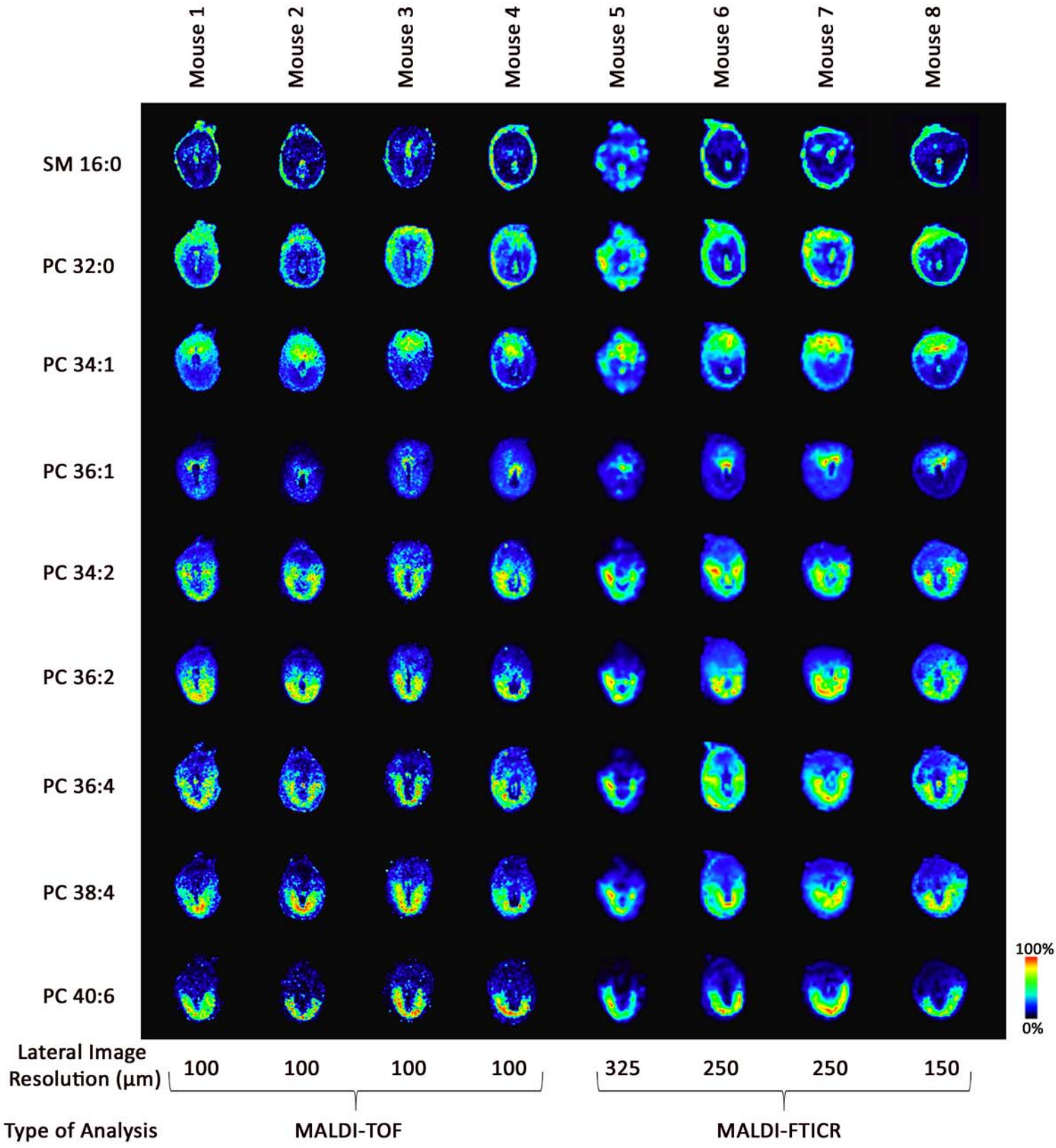
Assisted Laser Desorption/Ionization; FTICR, Fourier Transform Ion Cyclotron Resonance; AM, antimesometrial pole; M, mesometrial pole.

**Supplementary Figure 4. A Look at Positive Ion Mode MALDI-TOF Image Spectra.** All spectra is from representative "top" and "bottom" areas of a day 8 implantation site which are depicted as a green and blue circles on the optical image, respectively. Potassiated $[M+K]^+$ peaks were used to reconstitute images. SM, sphingomyelin; PC, phosphatidylcholine; M, mesometrial; AM, antimesometrial.

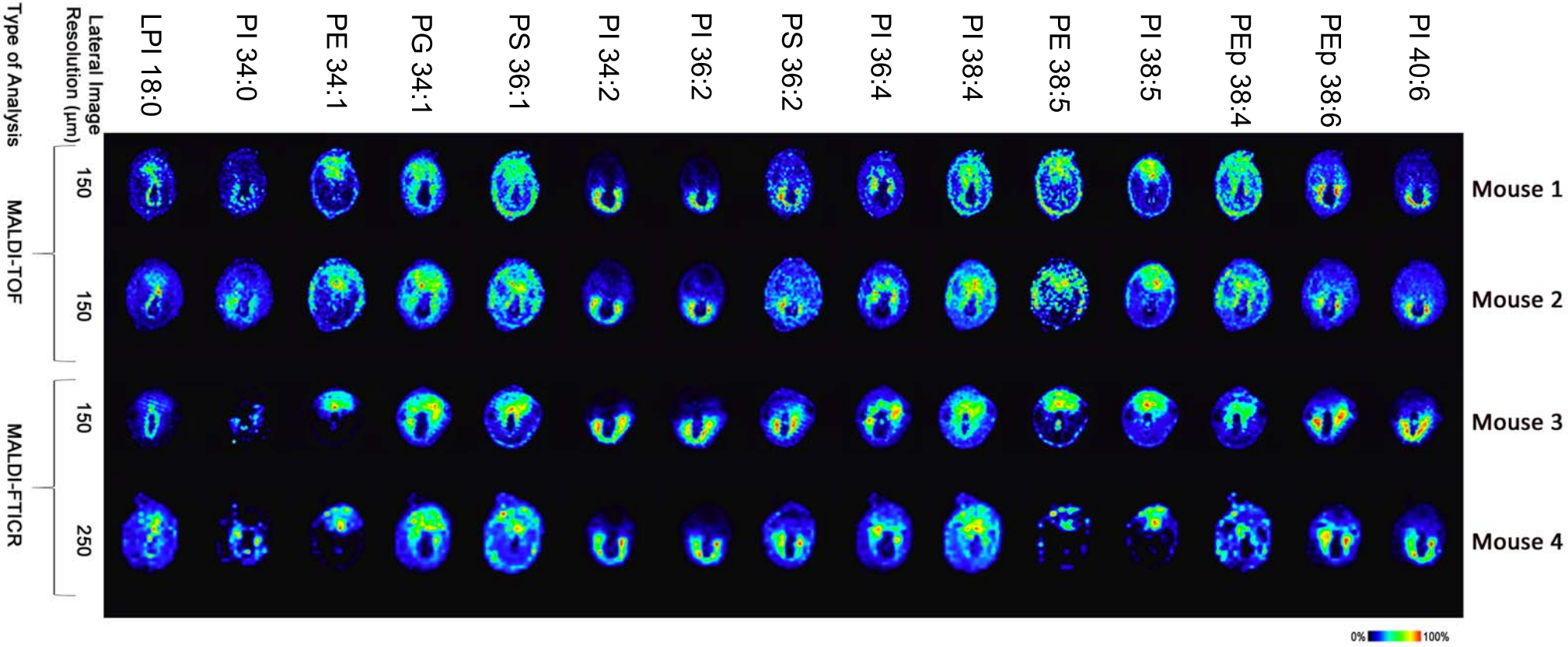
**Supplementary Figure 5. A Look at Negative Ion Mode MALDI-TOF Image Spectra.** All spectra is from representative "top" and "bottom" areas of a day 8 implantation site which are depicted as a green and blue circles on the optical image, respectively. PE, phosphatidylethanolamine; PEp, phosphatidylethanolamine plasmalogen; PI, phosphatidylinositol; LPI, lysophosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; M, mesometrial; AM, antimesometrial.

**Supplementary Figure 6. Identification of isobaric phospholipid species in day 8 implantation sites using high resolution MALDI-FTICR IMS.** MALDI-TOF images (***top***) representative of spectral peaks 100-200 mDa in width. MALDI-FTICR images (***bottom***) representative of spectral peaks 10-20 mDa in width. As an example of MALDI-FTICR spectra we have included a single FTICR spectrum (20 shots) underneath the corresponding FTICR images. The laser spot (purple spot) where this spectrum was obtained is denoted by a red circle on the matrix coated optical image. PC, phosphatidylcholine.

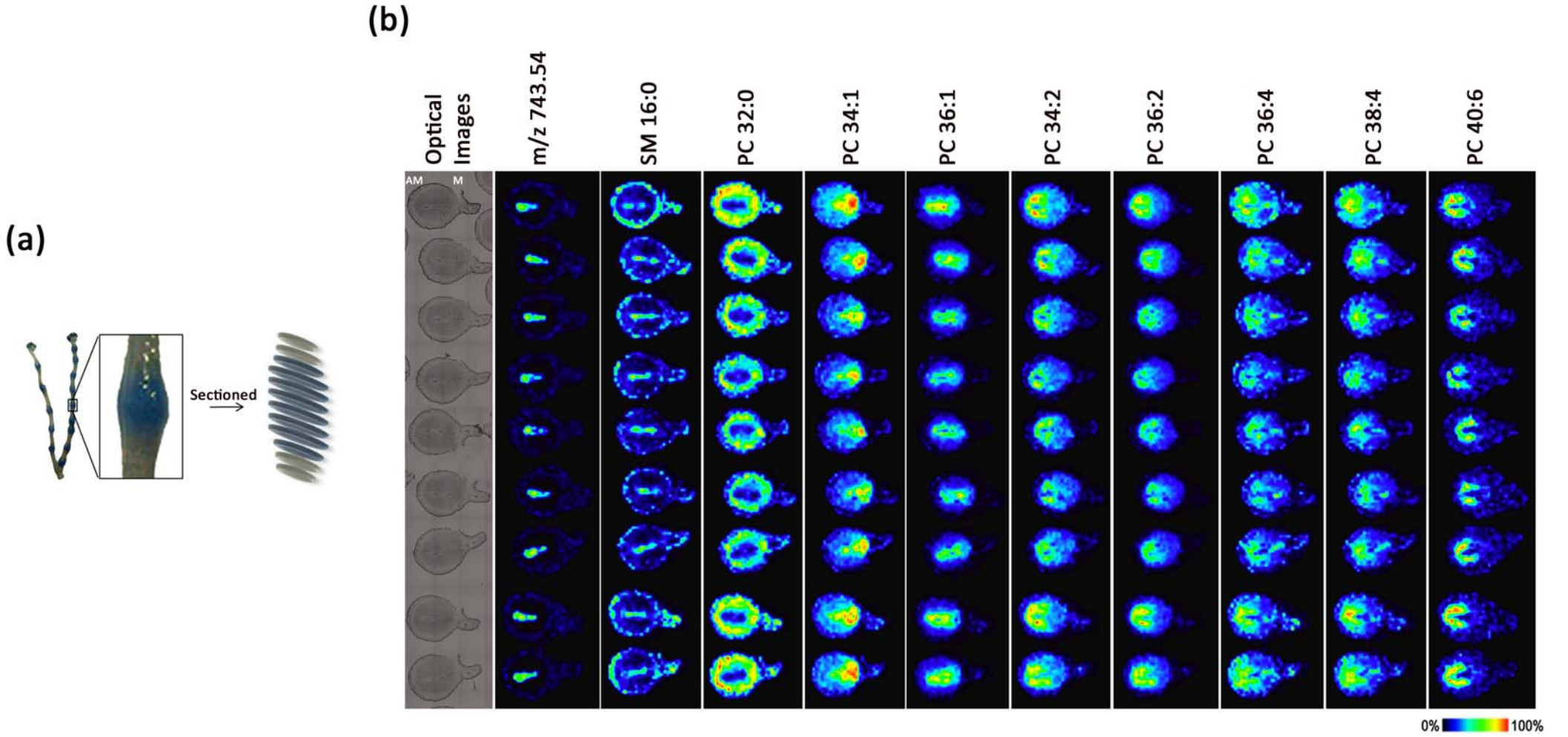
**Supplementary Figure 7. MALDI-MS/MS spectra. (a)** Fragmentation spectra of PI 36:2 in negative ion mode. **(b)** Fragmentation spectra of PC 36:2 in positive ion mode [matrix was doped with 100 mM lithium chloride]. GPIns (PI), phosphatidylinositol; GPC (PC), phosphatidylcholine.



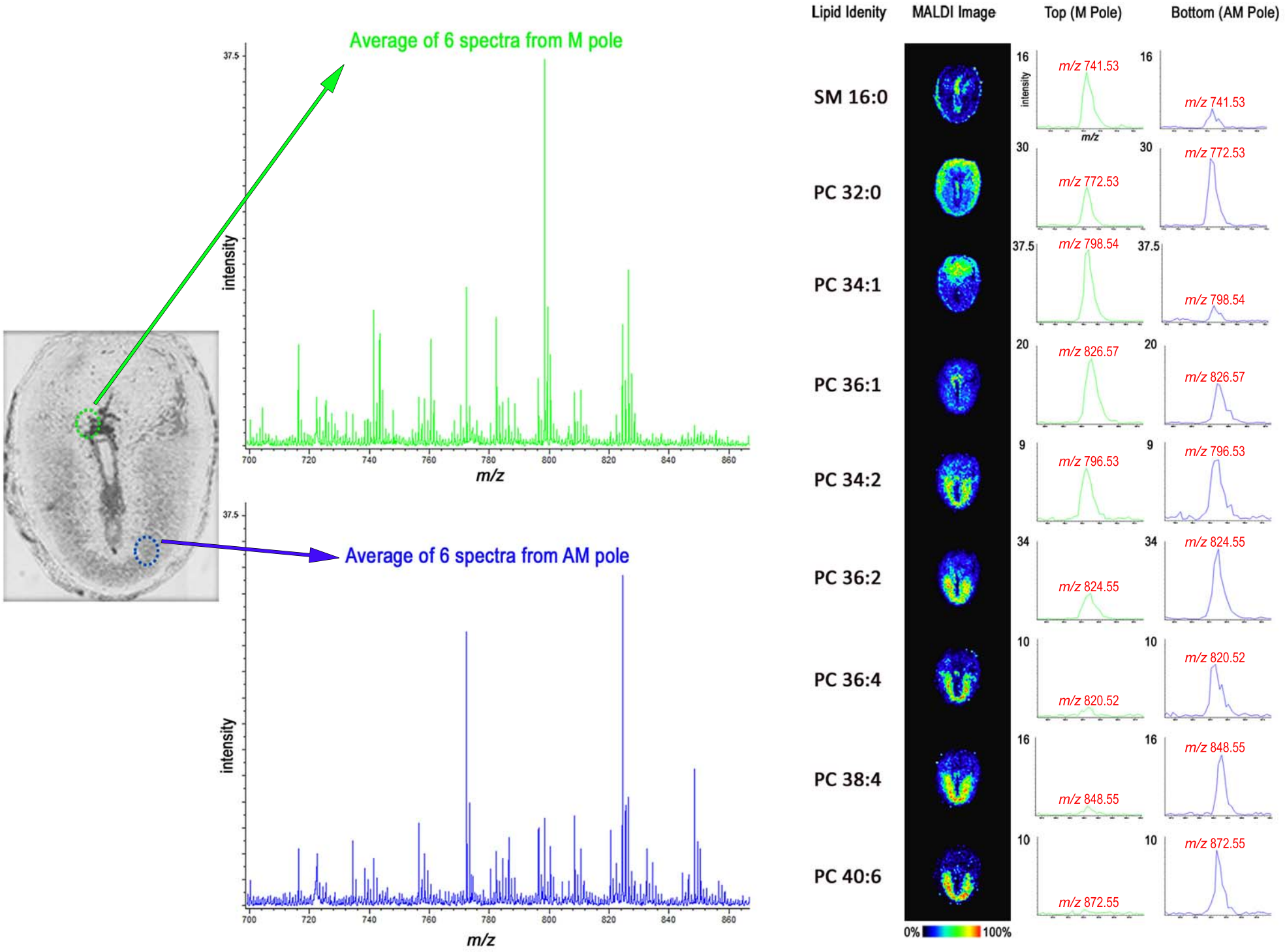

# Supplementary Figure 1



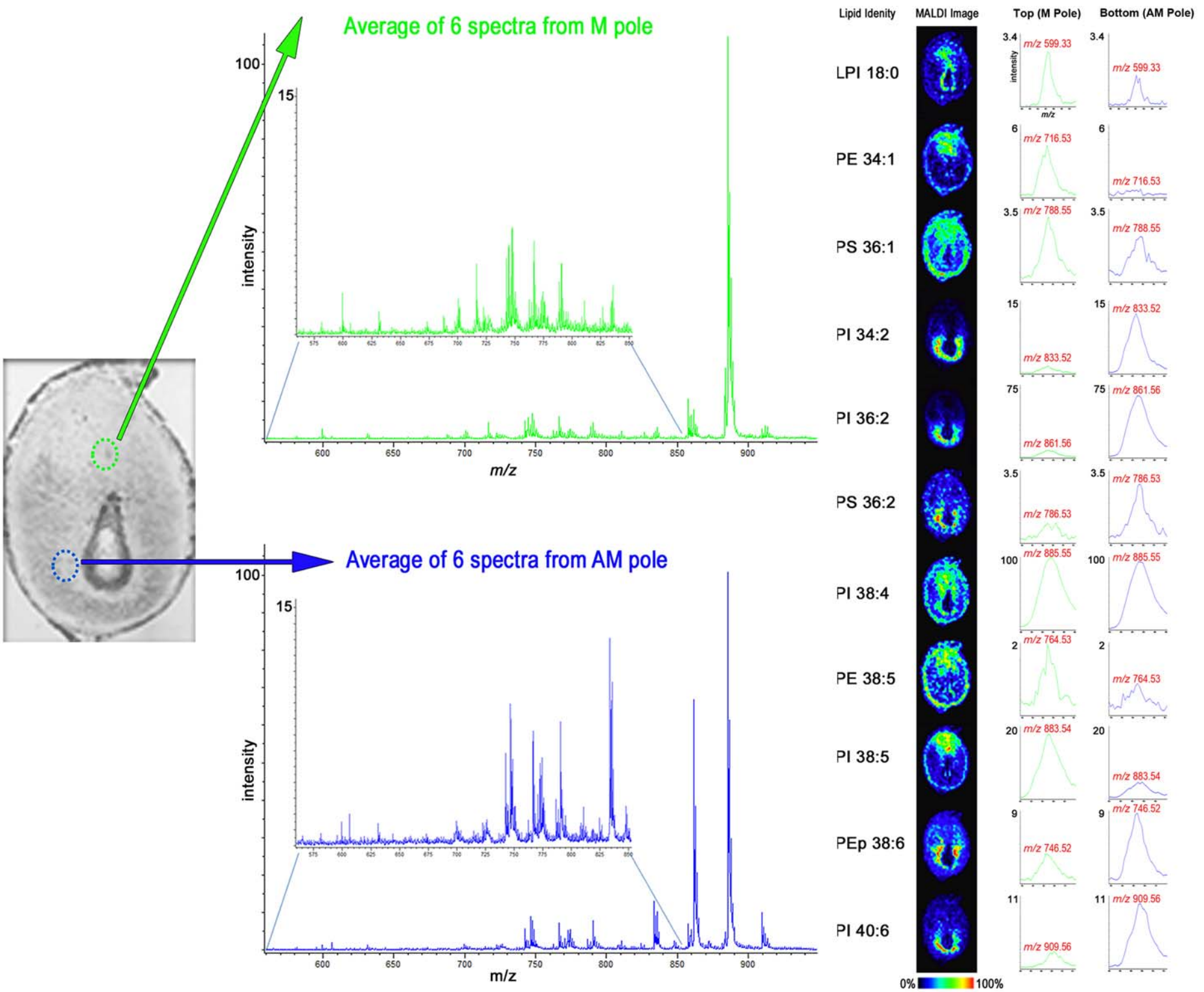

# Supplementary Figure 2



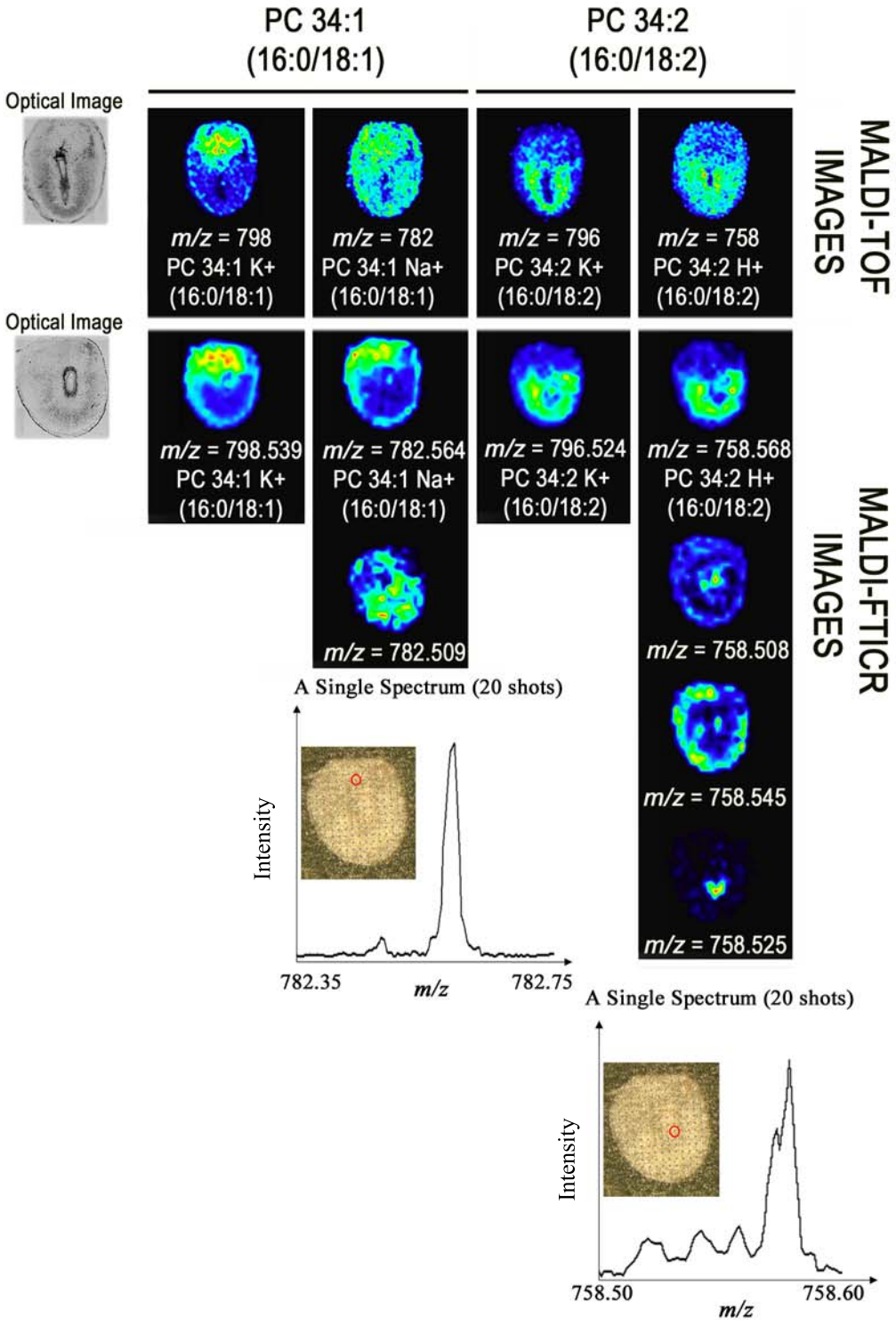

# Supplementary Figure 3



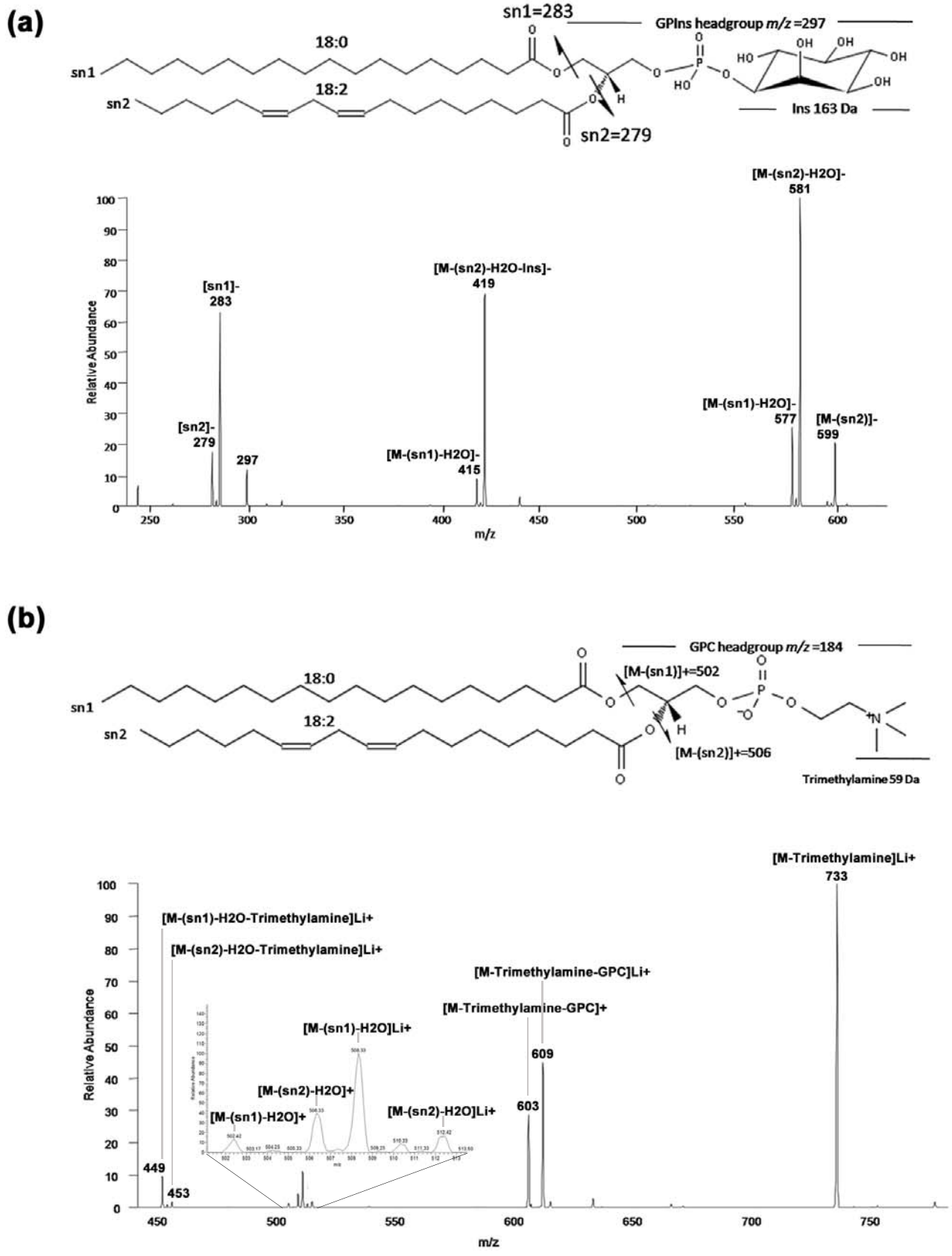

Supplementary Figure 4




Supplementary Figure 5




# Supplementary Figure 6




# Supplementary Figure 7

## Supplementary Table 1. Positive Ion Mode MALDI-MS/MS Data

Assignment of lipid molecular species in MS/MS positive ion mode

| Class | Molecular species (C1 acyl chain/ C2 acyl chain) | Ion Type | *m/z* [a] | Lithium induced MS/MS Peaks ^ |
|---|---|---|---|---|
| SM | **16:0** | $[M+H]^+$ | 703.573 | 650 |
| | | $[M+Na]^+$ | 725.555 | |
| | | $[M+K]^+$ | 741.529 | |
| PC | 32:0(16:0/**16:0**) | $[M+H]^+$ | 734.568 | 681, 551, 557, 484, 425, 478 |
| | | $[M+Na]^+$ | 756.551 | |
| | | $[M+K]^+$ | 772.526 | |
| PC | 34:0(16:0/**18:0**) | $[M+H]^+$ | 762.600 | 709, 579, 585, 484, 425, 512, 506, 453 |
| | | $[M+Na]^+$ | 784.581 | |
| | | $[M+K]^+$ | 800.554 | |
| PC | 34:1(16:0/**18:1**) | $[M+H]^+$ | 760.584 | 707, 583, 577, 510, 451, 478, 484, 425 |
| | | $[M+Na]^+$ | 782.564 | |
| | | $[M+K]^+$ | 798.539 | |
| PC | 36:1(18:0/**18:1**) | $[M+H]^+$ | 788.614 | 735, 611, 605, 510, 451, 506 |
| | | $[M+Na]^+$ | 810.594 | |
| | | $[M+K]^+$ | 826.569 | |
| PC | 34:2(16:0/**18:2**) | $[M+H]^+$ | 758.568 | 705, 581, 575, 508, 449, 478, 484, 425, 502 |
| | | $[M+Na]^+$ | 780.548 | |
| | | $[M+K]^+$ | 796.524 | |
| PC | 36:2(18:0/**18:2**) | $[M+H]^+$ | 786.600 | 733, 609, 603, 508, 449, 506, 512, 453, 502 |
| | | $[M+Na]^+$ | 808.581 | |
| | | $[M+K]^+$ | 824.554 | |
| PC | 36:4(16:0/**20:4**) | $[M+H]^+$ | hidden by 34:1 Na+ | 729, 605, 599, 532, 473, 478, 425, 484 |
| | | $[M+Na]^+$ | 804.550 | |
| | | $[M+K]^+$ | 820.524 | |
| PC | 38:4(18:0/**20:4**) | $[M+H]^+$ | no peak | 633, 757, 627, 532, 473, 506, 526, 512, 453 |
| | | $[M+Na]^+$ | 832.580 | |
| | | $[M+K]^+$ | 848.554 | |
| PC | 40:6(18:0/**22:6**) | $[M+H]^+$ | 834.596 | 781, 657, 651, 556, 497, 506, 453, 512 |
| | | $[M+Na]^+$ | 856.581 | |
| | | $[M+K]^+$ | 872.554 | |

[a] Data acquired on a MALDI-FTICR Mass Spectrometer

^ arrangement - highest intensity ion (left) to lowest intensity ion (right) in the MALDI-MS/MS spectra (decimal values not included)

**Note: All positive ion mode images depict the potassiated $[M+K]^+$ form of each phospholipid species since they had the highest intensity in this study**

## Supplementary Table 2. Negative Ion Mode MALDI-MS/MS Data

Assignment of lipid molecular species in MS/MS negative ion mode

| Class | *m/z* [a] | MS/MS Peaks ^ | Molecular species (C1 acyl chain/ C2 acyl chain) |
|---|---|---|---|
| LPI | 599.332 | 283, 419, 315 | **18:0** |
| PE | 716.528 | 281, 255, 452, 478 | 34:1(16:0/**18:1**) |
| PG | 747.521 | 281, 255, 391, 465, 483, 491, 509 | 34:1(16:0/**18:1**) |
| PS | 788.551 | 701, 419, 283, 417, 504 | 36:1(18:0/**18:1**) |
| PI | 857.522 | 553, 391, 571, 303, 255, 439, 601, 297 | 36:4(16:0/**20:4**) |
| PI | 885.553 | 581, 419, 283, 599, 601, 303, 439, 297, 619 | 38:4(18:0/**20:4**) |
| PE | 764.529 | 303, 281, 478 | 38:5(18:1/**20:4**) |
| PI | 883.541 | 579, 417, 281, 597, 601, 303, 297, 439, 619 | 38:5(18:1/**20:4**) |
| PEp | 750.549 | 331, 436 | 38:4(16:0p/**22:4**) |
| PI | 837.556 | 581, 419, 283, 553, 599, 391, 297 | 34:0(18:0/**16:0**) |
| PI | 833.523 | 553, 391, 577, 255, 571, 279, 297 | 34:2(16:0/**18:2**) |
| PI | 861.558 | 581, 419, 283, 577, 599, 415, 279, 297 | 36:2(18:0/**18:2**) |
| PS | 786.534 | 699, 419, 283, 415,279 | 36:2(18:0/**18:2**) |
| PEp | 746.517 | 327, 436, 418 | 38:6(16:0p/**22:6**) |
| PI | 909.556 | 581, 419, 283, 599, 625, 297, 327 | 40:6(18:0/**22:6**) |
| PE | 742.543 | 279, 283, 281, 480, 478 | 36:2(18:0/**18:2**, 18:1/**18:1**)* |
| PE | 770.575 | 279, 283, 281, 460, 508, 307 | 38:2(20:0/**18:2**, 18:0/**20:2**, 18:1/**20:1**)* |

[a] Data acquired on a MALDI-FTICR Mass Spectrometer

^ arrangement - highest intensity ion (left) to lowest intensity ion (right) in the MALDI-MS/MS spectra (decimal values not included)

* acyl chain identities confirmed by MS/MS imaging

## Supplementary Table 3. LC-MS/MS Data
## Predominantly bottom expression (AM pole)

| weights (mg tissue): | 15 | 15 | 15 | 14.5 | 15 | 15 |
|---|---|---|---|---|---|---|
| **values below are ng lipid/mg tissue** | **top1** | **top2** | **topM** | **bot1** | **bot2** | **BotM** |
| **GPA(36:2)** | 6 | 5.6 | 5.2 | 8.2 | 10.8 | 6.1 |
| **GPA(38:4)** | 4.6 | 4.3 | 4.2 | 6.1 | 6.7 | 5.7 |
| **GPA(40:6)** | 12.9 | 13.4 | 11.2 | 19.7 | 18.9 | 13.9 |
| **GPCho(32:1e)/GPCho(32:0p)** | 1.1 | 0.6 | 1.1 | 1.9 | 1.7 | 1.3 |
| **GPCho(34:2)*** | 44.4 | 41.1 | 43.6 | 55.2 | 55.4 | 50.9 |
| **GPCho(36:4)*** | 71.9 | 69.0 | 73.7 | 97.1 | 67.2 | 83.6 |
| **GPCho(36:4p)** | 3.5 | 2 | 4.2 | 5.1 | 5.1 | 5.4 |
| **GPCho(38:4)** | 166.1 | 157.7 | 152.3 | 160.1 | 177.5 | 168.7 |
| **GPCho(38:6e)/GPCho(38:5p)** | 5 | 4.4 | 5.1 | 6.8 | 13.1 | 7 |
| **GPCho(40:6)*** | 77.2 | 67.8 | 79.9 | 80.1 | 133.9 | 83.9 |
| **GPEtn(34:2)** | 6.4 | 6.5 | 5.5 | 7.2 | 6.4 | 8 |
| **GPEtn(36:0)** | 41.3 | 30.2 | 40.9 | 52.2 | 62.7 | 43.3 |
| **GPEtn(36:4e)/GPEtn(36:3p)** | 4.4 | 2.8 | 4 | 5.3 | 4.7 | 4.6 |
| **GPEtn(38:4)** | 81.2 | 76.5 | 71.3 | 97 | 80.8 | 94.4 |
| **GPGro(36:3)** | 16.7 | 14.1 | 15.3 | 21.1 | 30.5 | 17.5 |
| **GPGro(36:4)** | 10.6 | 8.6 | 8.8 | 18.1 | 26.7 | 14.5 |
| **GPGro(38:1)** | 1.4 | 1 | 1.3 | 1.8 | 2.5 | 1.5 |
| **GPGro(42:10)** | 4.9 | 4 | 5 | 8.1 | 14.4 | 7 |
| **GPGro(44:10)** | 4.2 | 3.3 | 3.8 | 4.8 | 6.4 | 4.3 |
| **GPGro(44:11)** | 6.5 | 5.2 | 6.5 | 10.1 | 10.5 | 8.2 |
| **GPGro(44:12)** | 21.3 | 16.4 | 21.1 | 31 | 39.5 | 27.2 |
| **GPIns(32:0)** | 0.3 | 0.5 | 0.4 | 1 | 1.2 | 1.1 |
| **GPIns(34:1)** | 3.9 | 3.6 | 3.4 | 6.2 | 9 | 6.5 |
| **GPIns(34:2)*** | 4.3 | 3.9 | 4.5 | 11.4 | 17.6 | 9.9 |
| **GPIns(36:1)** | 1.6 | 1.7 | 1.9 | 4.1 | 5.4 | 3.8 |
| **GPIns(36:2)** | 6.2 | 6 | 6.6 | 16 | 22.1 | 13.2 |
| **GPIns(38:6)** | 1.7 | 1.5 | 1.3 | 2.9 | 3 | 2.4 |
| **GPIns(40:4)** | 1.8 | 1.7 | 1.9 | 2.6 | 2 | 2.3 |
| **GPIns(40:6)*** | 2.4 | 2.3 | 2.5 | 4.7 | 5.4 | 4 |
| **GPSer(38:4)** | 49.8 | 48.4 | 44.2 | 66.4 | 61.1 | 50.6 |
| **GPSer(42:8)** | 0.8 | 0.4 | 0 | 0.9 | 1.2 | 0.8 |
| **GPThr(38:4)** | 2.4 | 2.7 | 2.5 | 3.8 | 3.2 | 3 |

***LC-MS/MS data correlates with MALDI images**

GPCho (PC) = phosphatidylcholine, GPEtn (PE) = phosphatidylethanolamine, GPGro (PG) = phosphatidylglycerol, GPIns (PI) = phosphatidylinositol, GPSer (PS) = phosphatidylserine

**Supplementary Table 3. LC-MS/MS Data**
**Predominantly top expression (M pole)**

| weights (mg tissue): | 15 | 15 | 15 | 14.5 | 15 | 15 |
|---|---|---|---|---|---|---|
| **values below are ng lipid/mg tissue** | **top1** | **top2** | **topM** | **bot1** | **bot2** | **BotM** |
| **GPA(32:1)** | 0.9 | 0.7 | 0.8 | 0.6 | 0 | 0 |
| **GPA(34:1)** | 4.6 | 4 | 4 | 3.4 | 3.8 | 2.6 |
| **GPCho(30:0)** | 10.9 | 10.7 | 10.2 | 8.9 | 6.9 | 7.3 |
| **GPCho(32:1)** | 30.8 | 29.7 | 29.5 | 23.7 | 20.4 | 20.3 |
| **GPCho(34:0)** | 19.4 | 17 | 19.5 | 14.4 | 12.5 | 14.4 |
| **GPCho(34:0e)** | 2.5 | 2 | 2.2 | 1.5 | 1.8 | 1.4 |
| **GPCho(34:1)*** | 57 | 55.7 | 56.5 | 46 | 33.3 | 42.1 |
| **GPCho(34:1e)/GPCho(34:0p)** | 11.3 | 12.1 | 10.2 | 9 | 9.2 | 7.1 |
| **GPCho(36:1)** | 74.1 | 73.3 | 73.2 | 54.2 | 60.8 | 55.2 |
| **GPCho(36:1e)/GPCho(36:0p)** | 1.4 | 1.2 | 1.1 | 0.7 | 0.8 | 0.7 |
| **GPCho(36:3)** | 49.2 | 48.7 | 49 | 40.9 | 39 | 41.6 |
| **GPCho(38:2)** | 8 | 8.3 | 7.5 | 3.3 | 4.4 | 3.4 |
| **GPCho(38:5)** | 48.7 | 45.8 | 45.3 | 39.9 | 33.8 | 38.8 |
| **GPCho(40:1)** | 29.8 | 24.3 | 29.1 | 25.8 | 22.9 | 22.5 |
| **GPCho(40:7)** | 20.5 | 15.6 | 17.4 | 12 | 10.4 | 12.6 |
| **GPEtn(32:0)** | 1.2 | 1.1 | 0.8 | 0.6 | 0 | 0.5 |
| **GPEtn(32:1)** | 0.8 | 0.7 | 0.9 | 0.4 | 0.3 | 0.5 |
| **GPEtn(34:0)** | 2.6 | 2.1 | 2.5 | 1.8 | 1.3 | 1.5 |
| **GPEtn(34:1)*** | 13.3 | 13.5 | 13.1 | 10.4 | 8.1 | 10.5 |
| **GPEtn(38:3e)/GPEtn(38:2p)** | 6.4 | 9.5 | 4.1 | 5.2 | 3.5 | 4 |
| **GPEtn(40:4e)/GPEtn(40:3p)** | 12.3 | 15.1 | 9.2 | 9.5 | 0 | 8.3 |
| **GPEtn(40:7)** | 19.5 | 18.3 | 18.4 | 18 | 16.3 | 16.4 |
| **GPEtn(42:10)** | 2.9 | 4 | 1.7 | 1.4 | 1.2 | 1.2 |
| **GPEtn(42:5)** | 1.8 | 1.9 | 1.6 | 1.4 | 1 | 1.3 |
| **GPGro(38:2)** | 0.9 | 0.8 | 0.7 | 0.5 | 0.6 | 0.5 |
| **GPIns(38:5)*** | 8.7 | 9.7 | 8.5 | 8.2 | 6.1 | 7.2 |
| **GPSer(34:1)** | 19.5 | 21.3 | 17.5 | 13.4 | 14.1 | 11.1 |
| **GPSer(38:1)** | 3.6 | 2.9 | 2.4 | 2.3 | 1.9 | 1.9 |
| **GPSer(40:2)** | 3 | 2.9 | 2.3 | 1.4 | 0.9 | 1.2 |
| **GPSer(40:3)** | 3.1 | 3.2 | 2.1 | 2.3 | 2.1 | 1.5 |
| **GPSer(42:3)** | 2.2 | 2.2 | 1.9 | 2 | 1.4 | 1.4 |

***LC-MS/MS data correlates with MALDI images**

GPCho (PC) = phosphatidylcholine, GPEtn (PE) = phosphatidylethanolamine, GPGro (PG) = phosphatidylglycerol, GPIns (PI) = phosphatidylinositol, GPSer (PS) = phosphatidylserine