# Supporting Information

# High-throughput single-cell mass spectrometry enabling lipid structure elucidation and in-depth lipid metabolic pathway studies

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## Experimental Procedures

**Reagents and materials.** Ammonium formate (LC-grade), ammonium acetate (LC-grade) and 2-acetylpyridine (2-AP, 99%) were purchased from Meryer Chemical (Shanghai, China). Glutaraldehyde (50% in $H_2O$, AR-grade) was obtained from Aladdin Chemicals (Shanghai, China). HPLC-grade MeOH, EtOH, acetone, ACN, water, and LC-MS grade formic acid were purchased from Fisher Scientific (NJ, USA). Internal standards (PC 26:0, PE 30:0 and PS 28:0) were purchased from Avanti Polar Lipids, Inc. Fused silica capillary tubes were purchased from Polymicro Technologies (Molex, USA), while metal tee connectors were obtained from Yijia Technology Co., Ltd. (Beijing, China). An industrial camera (MV-HP900GM, MicroVision, China) was used to observe the flow of cells in the capillary. The MDA-MB-468 cells were purchased from Shanghai Enzyme Research Biotechnology Co. Ltd (Shanghai, China), while the MCF-10A cell line and its specific epithelial culture medium (CL-0525) were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). All cell cultured reagents, including Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), fetal bovine serum (FBS), Trypsin-EDTA (0.25%) and penicillin−streptomycin (100 $U \cdot ml^{-1}$) were purchased from Gibco Life Technologies (Carlsbad, CA).

**Cell culture and pretreatment**. MDA-MB-468 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. The MCF-10A cell line was cultured in its specific culture medium. All three types of cells were cultured with breathable dishes in a humidified atmosphere containing 5% $CO_2$ at 37℃ and passaged every two or three days. Upon reaching 80%-90% confluence, cells were detached using Trypsin-EDTA (0.25%), collected by centrifugation, and then washed twice with phosphate-buffered saline (PBS) for later use. Prior to MS analysis, there are still some necessary processing steps:

(i) For the optimization of the CyESI-MS platform using unfixed and underivatized cells, a simple pretreatment was performed on the freshly prepared cell suspension. The cells were centrifuged (1500 rpm, 3 minutes) to remove PBS, followed by resuspension in 140 mM $HCOONH_4$ aqueous solution (pH = 7.3) at a concentration of approximately $5 \times 10^4$ cells/mL.

(ii) For CyESI MS-SCSL in-depth lipidomics analysis, cells were fixed and derivatized collectively first. Specifically, cells in 2 mL PBS were mixed with 2 mL 5% glutaraldehyde in PBS and incubated on a shaker for 20 minutes for fixation. Subsequently, the cells were washed with water and resuspended in 100 mM 2-AP aqueous solution. The cell derivatization device, consisting of a low-pressure mercury lamp with emission centered around 254 nm (BHK Inc., USA) and a quartz cell, is shown in Fig. S1a. The cells in quartz cell were exposed to UV lamp irradiation for 8 minutes, with shaking of the quartz cell

every 2 minutes to ensure even cell distribution. Afterwards, the water containing 2-AP was removed by centrifugation (2500 rpm, 3 min), and cells were resuspended in water. This process was repeated twice, and cell suspension was finally diluted with water to a concentration of ~ $5 \times 10^4$ cells/mL. Cell concentrations were finally determined using a hemocytometer.

**Device and single cell analysis.** Cell preparation, introduction, lipid extraction, electrospray ionization and MS analysis were achieved by the homemade CyESI MS-SCSL platform. Fig. S1 illustrates the key components of the device, which consisted of a quartz cell, a UV lamp, and three coaxial capillaries connected through metal tee connectors. The quartz cell and the UV lamp were employed to conduct batch PB derivatization for in-depth lipid structural analysis. The three-layered tubing structure of CyESI was used for high throughput single cell analysis, in which the inner capillary (150 μm O.D., 50 μm I.D.) was employed to introduce the cell suspension, which was driven by a pressure-based flow controller (Flow EZ, Fluigent, France). The middle capillary (360 μm O.D., 200 μm I.D.) was used to transported the sheath liquid, which was served for better extraction of lipid and formation of electrospray. The outer capillary (700 μm O.D., 530 μm I.D.) positioned 2 mm shorter than the end of the middle capillary, was responsible for delivering the carrier gas. Nitrogen, served as the carrier gas, could assist the electrospray formation to promote lipid ionization and improve signal intensity. High voltage direct current was applied to the sheath liquid for the electrospray ionization. The distance between the outlet end of inner and middle capillary could be adjusted flexibly between 2-6 mm to balance the number of cells passing per unit time and the duration of each cell signal. The ion source was placed approximately 1 cm away from the MS inlet. For the acquisition of single-cell lipid profiles, cell suspension ($5 \times 10^4$ cells/mL) was introduced into the inner capillary at the flow rate of 1 μL/min, while the flow rate of the sheath liquid (methanol containing 1% formic acid) in the middle capillary was set to 10 μL/min. For in-depth lipidomics analysis, cell suspension ($3 \times 10^4$ cells/mL) was introduced into the inner capillary at the flow rate of 0.8 μL/min, and the flow rate of the sheath liquid in the middle capillary was set to 6 μL/min. The pressure of nitrogen, acting as the carrier gas, was set at around 0.6 MPa. A high positive DC voltage of 3 kV was applied to the sheath liquid.

**Lipid extraction.** A modified Folch method was employed for phospholipid extraction from cultured MDA-MB-468 cells as previously reported[1,2]. In short, the cell suspension in methanol (1 mL) was mixed with deionized water (1 mL), and extracted by 2 mL of chloroform twice. The bottom layer of chloroform was collected together and dried under nitrogen flow. Finally, the dried lipid extract was redissolved with 1 mL of ACN containing 10 mM ammonium acetate and stored at -20°C before MS analysis for lipid chain information identification.

For PC recovery analysis, cells were divided into two equal subsets. For cell fixation step, one subset was treated with 2.5% glutaraldehyde in PBS for fixation while the other subset was treated with PBS as a control. For cell derivatization step, one subset was resuspended in 0.5 mL 100 mM 2-AP aqueous solution for derivatization while the other was resuspended in 0.5 mL water as a control. After centrifugation, cells were resuspended in the mixture of water (1 mL), methanol (1 mL) and chloroform (2 mL) for lipid extraction. The internal standard (PC 26:0, PE 30:0 and PS 28:0) was added at a concentration of 5 $\mu$M for quantification.

**Mass spectrometry.** Mostmass spectrometry measurements were performed on a LTQ XL linear ion trap (LIT) mass spectrometer (Thermo Scientific, San Jose, CA). The instrument parameters were set as follows: capillary temperature = 275 ℃, capillary voltage = 19 V, tube lens voltage = 110 V, microscans = 1. For SCSL MS settings, the maximum injection time was set to 10 ms. For lipid C=C location analysis by SCSL MS/MS in positive ion mode and lipid chain information analysis by nano-ESI MS/MS in negative ion mode, the maximum injection time was set to 100 ms, and a normalized collision energy of 35

eV was used, with a 2.0 Da isolation window for precursor ion selection. Lipid sum composition analysis was performed on QTRAP 4500 mass spectrometry (Sciex, Toronto, CA). Neutral loss scan (NLS) 141 for PEs, NLS 185 for PSs, and precursor ion scan (PIS) 184 for PCs in positive mode were applied.

**Data analysis.** The MS raw data generated from the mass spectrometer were recorded with Xcalibur version 2.2 software (Thermo Scientific, San Jose, CA), and converted to mzXML format by the MSConvert software (ProteoWizard, version 3.0). Then, the converted mzXML format file was imported into MATLAB (MathWorks, Natick, MA, version R2022a) and processed using self-developed MATLAB code. To identify successful single-cell detection, the pulse peaks of phosphatidylcholine (PC) 34:1 at *m/z* 760.58 were selected as markers, with a signal-to-noise ratio (S/N ratio) threshold of 3. The mass spectra corresponding to these pulse peaks were extracted as single-cell events, and ion signals associated with single cells, exhibiting a S/N ratio greater than 3, were retained for further analysis. For in-depth lipidomics analysis, the intensities of C=C-specific diagnostic ions within the single-cell events were utilized to quantify lipid C=C location isomers relatively. To ensure reliable quantitation, the S/N ratio of the diagnostic ions was required to be greater than 5. All data processing including histogram distribution analysis and kernel density estimation were performed using Python (version 3.7).

The convert yield of PB reaction was defined as:

$$\text{Yield} = \frac{\sum Int.(^{PB}(PCs))}{\sum Int.(^{PB}(PCs)) + \sum Int.(remaining\ PCs)} \times 100\%$$

The recovery ratio of PCs after fixation or derivatization was defined as:

$$\text{Recovery} = \frac{Int.(PC_{with\ treated})/Int.(IS_{with\ treated})}{Int.(PC_{without\ treated})/Int.(IS_{without\ treated})} \times 100\%$$

# Supplementary Figures

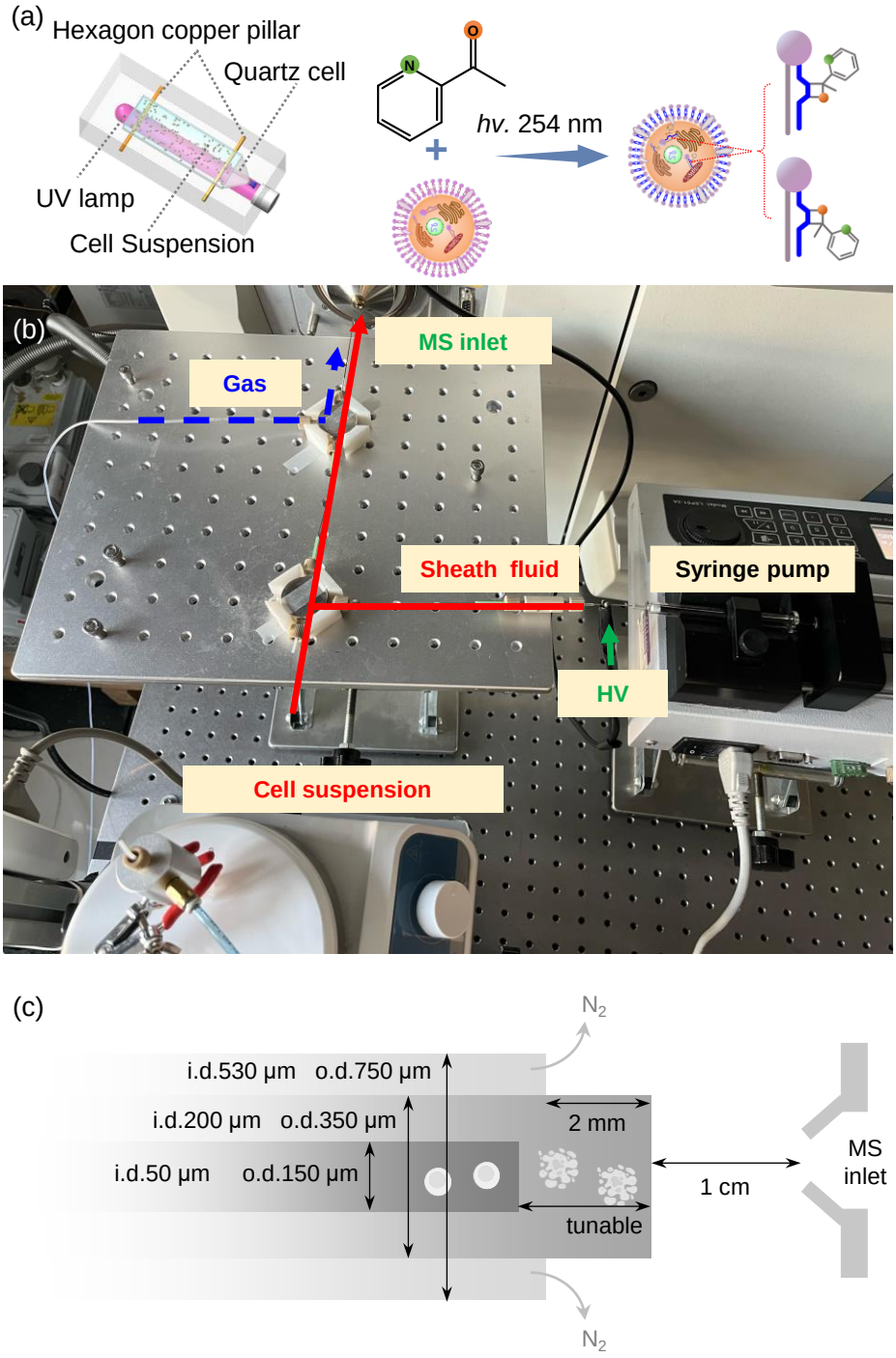


**Fig. S1. CyESI MS-SCSL device structure.** (a) Experiment setup and schematic of batch cell PB derivatization. (b) Physical diagram of the device. The solid red line indicates the direction of liquid flow, including cell suspension and sheath fluid; The blue dashed line indicates the sheath gas flow direction; The green label indicates mass spectrometry inlet and its high voltage direct current. (c) Dimensions and assembly instructions for the three-layered tubing structure.

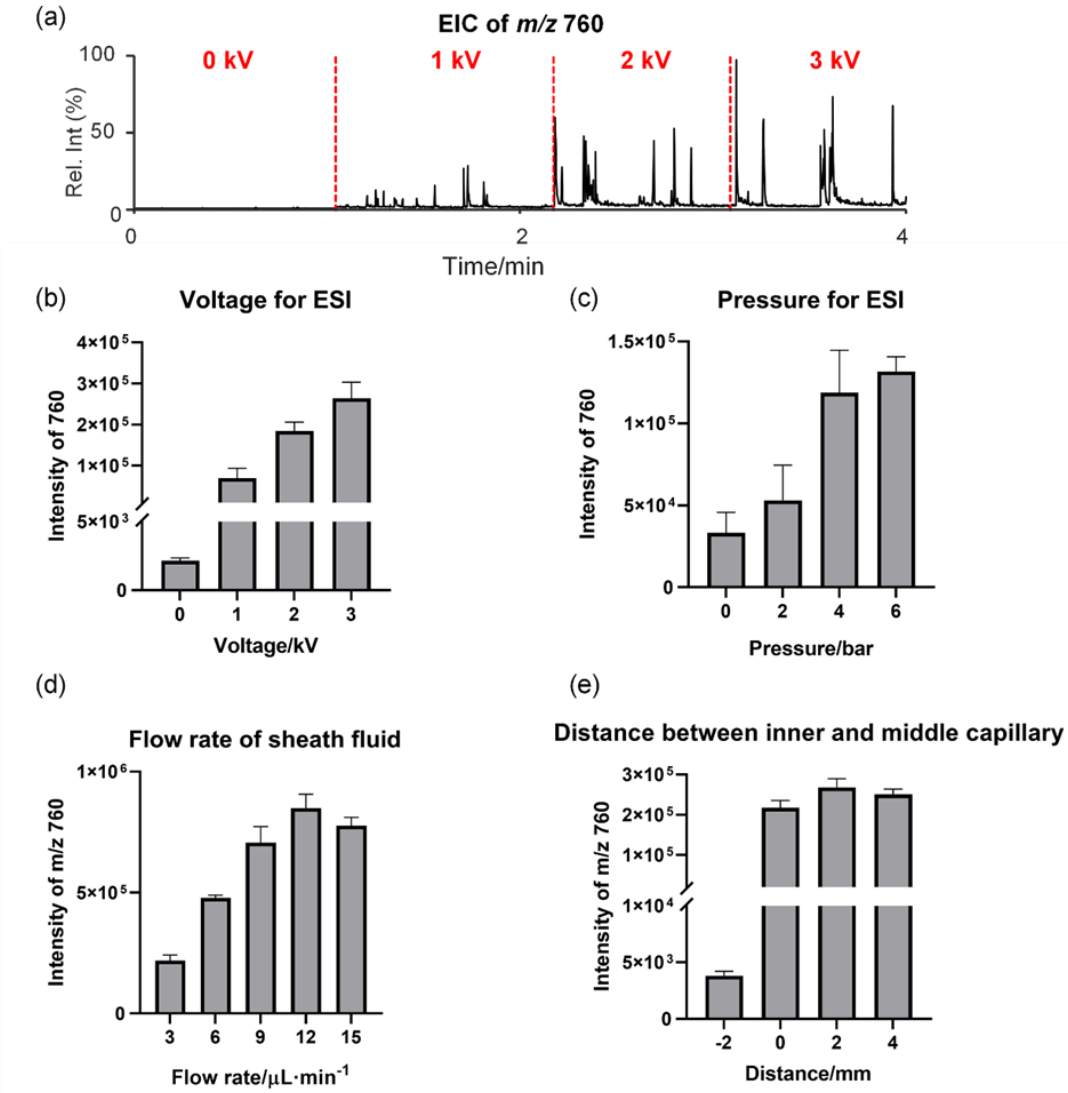


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**Fig. S2. CyESI MS-SCSL platform analysis performance optimization of MS intensity in single cell analysis characterized by m/z 760 ions**, including the influence of ESI voltages (a, b), the influence of gas pressure for ESI (c), the influence of sheath fluid flow rate (d), and the influence of the inner and the middle capillary distance (e).

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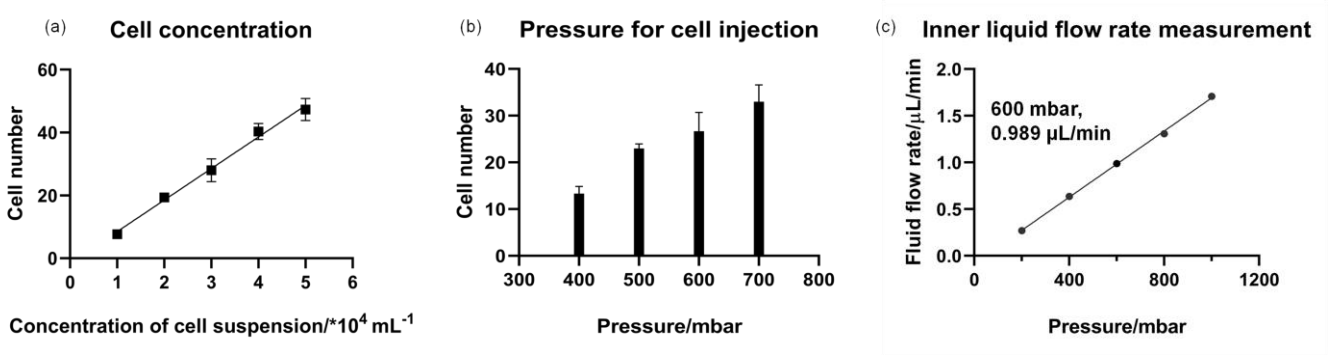


**Fig. S3. CyESI MS-SCSL platform analysis performance optimization of single cell analysis throughput,** including the influence of cell concentration (a) the influence of cell injection pressure (b), and the flow rate measurements of the precision pump that controls cell outflow (c).

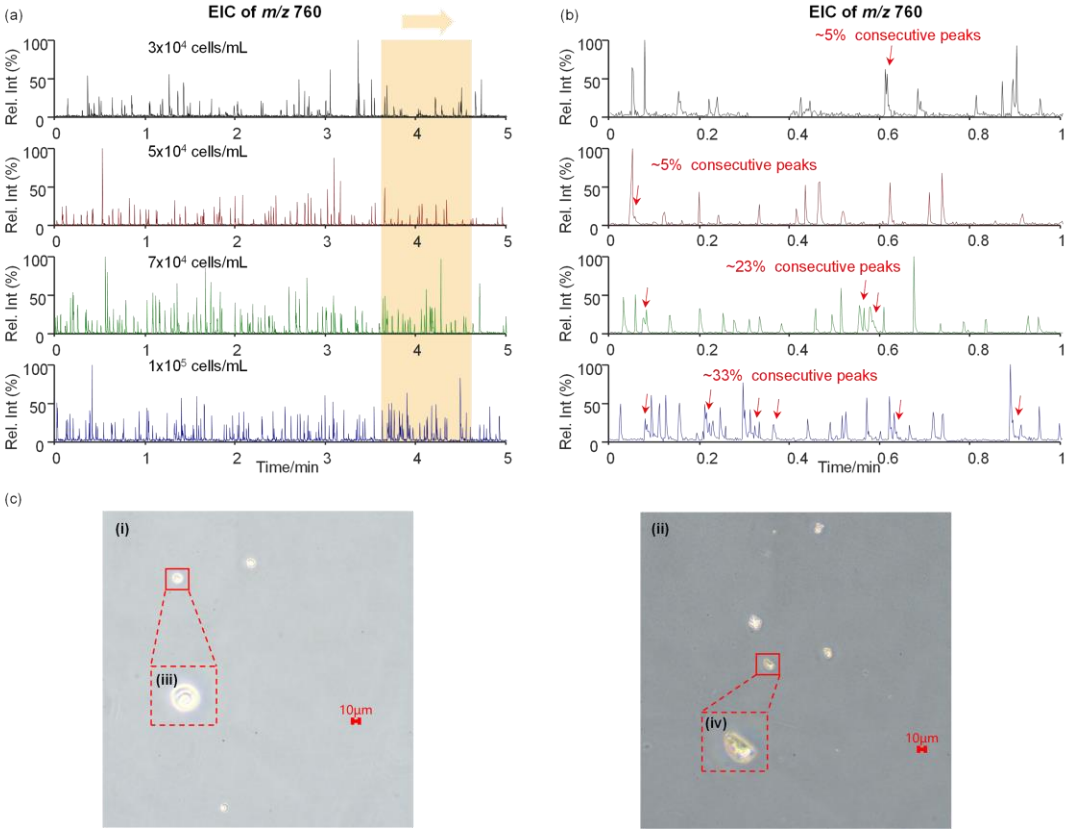


**Fig. S4. Single cell MS detection and outflow in the capillary.** (a) MS detection of single cells at different concentrations. ((b) Enlarged yellow area in (a). When the cell concentration is too high, such as $1 \times 10^5$ cells/mL, there will be many consecutive peaks. (c) Cells morphology after flowing out of the capillary, at $5 \times 10^4$ cells/mL concentration, 600 mbar flow pressure, with methanol as the sheath fluid. (i) fresh cells, no ESI high voltage and carrier gas; (ii) fresh cells disruption, with 3 kV high voltage and no carrier gas. (iii, iv) enlarged morphology of cells in (i) and (ii).

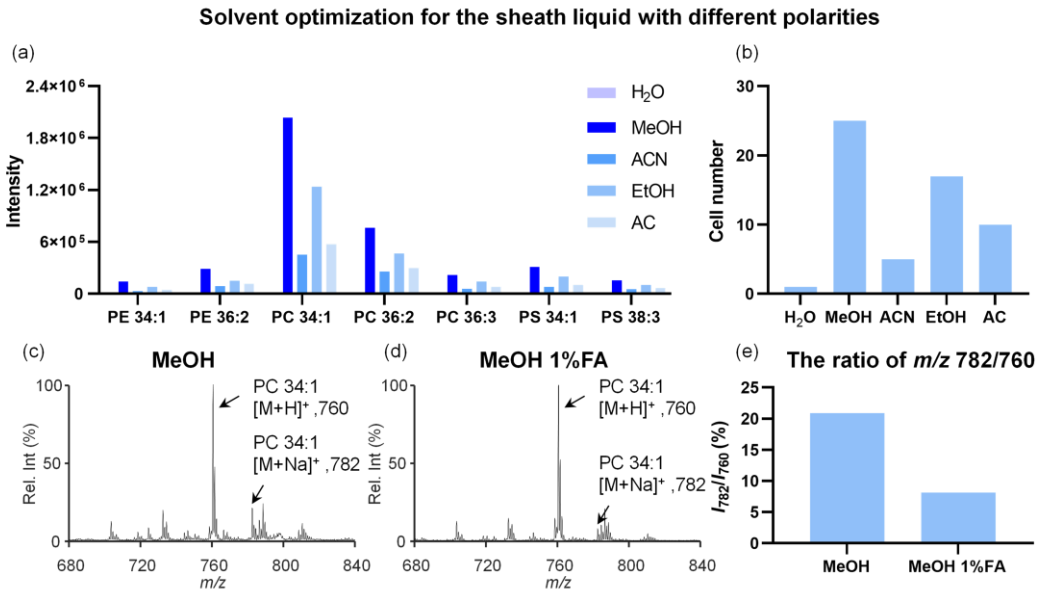


**Fig. S5. Solvent optimization for the sheath liquid.** (a, b) Single-cell lipids intensities and detected MS signals using sheath fluids of different polarities, with MeOH showing the highest intensities and cell numbers detected. (c-e) The addition of 1%FA suppressed [M+Na]+ while increased the intensities of [M+H]+ ions

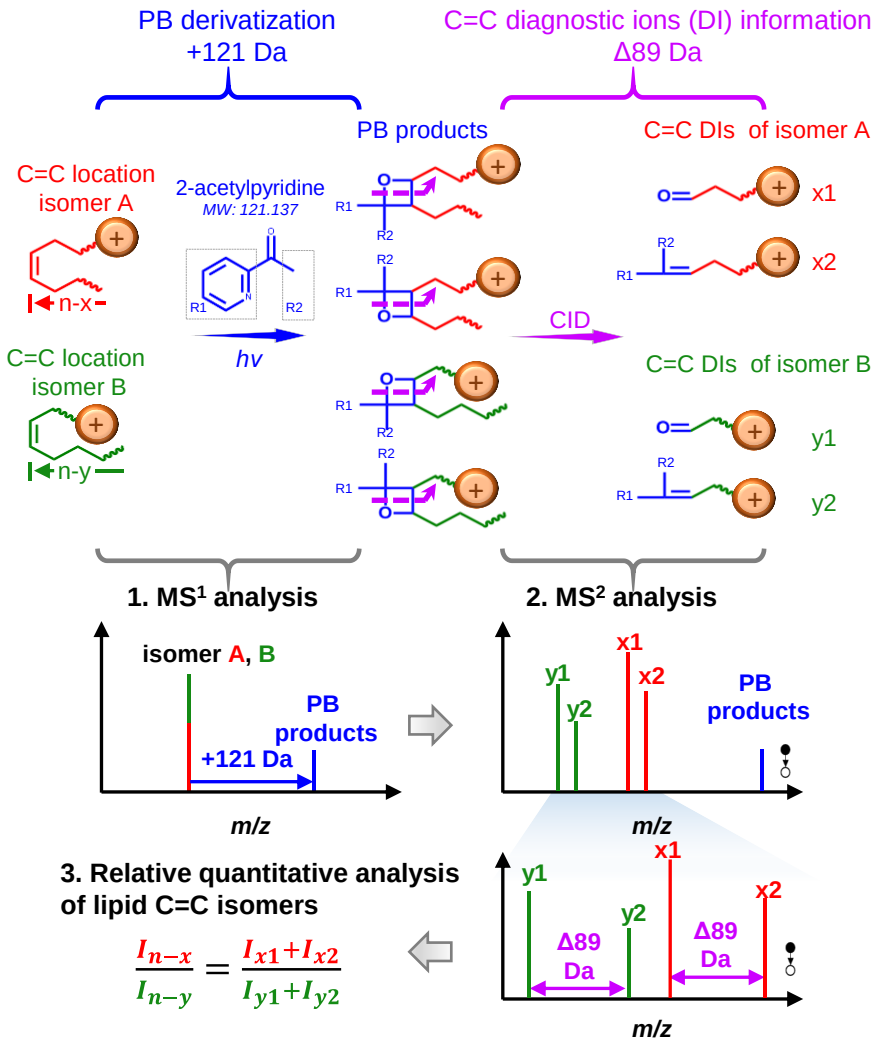


**Fig. S6. Schematic diagram for the identification of lipid C=C position and relative quantitative analysis of C=C isomers based on PB photochemical derivatization.**

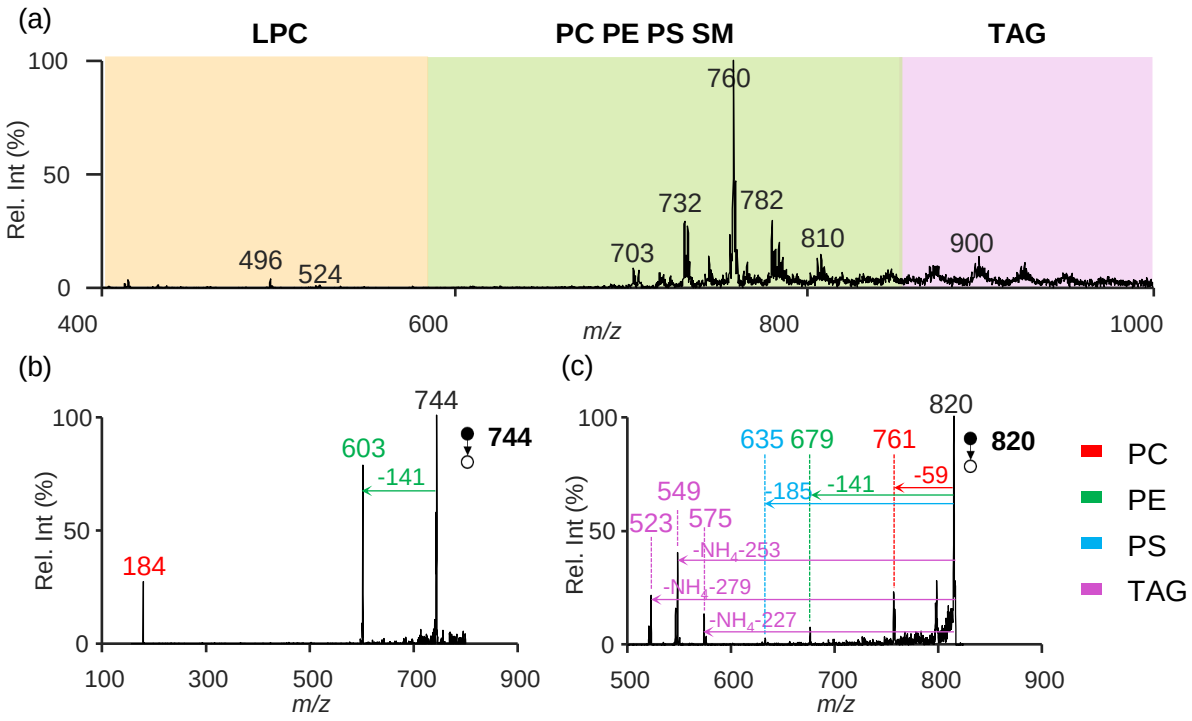


**Fig. S7. MS² analysis expanded single cell lipid identification scope.** (a) Lipid MS¹ spectrum of unfixed single cells. (b) MS² spectrum of *m/z* 744 ion reveals the simultaneous presence of PC and PE. (c) MS² spectrum of *m/z* 820 ion reveals the simultaneous presence of PC, PE, PS and TAG.

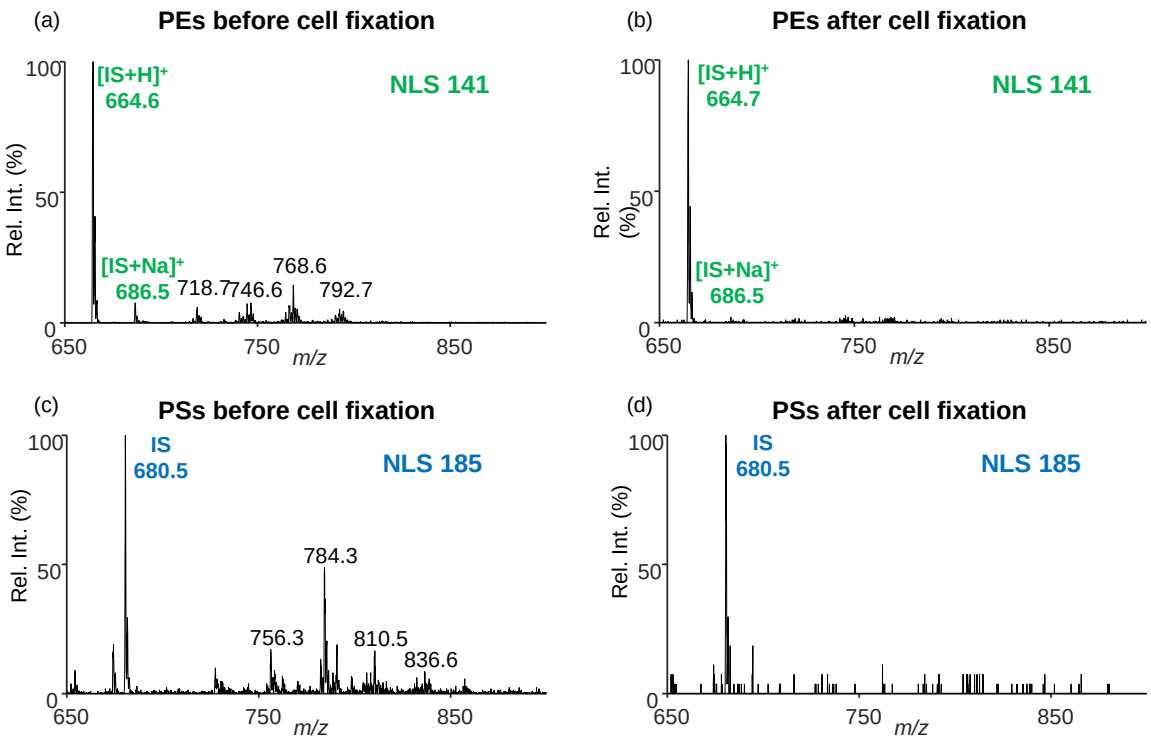


**Fig. S8. Spectra of neutral loss of 141 (PEs) and neutral loss of 185 (PSs) before after cell fixation**, which indicated PEs and PSs were lost. Internal standard (IS): PE 30:0 ($[M+H]^+$ *m/z* 664, $[M+Na]^+$ *m/z* 686), PS 28:0 (*m/z* 680).

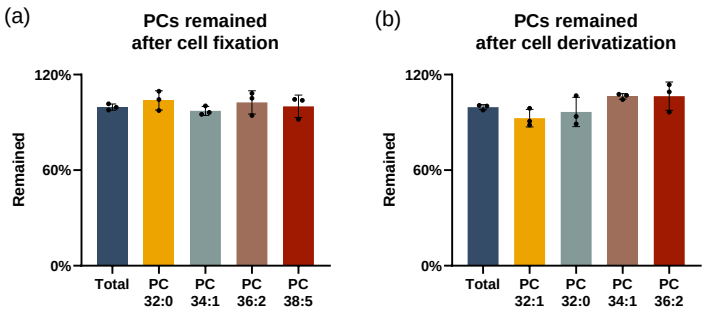


**Fig. S9**. The recovery rate of PCs after fixation (a) and derivatization (b).

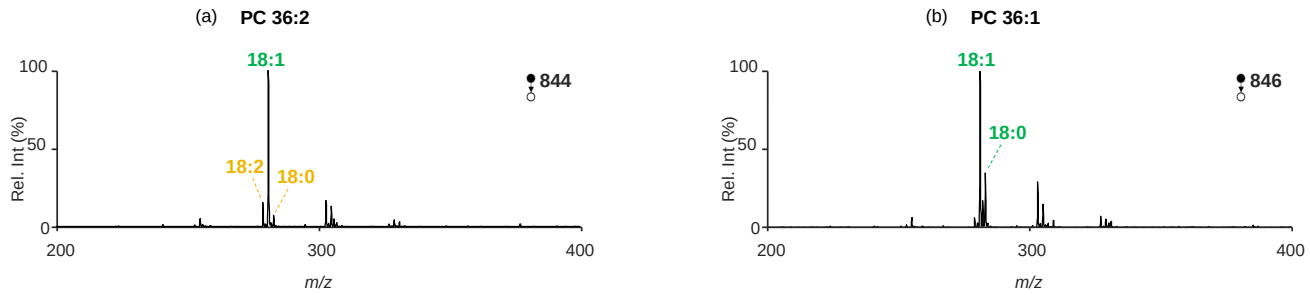


**Fig. S10. Chain composition of PC 36:2 (a) and PC 36:1 (b) analyzed by MS/MS in negative ion mode.**

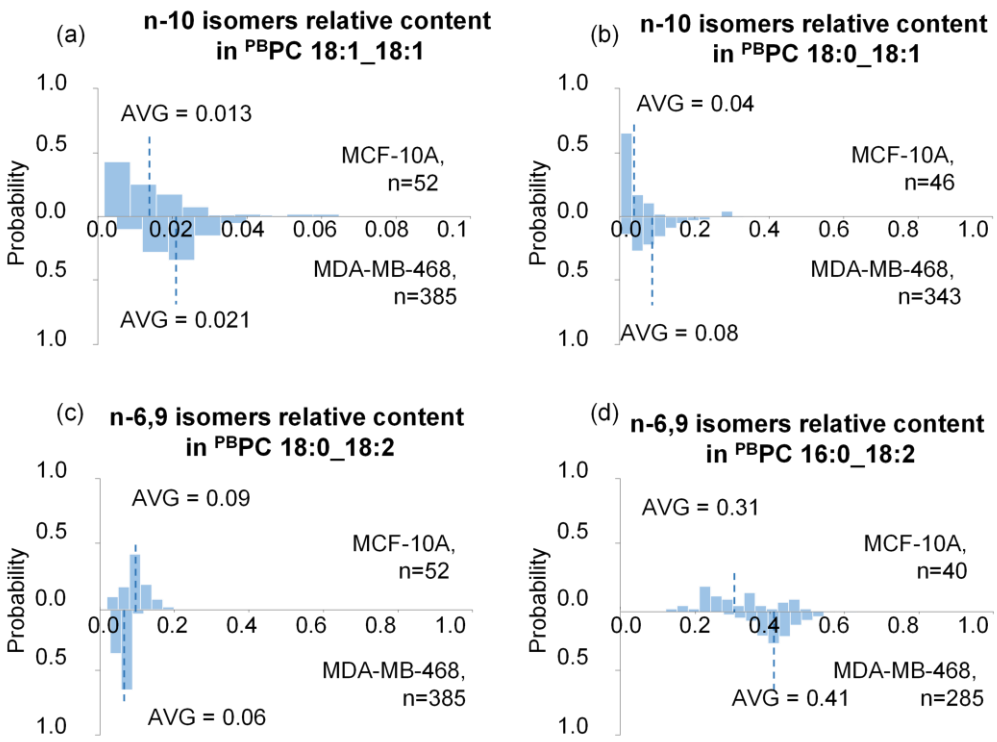


**Fig. S11. The heterogeneity analysis of lipid n-10 and n-6,9 C=C location isomers between breast epithelial cells MCF-10A and cancer cells MDA-MB-468.** (a, b) The distribution of n-10 isomers in PC 36:2 and PC 36:1. (c, d) The distribution of n-6,9 isomers in PC 36:2 and PC 34:2.

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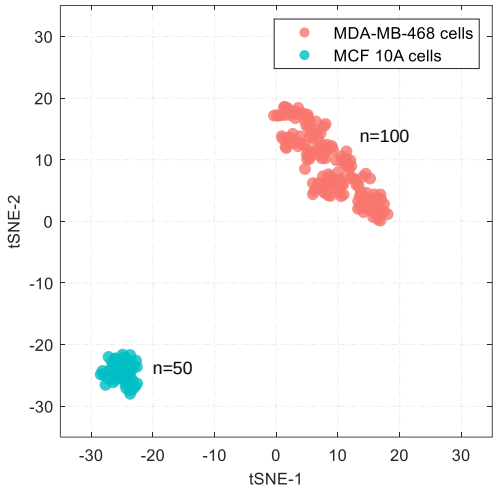
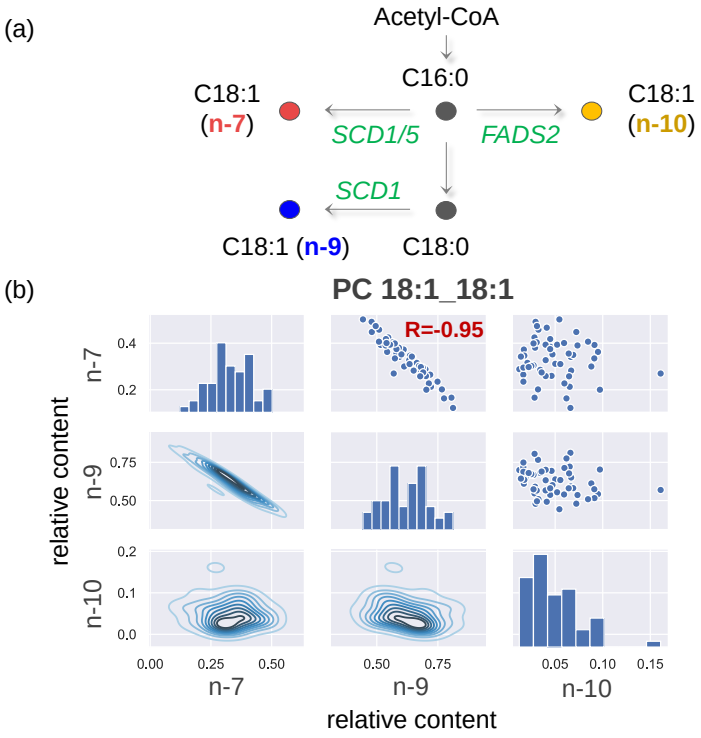



**Fig. S12. T-SNE plot of breast cancer single cells and normal breast epithelial cells calculated by relative quantitation of lipid C=C location isomers of PC 36:2.** T-SNE plot of 200 single cells selected randomly from each cell lines. Number of cells: MDA-MB-468: n = 150 single cells, MCF 10A: n = 50 single cells.




345 **Fig. S13. CyESI MS-SCSL enabled metabolic network analysis of lipid C=C location isomers.** (a) Biosynthetic pathways of monounsaturated FA 18:1. (b) Correlation analysis of n-7, n-9, and n-10 C=C isomers in lipid PC 36:2.

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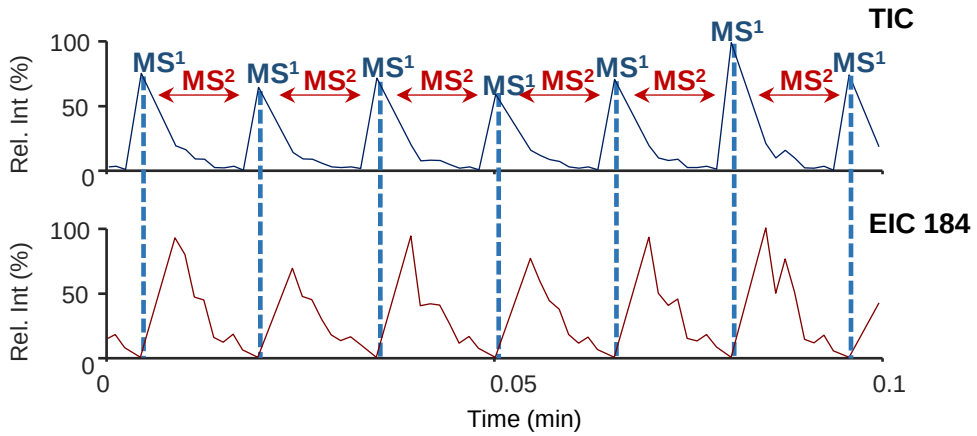


**Fig. S14. Segmental scanning between $MS^1$ and $MS^2$ of *m/z* 760, 758, 732, 788 and 786, corresponding to PC 34:1, PC 34:2, PC 32:1, PC 36:1 and PC 36:2 in lipid extracts.**

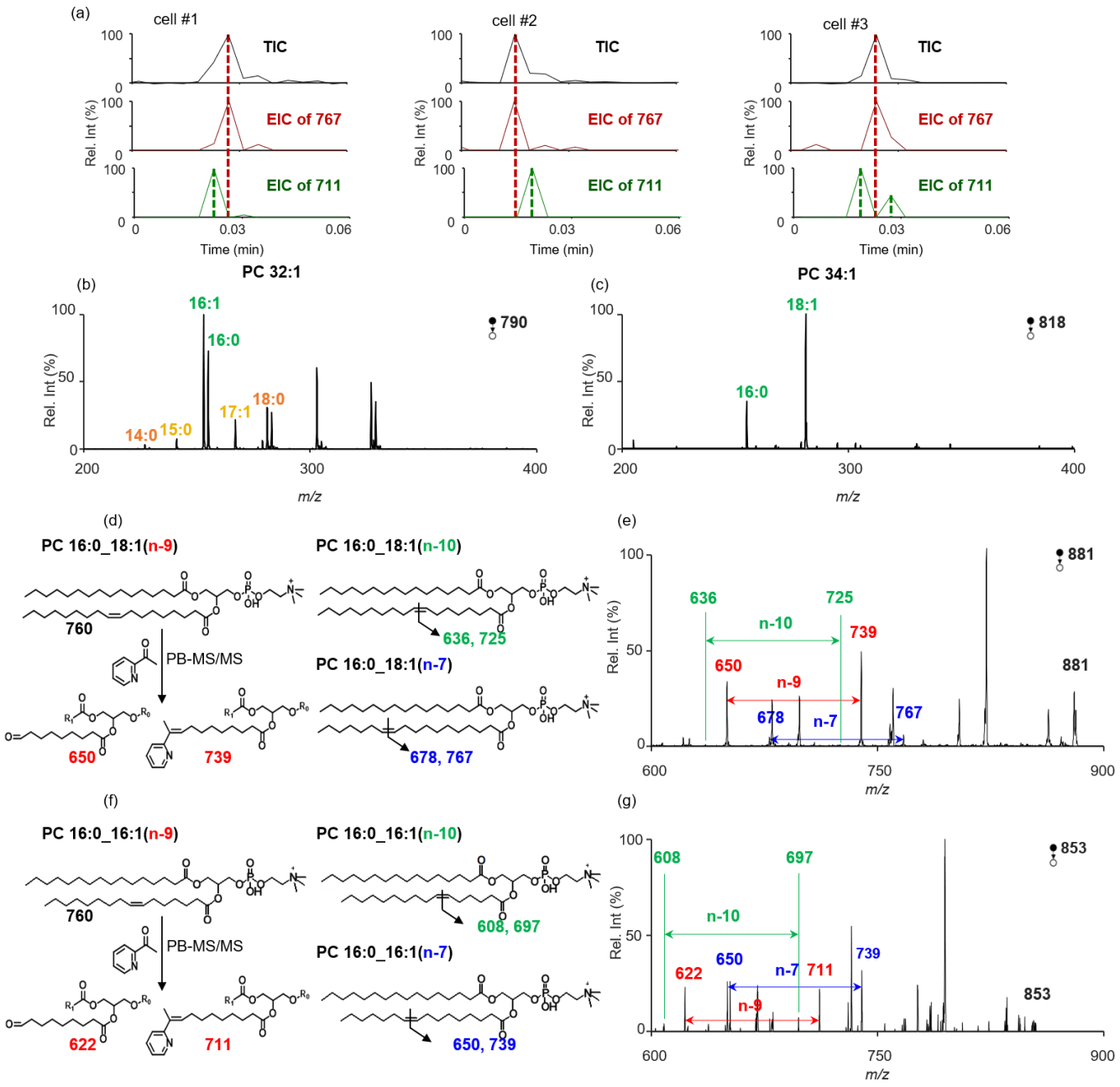


**Fig. S15. MS segmental scanning to multi-target lipid analysis for single cell analysis.** (a) $MS^2$ segmental scanning of $^{PB}$PC 34:1 and $^{PB}$PC 32:1 in single cells. (b, c) Chain composition of PC 32:1 (a) and PC 34:1 (b) analyzed by MS/MS in negative ion mode. (d) Chemical structures of C=C location and their specific diagnostic ions for PC 16:0_18:1, where the C=C in C18:1 was located at n-9, n-10, n-7. (e) MS/MS spectrum of $^{PB}$PC 16:0_18:1 (*m/z* 881) from a single MDA-MB-468 cell. (f) Chemical structures of C=C location and their specific diagnostic ions for PC 16:0_16:1, where the C=C in C16:1 was located at n-9, n-10, n-7. (g) MS/MS spectrum of $^{PB}$PC 16:0_16:1 (*m/z* 853) from a single MDA-MB-468 cell.

# Supplementary Tables

**Table S1. Lipid species detected by tandem MS at the single cell level in positive ion mode.**

| No. | Lipid Subclass | Name | Adduct | Observed m/z | MS/MS fragments |
|---|---|---|---|---|---|
| 1 | LPC | LPC 16:0 | M+H | 496 | 184 |
| 2 | | LPC 18:0 | M+H | 522 | 184 |
| 3 | | LPC 18:1 | M+H | 524 | 184 |
| 4 | SM | SM 34:2 | M+H | 701 | 184 |
| 5 | | SM 34:1 | M+H | 703 | 184 |
| 6 | | SM 36:2 | M+H | 729 | 184 |
| 7 | PC | PC 30:1 | M+H | 704 | 184 |
| 8 | | PC 30:0 | M+H | 706 | 184 |
| | | | M+Na | 728 | 669, 545 |
| 9 | | PC 31:2 | M+H | 716 | 184 |
| 10 | | PC 31:1 | M+H | 718 | 184 |
| | | | M+Na | 740 | 681, 577 |
| 11 | | PC 31:0 | M+H | 720 | 184 |
| | | | M+Na | 742 | 683 |
| 12 | | PC 32:2 | M+H | 730 | 184 |
| 13 | | PC 32:1 | M+H | 732 | 184 |
| | | | M+Na | 754 | 695, 571 |
| 14 | | PC 32:0 | M+H | 734 | 184 |
| | | | M+Na | 756 | 697, 573 |
| 15 | | PC 33:3 | M+Na | 764 | 705, 581 |
| 16 | | PC 33:2 | M+H | 744 | 184 |
| | | | M+Na | 766 | 707, 583 |
| 17 | | PC 33:1 | M+H | 746 | 184 |
| | | | M+Na | 768 | 709, 585 |
| 18 | | PC 33:0 | M+H | 748 | 184 |
| | | | M+Na | 770 | 711, 587 |
| 19 | | PC 34:3 | M+H | 756 | 184 |
| 20 | | PC 34:2 | M+H | 758 | 184 |
| | | | M+Na | 780 | 721, 597 |
| 21 | | PC 34:1 | M+H | 760 | 184 |
| | | | M+Na | 782 | 723, 599 |
| 22 | | PC 34:0 | M+H | 762 | 184 |
| | | | M+Na | 784 | 725, 601 |
| 23 | | PC 35:2 | M+H | 772 | 184 |
| | | | M+Na | 794 | 735, 611 |
| 24 | | PC 35:1 | M+H | 774 | 184 |
| | | | M+Na | 796 | 737 |
| 25 | | PC 35:0 | M+H | 776 | 184 |
| | | | M+Na | 798 | 739 |

| | | | | | |
|---|---|---|---|---|---|
| **26** | | **PC 36:4** | M+H | 782 | 184 |
| | | | M+Na | 804 | 745, 621 |
| **27** | | **PC 36:3** | M+H | 784 | 184 |
| | | | M+Na | 806 | 747, 623 |
| **28** | | **PC 36:2** | M+H | 786 | 184 |
| | | | M+Na | 808 | 749, 625 |
| **29** | | **PC 36:1** | M+H | 788 | 184 |
| | | | M+Na | 810 | 751, 627 |
| **30** | | **PC 37:5** | M+H | 794 | 184 |
| | | | M+Na | 816 | 757, 633 |
| **31** | | **PC 37:4** | M+H | 796 | 184 |
| | | | M+Na | 818 | 759, 635 |
| **32** | | **PC 37:3** | M+H | 798 | 184 |
| | | | M+Na | 820 | 761, 637 |
| **33** | | **PC 37:2** | M+H | 800 | 184 |
| **34** | | **PC 38:6** | M+H | 806 | 184 |
| **35** | | **PC 38:5** | M+H | 808 | 184 |
| **36** | | **PC 38:4** | M+H | 810 | 184 |
| | | | M+Na | 832 | 773, 649 |
| **37** | | **PC 38:3** | M+H | 812 | 184 |
| | | | M+Na | 834 | 775, 651 |
| **38** | | **PC 38:2** | M+H | 814 | 184 |
| **39** | **PS** | **PS 30:1** | M+H | 706 | 521 |
| **40** | | **PS 34:2** | M+H | 760 | 575 |
| **41** | | **PS 34:1** | M+H | 762 | 577 |
| **42** | | **PS 34:0** | M+H | 764 | 579 |
| **43** | | **PS 35:1** | M+H | 776 | 591 |
| **44** | | **PS 36:3** | M+H | 786 | 601 |
| **45** | | **PS 36:2** | M+H | 788 | 603 |
| **46** | | **PS 36:1** | M+H | 790 | 605 |
| **47** | | **PS 36:0** | M+H | 792 | 607 |
| **48** | | **PS 38:4** | M+H | 812 | 627 |
| **49** | | **PS 38:3** | M+H | 814 | 629 |
| **50** | | **PS 38:2** | M+H | 816 | 631 |
| **51** | | **PS 38:1** | M+H | 818 | 633 |
| **52** | | **PS 38:0** | M+H | 820 | 635 |
| **53** | | **PS 40:6** | M+H | 836 | 651 |
| **54** | | **PS 40:5** | M+H | 838 | 653 |
| **55** | **PE** | **PE 34:2** | M+H | 716 | 575 |
| **56** | | **PE 34:1** | M+H | 718 | 577 |
| **57** | | **PE 34:0** | M+H | 720 | 579 |
| **58** | | **PE 35:3** | M+H | 728 | 587 |
| **59** | | **PE 35:2** | M+H | 730 | 589 |
| **60** | | **PE 35:1** | M+H | 732 | 591 |
| **61** | | **PE 36:4** | M+H | 740 | 599 |
| **62** | | **PE 36:3** | M+H | 742 | 601 |

| | | | | | |
|---|---|---|---|---|---|
| **63** | | **PE 36:2** | M+H | 744 | 603 |
| **64** | | **PE 36:1** | M+H | 746 | 605 |
| **65** | | **PE 36:0** | M+H | 748 | 607 |
| **66** | | **PE 38:7** | M+H | 762 | 621 |
| **67** | | **PE 38:6** | M+H | 764 | 623 |
| **68** | | **PE 38:5** | M+H | 766 | 625 |
| **69** | | **PE 38:4** | M+H | 768 | 627 |
| **70** | | **PE 38:3** | M+H | 770 | 629 |
| **71** | | **PE 38:2** | M+H | 772 | 631 |
| **72** | | **PE 38:1** | M+H | 774 | 633 |
| **73** | | **PE 39:2** | M+H | 786 | 645 |
| **74** | | **PE 39:1** | M+H | 788 | 647 |
| **75** | | **PE 40:7** | M+H | 790 | 649 |
| **76** | | **PE 40:6** | M+H | 792 | 651 |
| **77** | | **PE 40:5** | M+H | 794 | 653 |
| **78** | | **PE 40:4** | M+H | 796 | 655 |
| **79** | | **PE 40:3** | M+H | 798 | 657 |
| **80** | | **PE 40:2** | M+H | 800 | 659 |
| **81** | | **PE 41:3** | M+H | 812 | 671 |
| **82** | | **PE 41:2** | M+H | 814 | 673 |
| **83** | | **PE 41:1** | M+H | 816 | 675 |
| **84** | | **PE 41:0** | M+H | 818 | 677 |
| **85** | | **PE 42:6** | M+H | 820 | 679 |
| **86** | | **PE 42:5** | M+H | 822 | 681 |
| **87** | | **PE 42:4** | M+H | 824 | 683 |
| **88** | | **PE 42:3** | M+H | 826 | 685 |
| **89** | | **PE 42:2** | M+H | 828 | 687 |
| **90** | **TAG** | **TAG 14:0_16:1_18:1** | M+NH4 | 818 | 573, 547, 519 |
| **91** | | **TAG 14:0_16:0_18:2** | M+NH4 | 818 | 573, 545, 521 |
| **92** | | **TAG 16:0_16:1_16:1** | M+NH4 | 820 | 547, 549 |
| **93** | | **TAG 14:0_16:1_18:1** | M+NH4 | 820 | 575, 549, 521 |
| **94** | | **TAG 14:0_16:0_18:2** | M+NH4 | 820 | 575, 547, 523 |
| **95** | | **TAG 14:0_16:0_18:1** | M+NH4 | 822 | 577, 549, 523 |
| **96** | | **TAG 14:0_16:1_18:0** | M+NH4 | 822 | 577, 551, 521 |
| **97** | | **TAG 14:0_17:0_18:1** | M+NH4 | 836 | 591, 551, 537 |
| **98** | | **TAG 14:0_17:1_18:0** | M+NH4 | 836 | 591, 553, 535 |
| **99** | | **TAG 15:0_16:0_18:1** | M+NH4 | 836 | 577, 563, 537 |
| **100** | | **TAG 15:0_16:1_18:0** | M+NH4 | 836 | 577, 565, 535 |
| **101** | | **TAG 15:0_17:0_17:1** | M+NH4 | 836 | 577, 551, 553 |
| **102** | | **TAG 16:0_16:0_17:1** | M+NH4 | 836 | 563, 553 |
| **103** | | **TAG 16:0_16:1_17:0** | M+NH4 | 836 | 563, 565, 551 |
| **104** | | **TAG 16:0_16:0_18:1** | M+NH4 | 850 | 551, 577 |
| **105** | | **TAG 16:0_16:1_18:0** | M+NH4 | 850 | 577, 579, 549 |
| **106** | | **TAG 16:1_18:1_18:1** | M+NH4 | 874 | 603, 575 |
| **107** | | **TAG 16:0_18:1_18:2** | M+NH4 | 874 | 601, 575, 577 |

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|---|---|---|---|---|---|
| **108** | | **TAG 16:0_18:0_18:3** | M+NH4 | 874 | 601, 579, 573 |
| **109** | | **TAG 14:0_18:0_20:3** | M+NH4 | 874 | 629, 579, 551 |
| **110** | | **TAG 16:0_18:0_18:2** | M+NH4 | 876 | 575, 579, 603 |
| **111** | | **TAG 16:0_18:1_18:1** | M+NH4 | 876 | 577, 603 |
| **112** | | **TAG 16:0_18:0_18:2** | M+NH4 | 876 | 603, 575, 579 |
| **113** | | **TAG 16:0_16:1_20:1** | M+NH4 | 876 | 603, 605, 551 |
| **114** | | **TAG 16:1_18:0_18:1** | M+NH4 | 876 | 605, 575, 577 |
| **115** | | **TAG 18:1_18:1_18:2** | M+NH4 | 900 | 601, 603 |
| **116** | | **TAG 16:0_18:1_20:3** | M+NH4 | 900 | 627, 601, 577 |
| **117** | | **TAG 16:1_18:1_20:2** | M+NH4 | 900 | 629, 601, 575 |
| **118** | | **TAG 16:1_18:0_20:3** | M+NH4 | 900 | 629, 599, 577 |
| **119** | | **TAG 16:0_16:0_22:4** | M+NH4 | 900 | 627, 551 |
| **120** | | **TAG 16:0_18:0_22:6** | M+NH4 | 924 | 651, 623, 579 |
| **121** | | **TAG 16:0_18:1_22:5** | M+NH4 | 924 | 651, 625, 577 |
| **122** | | **TAG 16:0_18:2_22:4** | M+NH4 | 924 | 651, 627, 575 |
| **123** | | **TAG 16:0_18:3_22:3** | M+NH4 | 924 | 651, 629, 573 |
| **124** | | **TAG 16:1_18:0_22:5** | M+NH4 | 924 | 653, 623, 577 |
| **125** | | **TAG 16:1_18:1_22:4** | M+NH4 | 924 | 653, 625, 575 |
| **126** | | **TAG 16:1_18:2_22:3** | M+NH4 | 924 | 653, 627, 573 |
| **127** | | **TAG 18:0_18:2_20:4** | M+NH4 | 924 | 623, 627, 603 |
| **128** | | **TAG 18:0_18:3_20:3** | M+NH4 | 924 | 623, 629, 601 |
| **129** | | **TAG 18:1_18:1_20:4** | M+NH4 | 924 | 625, 603 |
| **130** | | **TAG 18:1_18:2_20:3** | M+NH4 | 924 | 625, 627,601 |
| **131** | | **TAG 18:1_18:3_20:2** | M+NH4 | 924 | 625, 629, 599 |
| **132** | | **TAG 18:2_18:2_20:2** | M+NH4 | 924 | 627, 599 |
| **133** | | **TAG 18:2_18:3_20:1** | M+NH4 | 924 | 627, 629, 597 |
| **134** | | **TAG 16:0_20:5_22:3** | M+NH4 | 948 | 675, 629, 597 |
| **135** | | **TAG 16:0_20:4_22:4** | M+NH4 | 948 | 675, 627, 599 |
| **136** | | **TAG 16:0_20:3_22:5** | M+NH4 | 948 | 675, 625, 601 |
| **137** | | **TAG 16:0_20:2_22:6** | M+NH4 | 948 | 675, 625, 603 |
| **138** | | **TAG 18:0_20:3_20:5** | M+NH4 | 948 | 647, 625, 629 |
| **139** | | **TAG 18:0_20:4_20:4** | M+NH4 | 948 | 647, 627 |
| **140** | | **TAG 18:1_20:2_20:5** | M+NH4 | 948 | 649, 623, 629 |
| **141** | | **TAG 18:1_20:3_20:4** | M+NH4 | 948 | 649, 625, 627 |
| **142** | | **TAG 18:1_20:3_20:3** | M+NH4 | 950 | 651, 627 |
| **143** | | **TAG 18:1_20:2_20:4** | M+NH4 | 950 | 651, 625, 629 |
| **144** | | **TAG 18:1_18:2_22:4** | M+NH4 | 950 | 651, 653, 601 |
| **145** | | **TAG 18:1_18:1_22:5** | M+NH4 | 950 | 651, 603 |

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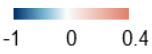
**Table S2. PCs' C=C location isomers identified at the single cell level.**

| PC Spieces | Adduct | Ob-served m/z | PB Product m/z | C=C location | MS/MS Fragments m/z |
|---|---|---|---|---|---|
| **PC 32:1** | M+H | 732 | 853 | n-10 | 608, 697 |
| | | | | n-9 | 622, 711 |
| | | | | n-7 | 650, 739 |
| **PC 34:2** | M+H | 758 | 879 | n-10 | 634, 723 |
| | | | | n-9 | 648, 737 |
| | | | | n-7 | 676, 765 |
| | | | | n-6,9 | 650, 739; 690, 779 |
| **PC 34:1** | M+H | 760 | 881 | n-10 | 636, 725 |
| | | | | n-9 | 650, 739 |
| | | | | n-7 | 678, 767 |
| **PC 36:2** | M+H | 786 | 907 | n-10 | 662, 751 |
| | | | | n-9 | 676, 765 |
| | | | | n-7 | 704, 793 |
| | | | | n-6,9 | 678, 767; 718, 807 |
| **PC 36:1** | M+H | 788 | 909 | n-10 | 664, 753 |
| | | | | n-9 | 678, 767 |
| | | | | n-7 | 706, 795 |

**Table S3. Correlation analysis of C16:1 and C18:1-containing lipids in single cells with C=C location isomers.**

| Correlation Analysis | | PC 32:1 | | | PC 34:1 | | |
|---|---|---|---|---|---|---|---|
| | | n-7 | n-9 | n-10 | n-7 | n-9 | n-10 |
| PC 32:1 | n-7 | 1 | -0.74984 | -0.51829 | 0.256567 | -0.0949 | -0.10563 |
| | n-9 | | 1 | -0.17718 | -0.11275 | 0.223452 | -0.01356 |
| | n-10 | | | 1 | -0.0679 | -0.00788 | 0.174649 |
| PC 34:1 | n-7 | | | | 1 | -0.90836 | -0.15012 |
| | n-9 | | | | | 1 | -0.27708 |
| | n-10 | | | | | | 1 |



## Reference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[2] S. Cheng, D. Zhang, J. Feng, Q. Hu, A. Tan, Z. Xie, Q. Chen, H. Huang, Y. Wei, Z. Ouyang, X. Ma, *Research* **2023**, *6*, research.0087.