Supporting Information

Single-cell Mass Spectrometry Enables Insight into Heterogeneity in Infectious Disease

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Experimental section

Parasite culture

Beta-galactosidase-expressing *T. cruzi* strain Tulahuen (clone C4) were obtained through BEI Resources, NIAID, NIH[1] and maintained in mouse C2C12 myoblasts by once-weekly passaging. Trypomastigotes were collected from culture supernatant and used for infections.

Cell culture

HeLa cells were cultivated in DMEM cell culture medium (Corning) supplemented with 10% iron-supplemented calf serum (HyClone) and 1% penicillin-streptomycin (Gibco) in 5% CO_2 at 37 °C. C2C12 cells were maintained in DMEM media supplemented with 5% iron-supplemented calf serum (HyClone) and 1% penicillin-streptomycin (Invitrogen), in 5% CO_2 and at 37 °C, as previously described[2].

Cell infection and staining

HeLa cells were infected at a host:parasite ratio of 1:10. Two days post-infection, cells were washed with ice-cold PBS and fixed with 0.7% glutaraldehyde for 5 min, fixing and killing the parasites. Cells were then rinsed three times with PBS for 4 min. Cells were then stained overnight with 1 mg/mL of X-Gal in PBS containing 2 mM $MgCl_2$, 4.98 mM potassium ferricyanide and 5.76 mM potassium ferrocyanide,[3] pH 7.3 at 37°C.

Single-probe single cell mass spectrometry (SCMS)

The single-probe SCMS setup includes a Single-probe, a digital microscope, a digital camera, a computer-controlled XYZ-translation stage system (CONEX-MFACC, Newport Co., Irvine, CA, USA) and a Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, USA). The fabrication of the Single-probe and the SCMS set-up were detailed in details in our previous studies.[4-10] Briefly, the Single-probe was fabricated using a laser-pulled (P-2000 Micropipette Laser Puller, Sutter Instrument Co., Novato, CA) dual-bore quartz tubing (outer diameter (OD) 500 μm; inner diameter (ID) 127 μm, Friedrich & Dimmock, Inc., Millville, NJ, USA) embedded with a fused silica capillary (OD 105 μm; ID 40 μm, Polymicro Technologies, Phoenix, AZ, USA) in one channel and a nano-ESI emitter, which is produced from the same fused silica capillary, in another channel. The three parts were sealed using UV curing resin (Light Cure Bonding Adhesive, Prime-Dent, Chicago, Il, USA).

Glass coverslips containing cells were washed three times with fresh DMEM and placed on the XYZ-stage system of the Single-probe SCMS set-up for measurement. The targeted single cells were selected for analysis by precisely moving the stage system guided by the microscope. Four groups of cells (infected, bystander, stained, and control cells) were analyzed. Although both bystander and infected cells were on the same glass coverslip, the bystander cells were clearly

distinct from infected cells, which contained the stained amastigote stage of parasite. Each type of cells were precisely sampled and separately analyzed using the Single-probe with small tip sizes (~9 μm). The sampling solvent (50% acetonitrile/50% methanol (v/v)) with 0.1% formic acid) was continuously delivered through the fused silica capillary to extract cellular contents followed by ionization via the nano-ESI emitter and real-time SCMS analysis. The SCMS experiments were conducted under the following parameters: 200 nL/min flow rate; mass resolution, 60,000; +4.5 kV ionization voltage; 1 microscan; 100 ms max injection time. MS/MS experiments of single cells were conducted under the following parameters: HCD mode, 200 nL/min flow rate; mass resolution 60,000; +4.5 kV ionization voltage; 3 microscan; 500 ms max injection time. Collision energies ranged between 33 and 35 NCE (normalized collision energy) as shown in Figure S3.

SCMS Data Analysis

The SCMS data pretreatment was conducted following our established protocols.[5, 8] MS data were exported with peaks (*m/z* values and relative intensities) generated by Thermo Xcalibur Qual Browser 3.0 (Thermo Scientific, Waltham, MA, USA). The exported raw data were subjected to noise subtraction by removing peaks with relative intensity < $3x10^3$. Background signals generated from organic solvent and cell culture medium were subtracted using an in-house R script as described in our prior work.[11-12] Normalization of ion intensities to total ion current (TIC) was subsequently performed. The normalized data were uploaded to *Geena2* online tool (http://proteomics.hsanmartino.it/geena2/geena2_ssi_norm.php)[13] for peak alignment (with a mass tolerance of 10 ppm) and subsequent analysis. *Geena2* parameters were as follows: analysis range from 150 to 1500 *m*/z, maximum number of isotopic replicas: 3, maximum delta between isotopic peaks: 0.01 Da, maximum delta for aligning replicates: 0.01 Da and maximum delta for aligning average spectra: 0.01 Da. After performing peak alignment, missing values (50%) were removed using an in-house Python script (SI Supporting File 1).

Pretreated SCMS data were then imported to Metaboanalyst 5.0[14-18] to perform principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and hierarchical clustering. Random Forest analysis[10] was used to classify cells in four different groups using an in-house R script (with 500 trees and 7 predictors) (SI Supporting File 2). To determine ions of interest (i.e., with significantly different abundances among cell groups), one-way analysis of variance (ANOVA)[19] was performed with an adjusted p-value cutoff of 0.05 using False Discovery Rate (FDR) correction. The hierarchical clustering heatmap[15] was generated using Ward's minimum variance clustering method and Euclidean distance method, from normalized data with auto-scale feature standardization. To minimize the technical variance[4, 20], two replicates were performed for comparison under similar experimental conditions. Boxplots display median, upper and lower quartiles, with whiskers extending to the highest and lowest quartiles and outliers beyond the whiskers represented as dots. Both single cell MS/MS and LC-MS/MS of cell lysates were conducted. Annotations of ions of interest were generated based on MS/MS of ions obtained from the ANOVA results of two combined replicates. Annotations were generated from the

resulting single cell MS/MS by comparing to data in METLIN (https://metlin.scripps.edu)[21], HMDB (http://www.hmdb.ca)[22] and GNPS (https://gnps.ucsd.edu/, see Table S3 for parameters)[23-24].

LC-MS/MS analysis

Metabolites were extracted from uninfected and infected HeLa cells using a two-step extraction with 50% methanol followed by 3:1 dichloromethane-methanol (all Fisher Optima LC-MS grade). Extracts were resuspended in 50% methanol, as in our prior work.[2] LC analysis was performed on a Thermo Vanquish LC equipped with a 1.7 µm Kinetex C18 50 x 2.1 mm column, 100 Å pore size, protected by a SecurityGuard ULTRA C18 Guard Cartridge (Phenomenex). Injection volume was 5 µL. Auto-injector was washed with 10% methanol at a rate of 10 µL/s for 2 seconds. LC gradient was composed of mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1% formic acid) at a flow rate of 0.5 mL/min (Table S1). The autosampler was maintained at 10 ℃ and the column compartment at 40 ℃.

MS data were acquired on a Thermo Fisher Q-Exactive Plus hybrid quadrupole orbitrap mass spectrometer operating in positive parallel reaction monitoring (PRM mode, Table S2). Instrument calibration was performed using Thermo Fisher Calmix. All samples underwent a 12.5-minute runtime elution gradient as follows: start at 5% solvent B for one minute, gradual increase to 100% solvent B for eight minutes, hold at 100% solvent B for two minutes, drop to 5% solvent B for 30 seconds, and hold at 5% solvent B for one minute (Table S1). Full PRM parameters were: scan range set to 100-1,500 *m/z*, default charge state was 1, resolution was 17,500, AGC target set to 2e5, maximum IT was 54 ms, isolation window set to 1 *m/z*, and normalized collision energy increased from 20-60%. MS source parameters were as previously described in[2].

Raw data files were converted to mzXML format using MSConvert.[25]

Data availability

Data from LC-MS (accession number MSV000087656) and SCMS (accession number MSV000089503) have been deposited in MassIVE.

Table S1. Solvent gradient used in the LC-MS/MS experiments.

Time	Flow (mL/min)	%A[1]	%B[2]
0.00	0.500	95.0	5.0
1.00	0.500	95.0	5.0
9.00	0.500	0	100.0
11.00	0.500	0	100.0
11.500	0.500	95.0	5.0
12.500	0.500	95.0	5.0

[1] *A: water with 0.1% formic acid.*

[2] *B: acetonitrile with 0.1% formic acid.*

Table S2. Parameters of the Thermo Fisher Q-Exactive Plus hybrid quadrupole orbitrap mass spectrometer used for Full MS/dd-MS^2 analysis.

General	
Runtime	0 to 12.5 min
Polarity	Positive
Default Charge	1
Inclusion	On
MS^2	
Resolution	17,500
AGC target	$2x10^5$
Maximum IT	54 ms
Isolation window	1.0 *m/z*
Fixed first mass	-
(N)CE/stepped	NCE: 20, 40, 60
ESI Ion Source	
ID	HESI
Sheath gas flow rate	35
Auxiliary gas flow rate	10
Sweep gas flow rate	0
Spray voltage	3.80 kV
S-lens RF level	50 V
Capillary temperature	320 ℃
Auxiliary gas temperature	350 ℃

Table S3. GNPS parameters used for annotation.

GNPS Search Single Spectrum	
Search Options	
Find Related Datasets	Do it
Select Databases to Search	All
Parent Mass Tolerance	0.02 Da
Ion Tolerance	0.02 Da
Min Matched Peaks	4
Score Threshold	0.7
Advanced Search Options	
Library Class	Bronze
Search Analogs	Do Search
Search Unclustered Data	Don’t Search
Top Hit Per spectrum	5
Maximum Analog Search Mass Difference	500.0
Advanced Filtering Options	
Filter StdDev Intensity	0.0
Minimum Peak Intensity	0.0
Min Peak Int	0.0
Filter Precursor Window	Filter
Filter Library	Filter Library
Filter peaks in 50 Da Window	Filter

Table S4. Metabolites differing between cell groups as determined by ANOVA (p-value <0.05, FDR-corrected)

m/z	Annotation	p value	FDR-corrected p value
267.0620	N/A	1.66E-10	4.34E-09
302.1440	N/A	0.001722	0.011115
322.885	N/A	2.01E-27	5.66E-24
359.0250	N/A	6.65E-16	3.99E-14
429.9390	N/A	0.001033	0.007073
431.9630	N/A	0.000185	0.001564
515.2590	N/A	0.005041	0.026922
523.2850	N/A	0.00346	0.019204
537.3000	N/A	1.60E-06	2.15E-05
665.3820	N/A	0.010602	0.048378
756.5470	PC(34:3) or LPC(34:4) or PC(O-34:4) (Library match to 1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (PC 34:1)) (*)	0.000168	0.001429
768.583	$[PC(P\text{-}20:0/14:0)+Na]^{+}$ (**)	0.000742	0.005306
780.5460	$[PC(16:0/18:2)+Na]^{+}$ (**)	2.59E-07	4.06E-06
782.5630	$[PC(16:0/18:1)+Na]^{+}$ (**)	9.58E-08	1.63E-06
808.5770	$[PC(18:1/18:1)+Na]^{+}$ (**)	2.57E-06	3.31E-05
810.5940	$[PC(18:0/18:1)+Na]^{+}$ (**)	0.000114	0.001031

(*)*Features were annotated by GNPS (cosine score = 0.92; number of shared peaks = 5; mass difference to library reference =4.03) and supported by the annotated spectrum in Figure 4b.*

(**) *Features were annotated manually and supported by the annotated spectra in the Figure S3.*

N/A: Metabolites were not annotatable.

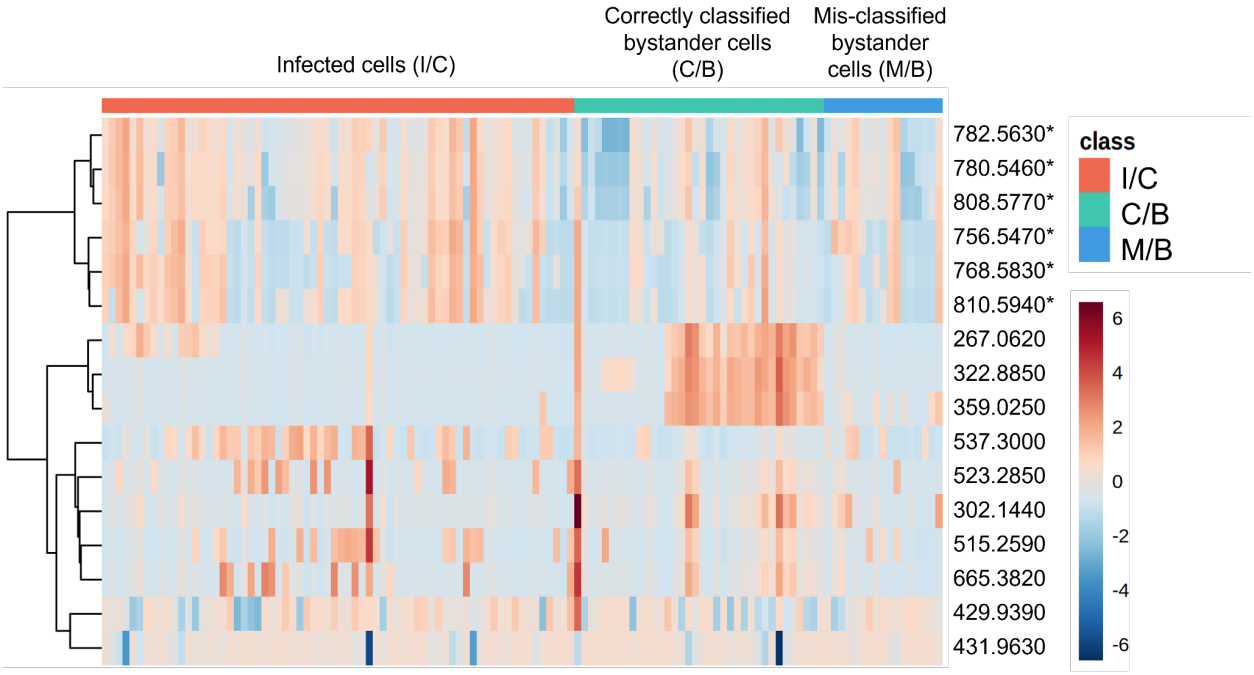


Figure S1: Hierarchical clustering of metabolite features differing between infected cells (I/C), correctly classified bystander cells (C/B), and mis-classified bystander cells (M/B). The annotated features are marked (*).

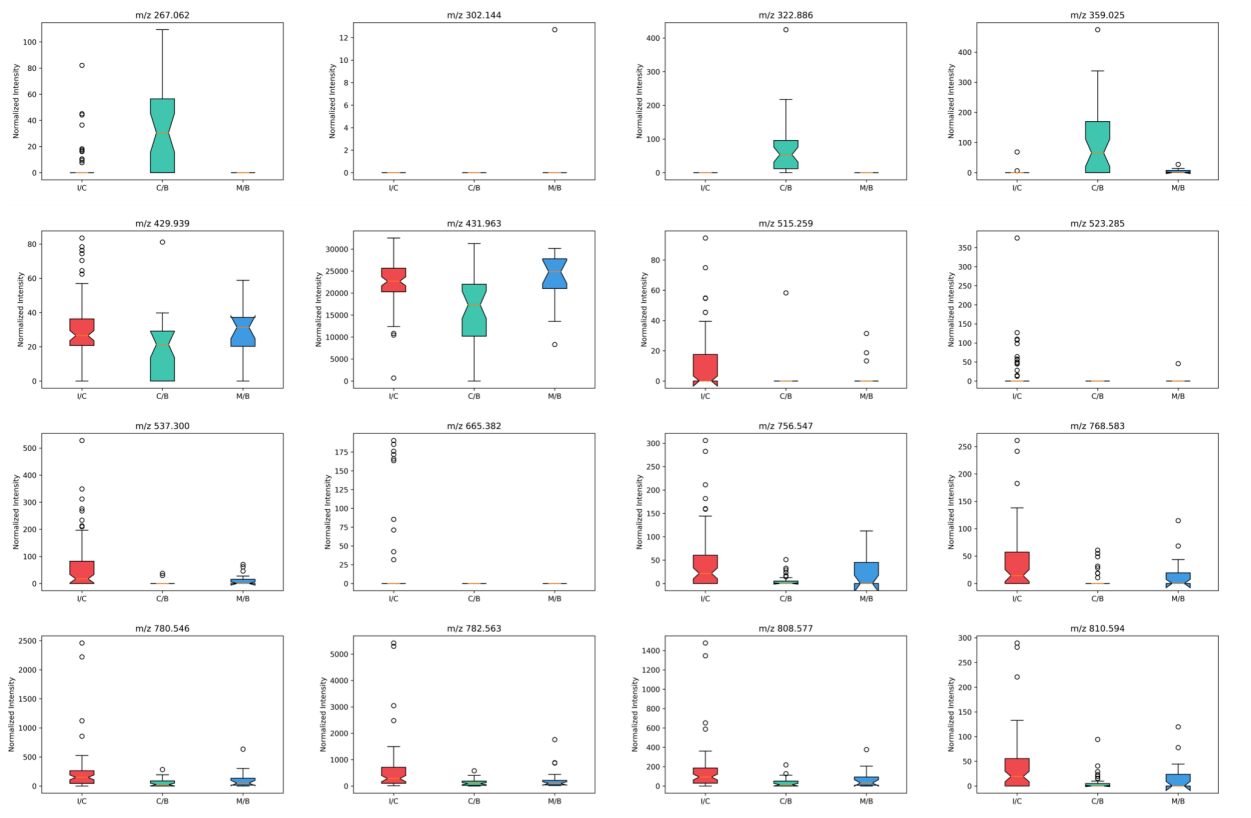
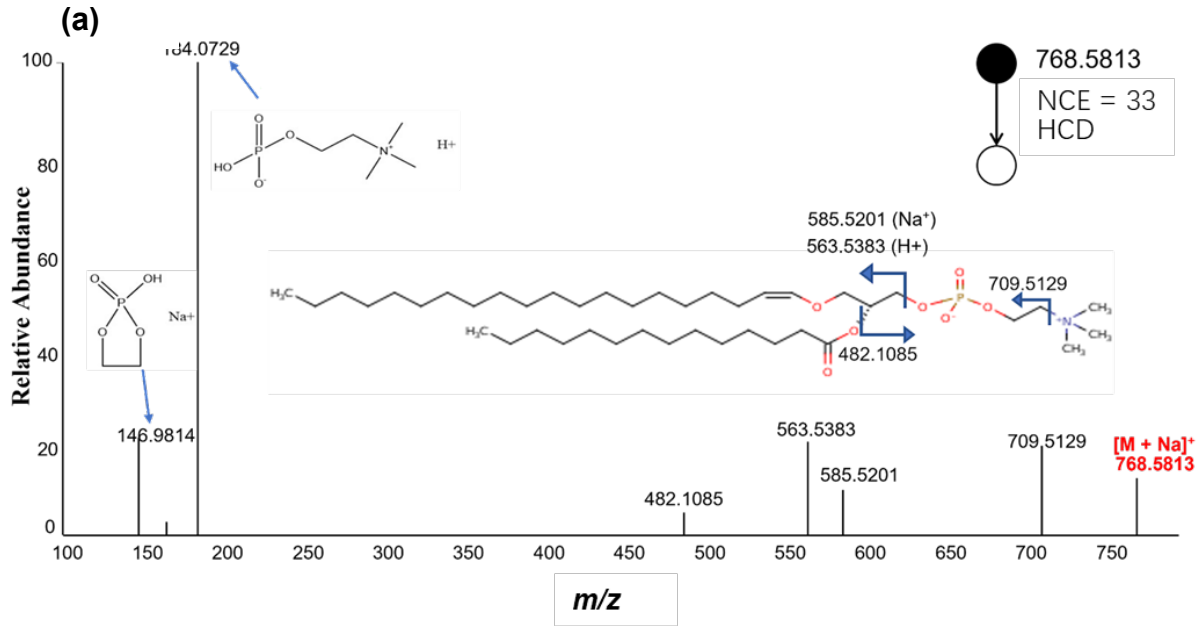
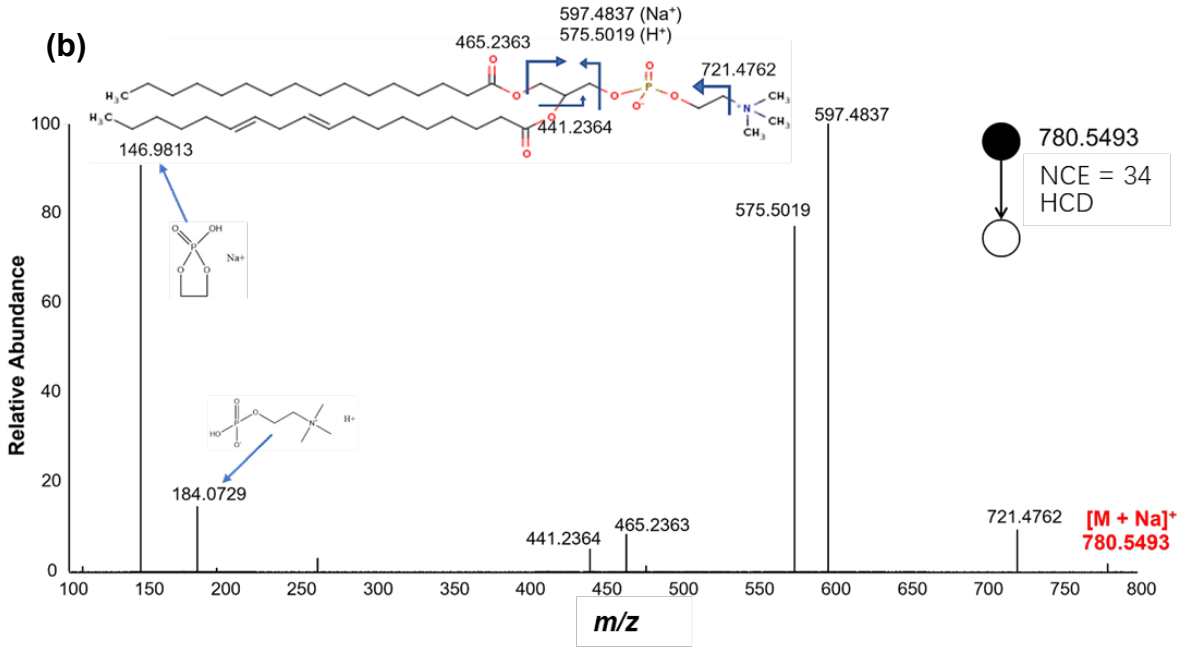
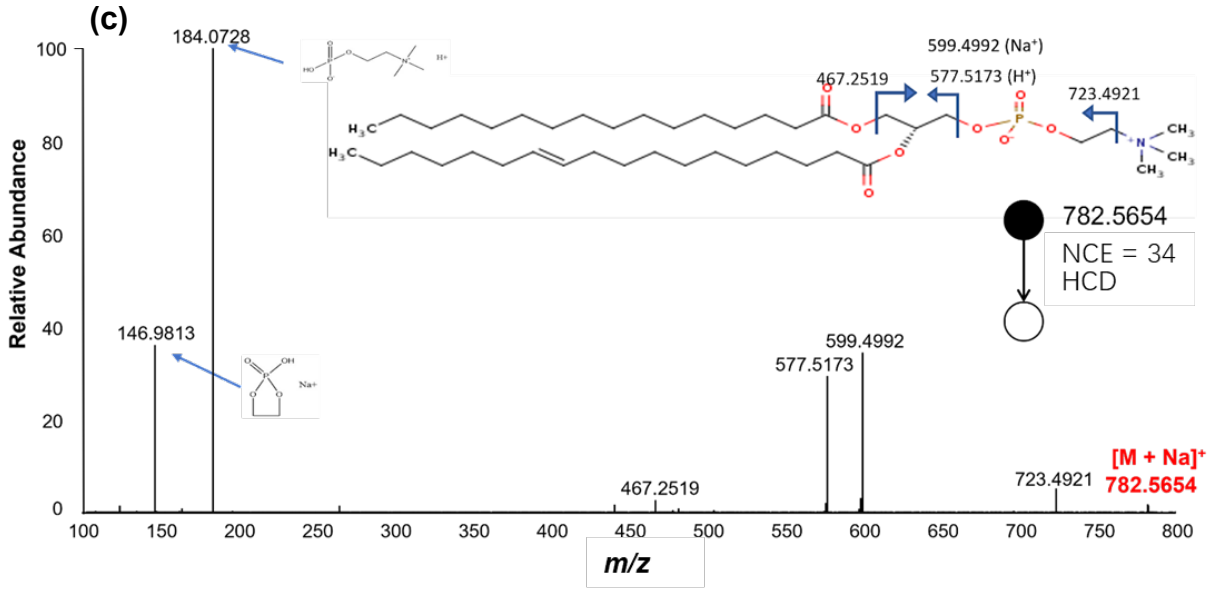
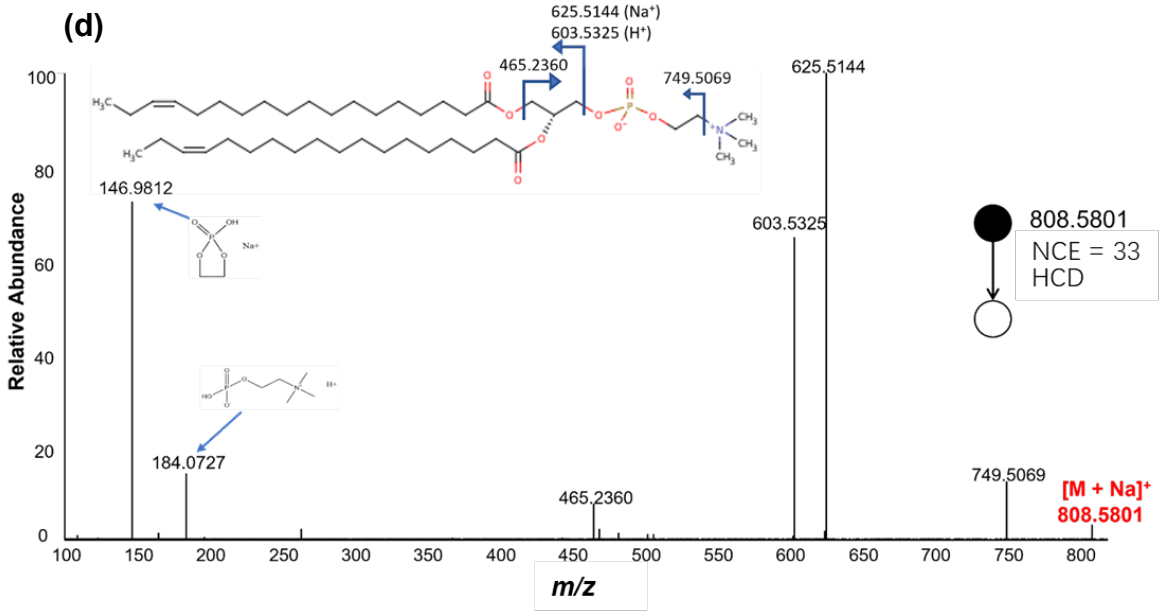


Figure S2. Boxplots for 16 metabolites that showed comparable behavior in infected cells (I/C) and in mis-classified bystander cells (M/B), as determined by ANOVA test with an adjusted p-value ≤ 0.05.


(a)
184.0729
768.5813
NCE = 33
HCD
585.5201 (Na+)
563.5383 (H+)
709.5129
482.1085
146.9814
563.5383
585.5201
709.5129
[M + Na]+
768.5813
482.1085
Relative Abundance
m/z


(b)
597.4837 (Na+)
575.5019 (H+)
465.2363
721.4762
441.2364
597.4837
146.9813
780.5493
NCE = 34
HCD
575.5019
184.0729
441.2364
465.2363
721.4762
[M + Na]+
780.5493
Relative Abundance
m/z


(c)
184.0728
599.4992 (Na+)
577.5173 (H+)
467.2519
723.4921
782.5654
NCE = 34
HCD
146.9813
599.4992
577.5173
467.2519
723.4921
[M + Na]+
782.5654
Relative Abundance
m/z


(d)
625.5144 (Na+)
603.5325 (H+)
465.2360
749.5069
625.5144
146.9812
603.5325
808.5801
NCE = 33
HCD
184.0727
465.2360
749.5069
[M + Na]+
808.5801
Relative Abundance
m/z

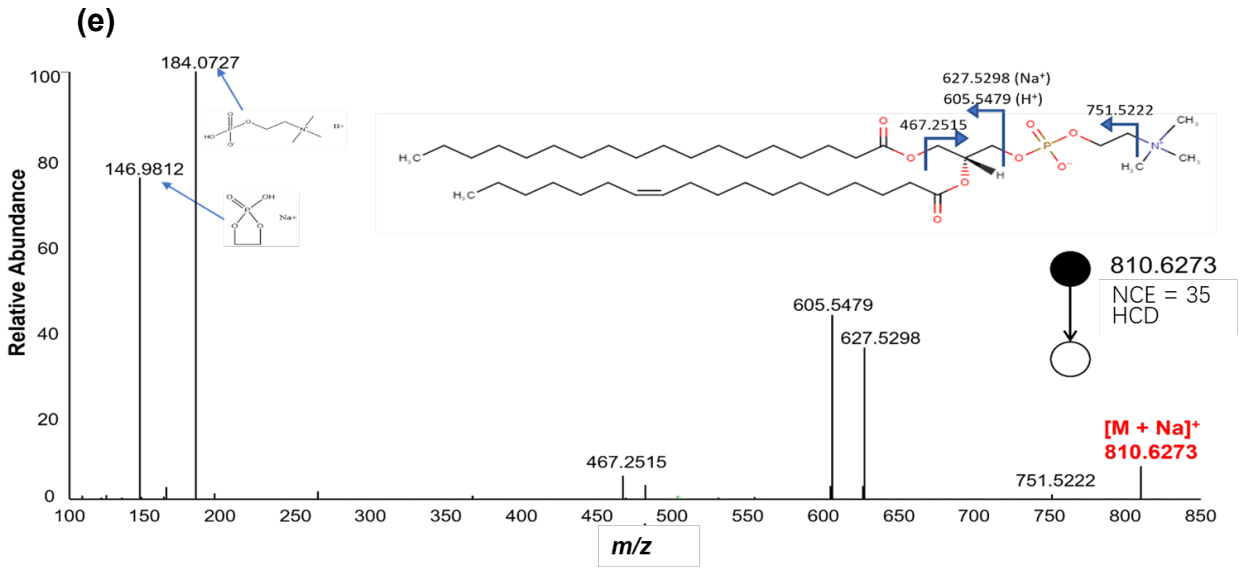


Figure S3. Annotated MS/MS spectra of (a) $[PC(P\text{-}20:0/14:0)+Na]^+$ (*m/z* 768.5813), (b) $[PC(16:0/18:2)+Na]^+$ (*m/z* 780.5493), (c) $[PC(16:0/18:1)+Na]^+$ (*m/z* 782.5654), (d) $PC(18:1/18:1)+Na]^+$ (*m/z* 808.5801), and (e) $[PC(18:0/18:1)+Na]^+$ (*m/z* 810.6273) from individual HeLa cells. NCE: normalized collision energy.

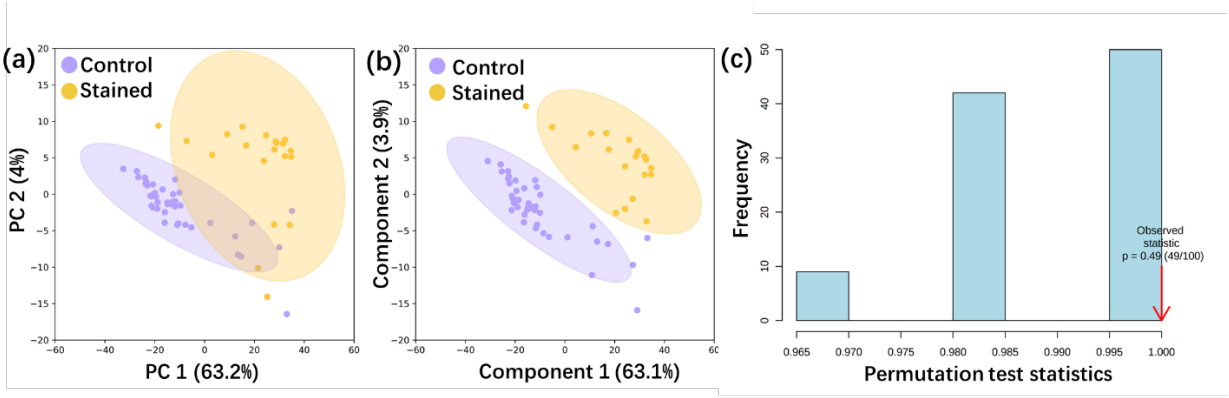


Figure S4. (a) PCA and (b) PLS-DA of SCMS data obtained from the control and stained (fixed) cells. (c) Permutation test statistics of PLS-DA model. The result (p = 0.49) indicates that fixation and staining processes have no significant influence on cellular metabolites.

Supporting file 1. Python script for missing value removal.

```
import pandas as pd
import numpy as np
from tqdm import tqdm
from tqdm._tqdm import trange

# read the data file
df = pd.read_csv('All_12-06.csv', header=None)

# show the format to check
df1.head()

# Set the missing value
missing_value = 0.5

# calculate the number of zero for each m/z. if it is higher than the set value, save it.
miss_list = []
for j in tqdm(range(len(df1) - 2)):
    m = 0
    for i in range(len(df1.iloc[1, :]) - 1):
        if df1.iloc[j+2, i+1] == 0 or df1.iloc[j+2, i+1] == '0':
            m+=1
    if m > missing_value * len(df1.iloc[1, :]):
        miss_list.append(j+2)

# Drop the row with more than set value data
miss_data = df1.drop(miss_list, axis=0)

# Save the file
miss_data.to_csv('12-06_50%.csv')
```

Supporting file 2. R script for identifying correctly classified and mis-classified adjacent uninfected cells.

```
install.packages('caret', dependencies=TRUE)
install.packages('klaR', dependencies=TRUE)
library(caret)
library(klaR)
library(randomForest)
setwd("E:/OU/Dr.Yang/project_3_BZ_McCall/11-10-20 analyzed data_new/After R/")
rawdata1 <- t(read.csv("blue_clear_1.csv", header=FALSE, sep = ","))
rawdata2 <- t(read.csv("blue_clear_2.csv", header=FALSE, sep = ","))
colnames(rawdata1) <- rawdata1[1,]
colnames(rawdata2) <- rawdata2[1,]
data1 <- rawdata1[2:123,]
data2 <- rawdata2[2:74,]
data1[is.na(data1)]<-0
data2[is.na(data2)]<-0
label1<-c(rep('Infected',69),rep('Aj_uninfected',53))
label2<-c(rep('Infected',48),rep('Aj_uninfected',25))
data_train<-cbind(as.data.frame(data1), label1)
data_test<-cbind(as.data.frame(data2), label2)
a <- dim.data.frame(data_train)[2]
b <- dim.data.frame(data_test)[2]
# train model
model <- randomForest(data_train$label~., data=data_train[,3:a], importance=TRUE,
ntree=5000)
#test model
predicted <- predict(model, newdata=data_test[ ,3:b])
actual <- data_test$OUTCOME
```

References

1. Buckner, F. S.; Verlinde, C. L.; La Flamme, A. C.; Van Voorhis, W. C., Efficient technique for screening drugs for activity against Trypanosoma cruzi using parasites expressing beta-galactosidase. *Antimicrob Agents Chemother* **1996,** *40* (11), 2592-7.
2. Hossain, E.; Khanam, S.; Dean, D. A.; Wu, C.; Lostracco-Johnson, S.; Thomas, D.; Kane, S. S.; Parab, A. R.; Flores, K.; Katemauswa, M.; Gosmanov, C.; Hayes, S. E.; Zhang, Y.; Li, D.; Woelfel-Monsivais, C.; Sankaranarayanan, K.; McCall, L. I., Mapping of host-parasite-microbiome interactions reveals metabolic determinants of tropism and tolerance in Chagas disease. *Sci Adv* **2020,** *6* (30), eaaz2015.
3. Monvoisin, A.; Alva, J. A.; Hofmann, J. J.; Zovein, A. C.; Lane, T. F.; Iruela-Arispe, M. L., VE-cadherin-CreERT2 transgenic mouse: A model for inducible recombination in the endothelium. *Developmental Dynamics* **2006,** *235* (12), 3413-3422.
4. Liu, R.; Sun, M.; Zhang, G.; Lan, Y.; Yang, Z., Towards early monitoring of chemotherapy-induced drug resistance based on single cell metabolomics: Combining single-probe mass spectrometry with machine learning. *Anal Chim Acta* **2019,** *1092*, 42-48.
5. Pan, N.; Rao, W.; Kothapalli, N. R.; Liu, R.; Burgett, A. W. G.; Yang, Z., The Single-Probe: A Miniaturized Multifunctional Device for Single Cell Mass Spectrometry Analysis. *Analytical Chemistry* **2014,** *86* (19), 9376-9380.
6. Standke, S. J.; Colby, D. H.; Bensen, R. C.; Burgett, A. W. G.; Yang, Z., Mass Spectrometry Measurement of Single Suspended Cells Using a Combined Cell Manipulation System and a Single-Probe Device. *Analytical Chemistry* **2019,** *91* (3), 1738-1742.
7. Pan, N.; Standke, S. J.; Kothapalli, N. R.; Sun, M.; Bensen, R. C.; Burgett, A. W. G.; Yang, Z., Quantification of Drug Molecules in Live Single Cells Using the Single-Probe Mass Spectrometry Technique. *Analytical Chemistry* **2019,** *91* (14), 9018-9024.
8. Sun, M.; Tian, X.; Yang, Z., Microscale Mass Spectrometry Analysis of Extracellular Metabolites in Live Multicellular Tumor Spheroids. *Anal Chem* **2017,** *89* (17), 9069-9076.
9. Standke, S. J.; Colby, D. H.; Bensen, R. C.; Burgett, A. W. G.; Yang, Z., Integrated Cell Manipulation Platform Coupled with the Single-probe for Mass Spectrometry Analysis of Drugs and Metabolites in Single Suspension Cells. *J Vis Exp* **2019,** (148), 10.3791/59875.
10. Tian, X.; Zhang, G.; Zou, Z.; Yang, Z., Anticancer Drug Affects Metabolomic Profiles in Multicellular Spheroids: Studies Using Mass Spectrometry Imaging Combined with Machine Learning. *Anal Chem* **2019,** *91* (9), 5802-5809.
11. Liu, R.; Pan, N.; Zhu, Y.; Yang, Z., T-Probe: An Integrated Microscale Device for Online In Situ Single Cell Analysis and Metabolic Profiling Using Mass Spectrometry. *Anal Chem* **2018,** *90* (18), 11078-11085.
12. Liu, R.; Zhang, G.; Sun, M.; Pan, X.; Yang, Z., Integrating a generalized data analysis workflow with the Single-probe mass spectrometry experiment for single cell metabolomics. *Anal Chim Acta* **2019,** *1064*, 71-79.
13. Romano, P.; Profumo, A.; Rocco, M.; Mangerini, R.; Ferri, F.; Facchiano, A., Geena 2, improved automated analysis of MALDI/TOF mass spectra. *BMC Bioinformatics* **2016,** *17* (4), 61.
14. Xia, J.; Broadhurst, D. I.; Wilson, M.; Wishart, D. S., Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics* **2013,** *9* (2), 280-299.
15. Xia, J.; Sinelnikov, I. V.; Wishart, D. S., MetATT: a web-based metabolomics tool for analyzing time-series and two-factor datasets. *Bioinformatics* **2011,** *27* (17), 2455-2456.
16. Xia, J.; Wishart, D. S., MetPA: a web-based metabolomics tool for pathway analysis and visualization. *Bioinformatics* **2010,** *26* (18), 2342-2344.
17. Xia, J.; Wishart, D. S., Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nature Protocols* **2011,** *6* (6), 743-760.
18. Pang, Z.; Chong, J.; Zhou, G.; de Lima Morais, D. A.; Chang, L.; Barrette, M.; Gauthier, C.; Jacques, P.-É.; Li, S.; Xia, J., MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research* **2021,** *49* (W1), W388-W396.
19. Quirk, T. J., One-Way Analysis of Variance (ANOVA). In *Excel 2007 for Educational and Psychological Statistics: A Guide to Solving Practical Problems*, Springer New York: New York, NY, 2012; pp 163-179.
20. Liu, R.; Yang, Z., Single cell metabolomics using mass spectrometry: Techniques and data analysis. *Anal Chim Acta* **2021,** *1143*, 124-134.

21. Smith, C. A.; Maille, G. O.; Want, E. J.; Qin, C.; Trauger, S. A.; Brandon, T. R.; Custodio, D. E.; Abagyan, R.; Siuzdak, G., METLIN: A Metabolite Mass Spectral Database. *Therapeutic Drug Monitoring* **2005,** *27* (6), 747-751.
22. Wishart, D. S.; Feunang, Y. D.; Marcu, A.; Guo, A. C.; Liang, K.; Vázquez-Fresno, R.; Sajed, T.; Johnson, D.; Li, C.; Karu, N.; Sayeeda, Z.; Lo, E.; Assempour, N.; Berjanskii, M.; Singhal, S.; Arndt, D.; Liang, Y.; Badran, H.; Grant, J.; Serra-Cayuela, A.; Liu, Y.; Mandal, R.; Neveu, V.; Pon, A.; Knox, C.; Wilson, M.; Manach, C.; Scalbert, A., HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Research* **2017,** *46* (D1), D608-D617.
23. Wang, M.; Carver, J. J.; Phelan, V. V.; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D.; Watrous, J.; Kapono, C. A.; Luzzatto-Knaan, T.; Porto, C.; Bouslimani, A.; Melnik, A. V.; Meehan, M. J.; Liu, W.-T.; Crüsemann, M.; Boudreau, P. D.; Esquenazi, E.; Sandoval-Calderón, M.; Kersten, R. D.; Pace, L. A.; Quinn, R. A.; Duncan, K. R.; Hsu, C.-C.; Floros, D. J.; Gavilan, R. G.; Kleigrewe, K.; Northen, T.; Dutton, R. J.; Parrot, D.; Carlson, E. E.; Aigle, B.; Michelsen, C. F.; Jelsbak, L.; Sohlenkamp, C.; Pevzner, P.; Edlund, A.; McLean, J.; Piel, J.; Murphy, B. T.; Gerwick, L.; Liaw, C.-C.; Yang, Y.-L.; Humpf, H.-U.; Maansson, M.; Keyzers, R. A.; Sims, A. C.; Johnson, A. R.; Sidebottom, A. M.; Sedio, B. E.; Klitgaard, A.; Larson, C. B.; Boya P, C. A.; Torres-Mendoza, D.; Gonzalez, D. J.; Silva, D. B.; Marques, L. M.; Demarque, D. P.; Pociute, E.; O'Neill, E. C.; Briand, E.; Helfrich, E. J. N.; Granatosky, E. A.; Glukhov, E.; Ryffel, F.; Houson, H.; Mohimani, H.; Kharbush, J. J.; Zeng, Y.; Vorholt, J. A.; Kurita, K. L.; Charusanti, P.; McPhail, K. L.; Nielsen, K. F.; Vuong, L.; Elfeki, M.; Traxler, M. F.; Engene, N.; Koyama, N.; Vining, O. B.; Baric, R.; Silva, R. R.; Mascuch, S. J.; Tomasi, S.; Jenkins, S.; Macherla, V.; Hoffman, T.; Agarwal, V.; Williams, P. G.; Dai, J.; Neupane, R.; Gurr, J.; Rodríguez, A. M. C.; Lamsa, A.; Zhang, C.; Dorrestein, K.; Duggan, B. M.; Almaliti, J.; Allard, P.-M.; Phapale, P.; Nothias, L.-F.; Alexandrov, T.; Litaudon, M.; Wolfender, J.-L.; Kyle, J. E.; Metz, T. O.; Peryea, T.; Nguyen, D.-T.; VanLeer, D.; Shinn, P.; Jadhav, A.; Müller, R.; Waters, K. M.; Shi, W.; Liu, X.; Zhang, L.; Knight, R.; Jensen, P. R.; Palsson, B. Ø.; Pogliano, K.; Linington, R. G.; Gutiérrez, M.; Lopes, N. P.; Gerwick, W. H.; Moore, B. S.; Dorrestein, P. C.; Bandeira, N., Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology* **2016,** *34* (8), 828-837.
24. Wang, M.; Jarmusch, A. K.; Vargas, F.; Aksenov, A. A.; Gauglitz, J. M.; Weldon, K.; Petras, D.; da Silva, R.; Quinn, R.; Melnik, A. V.; van der Hooft, J. J. J.; Caraballo-Rodríguez, A. M.; Nothias, L. F.; Aceves, C. M.; Panitchpakdi, M.; Brown, E.; Di Ottavio, F.; Sikora, N.; Elijah, E. O.; Labarta-Bajo, L.; Gentry, E. C.; Shalapour, S.; Kyle, K. E.; Puckett, S. P.; Watrous, J. D.; Carpenter, C. S.; Bouslimani, A.; Ernst, M.; Swafford, A. D.; Zúñiga, E. I.; Balunas, M. J.; Klassen, J. L.; Loomba, R.; Knight, R.; Bandeira, N.; Dorrestein, P. C., Mass spectrometry searches using MASST. *Nature Biotechnology* **2020,** *38* (1), 23-26.
25. Chambers, M. C.; Maclean, B.; Burke, R.; Amodei, D.; Ruderman, D. L.; Neumann, S.; Gatto, L.; Fischer, B.; Pratt, B.; Egertson, J.; Hoff, K.; Kessner, D.; Tasman, N.; Shulman, N.; Frewen, B.; Baker, T. A.; Brusniak, M.-Y.; Paulse, C.; Creasy, D.; Flashner, L.; Kani, K.; Moulding, C.; Seymour, S. L.; Nuwaysir, L. M.; Lefebvre, B.; Kuhlmann, F.; Roark, J.; Rainer, P.; Detlev, S.; Hemenway, T.; Huhmer, A.; Langridge, J.; Connolly, B.; Chadick, T.; Holly, K.; Eckels, J.; Deutsch, E. W.; Moritz, R. L.; Katz, J. E.; Agus, D. B.; MacCoss, M.; Tabb, D. L.; Mallick, P., A cross-platform toolkit for mass spectrometry and proteomics. *Nature Biotechnology* **2012,** *30* (10), 918-920.