Electronic Supplementary Material (ESI) for Molecular BioSystems.

Metabolomics study on inhibitory effect of 17 beta-estradiol on osteoclast proliferation and differentiation

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Supplementary information

Fig. S1 Data quality assessment. **a. c** The retention time deviation profile generated by XCMS. A positive deviation indicates that the sample was eluting after the median retention time, and a negative deviation indicates that the samples was eluting before the median retention time. **b.** d PCA first component for the QC samples versus time analyzed.

Fig. S2 Inhibition effect of 0.1uM estradiol to RAW264.7 differentiation. **a.** Cell morphology of differentiated cells, estradiol exposed differentiated cells and the control. The number of differentiated cells was evaluated by TRAP enzyme staining experiment. And differentiated cells showed deep black color. **b.** OD value of cells after culturing for 6days with 0.1uM estradiol (conditional control) and without estradiol (negative control). They did not have significant difference, which excluded the interferences by cell death.

Fig. S3 Pattern recognition analysis of metabolic changes caused by 0.1 μM of estradiol during RAW264.7 cell differentiation. **a.** PCA score plot of data obtained from RPLC-MS. **b.** PCA score plot of data obtained from HILIC-MS

Fig. S4 Characterization of LysoPC isomers. Taken a feature at m/z 496.3440 for example, it was first examined in extracted ion chromatography (EIC). Two peaks were extracted with retention time of 9.98 and 10.46 min, respectively **(a)**. Further MS/MS analyses were conducted for identify these two compounds. Overall, the two compounds have highly comparable MS/MS spectra **(b, c)**. Using exact mass matching, online database search against Metlin suggested the two compounds as lysophosphatidylcholine (16:0) isomers (sn-1LysoPCs (16:0), sn-2 LysoPCs (16:0)). As showed in **b** and **c**, these two isomers have different abundance ratios between the ions at m/z 184.1 and 104.1. Previous studies found that the abundance ratio of sn-1 LysoPCs is larger than 1, while abundance ratio of sn-2 LysoPCs is lower than 1 [22, 23]. Based on this principle, the two compounds eluted at 9.98 and 10.46 min were identified as sn-2LysoPCs (16:0) and sn-1 LysoPCs (16:0), respectively.

Fig. S5 Changes in the intensity of significant metabolites in estradiol treated RAW264.7 cells performed for differentiation inhibition experiment. **a.** Changes in the intensity of significant metabolites obtained in RPLC-MS

mode in estradiol treated RAW264.7 cells performed for differentiation inhibition experiment. The X axis markers represented to the Table 1. Bars and error bars represent the mean relative metabolite signal intensity and standard deviations, respectively. *, $p<0.01$; **, $p<0.001$. **b.** Changes in the intensity of significant metabolites obtained in HILIC-MS mode in estradiol treated RAW264.7 cells performed for differentiation inhibition experiment. The X axis markers represented to the Table 2. **, $p<0.01$.

Table S1 Feature screening process using statistical methods for differentiation inhibition experiment.

Fig. S1

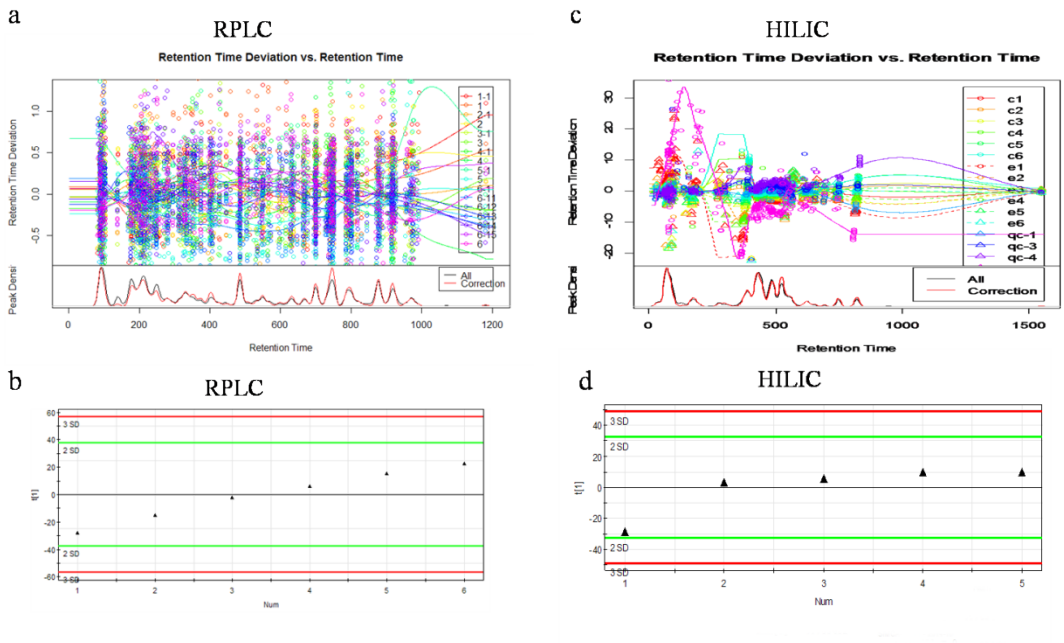
a
RPLC
Retention Time Deviation vs. Retention Time
Retention Time Deviation
Peak Densi
All
Correction
Retention Time
c
HILIC
Retention Time Deviation vs. Retention Time
c1
c2
c3
c4
c5
c6
e1
e2
e3
e4
e5
e6
qc-1
qc-3
qc-4
All
Correction
Retention Time
b
RPLC
3 SD
2 SD
t[1]
Num
d
HILIC
3 SD
2 SD
t[1]
Num

Fig. S2

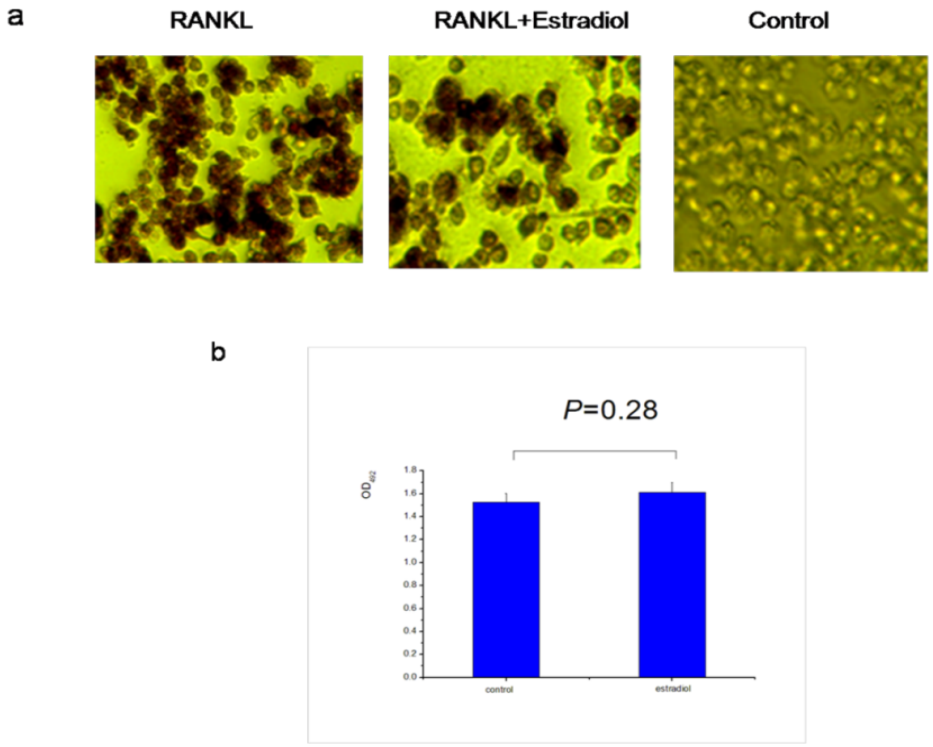
a
RANKL
RANKL+Estradiol
Control
b
P=0.28
OD450
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
control
estradiol

Fig. S3

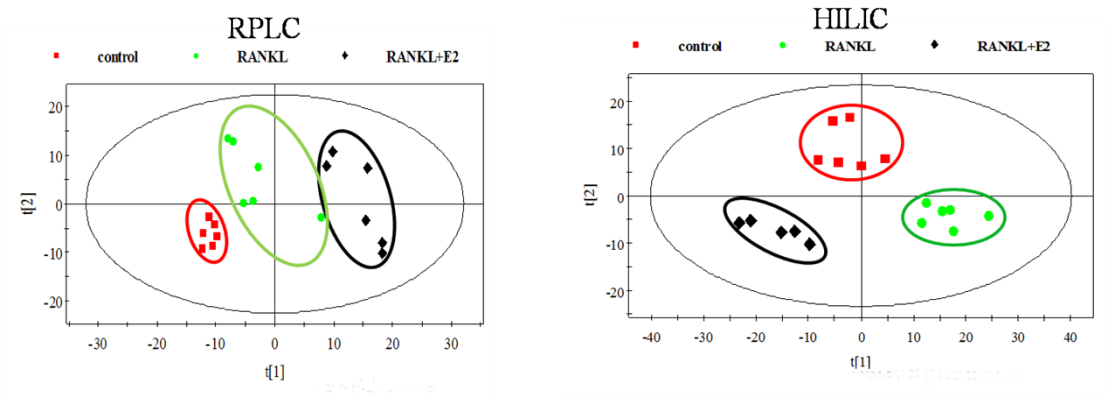
RPLC
control
RANKL
RANKL+E2
t[2]
t[1]
HILIC
control
RANKL
RANKL+E2
t[2]
t[1]

Fig. S4

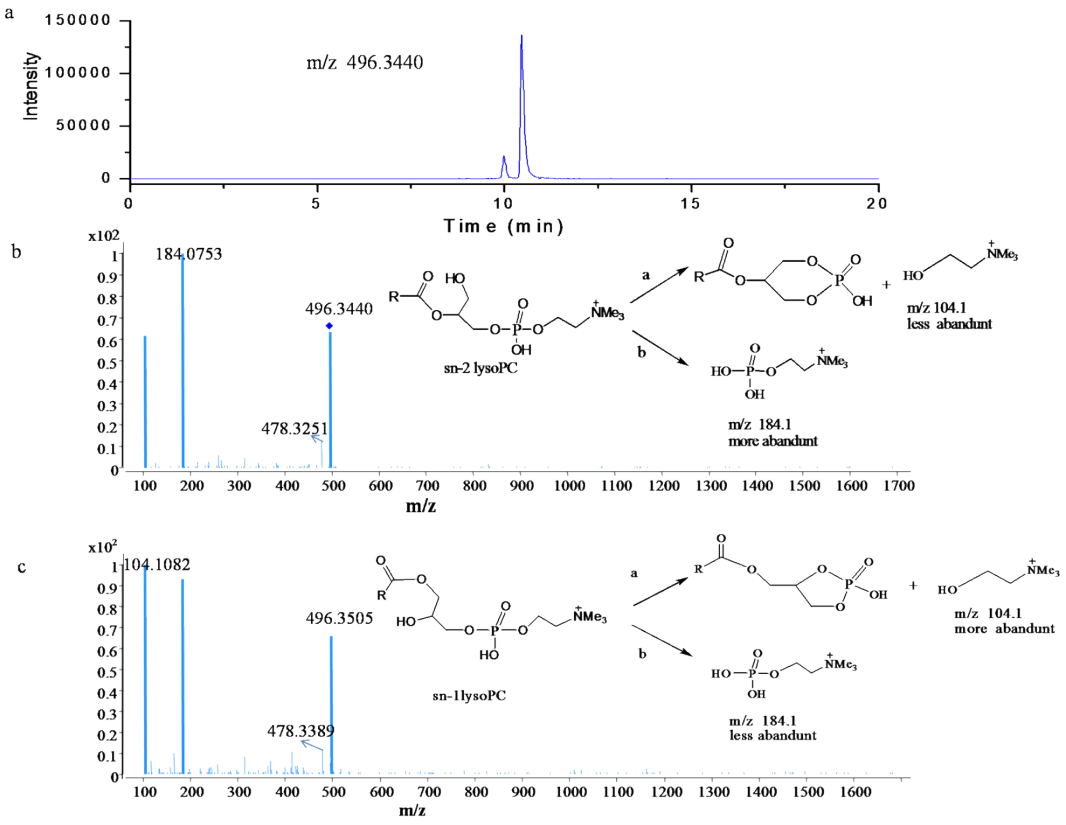
a
m/z 496.3440
Intensity
Time (min)
b
184.0753
496.3440
478.3251
m/z
sn-2 lysoPC
m/z 104.1
less abandunt
m/z 184.1
more abandunt
c
104.1082
496.3505
478.3389
sn-1lysoPC
m/z 104.1
more abandunt
m/z 184.1
less abandunt

Fig. S5

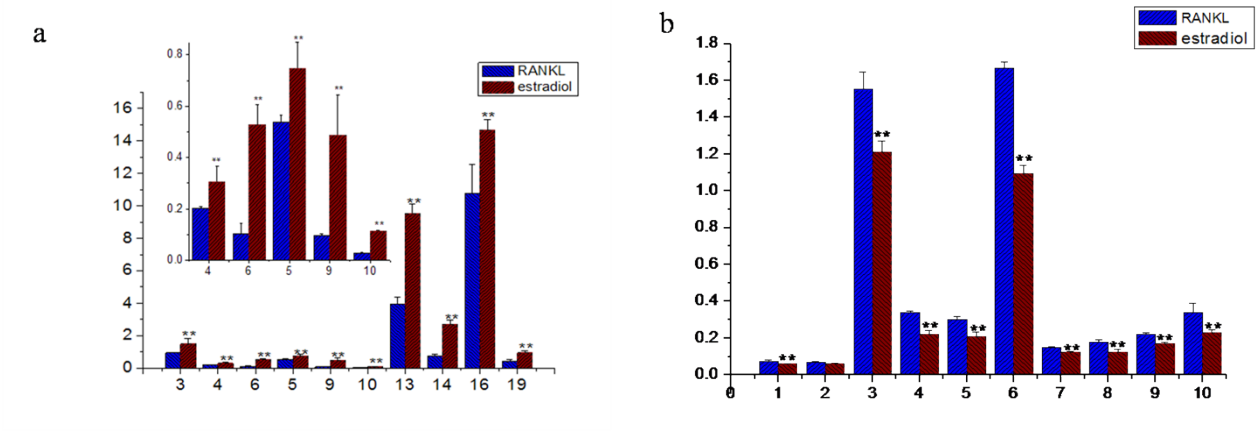
a
RANKL
estradiol
3
4
6
5
9
10
13
14
16
19
b
RANKL
estradiol
0
1
2
3
4
5
6
7
8
9
10

Table S1 Feature screening process using statistical methods for differentiation inhibition experiment.

Mode	Original features (CV%<30)	GIOS selected feature	Wilcoxon Mann-Whitney test selected feature
RPLC-MS	906	195	76
HILIC-MS	598	194	52