Supporting Information

Development of methionine methylation profiling and relative quantification in human breast cancer cells based on stable isotope metabolic labeling

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CONTENTS

Methods

Chemicals and Reagents. The metabolite standards including L-methionine, $^{13}C,d_3$-Met-L-methionine, creatine, 5-methylthioadenosine, 1-methyl-nicotinamide, monomethyl-L-arginine acetate, dimethylarginine, adenine, guanine, cytosine, taurine, arginine, dimethyl glycine, lysine, histidine, serine, thymine, cystathionine and N',N',N'-trimethyl-L-lysine chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA) and S-adenosyl methionine and S-adenosyl homocysteine were purchased from Ark Pharm (Chicago, US). All standards were stored at 4 ℃ or -20 ℃ before use according to their instructions.

The parameters for peak finding, filtering, alignment, scaling and identification using R software. (based on data of cell samples using LC-ESI (+)-MS, for example)

```
rm(list=ls(all=TRUE))
library(Biobase)
library(xcms)
library(multtest)
library(CAMERA)
sessionInfo()
xs<-xcmsSet(profmethod = "binlin",method="centWave",ppm = 2.5, peakwidth=c(5,15),snthresh
=30, prefilter=c(5,5000),integrate=1, mzdiff =0.02)
xs <-group(xs,bw=2,minfrac=0.8,mzwid=0.015)
save(xs,file="xs.Rda")
ret.xs.obiwarp <-retcor(xs,method="obiwarp",plottype="deviation")
ret.xs.obiwarp<-group(ret.xs.obiwarp, bw = 5,minfrac=0.8,mzwid=0.015)
ret.xs.obiwarp
fill.ret.xs.obiwarp<-fillPeaks(ret.xs.obiwarp)
fill.ret.xs.obiwarp
save(fill.ret.xs.obiwarp, file="fill.ret.xs.obiwarp.Rda")
an.1<annotate(fill.ret.xs.obiwarp,sigma=6,perfwhm=0.3,cor_eic_th=0.75,maxcharge=3,maxiso=3,m
zabs=0.03,multiplier=3,polarity="positive")
peaklist.1<-getPeaklist(an.1)
```

```
write.csv(peaklist.1,file='annotated.1.csv')
```

The parameters for possible $^{13}CD_3$-labeled compounds peak search using Python software (based on data of cell samples using LC-ESI(+)-MS, for example)

```
import pandas as pd
import numpy as np
PPM = 1e-6
def find_isotope(a,dif=4.015,rthreshold=10*PPM,rt_threshold=.05,abs_threshold=.1):
    abs_err = abs(abs(a['M/Z'].values-a['M/Z'].values[:,np.newaxis])-dif)
    mask_rt = abs(a['RT'].values-a['RT'].values[:,np.newaxis])<rt_threshold
    np.fill_diagonal(mask_rt,False)
    if rthreshold is not None:
        mask_mz = abs_err<a['M/Z'].values*rthreshold
        mask_ = mask_mz&mask_rt
        mask = np.any(mask_,axis=1)
    else:
        mask_mz = abs_err<abs_threshold
        mask_ = mask_mz&mask_rt
        mask = np.any(mask_,axis=1)
    return a.loc[mask]
def find_isotope(input_,output_,dif=4.015,rthreshold=20*PPM,rt_threshold=.05):
input_ ='input-data.xlsx'
output_ ='output-data.xlsx'
dif=4.022/6.044/12.066
mz_rthreshold=5*PPM
rt_threshold=0.02
df_ = pd.read_excel(input_).loc[:,['M/Z','RT']]
    result =find_isotope_(df_,dif=dif,rthreshold=rthreshold,rt_threshold=rt_threshold)
    result.sort_values(['M/Z','RT']).reset_index(drop=True).to_excel(output_)
    return result
```

Supplementary Tables and Figures

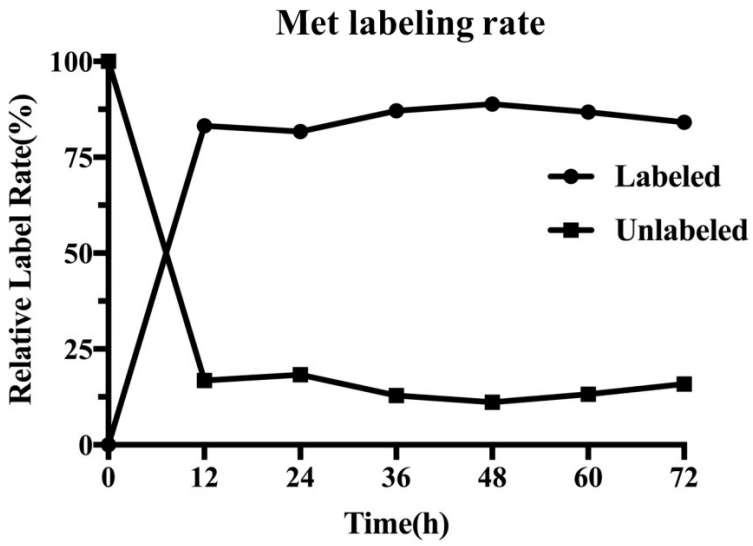


Fig. S1 Labeling rate of methionine. Labeled rate % =$^{13}CD_3$-Met/($^{13}CD_3$-Met+$^{12}CH_3$-Met) *100.

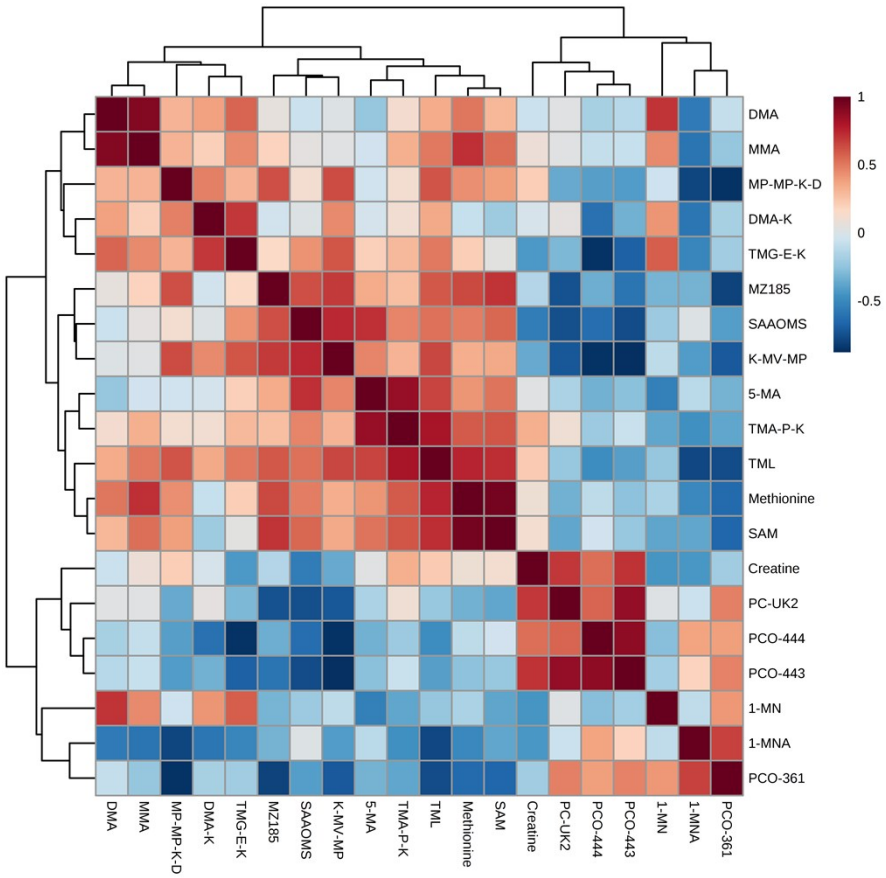


Fig. S2 Correlation analysis of methylated metabolites in BCs.

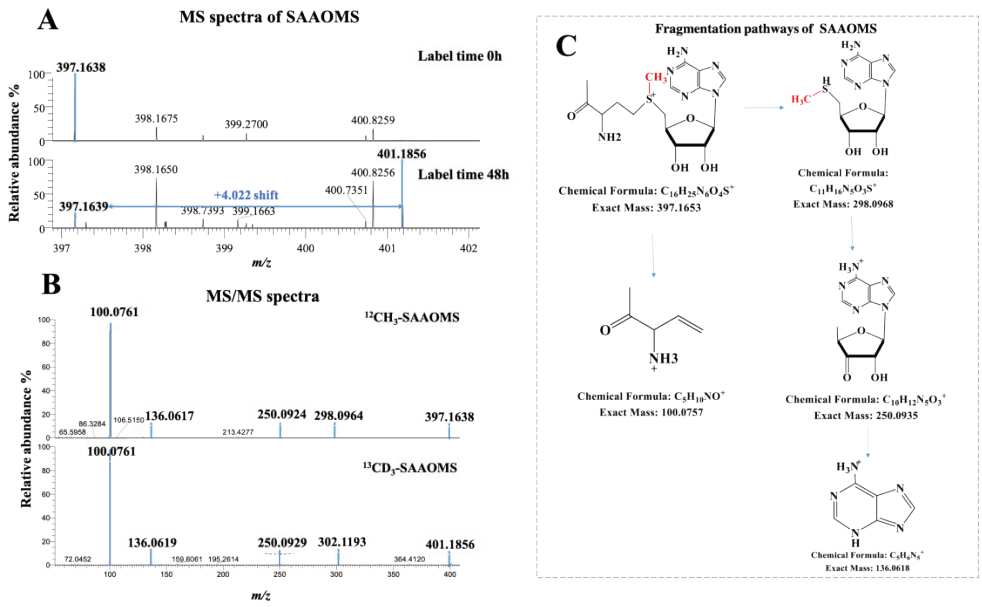


Fig. S3 MS and MS/MS spectra of SAAOMS and its identification. (A) MS spectra of SAAOMS. (B) MS/MS spectra of SAAOMS. (C) Fragmentation pathways of SAAOMS.

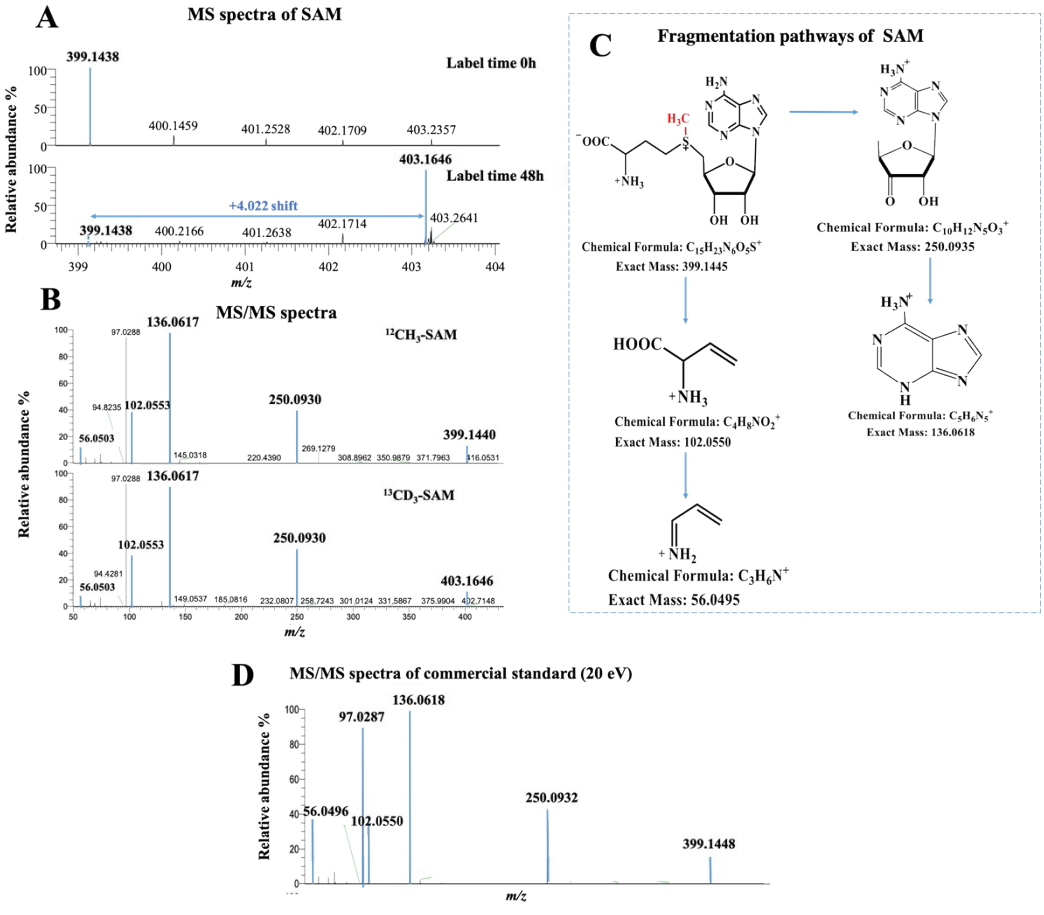
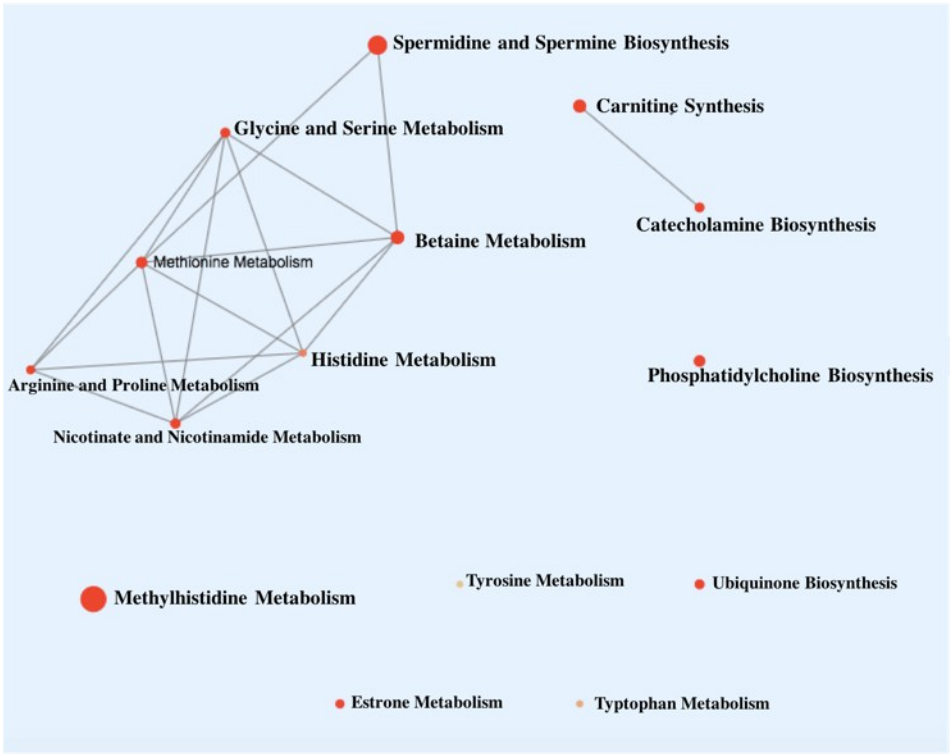


Fig. S4 MS and MS/MS spectra of SAM and its identification. (A) MS spectra of SAM. (B) MS/MS spectra of SAM. (C) Fragmentation pathways of SAAOMS. (D) MS/MS spectra of commercial standard.



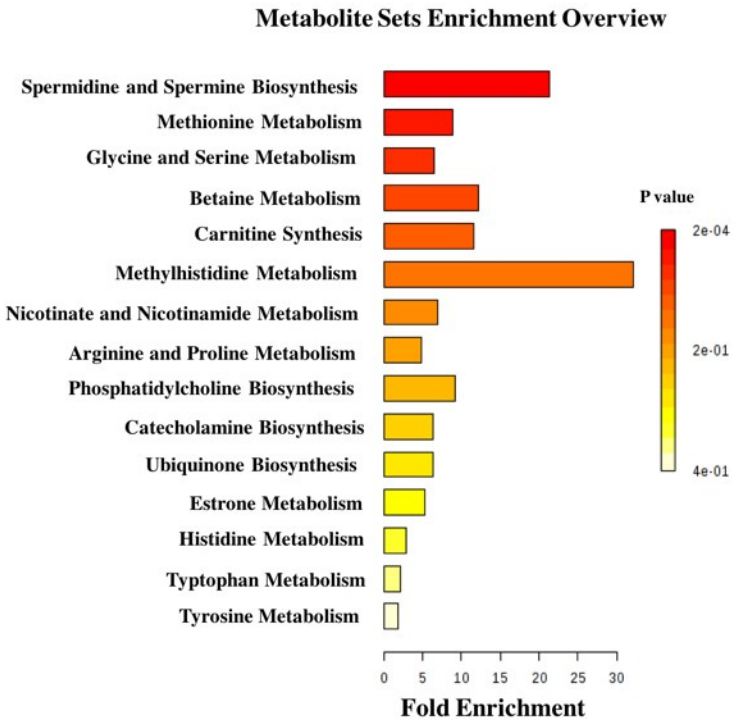


Fig. S5 Metabolic pathways enrichment analysis of methylated metabolites in BCs.

Table. S1 The information list, including compound name, *m/z*, RT, regression equation, correlation coefficient and accuracy of targeted methylation metabolites. UK, unknown.

ID	Compound Abbreviations	Quantification method	*m/z*	*m/z* (I.S)	RT(min)	Calibration curve	R^2	Weight	Precision(%)		
									Low	Medium	High
1	Creatine	XIC	132.0765	136.0985	12.45	y=7.107e-1x+5.785e1	0.9995	equal	13.92	3.03	6.78
2	1-MNA	XIC	137.0705	141.0925	6.57	y=1.383e-2x+4.286e-2	0.9980	equal	8.60	5.78	6.09
3	1-MN	SIM	138.0545	142.0765	10.88	y=1.136e-1x+8.903e-1	0.9951	equal	19.35	12.91	9.61
4	Methionine	XIC	150.0583	154.0802	9.9	y=2.536e-2x+1.548e-1	0.9991	equal	4.70	1.05	1.92
5	MZ185	XIC	185.128	189.15	11.91	y=2.159e-2x+6.521e-1	0.9946	equal	17.30	13.20	5.07
6	MMA	SIM	189.1342	193.1562	11.25	y=7.757e-2x+1.732e0	0.9979	equal	18.74	8.77	6.65
7	5-MA	XIC	298.0959	302.1183	3.43	y=1.202e-2x+1.74e-1	0.9999	equal	4.72	2.08	2.19
8	SAAOMS	SIM	397.1635	401.1855	8.33	y=2.265e-2x+2.009e-1	0.9824	equal	20.70	4.80	5.68
9	SAM	XIC	399.1434	403.1654	11.64	y=1.356e-2x+1.706e-1	0.9999	equal	6.59	2.65	3.11
10	DMA	XIC	203.1501	211.1941	10.81	y=2.635e-2x+4.384e-1	0.9994	equal	17.84	5.35	3.63
11	DMA-K	XIC	246.1804	254.2244	11.99	y=4.22e-1x+6.848e0	0.9884	equal	14.31	2.59	5.83
12	K-MV-MP	XIC	369.2486	377.2926	11.91	y=2.04e-2x+3.233e-1	0.9997	equal	18.74	6.57	6.01
13	MP-MP-K-D	SIM	484.2764	492.3213	17.28	y=1.876e-2x+4.41e-1	0.9998	equal	18.47	10.12	9.06

14	TML	XIC	189.1593	201.2253	12.23	y=7.487e-2x+7.166e-1	0.9926	equal	13.12	1.34	3.97
15	TMA-P-K	XIC	357.2486	369.3146	11.91	y=1.226e-1x+3.346e0	0.9935	equal	10.91	1.57	1.66
16	TMG-E-K	SIM	375.2225	387.2885	16.79	y=4.668e-1x+6.201e-1	0.9827	equal	18.82	10.19	7.39
17	PC(O-36:1)	XIC	788.6143	800.6793	13.54	y=4.686e0x+4.398e2	0.9987	equal	9.42	3.98	4.14
18	PC(O-44:4)	XIC	880.7155	892.7815	22.78	y=2.803e-2x+6.254e-1	0.9899	equal	20.36	5.50	2.94
19	PC(O-44:3)	XIC	882.7311	894.7971	22.11	y=1.761e-2x+5.789e-1	0.9980	equal	18.92	5.58	0.98
20	PC-UK2	XIC	887.6864	899.7538	22.11	y=1.807e-2x+5.855e-1	0.9983	equal	16.57	7.15	7.42

Table. S2 The parameters of MS for targeted metabolites relative quantification.

Assay	*m/z* scan range	Scan type	Resolution	AGC target	IT time
SIM assay	50-600	Full scan	17500	5e6	100ms
		SIM (for 3 times)	70000	5e6	200ms
Full MS assay	100-1200	Full scan	70000	5e6	100ms

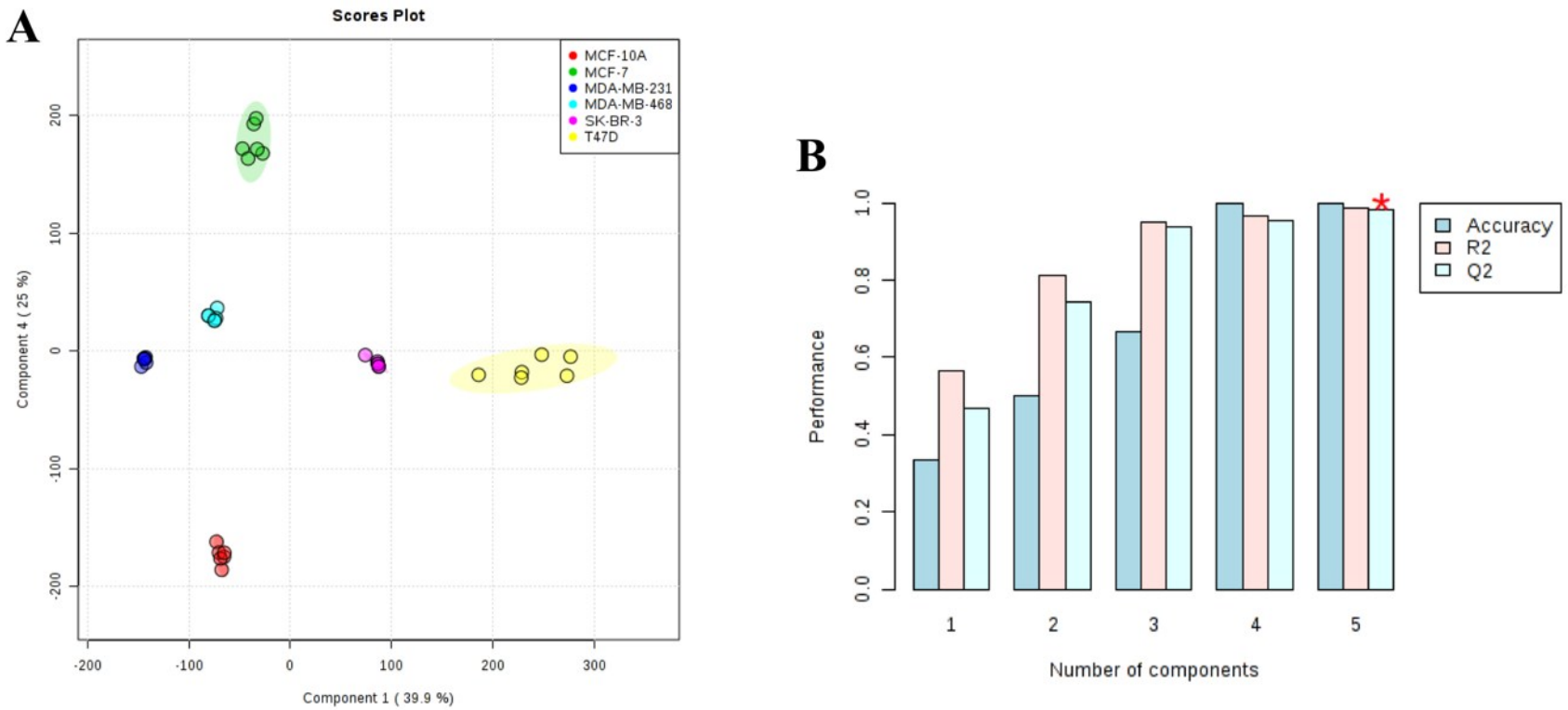


Fig. S6 Discrimination of investigated BC cell lines by OPLS-DA. (A) Based on relative metabolites abundance, the scores plot indicated similarities and differences between samples and groups. Confidence ellipses show confidence regions. Percentage of variance explained by individual component is indicated. (B) Performance measurement of OPLS-DA based on endometabolite levels using different numbers of components, showing the accuracy, multiple correlation coefficient R2 and the explained variance in prediction Q2: The red '*' indicates the best value of selected measure (Q2).

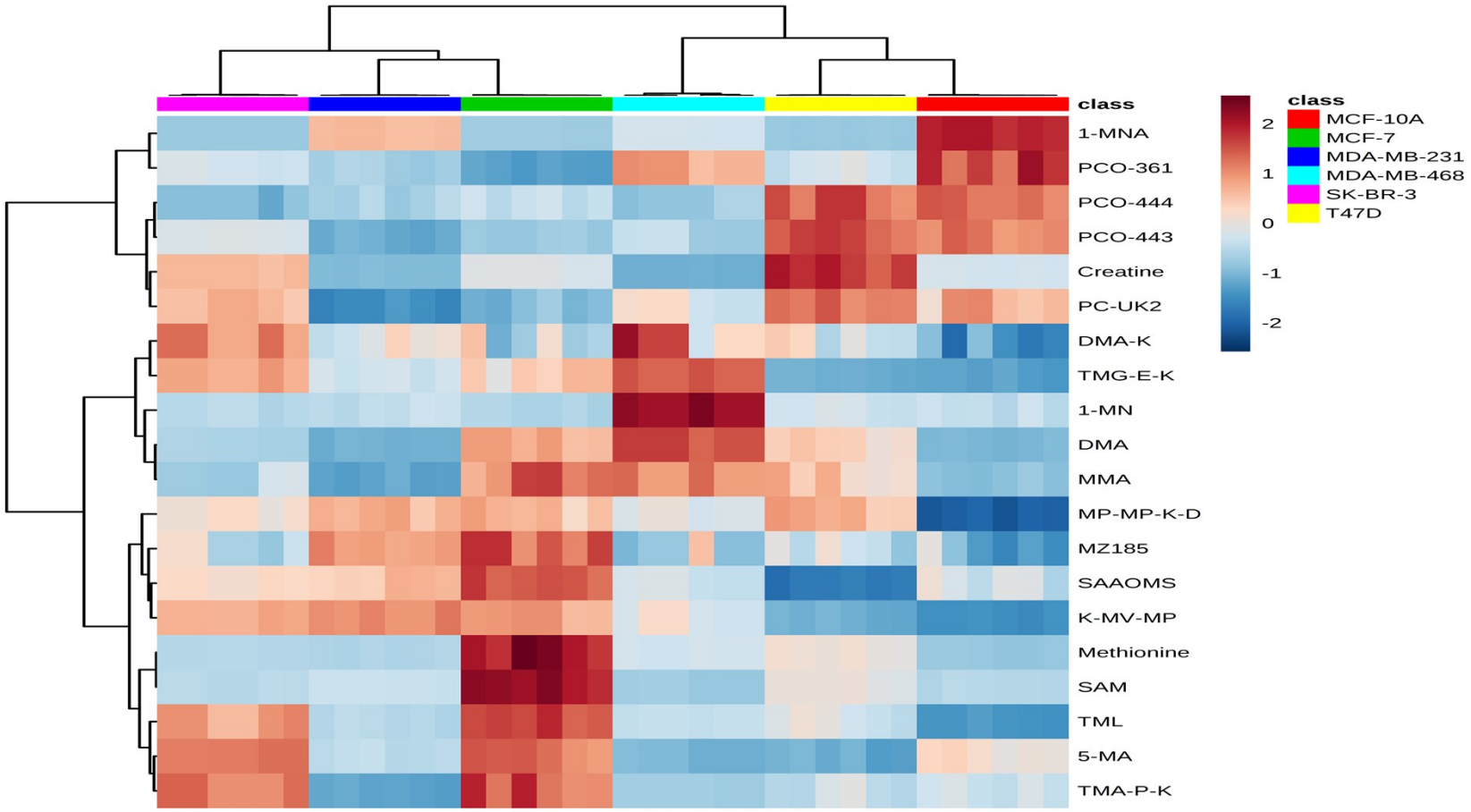


Fig. S7 Hierarchical cluster analysis of the labeled methylated metabolites in BCs and MEC. The relative abundance of MDA-MB-231 was used of normalized factor and other cell samples were corrected by the normalization