Supplementary Information

Metabolomic Studies of Live Single Cancer Stem Cells

Using Mass Spectrometry

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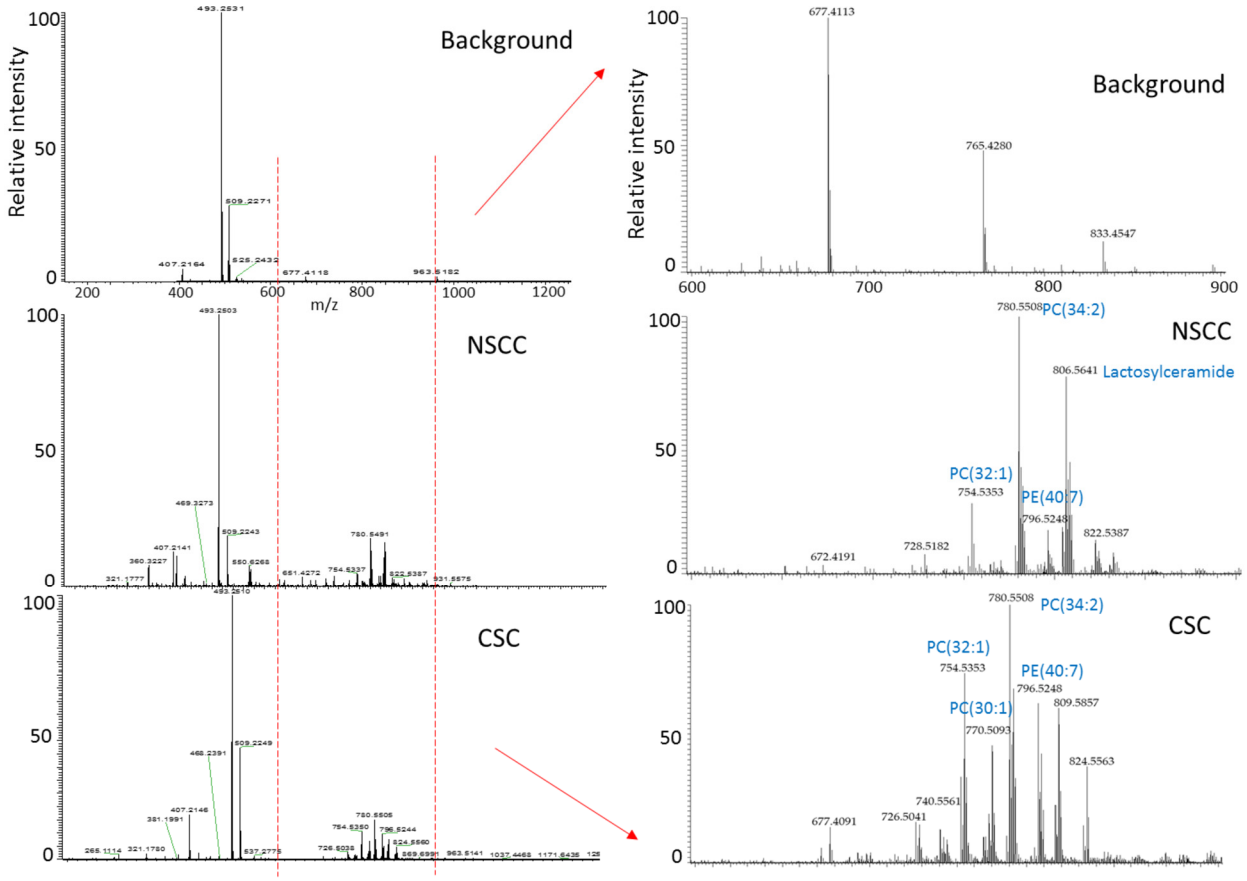

Figure S1. Mass spectra (positive ion mode) of (A) background, (B) a NSCC, and (C) a CSC obtained using the Single-probe SCMS technique. Figures on the right panel illustrate the zoomed-in regions of m/z 600 – 900.

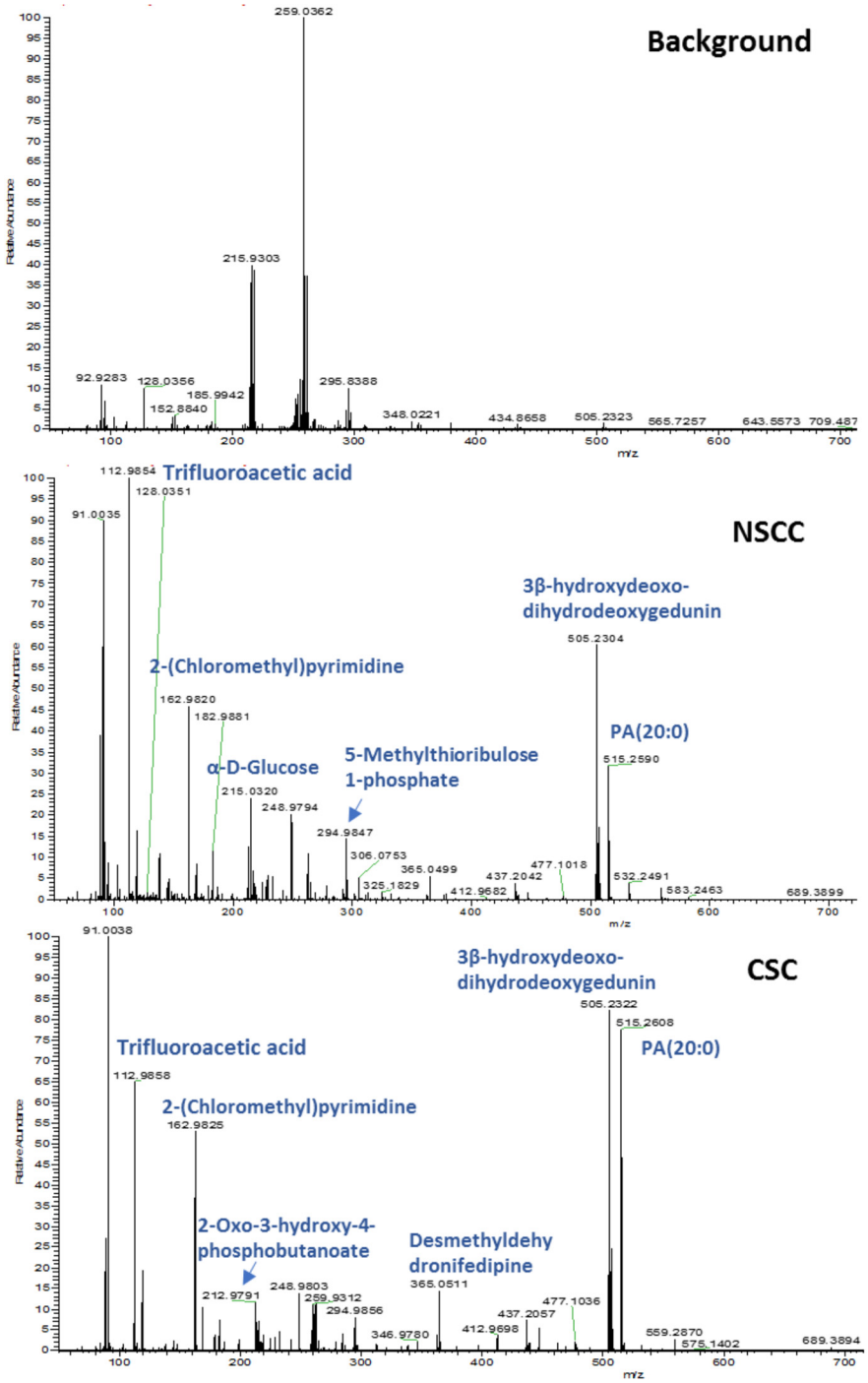

Figure S2. Mass spectra (negative ion mode) of (A) background, (B) a NSCC, and (C) a CSC obtained using the Single-probe SCMS technique.

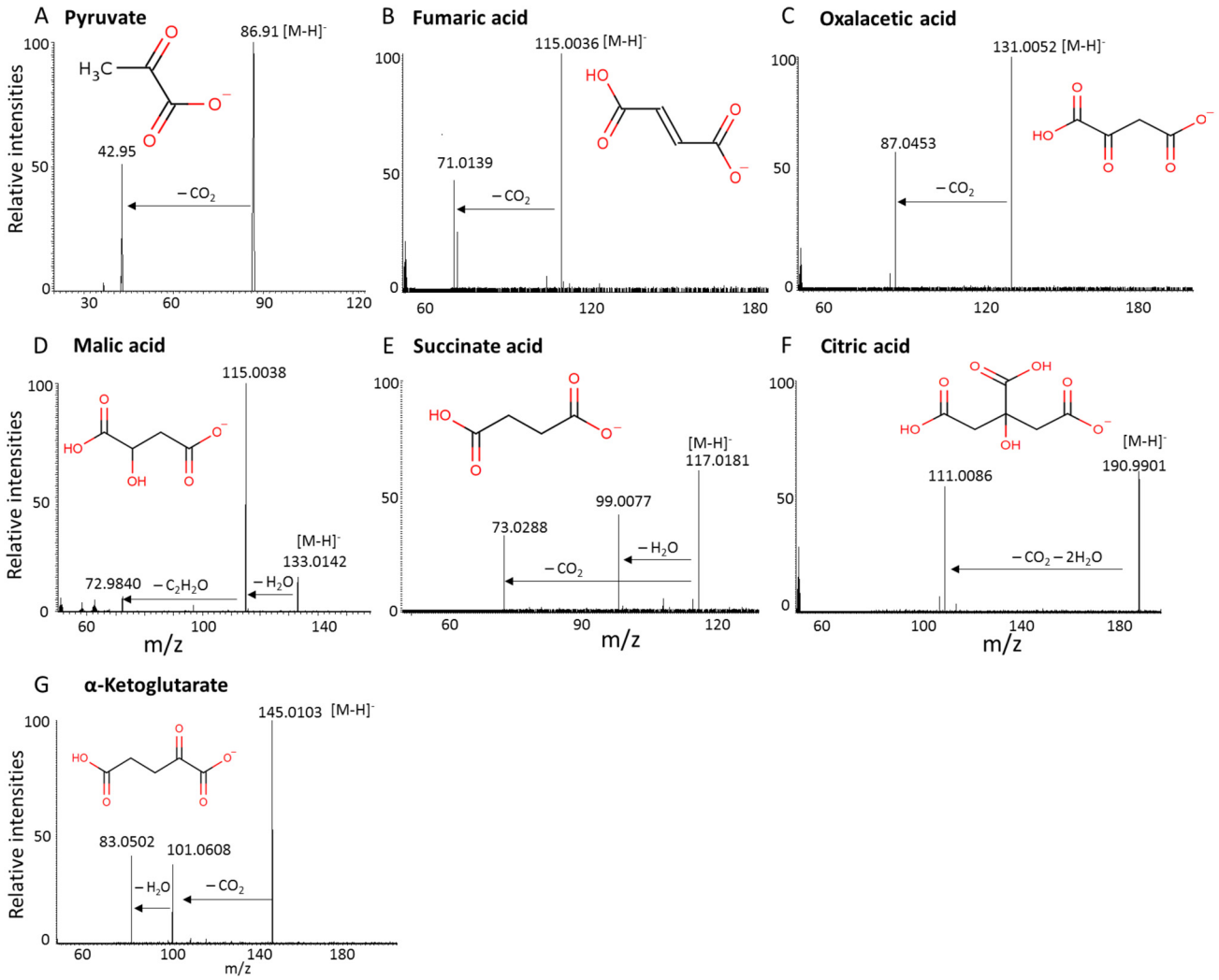

Figure S3. Identification of seven metabolites in the TCA cycle from MS^2 fragmentation (negative ion mode). (A) Pyruvate [pyruvate - $H]^-$ (m/z 86.91), (B) Fumaric acid [fumaric acid - $H]^-$ (m/z 115.0036), (C) Oxoglutaric acid [oxoglutaric acid - $H]^-$ (m/z 131.0052), (D) Malic acid [malic acid - $H]^-$ (m/z 133.0142), (E) Succinate acid [succinate acid - $H]^-$ (m/z 117.0181), (F) Citric acid [citric acid - $H]^-$ (m/z 190.9901), and (G) α-Ketoglutarate [α-ketoglutarate - $H]^-$ (m/z 145.0103). All MS^2 identification were confirmed through the comparison with online databases (METLIN and HMDB).

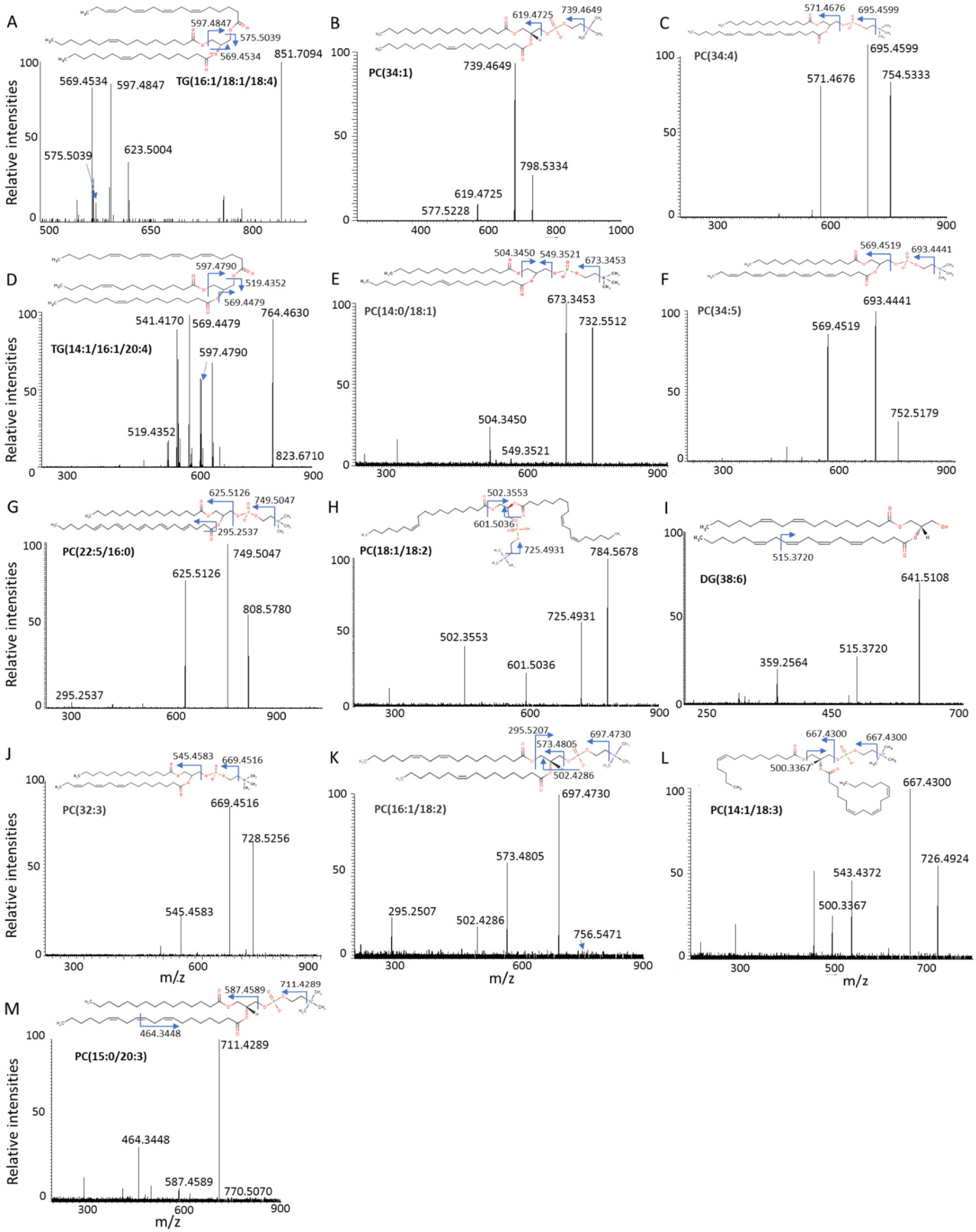

Figure S4. Identification of 13 unsaturated lipids from MS^2 fragmentation (positive ion mode). (A) TG (16:1/18:1/18:4) [TG(16:1/18:1/18:4) + $H]^+$ (m/z 851.7094), (B) PC(34:1) [PC(34:1) + $K]^+$ (m/z

798.5334), (C) PC(34:4), [PC(34:4) + H]$^+$ (m/z 754.5333), (D) TG(14:1/16:1/20:4) [TG(14:1/16:1/20:4) + H]$^+$ (m/z 823.6710), (E) PC(14:0/18:1) [PC(14:0/18:1) + H]$^+$ (m/z 732.5512), (F) PC(34:5) [PC(34:5) + H]$^+$ (m/z 752.5179), (G) PC(16:0/22:5) [PC(16:0/22:5) + H]$^+$ (m/z 808.5780), (H) PC(18:1/18:2) [PC(18:1/18:2) + H]$^+$ (m/z 784.5678), (I) DG(38:6) [DG(38:6)+H]$^+$ (m/z 641.5108), (J) PC(32:3) [PC(32:3) + H]$^+$ (m/z 728.5256) (K) PC(16:1/18:2) [PC(16:1/18:2) + H]$^+$ (m/z 756.5471), (L) PC(14:1/18:3) [PC(14:1/18:3) + H]$^+$ (m/z 726.4924), and (M) PC(15:0/20:3) [PC(15:0/20:3) + H]$^+$ (m/z 770.5070). All MS^2 identifications were confirmed through the comparison with online databases (METLIN and HMDB).

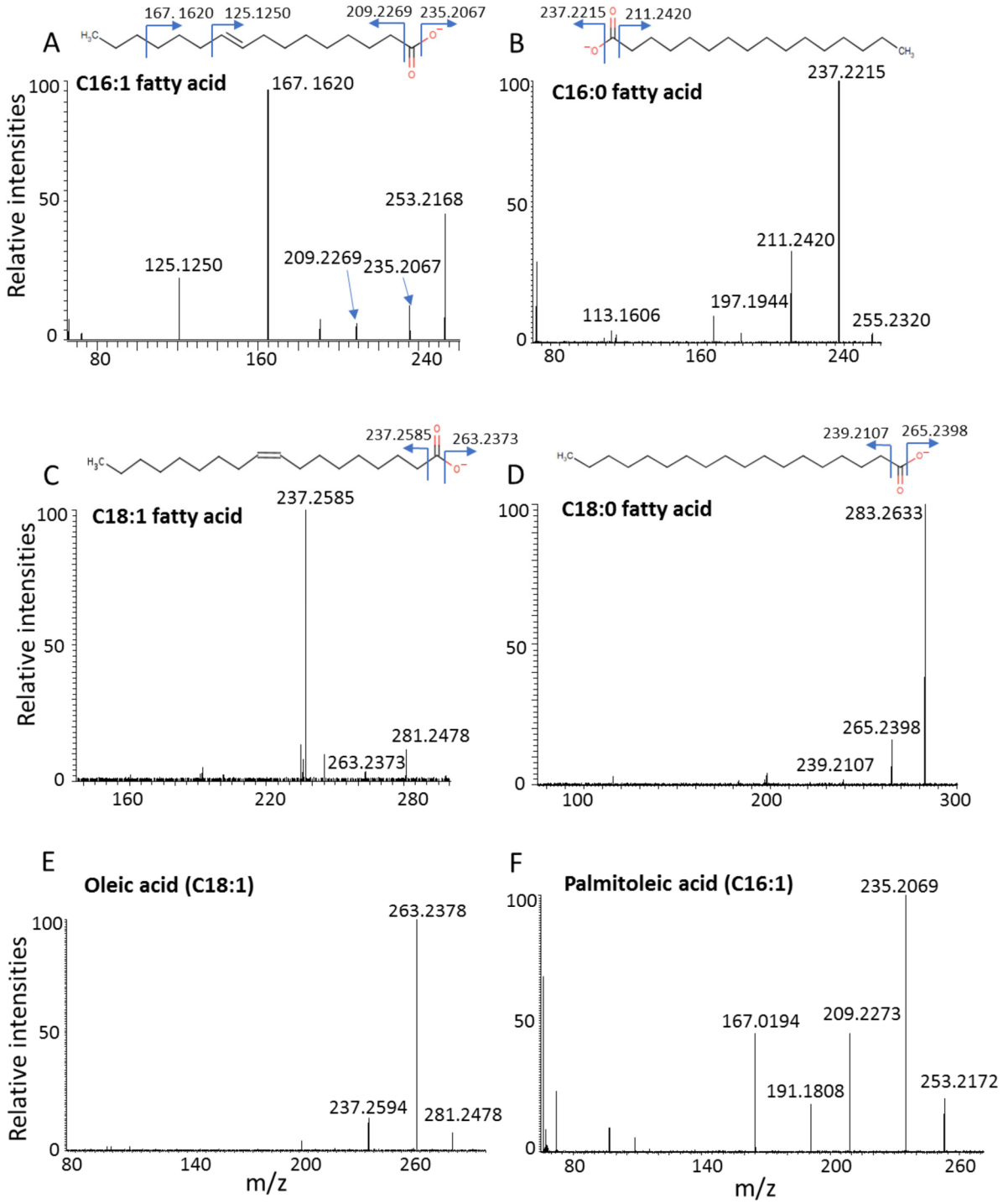

Figure S5. Identification of fatty acids from MS^2 fragmentation (negative ion mode). Single cell level identification of (A) Palmitoleic acid (C16:1) $[C16:1 - H]^-$ (m/z 253.2168), (B) Palmitic acid (C16:0) $[C16:0 - H]^-$ (m/z 255.2320), (C) Oleic acid (C18:1) $[C18:1 - H]^-$ (m/z 281.2478), (D) Stearic acid (C18:0) $[C18:0 - H]^-$ (m/z 283.2633) were. Further confirmation of (E) oleic acid $[C18:1 - H]^-$ (m/z 281.2478) and (F) palmitoleic acid ($[C16:0 - H]^-$ (m/z 253.2172) were carried out using the standard compounds. All MS^2 identifications were confirmed through the comparison with online databases (METLIN and HMDB) and reported studies[1].

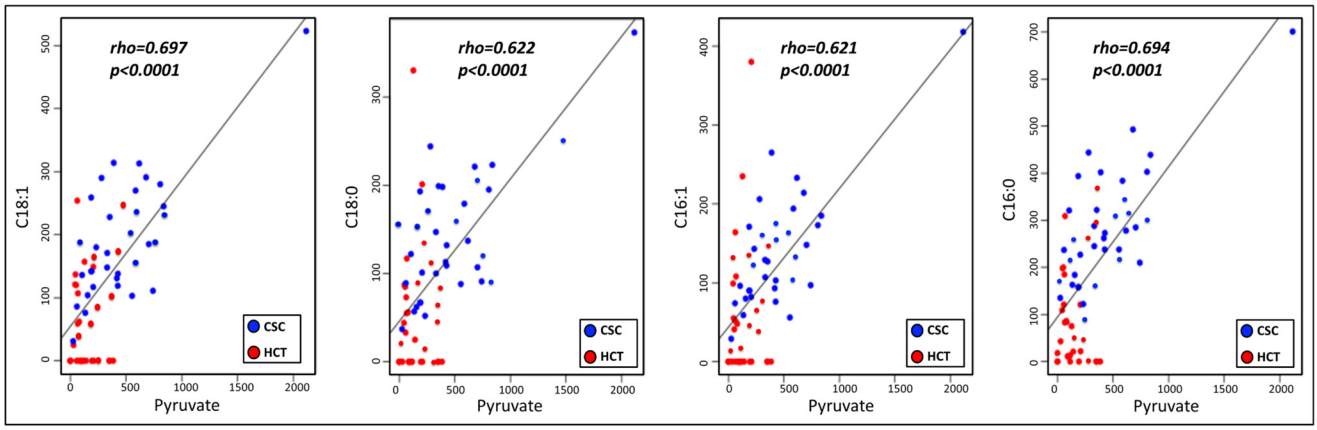

Figure S6. Correlation between the pyruvate and fatty acids in the CSCs and NSCCs. Pyruvate has good positive correlation with (A) C18:1, (B) C18:0, (C) C16:1, and (D) C16:0. (*roh*: correlation coefficient).

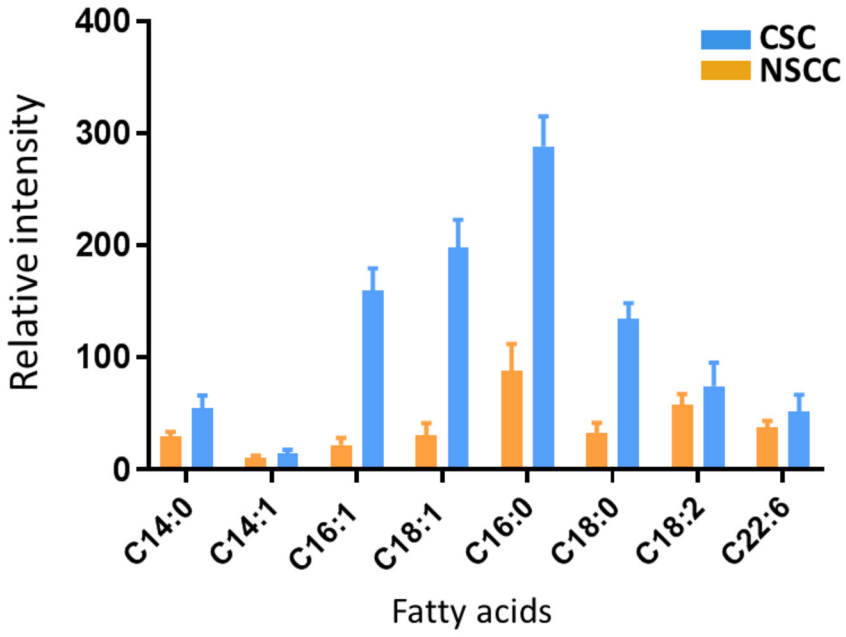

Figure S7. Relative abundances of fatty acids in CSCs and NSCCs.

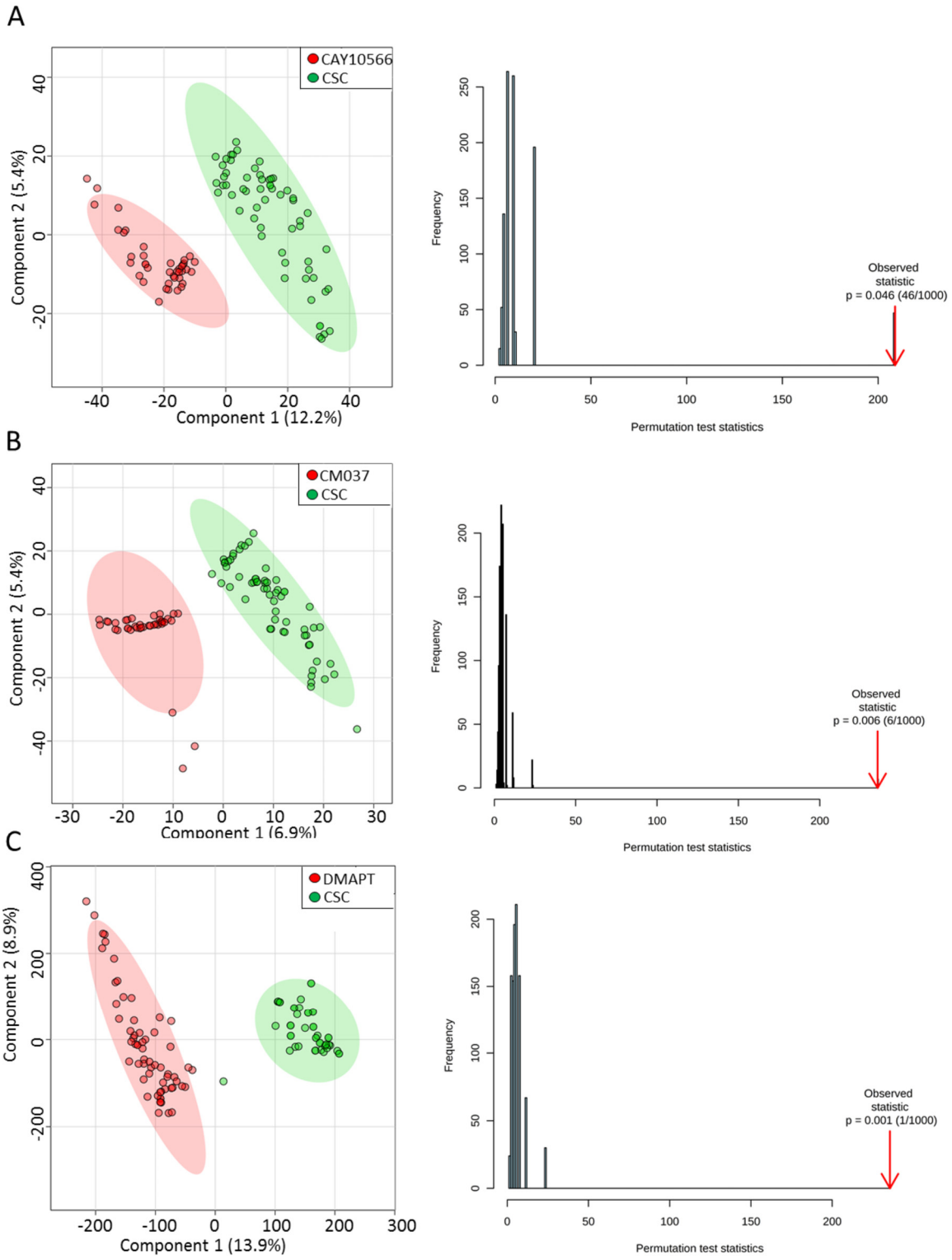

Figure S8. Results from Partial Least Squares Discriminant Analysis (PLS-DA) of SCMS data illustrate the overall difference of metabolites between CSCs and inhibitors with the permutation test (A) CAY10566, (B) CM037, and (C) DMAPT.

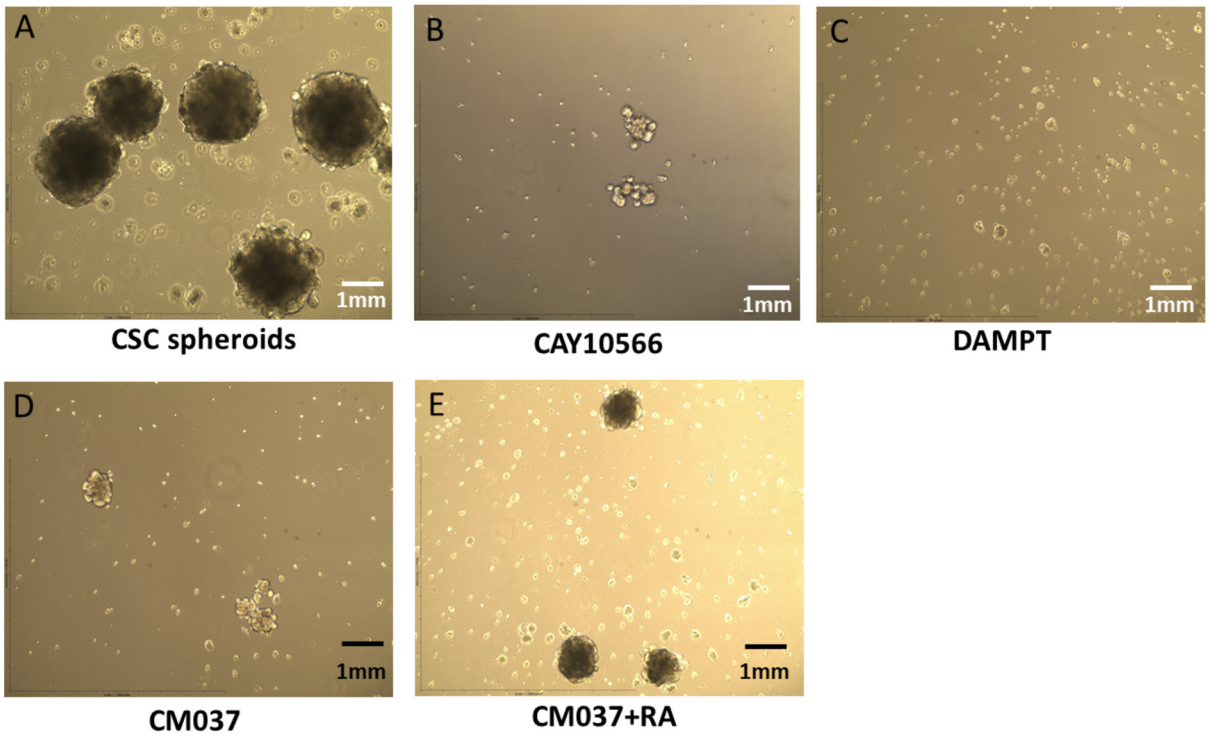

Figure S9. Inhibitors prevent the formation of CSC spheroids. (A) CSCs form spheroids in vitro after seven days of culture. Treatment using inhibitors of (B) SCD1 (CAY10566), (C) NF-κB (DMART), and (D) ALDH1A1 (CM037) suppresses the formation of spheroids. (E) Retinoic acid alleviates the inhibition of CM037 on ALDH1A1.

Reference:

(1) Zhou, Y.; Wu, Z.; Li, C.; Wang, N.; Zhang, X.; Chen, H.; Xiao, S. *Anal Methods.* **2014**, *6*, 1538-1544.