Supplementary data

Mass spectrometry imaging-based metabolomics to visualize the spatially resolved reprogramming of carnitine metabolism in breast cancer

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Analyte identification

The MS/MS experiment was carried out by performing LC-MS/MS analysis on Q-Qorbitrap mass spectrometer (Q Exactive, Thermo Scientific Bremen, Germany). Breast cancer tissue was weighed at approximately 50 mg and homogenated in 0.5 mL normal saline. Then 1000 μL acetonitrile was added to 200 μL homogenate and vortexed for 3 minutes. After centrifugation at 10000 rpm for 5 min, the supernatant was transferred to a sample vial for LC-MS/MS analysis. The ions of interest were listed as the targets, with the NCE value set at 25%, 35%, and 45% in targeted-MS2 scan mode. The resolving power was set at 17500 for MS/MS acquisition with the AGC value at 3E6 and maximum injection time at 200 ms. Representative results were shown in Figure S14 ~ Figure S23.
Segmentation analysis

The segmentation procedure including: (i) construct MS image of a certain tissue section; (ii) running the Tool “Find Peaks”, select work on all individual spectra; (iii) moving the results by running “Move Peaks to Local Max”; (iv) using the modified result to perform segmentation with bisecting k-means, weak denoising, and Correlation Distance; (v) visualizing the Segmentation Map in “Labels” window. We have added this procedure in the revised manuscript.

Figure S1. MALDI-MS spectra of matrix, cancer tissue, and normal tissue in breast cancer tissue section.
Figure S2. The PCA score plot based on the *in situ* MALDI-MS spectra of breast cancer regions and adjacent normal regions.

Figure S3. A) Calculated intensities of four carnitines in breast cancer regions and adjacent normal region; B) Correlation analysis of four carnitines in all pixels of the breast cancer tissue section. (The statistical data comes from all pixels of sample No. 535905); ***, $p < 0.001$. 

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Figure S4. MS spectra of acylcarnitine C6:0 and acylcarnitine C7:0 in breast cancer tissue section.

Figure S5. MS images of representative carnitines and other low-molecular-weight metabolites (LMWMs) in untreated and 70% - 95% ethanol-washed breast cancer tissue sections.
Figure S6. MS images of representative carnitines in untreated, 50% ethanol-, 70% ethanol-, 90% ethanol-, and 100% ethanol-washed breast cancer tissue sections.
Figure S7. MS images and statistical data of representative carnitines in untreated and 100% ethanol-washed breast cancer tissue sections.
Figure S8. MS images of acylcarnitine C7:0 and acylcarnitine C8:0 in untreated and 100% ethanol-washed breast cancer tissue sections.

Figure S9. MS images of 17 detected carnitines in 100% ethanol-washed breast cancer tissue sections.
Figure S10. The PCA score plots based on the *in situ* MALDI-MS spectra of cancer regions, stroma regions, and adipose regions (The statistical data comes from all pixels of mouse breast cancer tissues No. R001).

Figure S11. Statistical data of eight representative acylcarnitines in human breast cancer tissues. The scale of y-axis is log 2; ***, *p* < 0.001.
Figure S12. The content of acylcarnitine C4:0 are not completely consistent in the same cancer region.

Figure S13. The PCA score plot based on the metabolite profiles of six segmentation-derived regions. The statistical data comes from all pixels of sample No. 535437.
Figure S14. The MS/MS spectrum of L-carnitine.

Figure S15. The MS/MS spectrum of acetylcarnitine.
Figure S16. The MS/MS spectrum of acylcarnitine C3:0.

Figure S17. The MS/MS spectrum of acylcarnitine C4:0.
**Figure S18.** The MS/MS spectrum of acylcarnitine C6:0.

**Figure S19.** The MS/MS spectrum of acylcarnitine C16:0.
Figure S20. The MS/MS spectrum of acylcarnitine C16:1.

Figure S21. The MS/MS spectrum of acylcarnitine C18:0.
Figure S22. The MS/MS spectrum of acylcarnitine C18:1.

Figure S23. The MS/MS spectrum of acylcarnitine C18:2.
Table S1. Demographic and characteristics of 58 breast cancer patients.

| No.  | Age (year) | Lesion      | Tumor grade | Metastasis | Previous treatment       |
|------|------------|-------------|-------------|------------|--------------------------|
| 535431 | 56         | Left breast | T3          | No Metastasis | No                       |
| 535437 | 44         | Left breast | T2          | No Metastasis | No                       |
| 535425 | 53         | Left breast | T2          | No Metastasis | No                       |
| 535466 | 42         | Left breast | T3          | No Metastasis | No                       |
| 535512 | 49         | Left breast | T2          | No Metastasis | No                       |
| 535673 | 69         | Right breast | T3         | No Metastasis | No                       |
| 535905 | 40         | Right breast | T3         | No Metastasis | No                       |
| 535930 | 53         | Right breast | T2         | No Metastasis | No                       |
| 536171 | 48         | Right breast | T3         | No Metastasis | No                       |
| 536190 | 44         | Left breast | T1          | No Metastasis | No                       |
| 536208 | 51         | Left breast | T2          | No Metastasis | No                       |
| 536286 | 48         | Left breast | T2          | No Metastasis | No                       |
| 536348 | 47         | Left breast | T3          | No Metastasis | No                       |
| 536360 | 39         | Right breast | T3         | No Metastasis | Preoperative chemotherapy |
| 536262 | 46         | Left breast | T2          | No Metastasis | No                       |
| 536503 | 63         | Left breast | T3          | Metastasis   | No                       |
| 536522 | 65         | Right breast | T3         | No Metastasis | No                       |
| 536613 | 58         | Right breast | T1         | No Metastasis | No                       |
| 536645 | 66         | Left breast | T2          | No Metastasis | No                       |
| 536704 | 49         | Left breast | T2          | No Metastasis | No                       |
| 536719 | 59         | Left breast | T2          | No Metastasis | No                       |
| 536746 | 65         | Left breast | T3          | No Metastasis | No                       |
| 536916 | 69         | Right breast | T3         | No Metastasis | No                       |
| 537144 | 43         | Right breast | T2         | No Metastasis | No                       |
| 537187 | 49         | Right breast | T3         | Metastasis   | No                       |
| 537234 | 62         | Left breast | T1          | No Metastasis | No                       |
| 537266 | 59         | Right breast | T3         | No Metastasis | No                       |
| 537309 | 68         | Left breast | T2          | No Metastasis | No                       |
| 537355 | 62         | Left breast | T2          | No Metastasis | No                       |
| 537411 | 59         | Right breast | T1         | No Metastasis | No                       |
| 537435 | 67         | Right breast | T3         | No Metastasis | Preoperative chemotherapy |
| 537524 | 70         | Right breast | T2         | No Metastasis | No                       |
| 537533 | 59         | Left breast | T2          | No Metastasis | No                       |
| 537603 | 65         | Left breast | T2          | No Metastasis | No                       |
| 537629 | 61         | Left breast | T3          | No Metastasis | No                       |
| 537671 | 69         | Right breast | T2         | No Metastasis | No                       |
| 537739 | 40         | Right breast | T3         | No Metastasis | No                       |
| 537754 | 57         | Left breast | T1          | No Metastasis | No                       |
| 537807 | 49         | Right breast | T3         | No Metastasis | No                       |
| 537829 | 67         | Left breast | T3          | No Metastasis | Preoperative chemotherapy |
| 537857 | 59         | Left breast | T2          | No Metastasis | No                       |
| 537902 | 71         | Left breast | T2          | No Metastasis | No                       |
| 537933 | 64         | Right breast | T3         | No Metastasis | No                       |
| 537965 | 48         | Left breast | T2          | No Metastasis | No                       |
| 538008 | 64         | Left breast | T2          | No Metastasis | No                       |
| 538025 | 62         | Right breast | T3         | Metastasis   | No                       |
| 538054 | 52         | Left breast | T2          | No Metastasis | No                       |
| 538107 | 64         | Right breast | T2         | No Metastasis | No                       |
| 538144 | 68         | Right breast | T3         | No Metastasis | No                       |
| 538163 | 63         | Left breast | T2          | No Metastasis | No                       |
| 548305 | 47         | Right breast | T2         | No Metastasis | No                       |
| 548337 | 53         | Left breast | T3          | No Metastasis | Preoperative chemotherapy |
| ID    | Age | Side     | Stage | Metastasis | Result |
|-------|-----|----------|-------|------------|--------|
| 548412| 69  | Left     | T2    | No Metastasis | No     |
| 548476| 63  | Left     | T3    | No Metastasis | No     |
| 548508| 56  | Right    | T1    | No Metastasis | No     |
| 548559| 52  | Left     | T2    | No Metastasis | No     |
| 548602| 68  | Left     | T3    | No Metastasis | No     |
| 548635| 62  | Left     | T3    | No Metastasis | No     |