



Figure S1. Workflow of small extracellular vesicles (EVs) biomarker detection using SALDI-MS for CRC diagnosis. (A) Plasma EVs were isolated from metastatic CRC and CFI individuals. Then trypsin-digestion proteins and lithium adduct lipid samples were spotted on NIMS, and DIOS surfaces, respectively. (B) SALDI surfaces functionalised with a fluorinated silane are then coupled with a high-resolution mass spectrometer. CRC - colorectal cancer stage-I to stage-IV; CFI - cancer free individual and healthy participants; pSi - Porous silicon; NIMS - Nanostructure-initiator mass spectrometry; SALDI - Surface-assisted laser desorption/ionisation mass spectrometry; DIOS - Desorption/ionisation on porous silicon.

Table S1. Top 63 peptide ions discriminate CRC and CFI group. CRC - colorectal cancer stage-I to stage-IV; CFI - cancer free individuals and healthy participants.

| <i>m/z</i> | Interval width (\pm Da) | <i>m/z</i> | Interval width (\pm Da) | <i>m/z</i> | Interval width (\pm Da) |
|------------|----------------------------|------------|----------------------------|------------|----------------------------|
| 462.15 | 0.023 | 572.11 | 0.029 | 796.15 | 0.04 |
| 478.14 | 0.024 | 588.11 | 0.029 | 838.51 | 0.042 |
| 480.16 | 0.024 | 589.11 | 0.03 | 845.62 | 0.042 |
| 481.17 | 0.024 | 590.12 | 0.03 | 846.63 | 0.042 |
| 482.18 | 0.024 | 591.13 | 0.03 | 855.04 | 0.043 |
| 500.13 | 0.025 | 606.09 | 0.03 | 873.65 | 0.044 |
| 502.14 | 0.025 | 628.07 | 0.031 | 874.66 | 0.044 |
| 522.13 | 0.026 | 631.63 | 0.032 | 901.69 | 0.045 |
| 522.6 | 0.026 | 644.04 | 0.032 | 902.69 | 0.045 |
| 524.16 | 0.026 | 666.13 | 0.033 | 964.91 | 0.048 |
| 525.14 | 0.026 | 667.18 | 0.033 | 965.9 | 0.048 |
| 526.16 | 0.026 | 669.19 | 0.034 | 1425.1 | 0.071 |
| 544.11 | 0.027 | 669.21 | 0.034 | 1452.11 | 0.073 |
| 547.14 | 0.027 | 670.11 | 0.034 | 2002.54 | 0.1 |
| 550.12 | 0.028 | 689.17 | 0.035 | 2003.55 | 0.1 |
| 550.63 | 0.028 | 713.2 | 0.036 | 2691.47 | 0.135 |
| 551.13 | 0.028 | 733.14 | 0.037 | 2691.82 | 0.135 |
| 551.62 | 0.028 | 735.18 | 0.037 | 2691.99 | 0.135 |
| 568.12 | 0.028 | 777.14 | 0.039 | 3408.43 | 0.171 |
| 569.14 | 0.029 | 779.18 | 0.039 | 3582.63 | 0.18 |
| 570.15 | 0.029 | 795.14 | 0.04 | 3644.1 | 0.183 |

Table S2. Top 56 peptide ions discriminate CLM and CFI group. CLM - colorectal cancer liver metastasis; CFI - cancer free individuals and healthy participants.

| <i>m/z</i> | Interval width (±Da) | <i>m/z</i> | Interval width (±Da) | <i>m/z</i> | Interval width (±Da) | <i>m/z</i> | Interval width (±Da) |
|------------|----------------------|------------|----------------------|------------|----------------------|------------|----------------------|
| 453.9 | 4.860 | 524.16 | 0.014 | 1660.48 | 0.877 | 929.36 | 166.434 |
| 458.35 | 4.014 | 527.72 | 7.099 | 1661.47 | 0.929 | 1012.59 | 0.007 |
| 469.36 | 17.993 | 560.7 | 58.850 | 1661.99 | 0.059 | 1012.6 | 0.004 |
| 478.36 | 0.001 | 604.12 | 27.982 | 1671.95 | 19.857 | 1013.1 | 0.958 |
| 482.83 | 8.926 | 618.12 | 0.002 | 1681.91 | 0.007 | 1034.87 | 42.556 |
| 491.84 | 9.054 | 621.12 | 5.981 | 1681.92 | 0.052 | 1347.06 | 581.802 |
| 496.37 | 0.016 | 629.11 | 9.935 | 1715.44 | 66.992 | 1637.99 | 0.009 |
| 496.86 | 0.963 | 634.09 | 0.001 | 1749.47 | 0.960 | 1638 | 0.050 |
| 503.32 | 11.932 | 637.09 | 6.003 | 1750.01 | 0.050 | 1638.49 | 0.927 |
| 509.79 | 0.987 | 640.11 | 0.018 | 1760.48 | 20.921 | 1639.49 | 0.939 |
| 510.3 | 0.002 | 640.61 | 0.976 | 1770.98 | 0.083 | 1640 | 0.050 |
| 514.33 | 8.046 | 732.12 | 182.016 | 1815.02 | 87.991 | 1649.96 | 19.876 |
| 518.36 | 0.003 | 826.14 | 5.995 | 1879.54 | 41.036 | 1659.92 | 0.074 |
| 521.26 | 5.786 | 837.64 | 16.972 | 1900.56 | 0.977 | 1660 | 0.020 |

Table S3. Top 4 discriminative ions identified by NIMS-FT-ICR-MS were applied in the Mascot peptide mass fingerprint for protein matching. We identified the top-five potential protein signatures that can differentiate CRC and CFI group. CRC - colorectal cancer stage-I to stage-IV; CFI - cancer free individuals and healthy participants; NIMS-FT-ICR-MS - Nanostructure-initiator mass spectrometry - Fourier-transform ion cyclotron resonance mass spectrometry.

| Uniprot Accession | Mass | Score | Observed (<i>m/z</i>) | MASCOT Calculated and Mass Tolerance | Peptide Sequence Match (start to end) |
|-------------------|--|-------|-------------------------|--------------------------------------|---------------------------------------|
| E5RHQ2 | 3539 | 18 | 3408.4300 | 3407.5001 and -22.71 ppm | 2 to 33 |
| | <i>Sequence matched:</i> M.AGAGGGNDIQWCFSQVKGAVDDDVAEDHLFET.- <i>Identified protein:</i> Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform OS=Homo sapiens OX=9606 GN=PPP2R2A PE=1 SV=1 | | | | |
| D6RF68 | 2821 | 15 | 2691.4700 | 2690.4869 and -9 ppm | 2 to 23 |
| | <i>Sequence matched:</i> M.TSIFHFAIFMLILQIRIQLSE.- + Acetyl (Protein N-term); Oxidation (M) <i>Identified protein:</i> Toll-like receptor 1 (Fragment) OS=Homo sapiens OX=9606 GN=TLR1 PE=4 SV=1 | | | | |
| D6RBG6 | 5542 | 15 | 3582.6300 | 3581.6086 and 3.96 ppm | 1 to 30 |

| | | | | | |
|--------|--|----|-----------|--------------------------|-----------|
| | <i>Sequence matched:</i> -.MDLTGIESMDQCRHTLEQHNWNIEAAVQDR.L + Acetyl (Protein N-term) <i>Identified protein:</i> FAS-associated factor 2 (Fragment) OS=Homo sapiens OX=9606 GN=FAF2 PE=1 SV=1 | | | | |
| H3BR52 | 13975 | 13 | 3792.1300 | 3791.2038 and -21 ppm | 91 to 128 |
| | <i>Sequence matched:</i> R.AVLHLLLSVPAGFLLATILGTACLAIASGIYLLVSGAL.- <i>Identified protein:</i> Cytochrome b(558) alpha chain OS=Homo sapiens OX=9606 GN=CYBA PE=1 SV=1 | | | | |

Table S4. Top 4 discriminative ions identified by NIMS-FT-ICR-MS were applied in the Mascot peptide mass fingerprint for the protein match. We identified top-five potential protein signatures that can differentiate CLM and CFI group. CLM - colorectal cancer liver metastasis; CFI - cancer free individuals and healthy participants; NIMS-FT-ICR-MS - Nanostructure-initiator mass spectrometry - Fourier-transform ion cyclotron resonance mass spectrometry.

| Uniprot Accession | Mass | Score | Observed (m/z) | MASCOT Calculated and mass tolerance | Peptide Sequence Match (start to end) |
|-------------------|---|-------|----------------|--------------------------------------|---------------------------------------|
| F2Z356 | 15548 | 19 | (a) 3822.0500 | 1769.9709 and 2.50 ppm | 85 to 119 |
| | | | (b) 1770.9800 | 1769.9709 and 1.01 ppm | 107 to 123 |
| | Sequence matched (a): R.SIAVLELICDVHNPGQDLVIHRTSVPAPLNSCLLK.V Sequence matched (b): R.TSVPAPLNSCLLKVGSK.T Identified protein: General transcription factor 3C polypeptide 4 OS=Homo sapiens OX=9606 GN=GTF3C4 PE=1 SV=1 | | | | |
| | | | | | |
| X6RKG5 | 11905 | 16 | (a) 1671.9500 | 1670.9063 and 21.8 ppm | 49 to 64 |
| | | | (b) 2391.2900 | 2390.2827 and 7.80 ppm | 87 to 106 |
| | Sequence matched (a): R.ARLQGSATAAEASLLR.R Sequence matched (b): R.SSRALRPASMDLLRPHWLEV.- Identified protein: Lck-interacting transmembrane adapter 1 (Fragment) OS=Homo sapiens OX=9606 GN=LIME1 PE=4 SV=7 | | | | |
| | | | | | |
| S4R3D0 | 7403 | 15 | 3822.0500 | 3821.0801 and -9.78 ppm | 2 to 37 |
| | Sequence matched: M.ESSGTPSVTLIVGSGLSCLALITLAVVYAALWRYIR.S + Acetyl (Protein N-term) Identified protein: Adhesion G protein-coupled receptor B3 (Fragment) OS=Homo sapiens OX=9606 GN=ADGRB3 PE=4 SV=1 | | | | |
| F8WCL0 | 6018 | 14 | 3822.0500 | 3821.0067 and 9.44 ppm | 2 to 37 |
| | Sequence matched: M.MPTPVILLKEGTDSSQGIPQLVSNISACQVIAEAVR.T Identified protein: T-complex protein 1 subunit eta OS=Homo sapiens OX=9606 GN=CCT7 PE=1 SV=1 | | | | |

Table S5. Top discriminative ions were identified by DIOS-FT-ICR-MS, which was applied in the LipidMaps database for annotation. We identified a profile of lithiated-lipids that can differentiate CRC from the CFI group. CRC - colorectal cancer stage-I to stage-IV; CFI - cancer free individuals and healthy participants; DIOS-FT-ICR-MS - desorption/ionisation on porous silicon fourier-transform ion cyclotron resonance mass spectrometry. LPE – lysophosphatidylethanolamine, Cer – Ceramide, NAE - N-acylethanolamines, CerP - Ceramide 1-phosphates, LPS – lysophosphatidylserine, PC – phosphatidylcholine, PS – phosphatidylserine, LPC – lysophosphatidylcholine, SM – sphingomyelin, PE – phosphatidylethanolamine, DG – diacylglycerol, PG – phosphatidylglycerol, LPG – lysophosphatidylglycerol, LPI –lysophosphatidylionisitol, TG- triacylglycerol.

| Input Mass | Matched Mass in Lipid Maps | Delta (Da) | Putative Lipids | Formula | Ion |
|------------|----------------------------|------------|-----------------|---------------|---------|
| 496.340 | 496.337 | 0.0026 | LPE O-20:3 | C25H48NO6PLi | [M+Li]+ |
| 526.517 | 526.517 | 0 | Cer 34:2;O | C34H65NO2Li | [M+Li]+ |
| 526.517 | 526.517 | 0 | NAE 32:2 | C34H65NO2Li | [M+Li]+ |
| 636.627 | 636.626 | 0.0005 | Cer 42:3;O | C42H79NO2Li | [M+Li]+ |
| 640.416 | 640.416 | 0 | CerP 32:3;O5 | C32H60NO9PLi | [M+Li]+ |
| 640.416 | 640.416 | 0 | LPS 26:2 | C32H60NO9PLi | [M+Li]+ |
| 640.416 | 640.416 | 0 | LPS O-26:3;O | C32H60NO9PLi | [M+Li]+ |
| 640.416 | 640.416 | 0 | PC 24:2;O | C32H60NO9PLi | [M+Li]+ |
| 640.416 | 640.416 | 0 | PS O-26:2 | C32H60NO9PLi | [M+Li]+ |
| 650.510 | 650.509 | 0.0005 | CerP 36:2;O2 | C36H70NO6PLi | [M+Li]+ |
| 650.510 | 650.509 | 0.0005 | LPC O-28:3 | C36H70NO6PLi | [M+Li]+ |
| 678.540 | 678.540 | 0.0008 | CerP 38:2;O2 | C38H74NO6PLi | [M+Li]+ |
| 678.540 | 678.540 | 0.0008 | LPC O-30:3 | C38H74NO6PLi | [M+Li]+ |
| 735.595 | 735.598 | 0.0037 | SM 36:2;O2 | C41H81N2O6PLi | [M+Li]+ |
| 737.614 | 737.614 | 0.0003 | SM 36:1;O2 | C41H83N2O6PLi | [M+Li]+ |
| 758.570 | 758.567 | 0.003 | PE O-38:5 | C43H78NO7PLi | [M+Li]+ |
| 758.603 | 758.603 | 0.0004 | CerP 44:4;O2 | C44H82NO6PLi | [M+Li]+ |
| 759.574 | 759.574 | 0.0006 | DG 44:8;O2 | C47H76O7Li | [M+Li]+ |
| 759.574 | 759.572 | 0.0018 | LPG 34:0;O | C40H81O10PLi | [M+Li]+ |
| 759.574 | 759.572 | 0.0018 | PG O-34:0;O | C40H81O10PLi | [M+Li]+ |
| 759.574 | 759.574 | 0.0006 | TG 44:7;O | C47H76O7Li | [M+Li]+ |
| 759.574 | 759.574 | 0.0006 | TG O-44:8;O2 | C47H76O7Li | [M+Li]+ |
| 760.619 | 760.619 | 0.0001 | CerP 44:3;O2 | C44H84NO6PLi | [M+Li]+ |
| 763.634 | 763.630 | 0.004 | SM 38:2;O2 | C43H85N2O6PLi | [M+Li]+ |
| 765.645 | 765.645 | 0.0006 | SM 38:1;O2 | C43H87N2O6PLi | [M+Li]+ |
| 781.536 | 781.535 | 0.0006 | PA 42:7 | C45H75O8PLi | [M+Li]+ |
| 781.536 | 781.535 | 0.0006 | PA O-42:8;O | C45H75O8PLi | [M+Li]+ |
| 782.571 | 782.567 | 0.004 | PE O-40:7 | C45H78NO7PLi | [M+Li]+ |
| 786.602 | 786.598 | 0.0037 | PE O-40:5 | C45H82NO7PLi | [M+Li]+ |
| 788.578 | 788.577 | 0.0004 | CerP 44:5;O4 | C44H80NO8PLi | [M+Li]+ |
| 788.578 | 788.577 | 0.0004 | PC 36:4 | C44H80NO8PLi | [M+Li]+ |
| 788.578 | 788.577 | 0.0004 | PC O-36:5;O | C44H80NO8PLi | [M+Li]+ |
| 789.580 | 789.582 | 0.0028 | LPI O-32:0 | C41H83O11PLi | [M+Li]+ |
| 802.592 | 802.593 | 0.0013 | PE 40:4 | C45H82NO8PLi | [M+Li]+ |

| | | | | | |
|---------|---------|--------|--------------|---------------|---------|
| 802.592 | 802.593 | 0.0013 | PE O-40:5;O | C45H82NO8PLi | [M+Li]+ |
| 812.578 | 812.577 | 0.0004 | PC 38:6 | C46H80NO8PLi | [M+Li]+ |
| 812.578 | 812.577 | 0.0004 | PC O-38:7;O | C46H80NO8PLi | [M+Li]+ |
| 816.609 | 816.608 | 0.0001 | CerP 46:5;O4 | C46H84NO8PLi | [M+Li]+ |
| 816.609 | 816.608 | 0.0001 | PC 38:4 | C46H84NO8PLi | [M+Li]+ |
| 816.609 | 816.608 | 0.0001 | PC O-38:5;O | C46H84NO8PLi | [M+Li]+ |
| 817.613 | 817.614 | 0.0011 | LPI O-34:0 | C43H87O11PLi | [M+Li]+ |
| 817.677 | 817.676 | 0.0001 | SM 42:3;O2 | C47H91N2O6PLi | [M+Li]+ |
| 819.693 | 819.692 | 0.0004 | SM 42:2;O2 | C47H93N2O6PLi | [M+Li]+ |
| 838.593 | 838.593 | 0.0003 | PC 40:7 | C48H82NO8PLi | [M+Li]+ |
| 838.593 | 838.593 | 0.0003 | PC O-40:8;O | C48H82NO8PLi | [M+Li]+ |
| 840.608 | 840.608 | 0.0009 | PC 40:6 | C48H84NO8PLi | [M+Li]+ |
| 840.608 | 840.608 | 0.0009 | PC O-40:7;O | C48H84NO8PLi | [M+Li]+ |

Table S6. DIOS-FT-ICR-MS identified top discriminative ions, annotated using LipidMaps. We identified a profile of lithiated-lipids that can differentiate CLM and CFI group. CLM - colorectal cancer liver metastasis; CFI - cancer free individual and healthy participants; DIOS-FT-ICR-MS - desorption/ionisation on porous silicon fourier-transform ion cyclotron resonance mass spectrometry; PS – phosphatidylserine, PG – phosphatidylglycerol, LPG – lysophosphatidylglycerol , PI – phosphatidylinositol, TG- triacylglycerol, CL – cholesterol, PA - phosphatidicacid

| Input Mass | Matched Mass in Lipid Maps | Delta (Da) | Putative Lipids | Formula | Ion |
|------------|----------------------------|------------|-----------------|----------------|---------|
| 639.377 | 639.384 | 0.0074 | LPG 26:4;O | C32H57O10PLi | [M+Li]+ |
| 639.377 | 639.384 | 0.0074 | PG 26:3 | C32H57O10PLi | [M+Li]+ |
| 639.377 | 639.384 | 0.0074 | PG O-26:4;O | C32H57O10PLi | [M+Li]+ |
| 711.435 | 711.441 | 0.0069 | PG 30:3;O | C36H65O11PLi | [M+Li]+ |
| 711.435 | 711.444 | 0.0093 | TG 40:11;O2 | C43H60O8Li | [M+Li]+ |
| 753.445 | 753.454 | 0.0098 | TG 42:12;O3 | C45H62O9Li | [M+Li]+ |
| 771.456 | 771.457 | 0.0012 | PA 42:12 | C45H65O8PLi | [M+Li]+ |
| 843.513 | 843.514 | 0.0017 | PA 46:12;O | C49H73O9PLi | [M+Li]+ |
| 932.571 | 932.562 | 0.0086 | PS 46:11;O | C52H80NO11PLi | [M+Li]+ |
| 991.593 | 991.588 | 0.0048 | PI 46:11 | C55H85O13PLi | [M+Li]+ |
| 991.593 | 991.588 | 0.0048 | PI O-46:12;O | C55H85O13PLi | [M+Li]+ |
| 1017.600 | 1017.604 | 0.0039 | PI 48:12 | C57H87O13PLi | [M+Li]+ |
| 1035.610 | 1035.614 | 0.0044 | PI 48:11;O | C57H89O14PLi | [M+Li]+ |
| 1387.832 | 1387.831 | 0.0012 | CL 68:14 | C77H122O17P2Li | [M+Li]+ |
| 1415.861 | 1415.863 | 0.0025 | CL 70:14 | C79H126O17P2Li | [M+Li]+ |