Electronic Supplementary Information for

In Situ Metabolic Analysis of Single Plant Cells by Capillary Microsampling and Electrospray Ionization Mass Spectrometry with Ion Mobility Separation

Linwen Zhang,[a] Daniel P. Foreman,[a] Paaqua A. Grant,[b] Bindesh Shrestha,[a] Sally A. Moody,[c] Florent Villiers,[d] June M. Kwak[e] and Akos Vertes*[,a]

[a]*Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, The George Washington University, Washington, DC 20052, USA*

[b]*Department of Biological Sciences, The George Washington University, Washington, DC 20052, USA*

[c]*Department of Anatomy and Regenerative Biology, The George Washington University, Washington, DC 20037, USA*

[d]*Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742, USA*

[e]*Center for Plant Aging Research, Institute for Basic Science, Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, Daegu 711-873, Republic of Korea*

**Corresponding author. E-mail: vertes@gwu.edu, Phone: +1 (202) 994-2717, Fax: +1 (202) 994-5873.*

Fig. S1 Capillaries for sampling of (a) pavement or basal cells, and trichomes (b) before cutting and (c) after cutting. The insets show the capillary tips at higher magnifications (inset scale bars are 10 µm). The tip diameters are ~0.7 µm, ~0.2 µm, and ~5 µm, respectively.

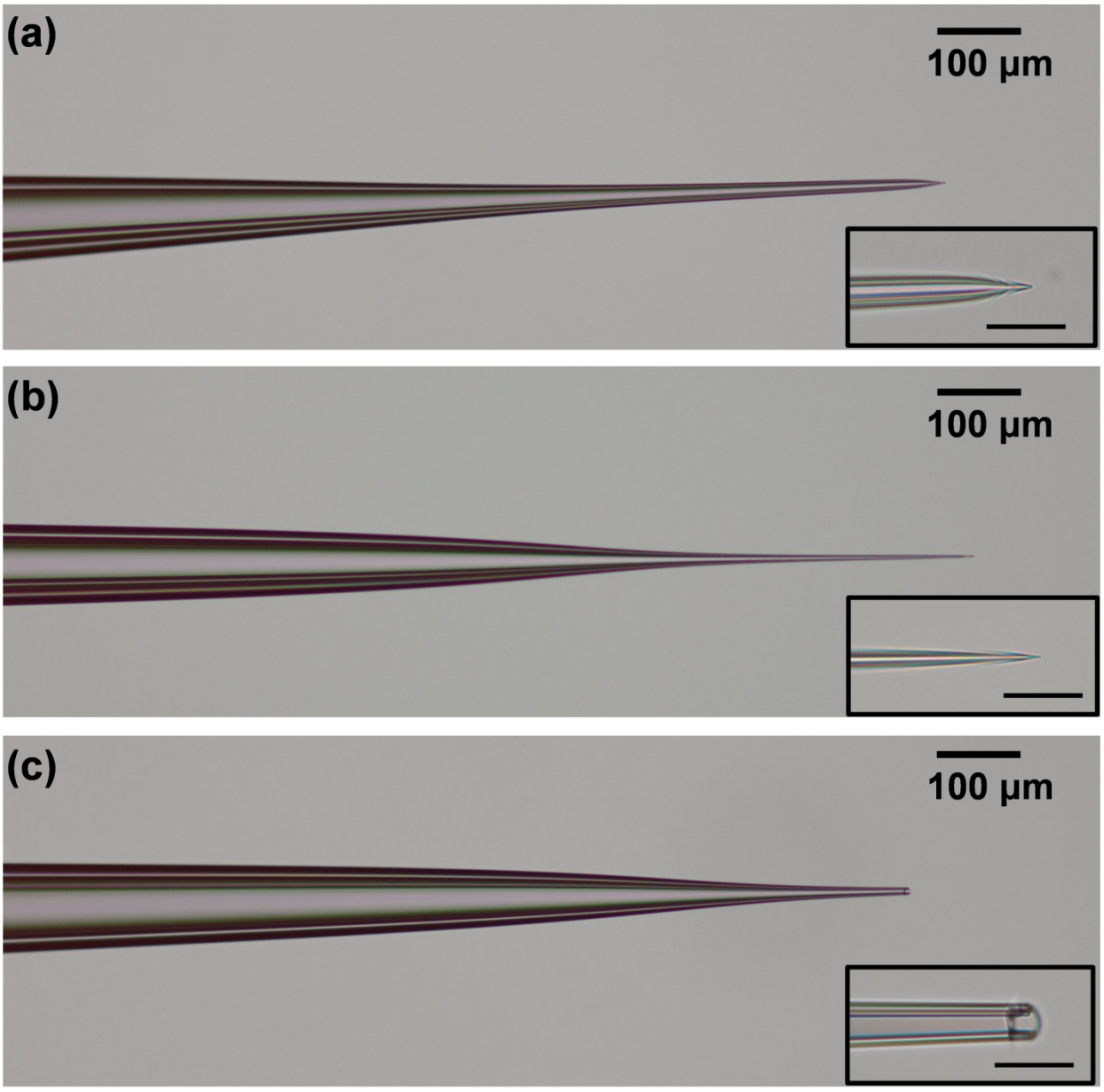

Fig. S2 Single cell tandem mass spectra of ions (a) *m/z* 763 and (b) *m/z* 779, with corresponding structures. In spectrum (a), the fragments *m/z* 287.056, 471.081, and 617.149 are derived from the *m/z* 763.217 precursor ion by loss of all the glycones, two rhamnosides, and one rhamnoside, respectively. In spectrum (b), the precursor ion, *m/z* 779.181, fragments to produce *m/z* 287.055, 471.079, 617.137, and 633.124 by the loss of all glycones, one glucoside and one rhamnoside, one glucoside, and one rhamnoside, respectively.

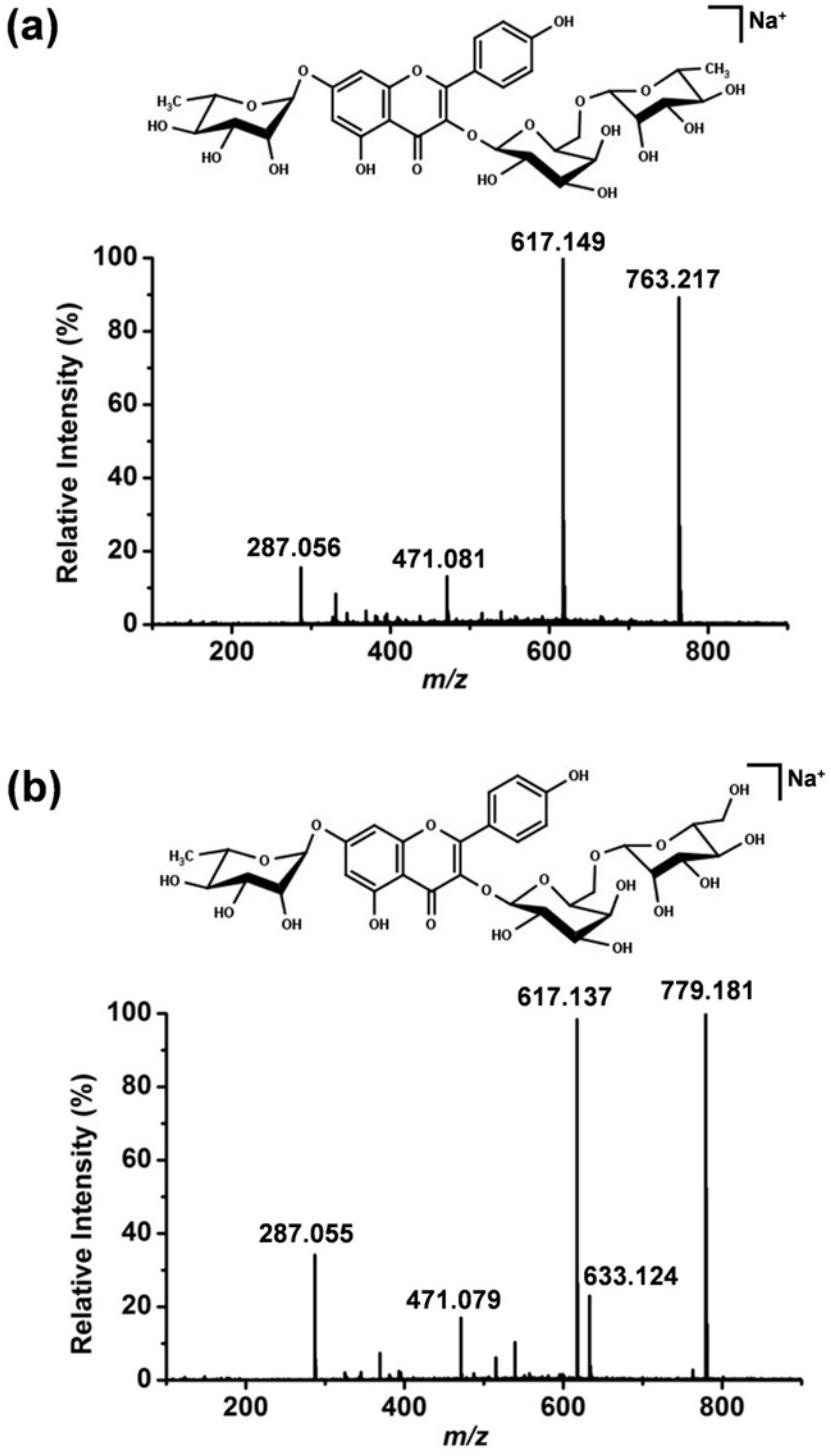

Fig. S3 Normalized intensities of selected metabolite ions detected in pavement cells (green), basal cells (red) and trichomes (blue). K, Rha and Glc denote kaempferol, rhamnoside and glucoside, respectively. Sinapic acid esters and kaempferol glycosides are separated by a dashed line. Significantly different metabolite levels are observed in the three cell types.

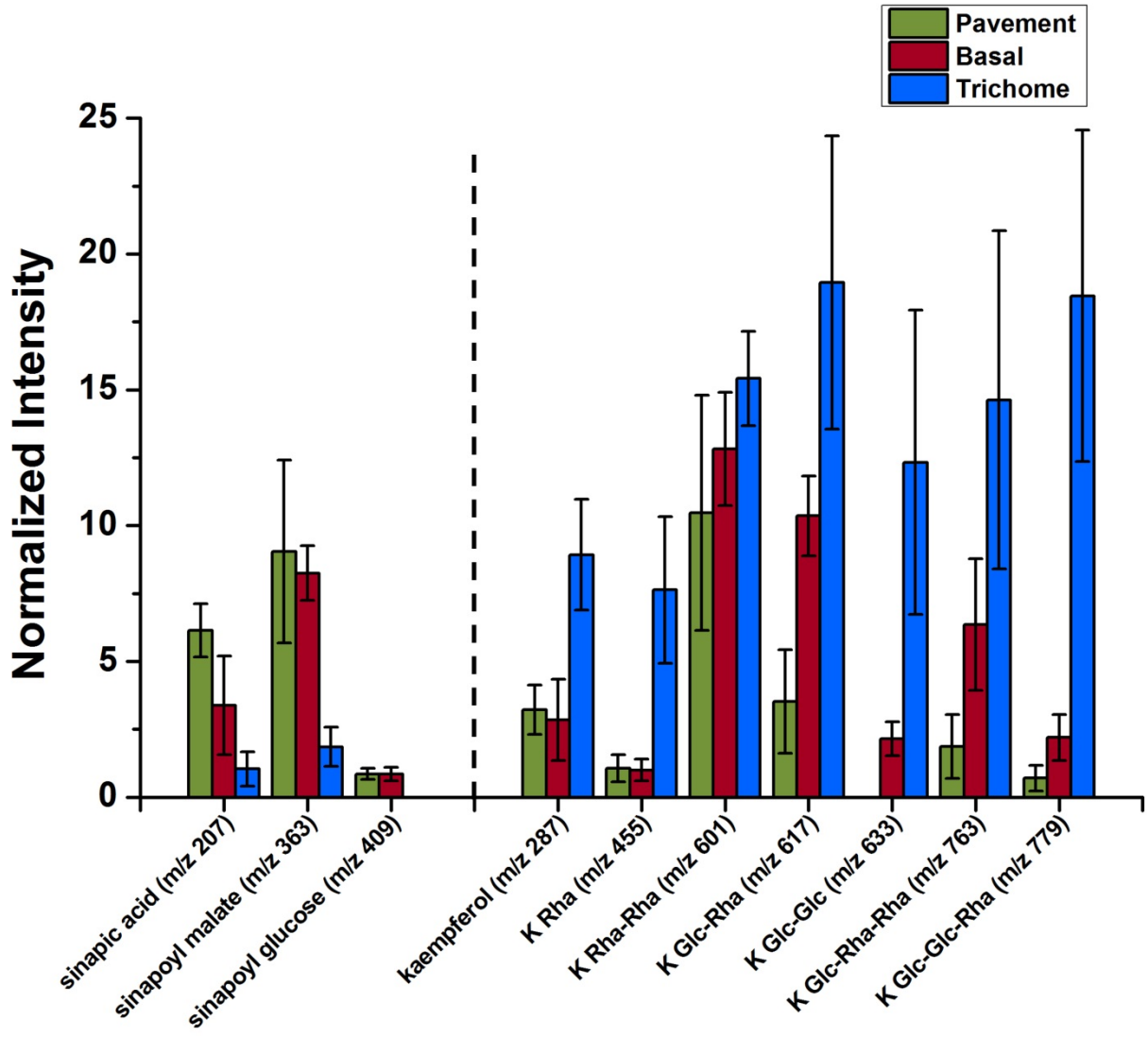

Fig. S4 A DT vs. *m/z* plot produced by the analysis of a single trichome using capillary microsampling ESI-IMS-MS. The mass spectra shown on the right, corresponding to the framed regions in the 2D plot, indicate the presence of mono-, di- and triglycosides of kaempferol (K).

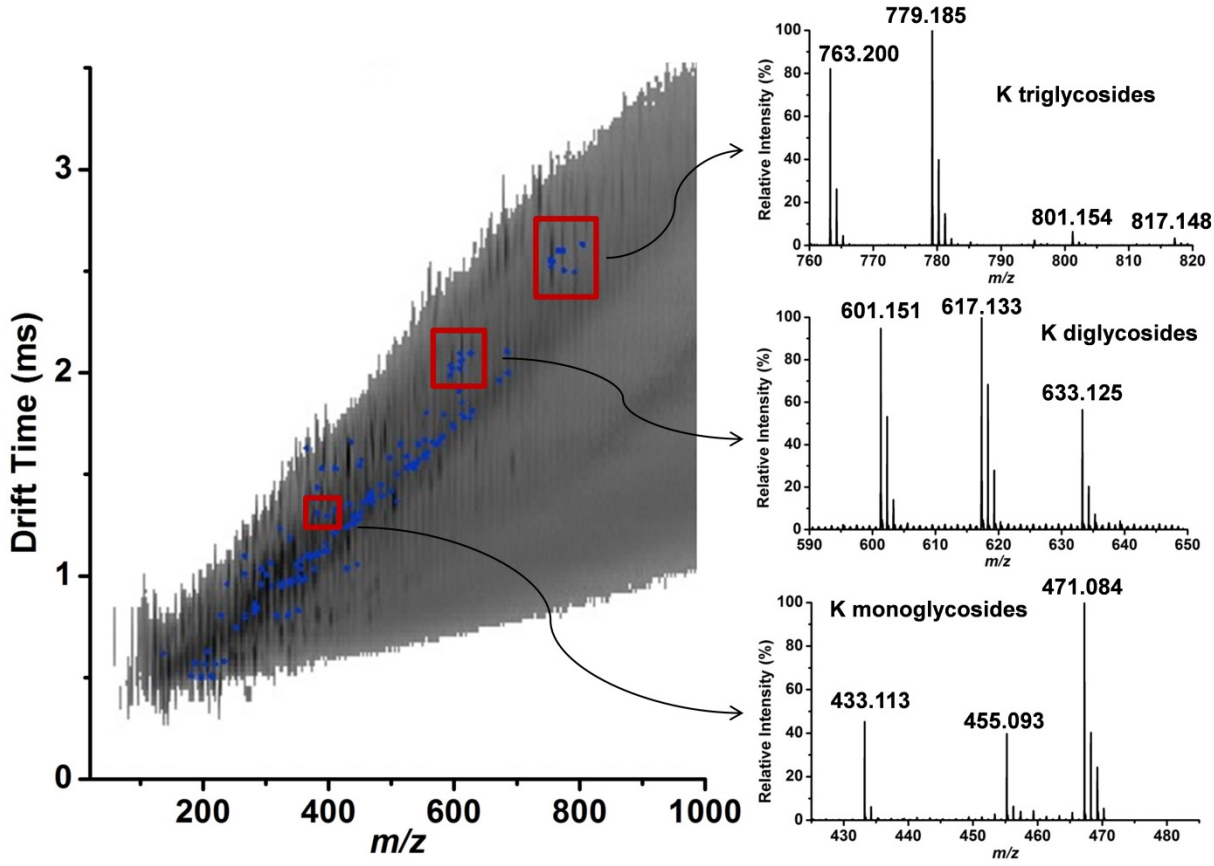

Fig. S5 Part of phenylpropanoid metabolism pathways in *A. thaliana* with the phenylpropanoid acid biosynthesis, sinapic acid ester biosynthesis, and kaempferol glycoside biosynthesis subpathways framed by dashed rectangles (K = kaempferol, Rha = rhamnoside and Glc = glucoside). Metabolites detected at higher levels in pavement and basal cells, and in trichomes are marked by blue and yellow frames, respectively. The detected ions are indicated by the measured *m/z* values.

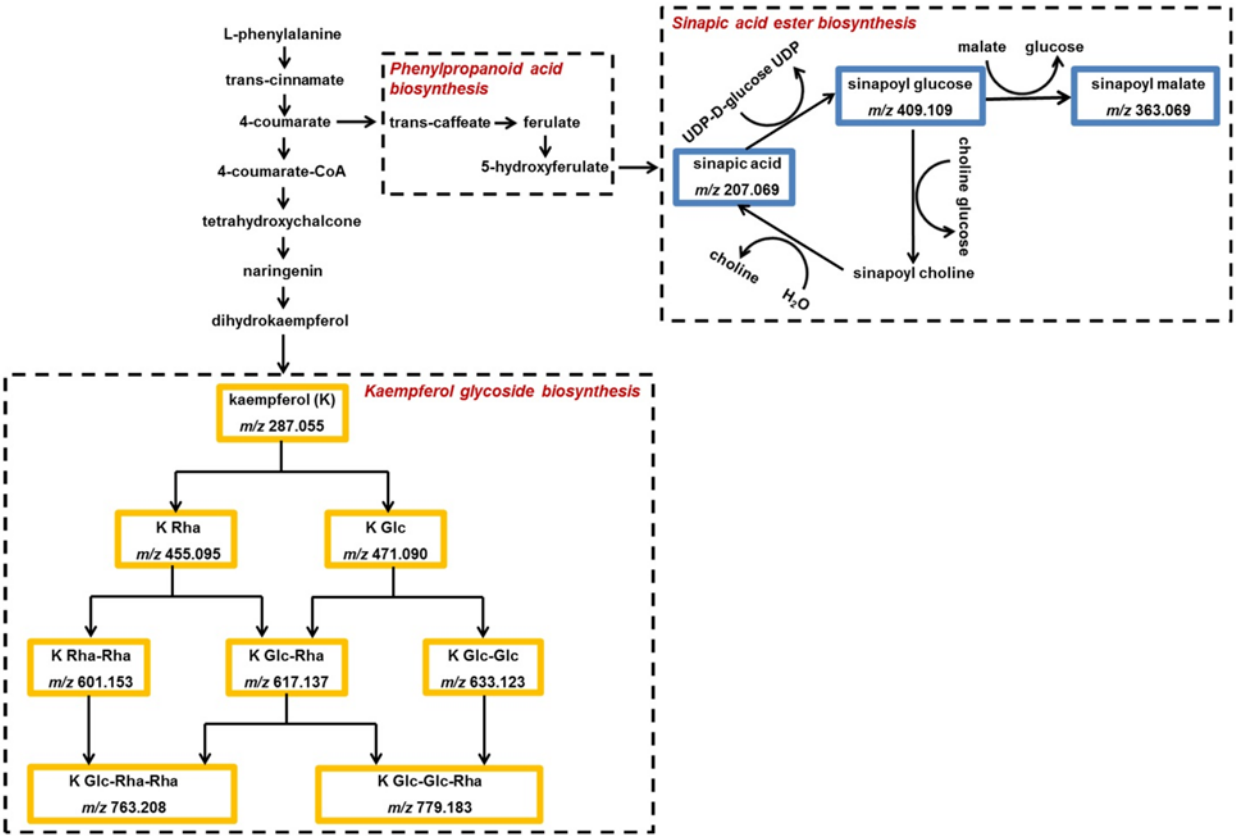

Table S1. Tentative assignments of metabolite ions detected in the three *A. thaliana* epidermal cell types. The ratios of the normalized ion abundances from trichomes and pavement cells ($I_{T:P}$), and from trichomes and basal cells ($I_{T:B}$) are shown. Symbols "T", "P" and "B" indicate that the ion was only detected in trichomes, pavement cells or basal cells, respectively.

Compound	Formula	Measured Mass	Calculated Mass	Δm (mDa)	$I_{T:P}$	$I_{T:B}$
coumarin	$C_9H_6O_2$	147.046 ($+H^+$)	147.0441	1.9	0.15	0.47
aconitic acid[b]	$C_6H_6O_6$	175.038 ($+H^+$)	175.0237	14.3	0.15	0.27
sinapic acid[b]	$C_{11}H_{12}O_5$	207.069 ($-H_2O+H^+$) 247.049 ($+Na^+$) 263.024 (K^+)	207.0652 247.0577 263.0316	3.8 -8.7 -7.6	0.17 P P	0.31 B B
kaempferol[c]	$C_{15}H_{10}O_6$	287.055 ($+H^+$) 325.003 ($+K^+$)	287.0550 325.0109	0 -7.9	2.77 T	3.13 T
sinapoyl malate[b]	$C_{15}H_{16}O_9$	363.069 (Na^+)	363.0686	0.4	0.20	0.22
disaccharide[b]	$C_{12}H_{22}O_{11}$	365.113 ($+Na^+$) 381.078 ($+K^+$)	365.1054 381.0794	7.6 -1.4	0.75 0.87	0.24 0.14
sinapoyl glucose[b]	$C_{17}H_{22}O_{10}$	409.110 (Na^+)	409.1105	-0.5	P	B
kaempferol rhamnoside[a,d]	$C_{21}H_{20}O_{10}$	433.114 ($+H^+$) 455.095 ($+Na^+$)	433.1129 455.0949	1.1 0.1	T 7.13	T 7.63
kaempferol glucoside[a,d]	$C_{21}H_{20}O_{11}$	471.090 ($+Na^+$)	471.0898	0.2	T	T
kaempferol dirhamnoside[a,d]	$C_{27}H_{30}O_{14}$	601.153 ($+Na^+$)	601.1528	0.2	1.47	1.20
kaempferol glucoside-rhamnoside[a,d]	$C_{27}H_{30}O_{15}$	617.137 ($+Na^+$)	617.1477	-10.7	5.38	1.83
kaempferol diglucoside[a,d]	$C_{27}H_{30}O_{16}$	633.123 ($+Na^+$)	633.1426	-19.6	T	5.71
PC (34:4)[b]	$C_{42}H_{76}NO_8P$	754.552 ($+H^+$)	754.5381	13.9	P	B
PC (34:3)[b]	$C_{42}H_{78}NO_8P$	756.568 ($+H^+$) 794.510 ($+K^+$)	756.5538 794.5096	14.2 0.4	P P	B B
PC (34:2)[b]	$C_{42}H_{80}NO_8P$	758.581 ($+H^+$) 796.526 ($+K^+$)	758.5694 796.5253	11.6 0.7	P P	B B
PC (34:1)[b]	$C_{42}H_{82}NO_8P$	760.597 ($+H^+$)	760.5851	11.9	P	B
kaempferol glucoside-rhamnoside-rhamnoside[a,d]	$C_{33}H_{40}O_{19}$	763.208 ($+Na^+$) 801.162 ($-H^++Na^++K^+$)	763.2056 801.1615	2.4 0.5	7.84 T	2.30 T
PC (36:6)[b]	$C_{44}H_{76}NO_8P$	778.549 ($+H^+$)	778.5381	10.9	P	B
kaempferol glucoside-glucoside-rhamnoside[a,d]	$C_{33}H_{40}O_{20}$	779.183 ($+Na^+$) 817.157 ($-H^++Na^++K^+$)	779.2005 817.1564	-17.5 0.6	26.17 T	8.39 T
PC (36:5)[b]	$C_{44}H_{78}NO_8P$	780.566 (H^+) 802.552 (Na^+)	780.5538 802.5357	12.2 16.3	P P	B B
PC (36:4)[b]	$C_{44}H_{80}NO_8P$	782.580 (H^+) 804.559 (Na^+)	782.5694 804.5513	10.6 7.7	P P	B B
PC (36:3)[b]	$C_{44}H_{82}NO_8P$	784.597 (H^+) 806.578 (Na^+)	784.5851 806.5670	11.9 11.0	P P	B B
PC (36:2)[b]	$C_{44}H_{84}NO_8P$	786.601 (H^+) 808.580 (Na^+)	786.6007 808.5826	0.3 -2.6	P P	B B

[a]Chemical species assigned based on tandem MS from capillary microsampling of single cell.
[b]Chemical species assigned based on tandem MS from LAESI of multiple cells.
[c]Kaempferol and its structural isomers are consistent with the measured *m/z*.
[d]Kaempferol and its structural isomers are consistent with the tandem MS data.