

Supplementary Information

Profiling and quantifying endogenous molecules in single cells using nano-DESI MS

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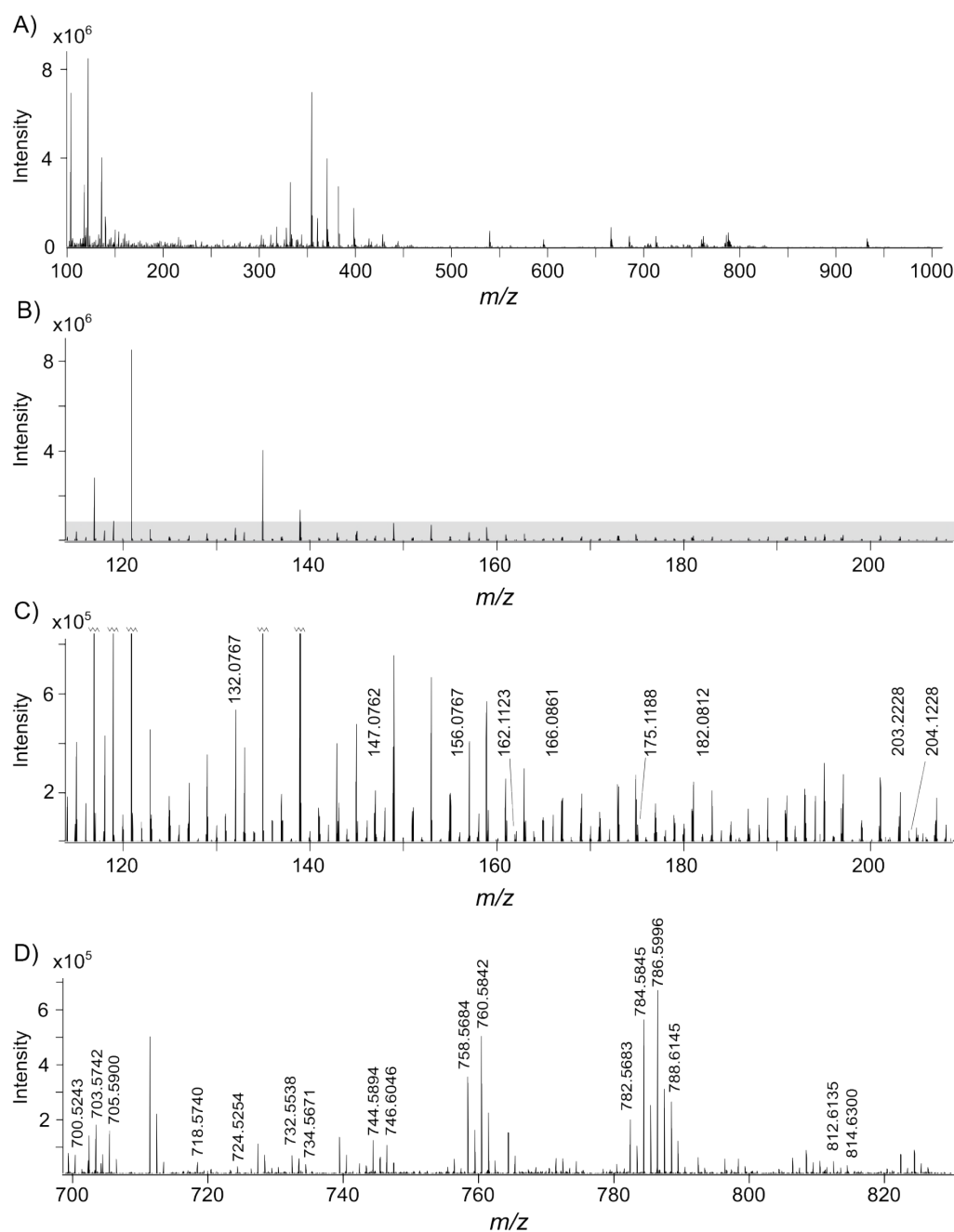


Figure S1 – Mass spectrum from a single cheek cell, analyzed by nano-DESI MS. Eight mass spectra from the extraction were averaged. A) Mass spectrum showing the mass range m/z 100-1000. B) Mass spectrum showing the mass range m/z 110-215. The grey box shows the intensity range in which endogenous compounds are detected. C) Zoom-in of the mass range m/z 110-215, corresponding to the grey box shown in B). Some peaks corresponding to identified compounds are labeled to facilitate inspection of the spectrum. D) Mass spectrum showing the mass range m/z 700-830. Some peaks corresponding to identified compounds have been labeled to facilitate inspection of the spectrum.

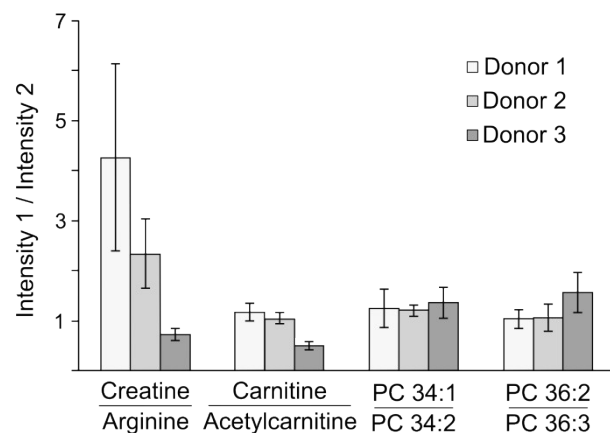


Figure S2 – Relative signal intensities of creatine (m/z 132.0768 [M+H]⁺) vs arginine (m/z 175.1188 [M+H]⁺); carnitine (m/z 162.1124 [M+H]⁺ vs acetylcarnitine (m/z 204.1229 [M+H]⁺); PC 34:1 (m/z 798.5404 [M+K]⁺) vs PC 34:2 (m/z 796.5277 [M+K]⁺); and PC 36:2 (m/z 824.5575 [M+K]⁺) vs PC 36:3 (m/z 822.5416 [M+K]⁺). Relative signal intensities were extracted from five cells from each of three healthy volunteers (n=5). Error bars represent ± 1 standard deviation, the typical relative standard deviation (RSD) is 20%.

Table S1 - Tandem mass spectrometry data of metabolites from human cheek cells (end), compared to fragmentation pattern of standard (std) or database values (https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage).

Compound [M+H] ⁺	Measured <i>m/z</i>	Theoretical <i>m/z</i>	Detected fragment <i>m/z</i> (Relative ratio in percent at NCE 40 unless otherwise stated)
Proline_{std}	116.0707	116.1706	70.0657 (100), 68.0502 (0.4)
Proline_{end}	116.0708	116.0706	70.0658 (100), 68.0503 (0.15)
Taurine_{std}	126.0220	126.0219	126.0220 (100) 108.0117 (100)
Taurine_{end}	126.0222	126.0219	126.0222 (100), 108.0117 (50)
Creatine (Metlin)	132.0768	132.0768	90.0555 (100), 87.0607 (12) Spectrum at 20V
Creatine_{end}	132.0768	132.0768	90.0554 (100)
Leu/Ile_{std}	132.1017	132.1019	86.0969 (100), 69.0705 (11), 68.0501 (7)
Leu/Ile_{end}	132.1019	132.1019	86.0969 (100), 69.0705 (8), 68.0501 (2)
Asparagine (Metlin)	133.0602	133.0608	74.0242 (100), 87.0558 (65), 116.0353 (15), 88.0392 (13) Spectrum at 10V
Asparagine_{end}	133.0606	133.0608	87.0557 (100), 74.0242 (90), 88.0397 (50), 116.0344 (13)
Aspartic acid_{std}	134.0446	134.0448	74.0242 (100), 88.0397 (85), 86.0609 (35), 71.0691 (25), 117.0741 (16), 116.0344 (12), 70.0658 (10)
Aspartic acid_{end}	134.0447	134.0448	74.0243 (100), 88.0398 (80), 116.0344 (12) 86.0609 (2), 71.0691 (2), 117.0741 (1)
Spermidine (Metlin)	146.1649	146.1652	72.0814 (100), 84.0811 (20), 112.1119 (16), 58.0663 (15) Spectrum at 20V

Spermidine_{end}	146.1651	146.1652	72.0814 (100), 84.0813 (50), 112.1123 (30), 58.0659 (4) Spectrum at NCE 10
Glutamine (Metlin)	147.0762	147.0764	84.0449 (100), 130.0496 (95), 56.0502 (2) Spectrum at 10 V 84.0449 (100), 56.0504 (12), 130.0496 (4) Spectrum at 20 V
Glutamine_{end}	147.0764	147.0764	84.0449 (100), 130.0499 (70) , 56.0502 (1)
Lysine_{std}	147.1126	147.1128	84.0813 (100), 130.0862 (30), 102.0553 (6), 118.0653 (3), 104.0709 (2.5)
Lysine_{end}	147.1126	147.1128	84.0813 (100), 102.0553 (40), 130.0862 (30)
Methionine_{std}	150.0582	150.0583	56.0502 (100), 104.0531 (90), 133.0317 (65), 61.0114 (30), 102.0553 (25), 87.0268 (13)
Methionine_{end}	150.0581	150.0583	56.0502 (100), 104.0531 (100), 133.0318 (75), 102.0553 (50), 61.0114 (35), 87.0267 (12)
Valine_{std}	118.0863	118.0863	72.0815 (100), 55.0551 (40) Spectrum at NCE 80
Valine_{end}	156.0420	156.0421	72.0815 (100), 55.0551 (95) Spectrum at NCE 80
Histidine_{std}	156.0765	156.0768	110.0715 (100), 95.0608 (6), 83.0609 (3), 93.0452 (2.5), 138.1100 (1)
Histidine_{end}	156.0768	156.0768	110.0716 (100), 95.0609 (5), 83.0610 (4), 93.0453 (1.5)

Carnitine (Metlin)	162.1125	162.1125	103.0396 (12), 60.0821 (11), 102.0919 (3), 85.0291 (2) Spectrum at 10V
Carnitine_{end}	162.1124	162.1125	103.0394 (40), 60.0815 (25), 102.0918 (7), 85.0290 (4)
Phenylalanine_{std}	166.0860	166.0863	103.0545 (100), 107.0494 (30), 93.0703 (25), 79.0548 (5), 91.0547 (4)
Phenylalanine_{end}	166.0862	166.0863	166.0862 (100), 103.0546 (35), 107.0495 (20), 93.0704 (20), 79.0549 (10), 91.0548 (8)
Arginine_{std}	175.1186	175.1190	70.0657 (100), 60.0564 (50), 116.0708 (45), 130.0975 (15), 158.0923 (13), 113.0712 (12), 112.0872 (8), 159.0762 (8)
Arginine_{end}	175.1188	175.1190	70.0658 (100), 60.0564 (30), 116.0709 (30), 130.0976 (13), 158.0924 (10), 112.0873 (6)
Tyrosine_{std}	182.0813	182.0812	136.0756 (100), 123.0441 (50), 119.0493 (40), 147.0439 (25), 137.0894 (20), 165.0545 (20), 129.0556 (15), 91.0547 (12)
Tyrosine_{end}	182.0811	182.0812	136.0757 (100), 123.0442 (55), 119.0494 (30), 165.0546 (25), 147.0440 (20), 91.0547 (11)
Spermine (Metlin)	203.2230	203.2230	129.1385 (100), 112.1122 (30) Spectrum at 10 V 112.1122 (100), 129.1381 (25), 84.0811 (25), 58.0661 (10) Spectrum at 20 V
Spermine_{end}	203.2225	203.2230	112.1122 (100), 129.1386 (50), 84.0813 (9), 58.0659 (6)

Acetylcarnitine (Metlin)	204.1223	204.1230	204.1223 (100), 85.0283 (53), 145.0506 (16), 60.0821 (14) Spectrum at 10 V
Acetylcarnitine_{end}	204.1231	204.1230	204.1231 (100), 85.0290 (65), 145.0496 (25), 60.0816 (12) Spectrum at NCE 20
Tryptophan (Metlin)	205.0966	205.0972	188.0704 (100), 146.0598 (20) Spectrum at 10 V 146.0595 (100), 118.0653 (30), 144.0804 (21), 188.0702 (18), 159.0904 (11) Spectrum at 20 V
Tryptophan_{end}	205.0967	205.0972	146.0599 (100), 188.0704 (35), 118.0652 (15), 144.0808 (15), 159.0914 (9)

Table S2 - Tandem mass spectrometry data of lipids from human cheek cells

Compound [M+H] ⁺	Measured <i>m/z</i>	Theoretical <i>m/z</i>	Detected fragment <i>m/z</i>
ePE 34:3	700.5257	700.5252	337.2732, ^{a)} 364.2606, ^{a)} 559.5077 ^{a)}
ePE 34:2	702.5420	702.5432	339.2889, ^{a)} 364.2606, ^{a)} 561.6236 ^{a)}
SM 34:1	703.5740	703.5749	86.0969, 104.1072, 184.0732
SM 34:0	705.5892	705.5905	86.0969, 104.1073, 184.0732
ePC 32:1	718.5725	718.5745	86.0969, 104.1073, 184.0732
ePE 36:5	724.5268	724.5276	361.2737, ^{a)} 364.2610, ^{a)} 583.5089 ^{a)}
ePE 36:4	726.5396	726.5432	337.2732, ^{a)} 390.2763, ^{a)} 585.5236 ^{a)}
ePE 36:3	728.5567	728.5589	337.2732, ^{a)} 339.2889, ^{a)} 390.2763, ^{a)} 392.2919, ^{a)} 587.5386 ^{a)}
ePE 36:2	730.5726	730.5745	339.2889, ^{a)} 392.2920, ^{a)} 589.5549 ^{a)}
PC 32:1	732.5532	732.5538	86.0969, 104.1073, 184.0731
SM 36:0	733.6206	733.6218	86.0969, 104.1073, 184.0731
PC 32:0	734.5664	734.5694	86.0969, 104.1073, 184.0732
ePC 34:3	742.5740	742.5745	86.0969, 104.1073, 184.0732
ePC 34:2	744.5891	744.5902	86.0968, 104.1072, 184.0731
PE 36:1	746.5707	746.5694	605.5507
ePC 34:1	746.6050	746.6058	86.0970, 104.1074, 184.0733
PC 34:3	756.5537	756.5538	86.0968, 104.1073, 184.0731
PC 34:2	758.5695	758.5694	86.0969, 104.1073, 184.0732
PC 34:1	760.5845	760.5851	86.0969, 104.1073, 184.0732
PC 36:5 [PC 34:2+Na] ⁺	780.5516	780.5538 780.5514	86.09670, 104.1074, 184.0733

PC 36:4 [PC 34:2+Na]⁺	782.5683	782.5694 782.5670	86.09670, 104.1074, 184.0733
PC 36:3	784.5844	784.5851	86.0969, 104.1073, 184.0732
PC 36:2	786.5996	786.6007	86.0969, 104.1073, 184.0732
PC 36:1	788.6135	788.6164	86.0967, 104.1070, 184.0730
PC 38:6 [PC 36:3+Na]⁺	806.5657	806.5694 806.5670	86.09670, 104.1074, 184.0732
PC 38:5 [PC 36:2+Na]⁺	808.5827	808.5851 808.5827	86.09670, 104.1074, 184.0733
PC 38:4 [PC 36:1+Na]⁺	810.5979	810.6007 810.5983	86.0969, 104.1073, 184.0732
PC 38:3	812.6146	812.6164	86.0969, 104.1073, 184.0732
PC 38:2	814.6300	814.6320	86.0969, 104.1073, 184.0732

a) Fragments detected in previous tandem mass spectrometry studies of glycerophosphoethanolamine plasmalogen species¹

Table S3. Data for seven different PC species quantified in seven single cells, including total amount PC quantified and cell area. Amounts are provided in fmoles per cell.

	PC 34:1	PC 36:2	PC 34:2	PC 36:3	PC 36:4	PC 36:1	Total PC	Cell area (μm²)
cell 1	380	280	260	250	206	90	1466	4900
cell 2	330	390	180	180	160	230	1470	3500
cell 3	245	220	150	160	160	70	1005	4360
cell 4	220	220	160	140	160	130	1030	2900
cell 5	170	100	70	50	60	100	550	2400
cell 6	300	290	240	240	240	110	1420	3150
cell 7	250	310	160	180	140	190	1230	5900
Average	270	260	170	170	160	130	1160	3880
RSD	26%	34%	36%	39%	35%	44%	29%	32%

Table S4. Relative abundance of seven different PC species quantified in seven single cells, calculated by dividing the amount for each PC species in Table S3 with the Total amount quantified PC in Table S3.

	PC 34:1	PC 36:2	PC 34:2	PC 36:3	PC 36:4	PC 36:1
cell 1	26%	19%	17%	17%	14%	6%
cell 2	23%	26%	12%	12%	11%	16%
cell 3	24%	22%	15%	16%	16%	7%
cell 4	21%	22%	16%	14%	15%	12%
cell 5	31%	19%	12%	10%	10%	18%
cell 6	21%	20%	17%	17%	17%	8%
cell 7	21%	25%	13%	14%	12%	15%
RSD	16%	13%	15%	20%	19%	40%

References

1. K. A. Zemski Berry and R. C. Murphy, *Journal of the American Society for Mass Spectrometry*, 2004, **15**, 1499-1508.