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Supporting Information

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Metabolome-Based Genome-Wide Association Study of Duck Meat Leads to Novel Genetic and Biochemical Insights

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Supplemental Information for

Metabolome-based genome-wide association study of duck meat leads to novel genetic and biochemical insights

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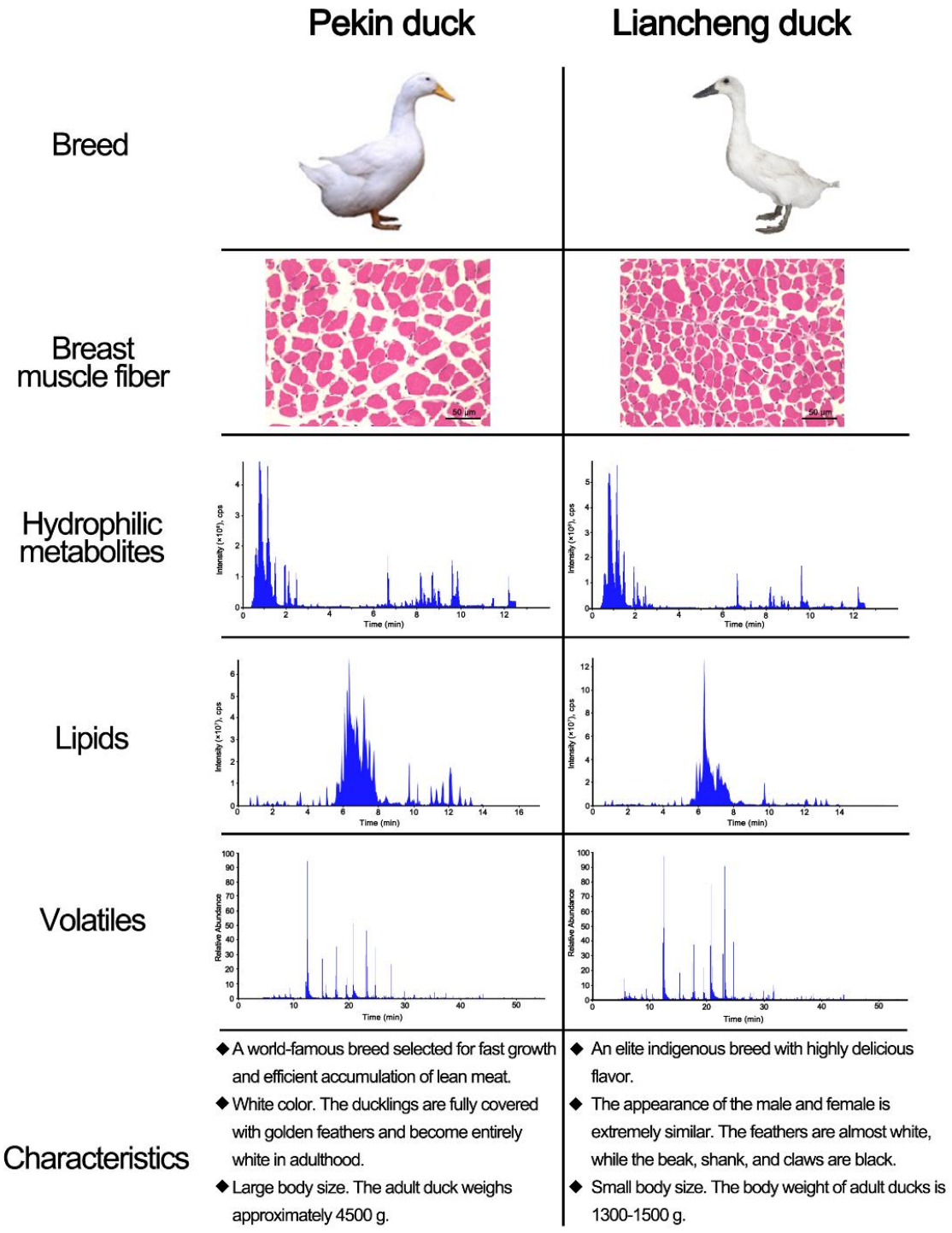
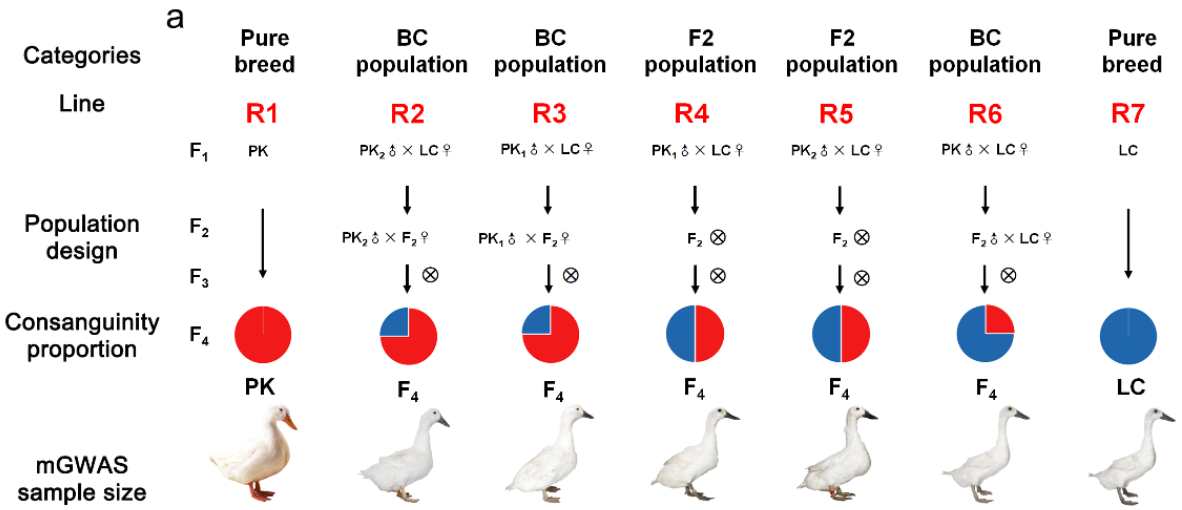


Supplementary Figure 1 Breed information for Pekin duck and Liancheng duck. The differences characteristics between Pekin duck and Liancheng duck, including breast muscle fiber trait, hydrophilic metabolites were analyzed by LC–MS/MS and volatiles were analyzed by SPME-GC–MS.

a



								sum
n	30	74	75	75	75	64	30	423
♂	18	33	37	38	41	32	15	214
♀	12	41	38	37	34	32	15	209

b

RNA-seq samples of 7 development stages

Time	1d	1w	2w	5w	4w	5w	6w
Pekin duck	3	3	3	3	3	3	3
Liancheng duck	3	3	3	3	3	3	3

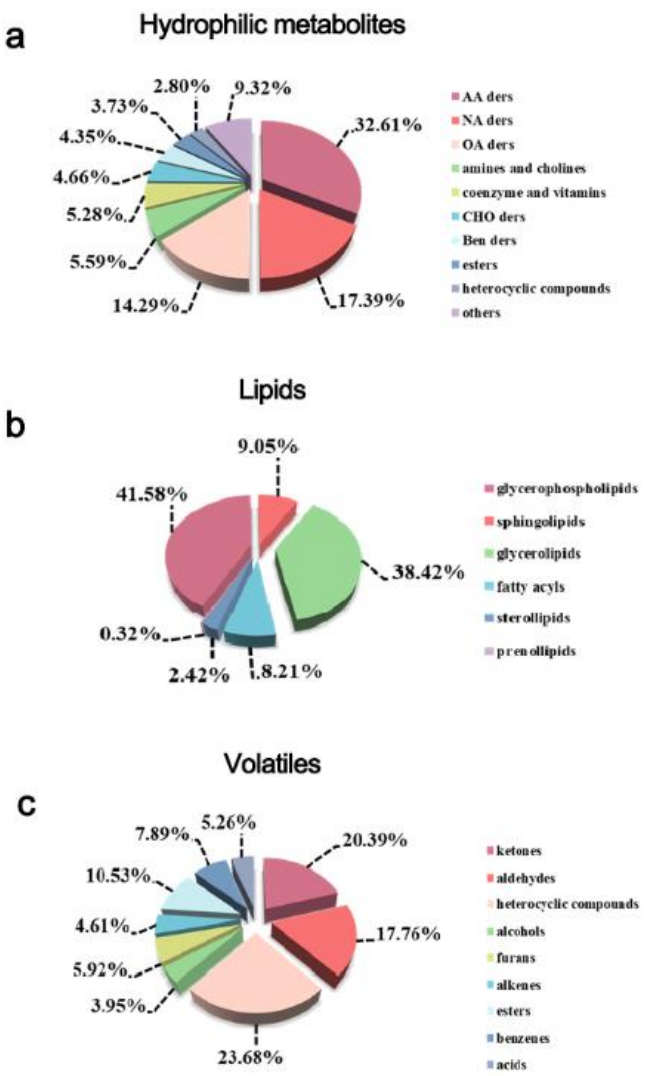
c

Hydrophilic metabolites and Lipids samples of 7 development stages

Time	1d	1w	2w	3w	4w	5w	6w
Pekin duck	5	5	5	5	5	5	5
Liancheng duck	5	5	5	5	5	5	5

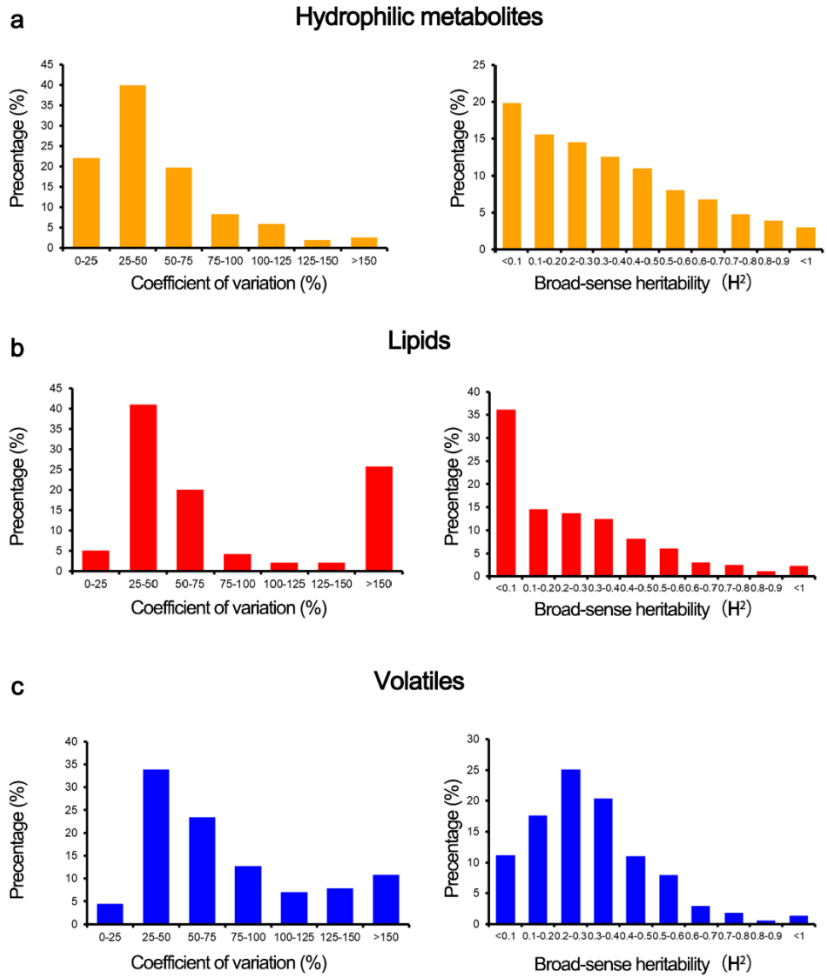
Supplementary Figure 2 The detailed population and sample design used in this study.

(a) Gradient consanguinity design used for the two purebreds and the fourth generation of a large segregating duck population used for the genetic and biochemical basis of metabolites and volatiles. (b) Detailed information on the 7 developmental stages of RNA-seq samples used in this study. (c) Detailed information on the 7 development stages of hydrophilic metabolites and lipid samples used in this study.



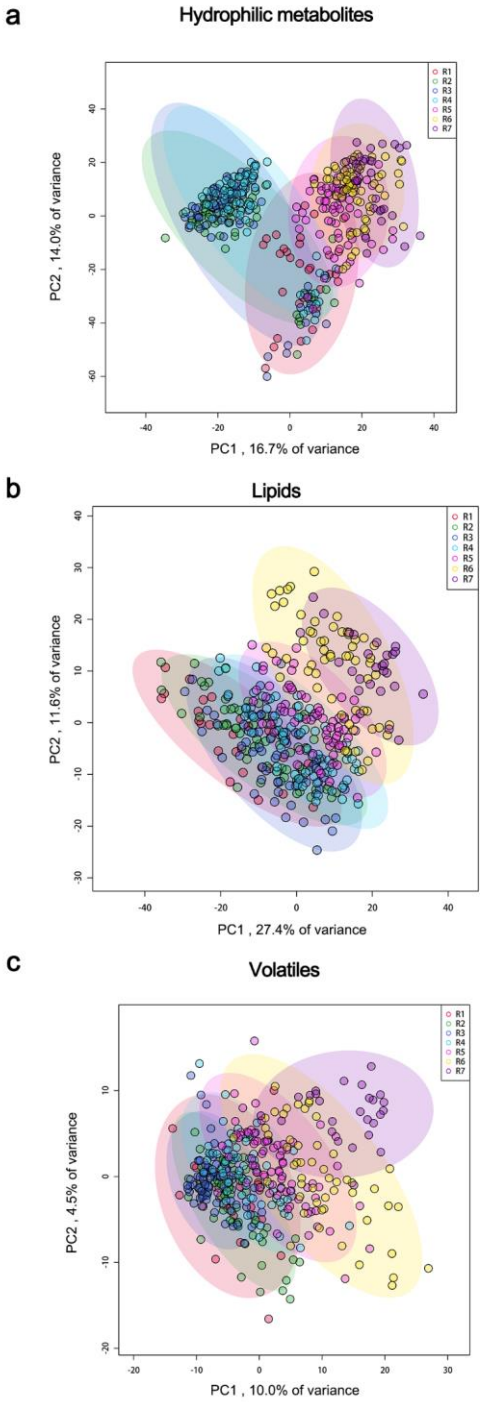
Supplementary Figure 3 Classification of annotated metabolites identified in hydrophilic metabolites, lipids and volatiles of the 423 ducks.

(a) 10 subclasses of 321 annotated metabolites identified in hydrophilic metabolites. (b) 6 categories of 950 annotated metabolites identified in lipids. (c) 9 subclasses of 152 annotated metabolites identified in volatiles.



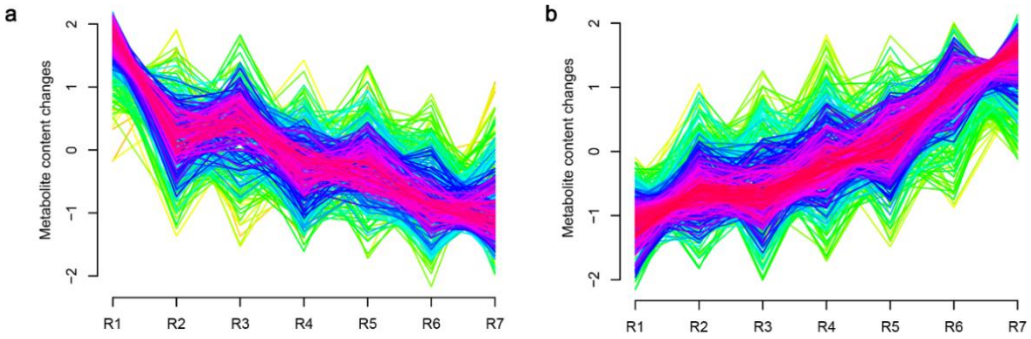
Supplementary Figure 4 The broad-sense heritability (H2) and the coefficients of variation (CV) results for each metabolite.

(a) Distribution of broad-sense heritability (H^2) and coefficients of variation (CV) of hydrophilic metabolites (n= 2481). (b) Distribution of broad-sense heritability (H^2) and coefficients of variation (CV) of lipids (n= 950). (c) Distribution of broad-sense heritability (H^2) and coefficients of variation (CV) of volatiles (n= 702).



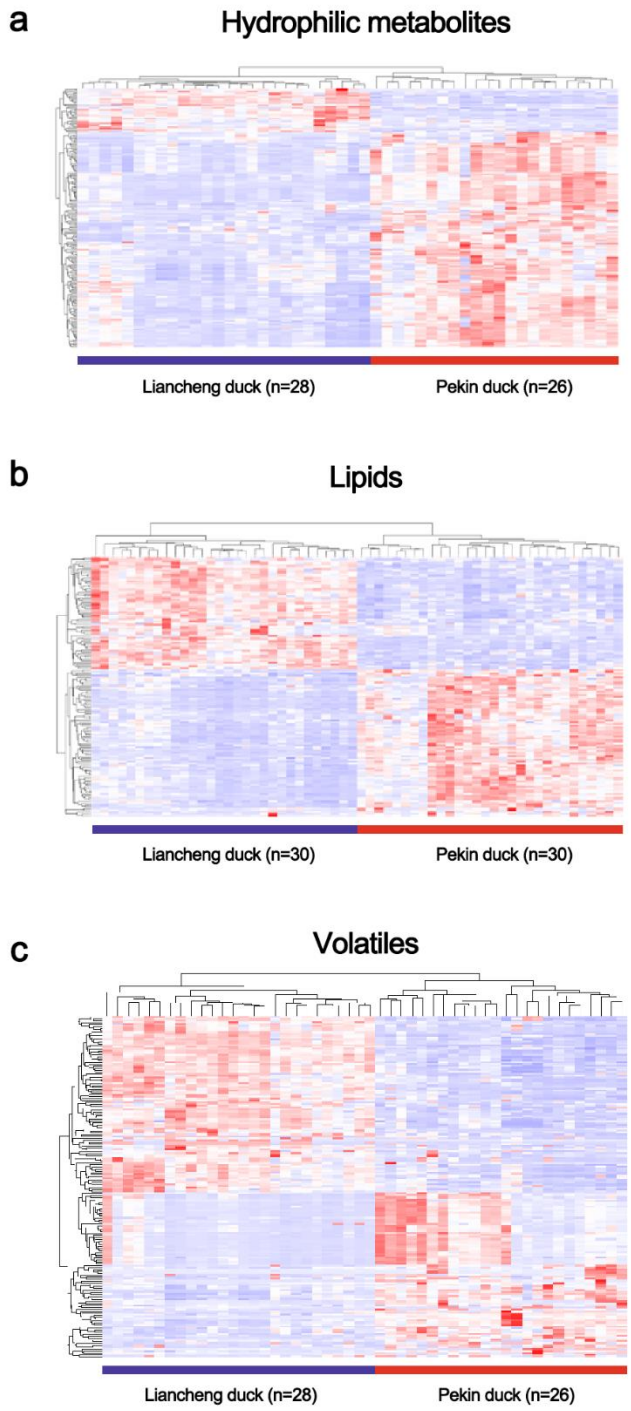
Supplementary Figure 5 Partial least squares discriminant analysis (PLS-DA) based on the levels of hydrophilic metabolites, lipids and volatiles.

(a) 423 individuals could be divided into seven independent clusters by the levels of hydrophilic metabolites (n=2481). (b) 423 individuals could be divided into seven independent clusters by the levels of lipids (n= 950). (c) 423 individuals could be divided into seven independent clusters by the levels of volatiles (n= 702).



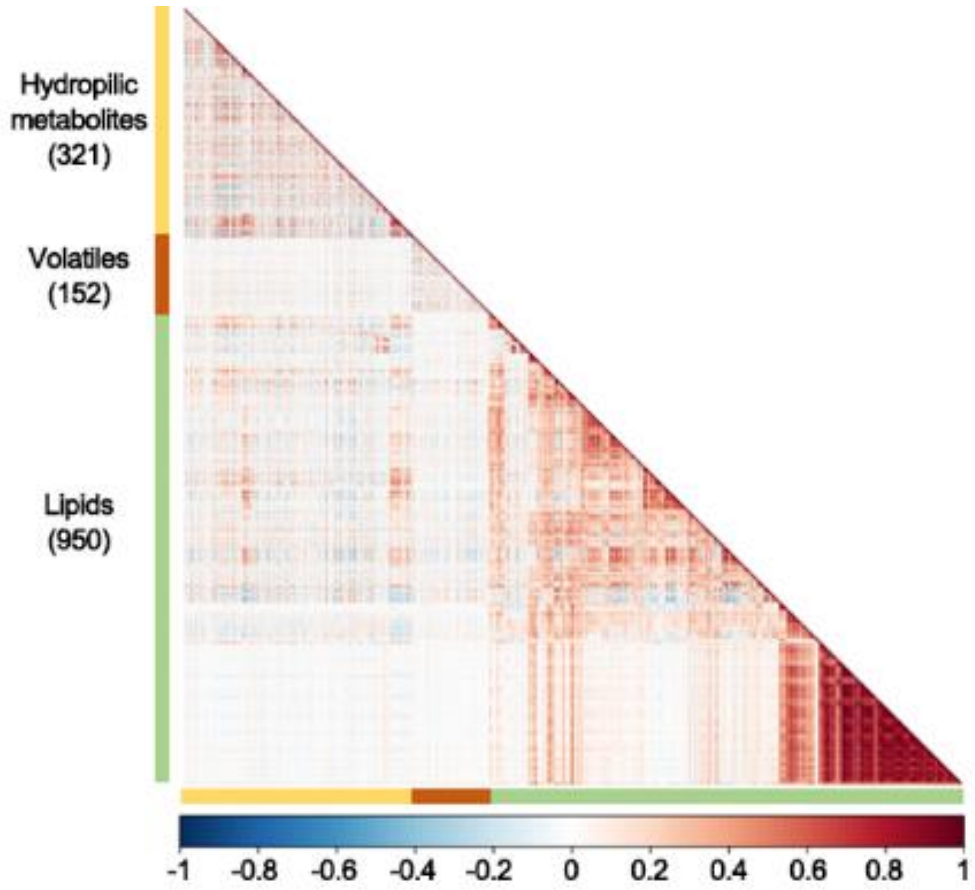
Supplementary Figure 6 The metabolites and volatiles with increasing or decreasing trends from R1 to R7.

(a) The metabolites and volatiles showing a decreasing trend from R1 to R7. (b) The metabolites and volatiles showing an increasing trend from R1 to R7.

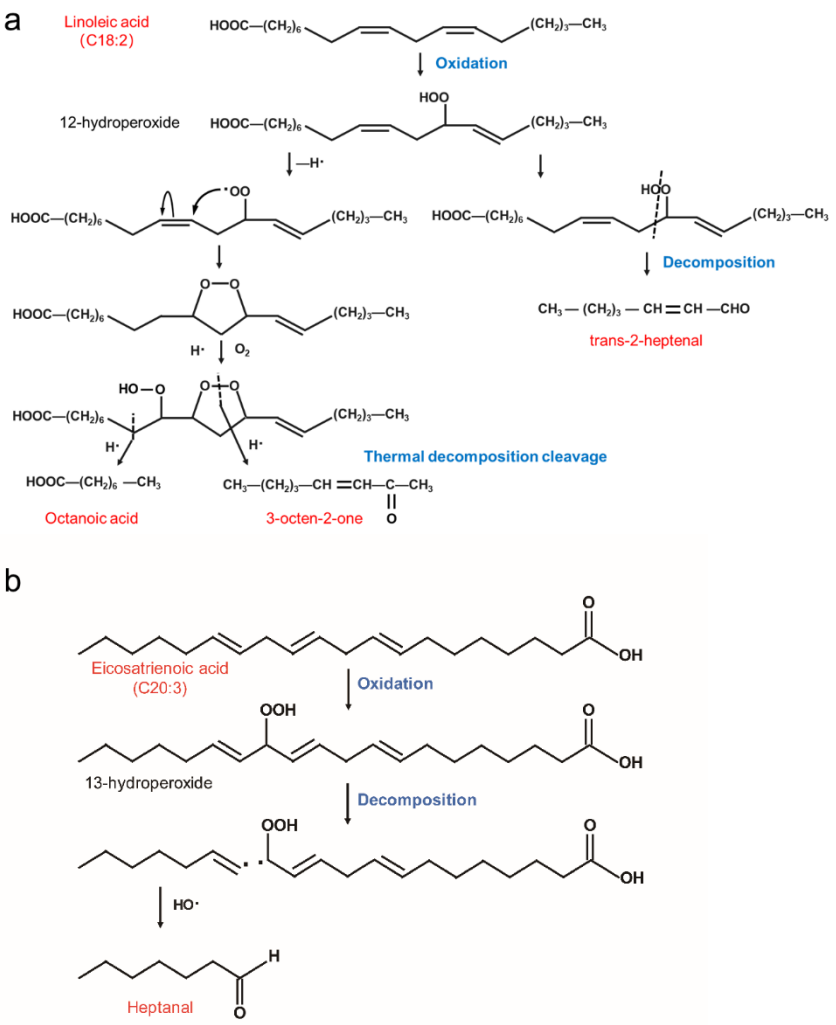


Supplementary Figure 7 Differential hydrophilic metabolites, lipids and volatiles between Pekin ducks and Liancheng ducks at 6 weeks.

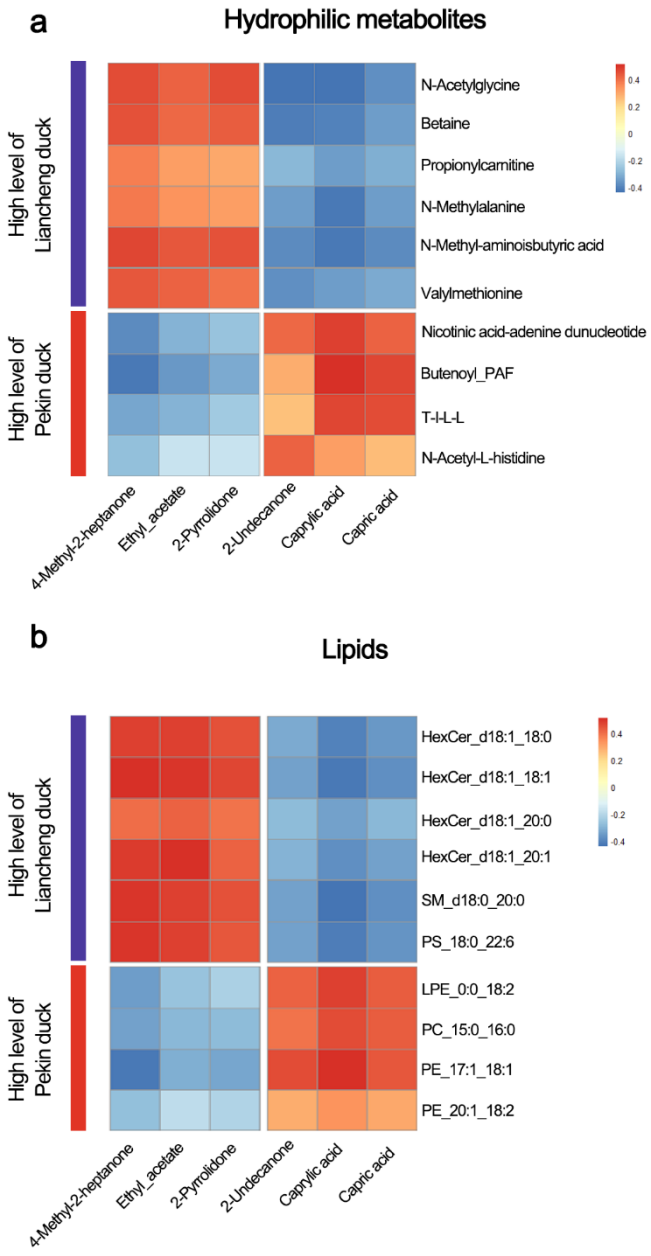
(a) 145 differential hydrophilic metabolites were identified. (b) 144 differential lipids were identified. (c) 180 differential volatiles were identified.



Supplementary Figure 8 A heatmap depicting Pearson's correlation between annotated hydrophilic metabolites, lipids and volatiles.

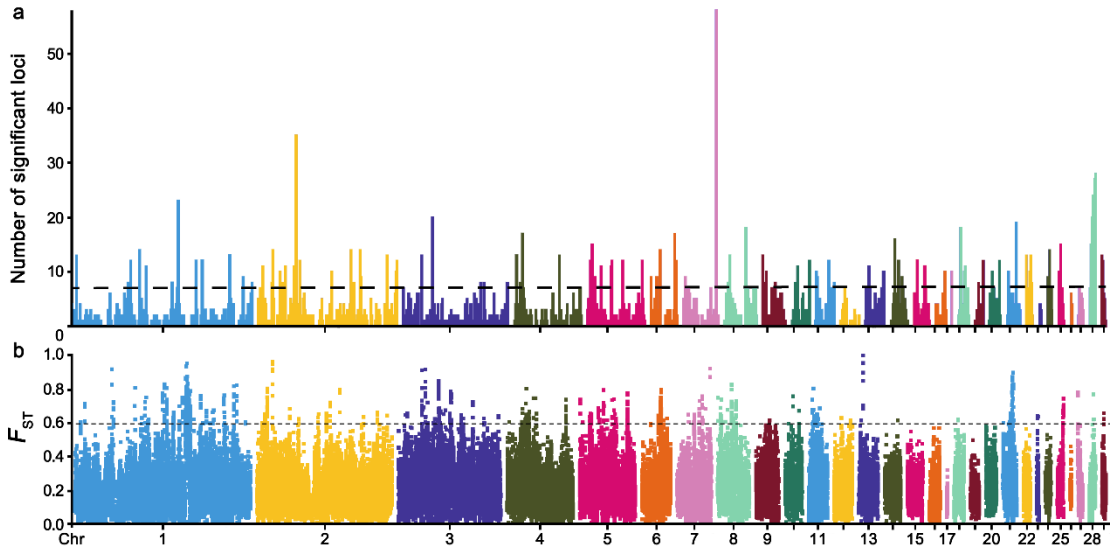


Supplementary Figure 9 Pathways for the formation of heptanal and 3-octen-2-one. (a) Linoleic acid can be generated 3-octen-2-one by automatic oxidation and (b) Heptanal was generated by eicosatrienoic acid automatic oxidation and thermal decomposition cleavage.

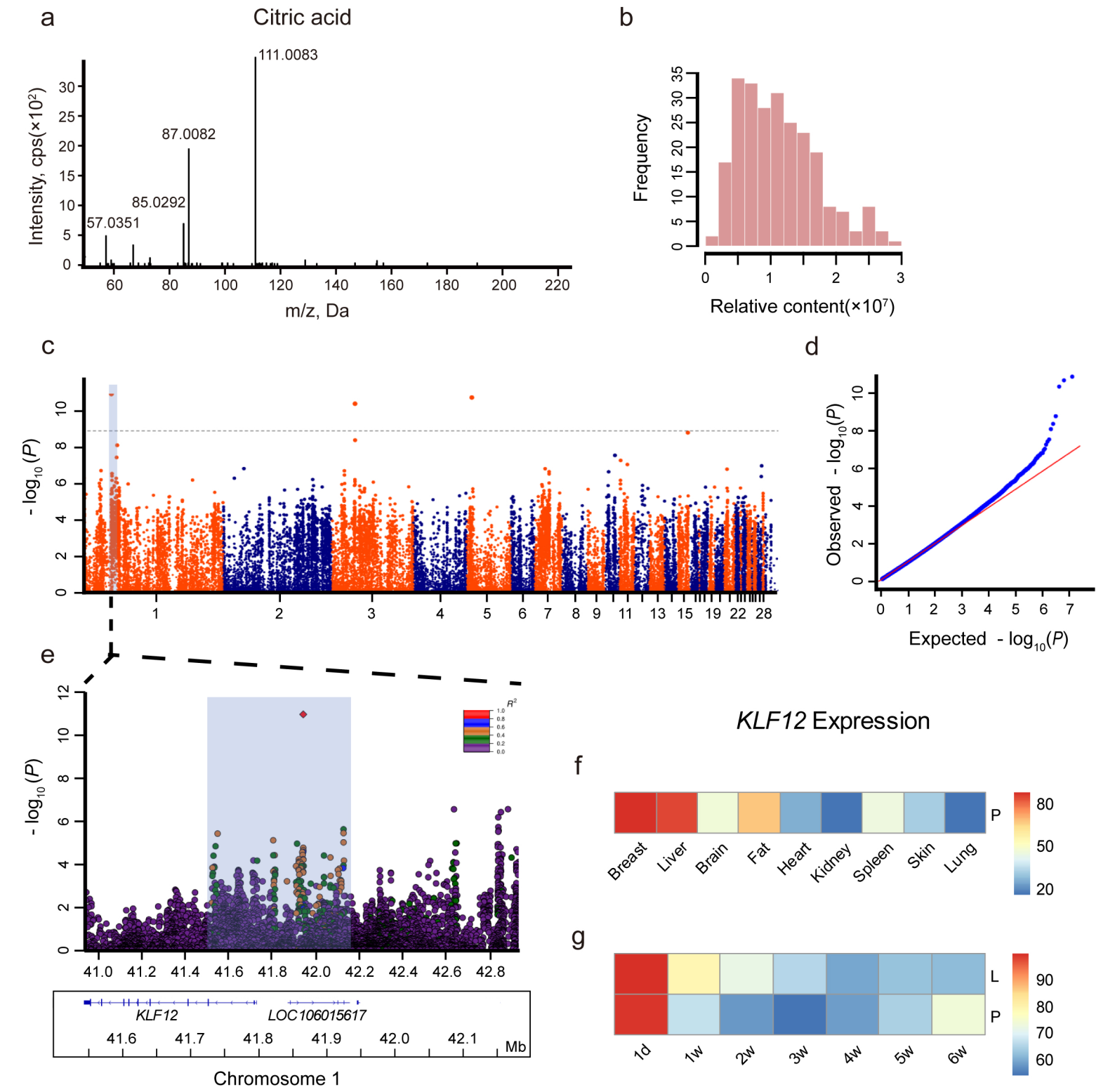


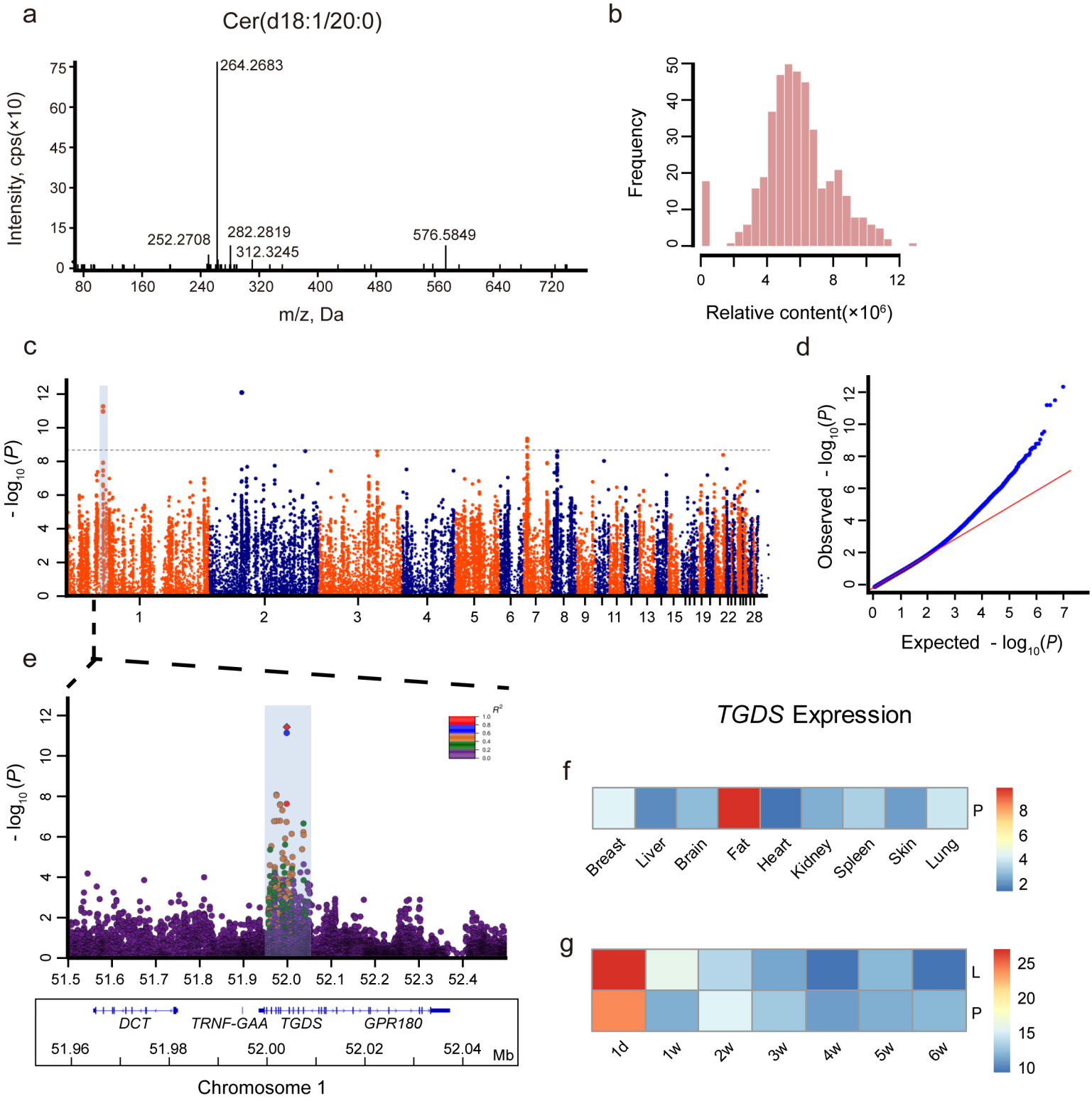
Supplementary Figure 10 Correlations in the systematic relationship between differential hydrophilic metabolites, lipids and volatiles.

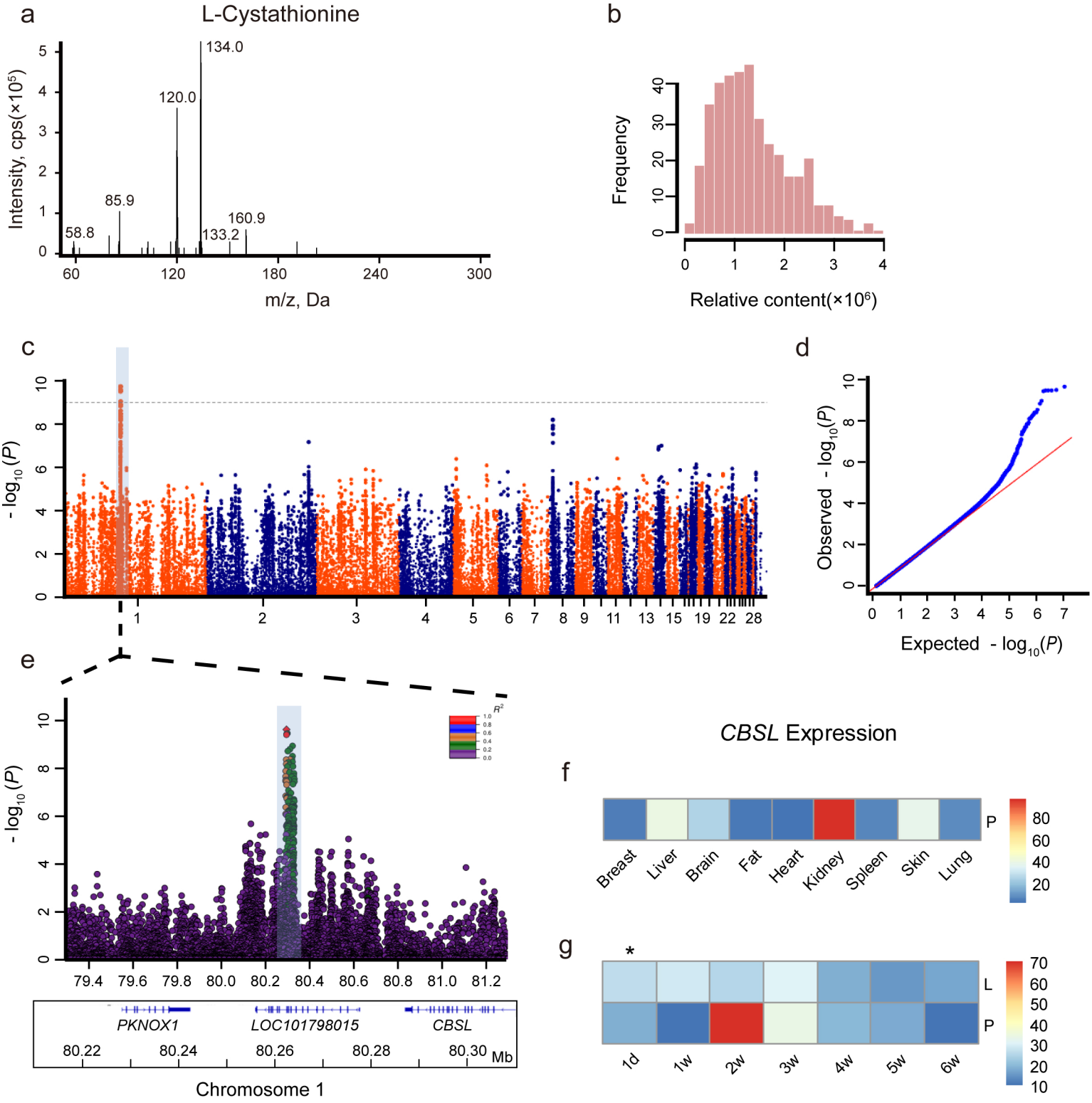
(a) Differential hydrophilic metabolites was correlated with characteristical volatiles between Pekin duck and Liancheng duck. (b) Differential lipids was correlated with characteristical volatiles between Pekin duck and Liancheng duck.

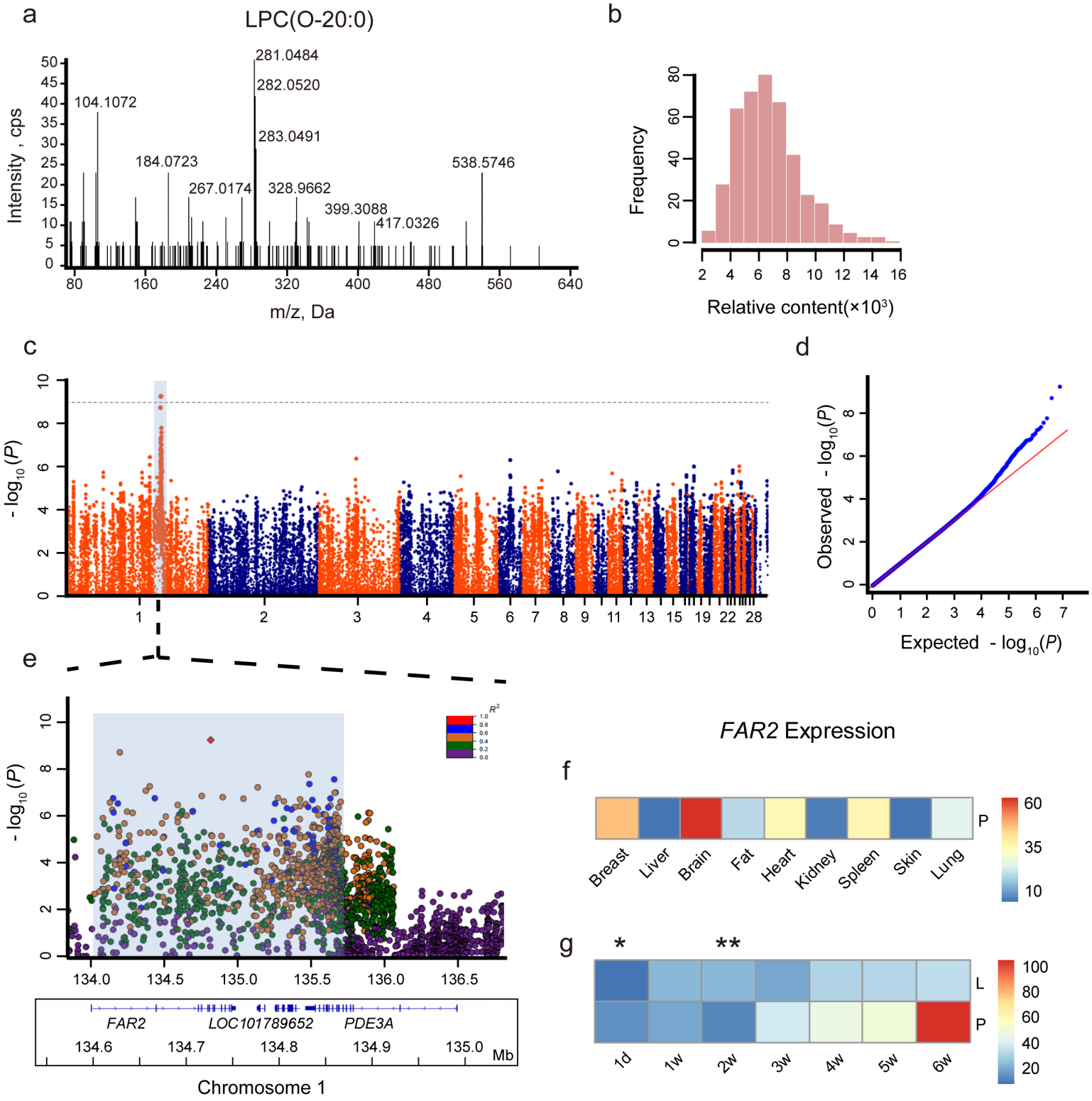


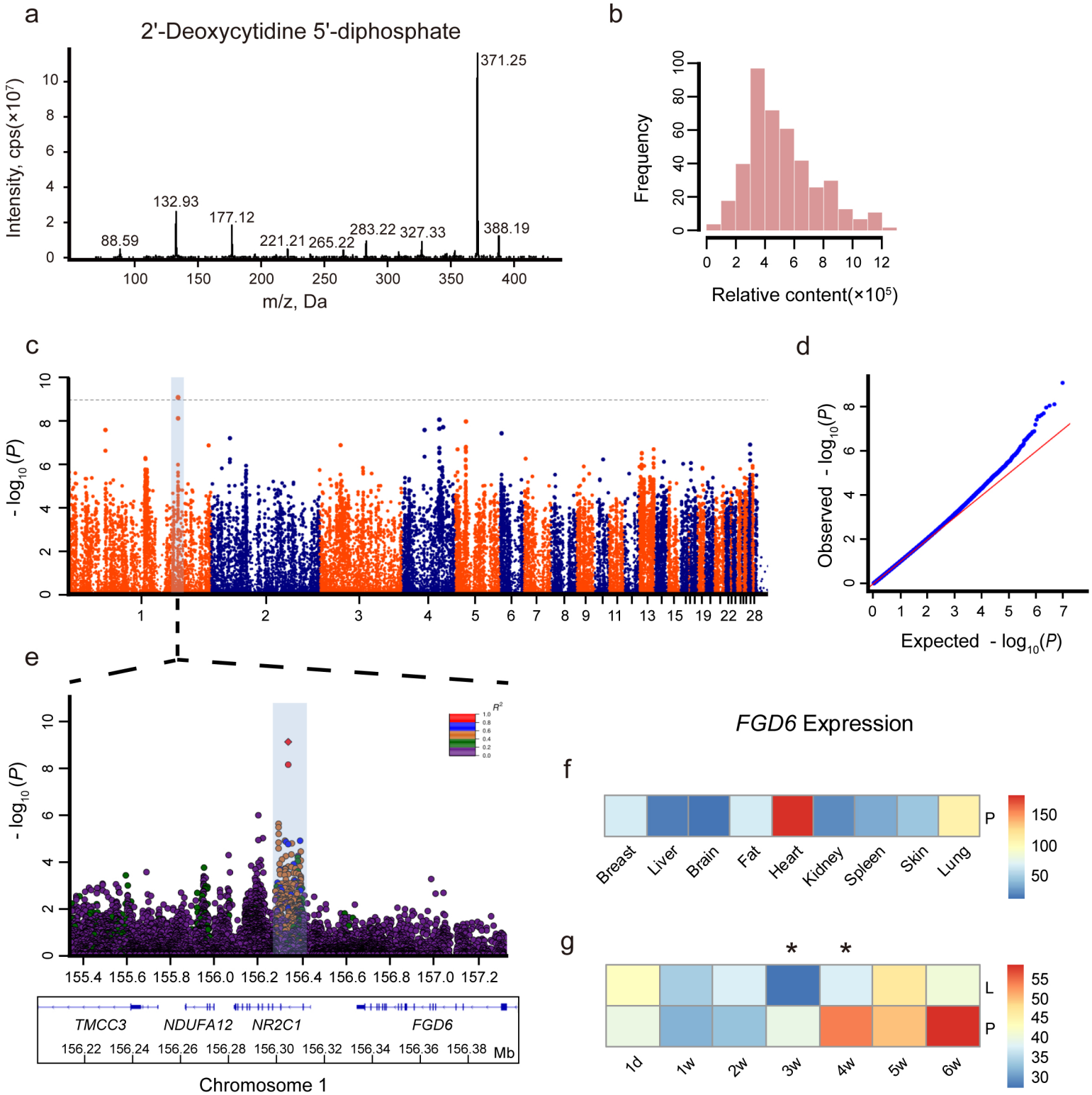
Supplementary Figure 11 Distribution of significant signals for the mGWAS results and scanned the extreme genetic divergence between Pekin duck and Liancheng duck. (a) Statistics of the number of the significant signals for the mGWAS results, the horizontal dashed line indicates the threshold (permutation test < 0.01) for signals hotspots. (b) The genetic divergence between Pekin duck and Liancheng duck, the horizontal dashed line indicates the threshold (TOP 1%) for genetic divergence region.

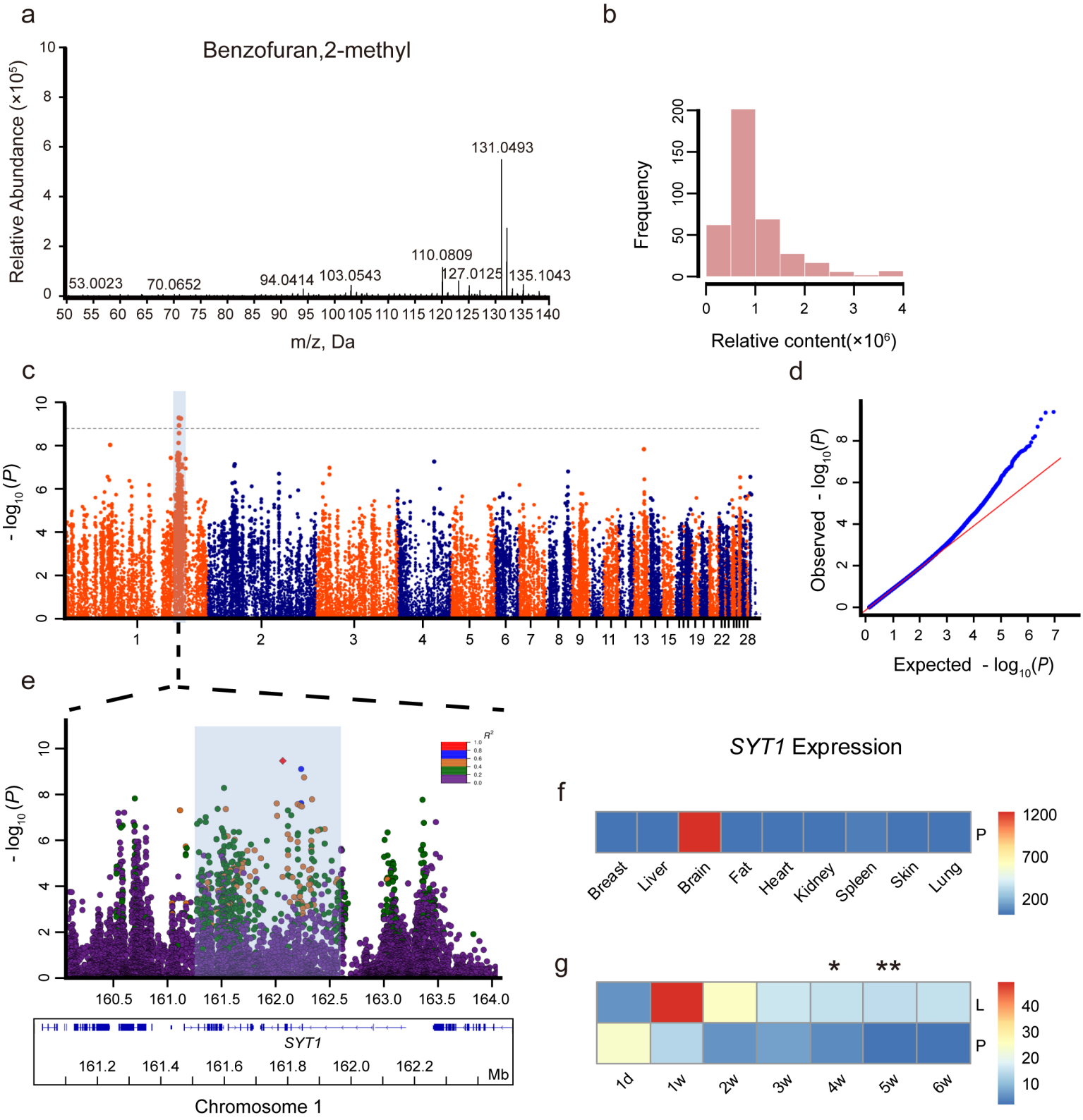

a
Citric acid
111.0083
87.0082
85.0292
57.0351
Intensity, cps(×10²)
m/z, Da
b
Frequency
Relative content(×10⁷)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
KLF12
LOC106015617
Mb
Chromosome 1
KLF12 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
1d
1w
2w
3w
4w
5w
6w
L
P

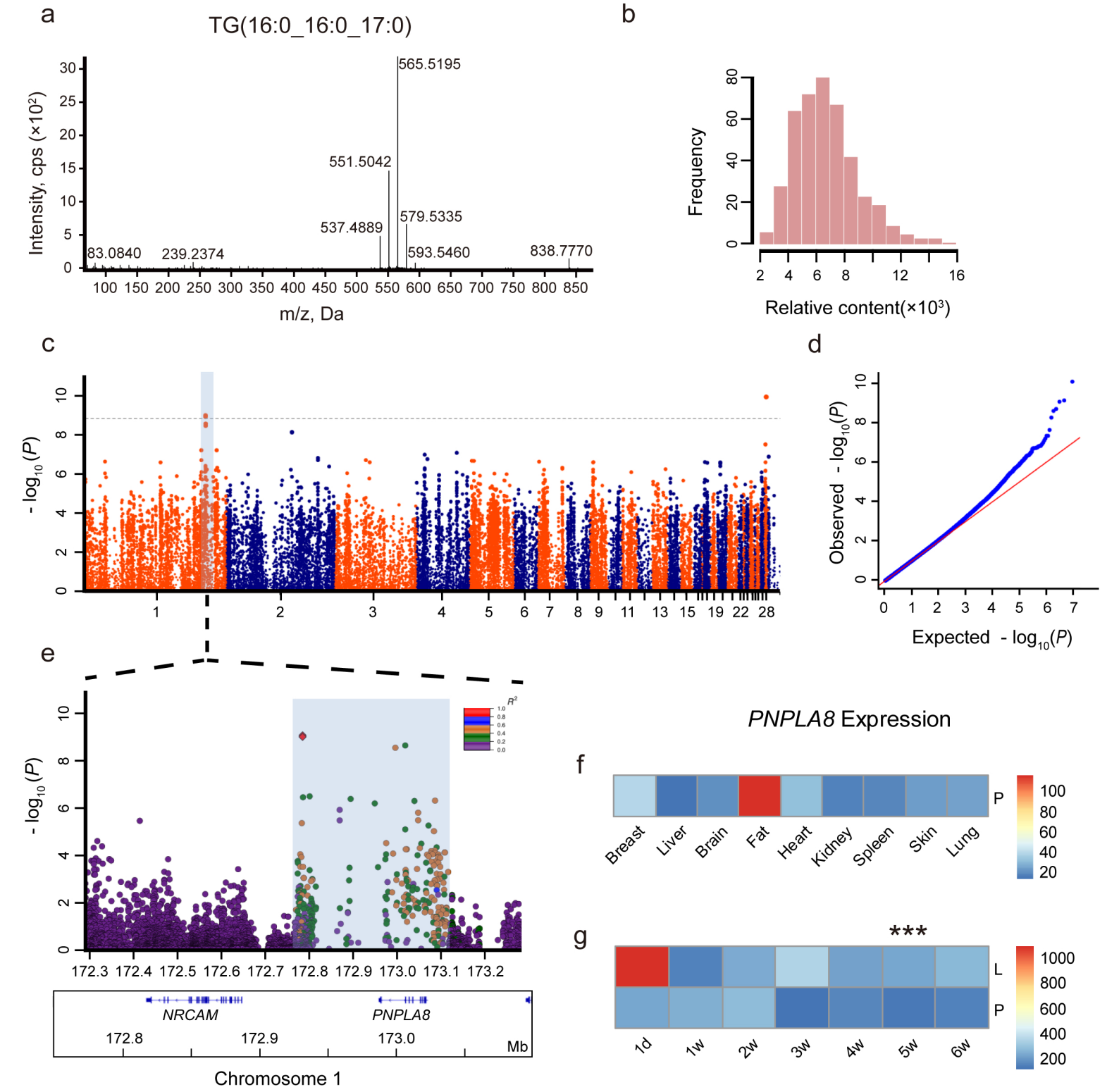

a
Cer(d18:1/20:0)
Intensity, cps(×10)
m/z, Da
264.2683
252.2708
282.2819
312.3245
576.5849
b
Frequency
Relative content(×10⁶)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
DCT
TRNF-GAA
TGDS
GPR180
Mb
Chromosome 1
TGDS Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
1d
1w
2w
3w
4w
5w
6w
L
P

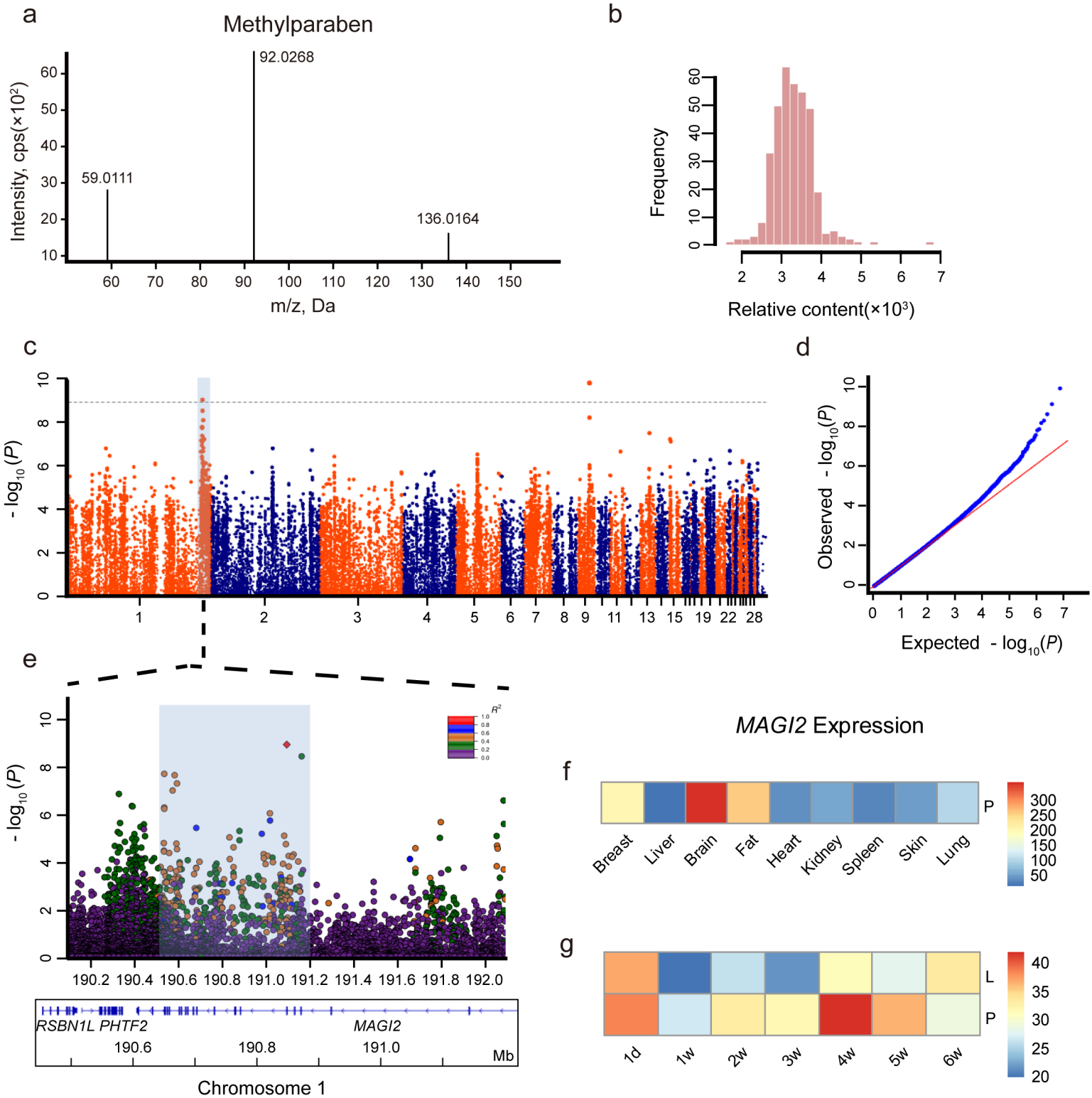

a
L-Cystathionine
Intensity, cps(×10⁵)
134.0
120.0
85.9
160.9
58.8
133.2
m/z, Da
b
Frequency
Relative content(×10⁶)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
PKNOX1
LOC101798015
CBSL
Mb
Chromosome 1
CBSL Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
*
L
P
1d
1w
2w
3w
4w
5w
6w

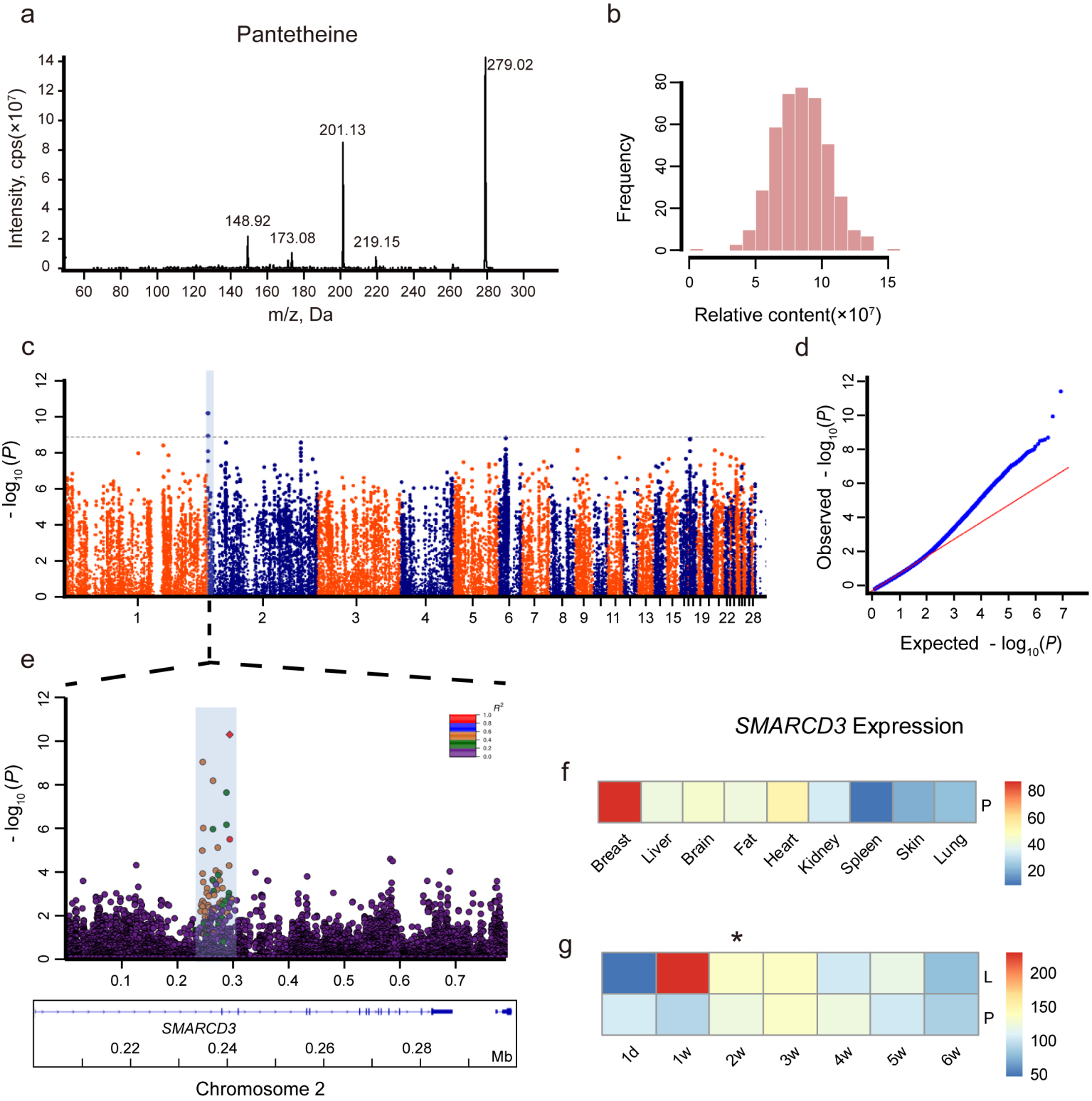

a
LPC(O-20:0)
Intensity , cps
m/z, Da
281.0484
282.0520
283.0491
104.1072
184.0723
267.0174
328.9662
399.3088
417.0326
538.5746
b
Frequency
Relative content(×10³)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
FAR2
LOC101789652
PDE3A
Mb
Chromosome 1
FAR2 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
*
**
L
P
1d
1w
2w
3w
4w
5w
6w

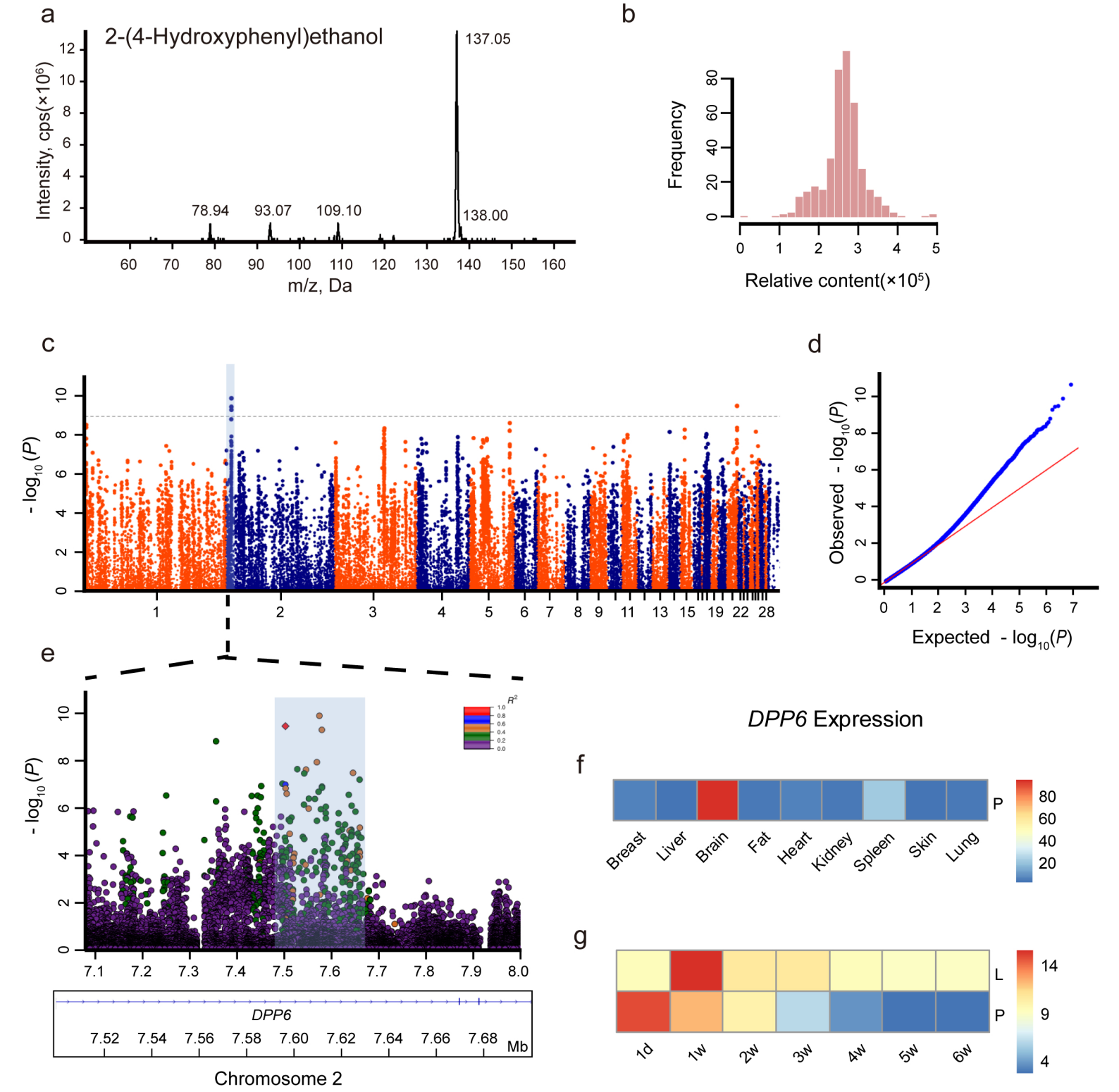

a
2'-Deoxycytidine 5'-diphosphate
Intensity, cps(×10⁷)
m/z, Da
371.25
132.93
177.12
88.59
221.21
265.22
283.22
327.33
388.19
b
Frequency
Relative content(×10⁵)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
TMCC3
NDUFA12
NR2C1
FGD6
Mb
Chromosome 1
FGD6 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
1d
1w
2w
3w
4w
5w
6w
L
P


a
Benzofuran,2-methyl
Relative Abundance (×10^5)
m/z, Da
131.0493
110.0809
127.0125
135.1043
53.0023
70.0652
94.0414
103.0543
b
Frequency
Relative content(×10^6)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
SYT1
Chromosome 1
Mb
f
SYT1 Expression
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
1200
700
200
g
*
**
L
P
1d
1w
2w
3w
4w
5w
6w
40
30
20
10


a
TG(16:0_16:0_17:0)
Intensity, cps (×10²)
m/z, Da
565.5195
551.5042
579.5335
537.4889
593.5460
83.0840
239.2374
838.7770
b
Frequency
Relative content(×10³)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
NRCAM
PNPLA8
Mb
Chromosome 1
PNPLA8 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
***
L
P
1d
1w
2w
3w
4w
5w
6w


a
Methylparaben
Intensity, cps(×10²)
92.0268
59.0111
136.0164
m/z, Da
b
Frequency
Relative content(×10³)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
RSBN1L PHTF2
MAGI2
Mb
Chromosome 1
MAGI2 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
L
P
1d
1w
2w
3w
4w
5w
6w


a
Pantetheine
Intensity, cps(×10⁷)
m/z, Da
279.02
201.13
148.92
173.08
219.15
b
Frequency
Relative content(×10⁷)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
SMARCD3
Mb
Chromosome 2
f
SMARCD3 Expression
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
*
L
P
1d
1w
2w
3w
4w
5w
6w


a
2-(4-Hydroxyphenyl)ethanol
137.05
138.00
78.94
93.07
109.10
Intensity, cps(×10⁶)
m/z, Da
b
Frequency
Relative content(×10⁵)
c
- log10 (P)
d
Observed - log10(P)
Expected - log10(P)
e
- log10 (P)
DPP6
Mb
Chromosome 2
DPP6 Expression
f
Breast
Liver
Brain
Fat
Heart
Kidney
Spleen
Skin
Lung
P
g
L
P
1d
1w
2w
3w
4w
5w
6w