Supplementary

Changes of serum amino acid profiles by epidermal growth factor receptor mutation and benzo[a]pyrene in mouse lung tumorigenesis

Pinpin Lin [1], Yi-Rong Chen[2], Chao-Yu Chen[3], Ya-Ting Chang[3], Jhih-Sheng Chen[3], Ming-Hsien Tsai[1], Cheng-Chin Kuo[4], Hui-Ling Lee[3*]

[1]*National Institute of Environmental Health Sciences, National Health Research Institutes, Zhunan, Miaoli County 350, Taiwan*

[2]*Institute of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Miaoli County 350, Taiwan*

[3]*Department of Chemistry, Fu Jen Catholic University, Xinzhuang Dist, New Taipei City 24205 Taiwan*

[4]*Institute of Cellular and System Medicine, National Health Research Institutes, Zhunan, Miaoli County 350, Taiwan*

**Corresponding authors: Hui-Ling Lee, Ph.D.*

Department of Chemistry, Fu Jen Catholic University, No.510 Zhongzheng Rd. Xinzhuang Dist, New Taipei City 24205 Taiwan.

Tel: 886-2-29053573; Fax: 886-2-29023209; e-mail:076308@mail.fju.edu.tw

Supplementary Figure legends

Fig. S1 The results of principal component analysis (PCA) of serum from the vehicle mutant control (circle ○) and vehicle wild-type (filled triangle ▲) (A) 16 weeks old, (B) 32 weeks old, (C) 16 weeks old, and (D) 32 weeks old using positive ion modes and negative ion mode, respectively (n = 9).

Fig. S2 Typical LC−ESI−TOF total ion chromatograms of serum. Metabolites were extracted from serum from (A) mutant control, (B) 0.1 mg BaP-treated mice, (C) 2.0 mg BaP-treated mice for positive ion mode, (D) mutant control, (E) 0.1 mg BaP-treated mice ,and (F) 2.0 mg BaP-treated mice for negative ion mode.

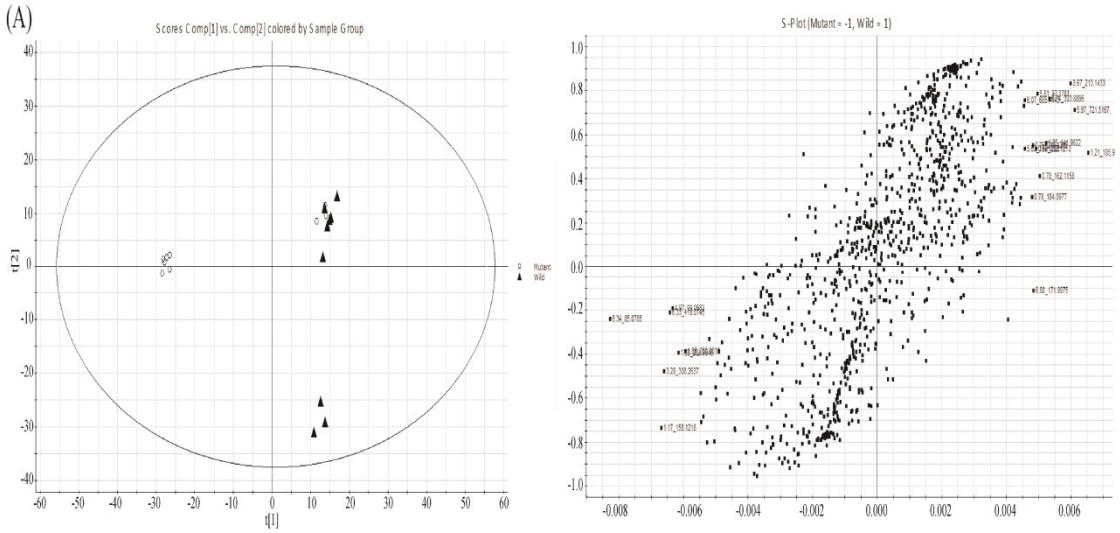
Table S1A The list of significantly changed metabolites ($p < 0.05$) in serum of vehicle groups, 0.1 mg/mouse BaP and 2 mg/mouse BaP for 16 weeks using positive ion modes

Table S1B The list of significantly changed metabolites ($p < 0.05$) in serum of vehicle groups, 0.1 mg/mouse BaP and 2 mg/mouse BaP for 16 weeks using negative ion modes.

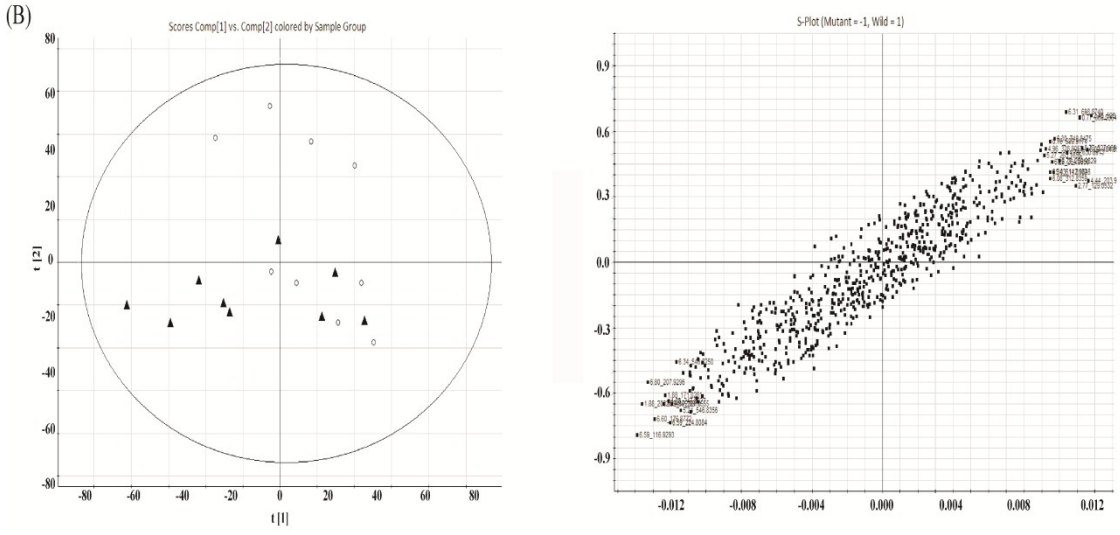
Table S2 Amino acid concentrations in the sera of EGFR mutant control and wild-type control mice at 16 and 32 weeks old.

Supplementary

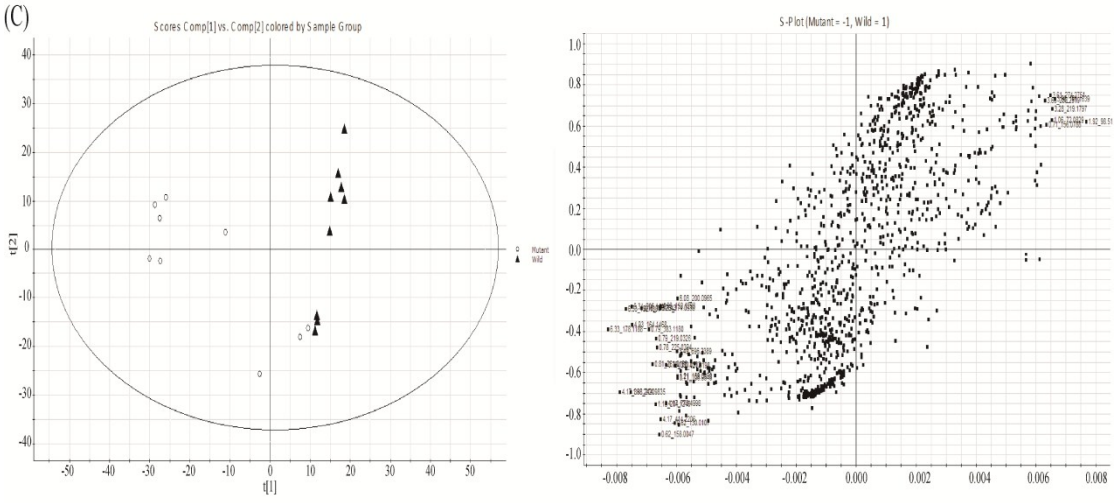
1


(A)
Scores Comp[1] vs. Comp[2] colored by Sample Group
t[2]
t[1]
Mutant
Wild
S-Plot (Mutant = -1, Wild = 1)

2


(B)
Scores Comp[1] vs. Comp[2] colored by Sample Group
t[2]
t[1]
S-Plot (Mutant = -1, Wild = 1)

3



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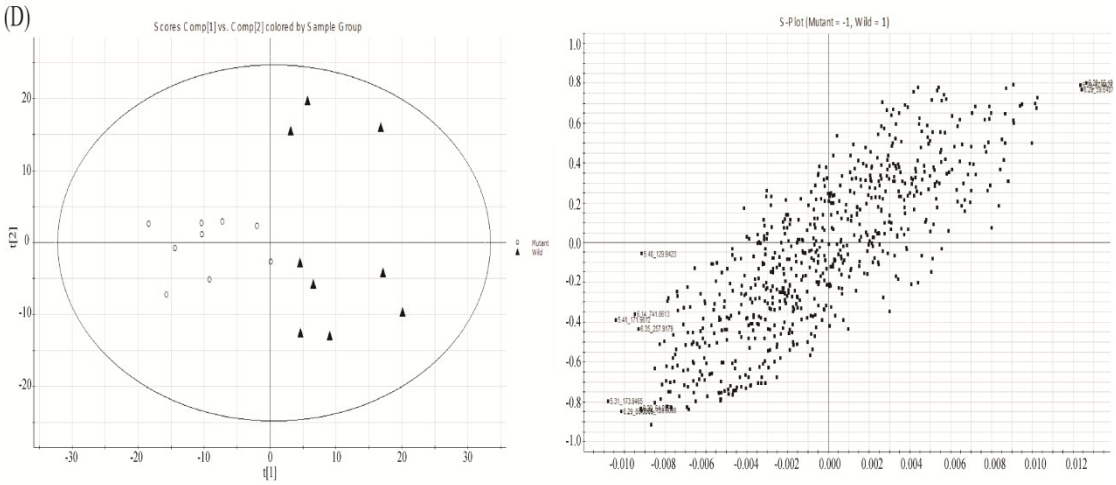


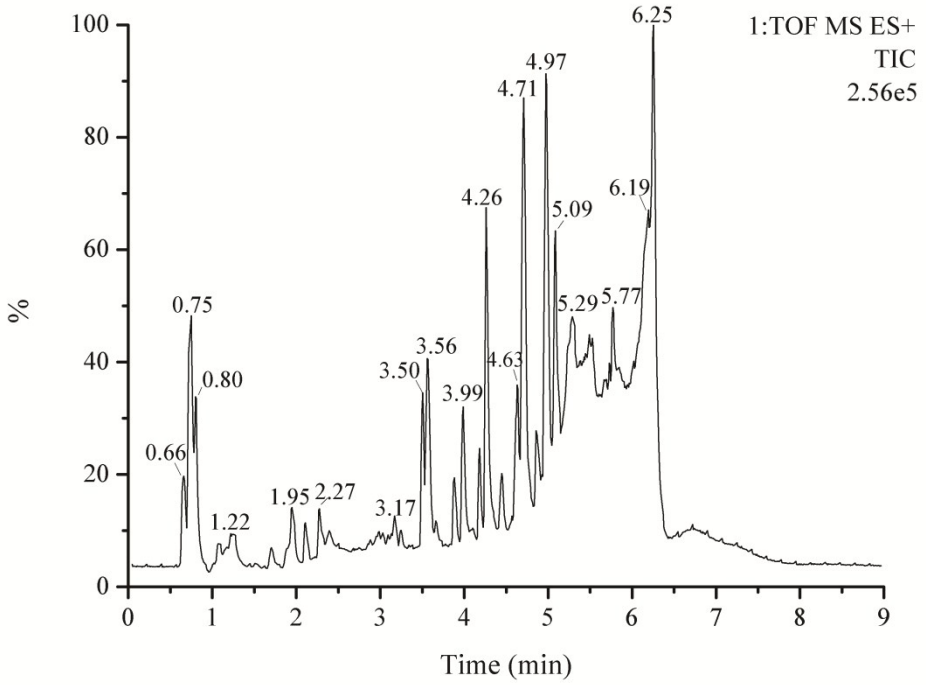
2

3 **Fig. S1** The results of principal component analysis (PCA) of serum from the vehicle
4 mutant control (circle ○) and vehicle wild-type (filled triangle ▲) (A) 16 weeks old,
5 (B) 32 weeks old, (C) 16 weeks old, and (D) 32 weeks old using positive ion modes
6 and negative ion mode, respectively (n = 9).

7

8

(A)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TIC
2.56e5
%
Time (min)
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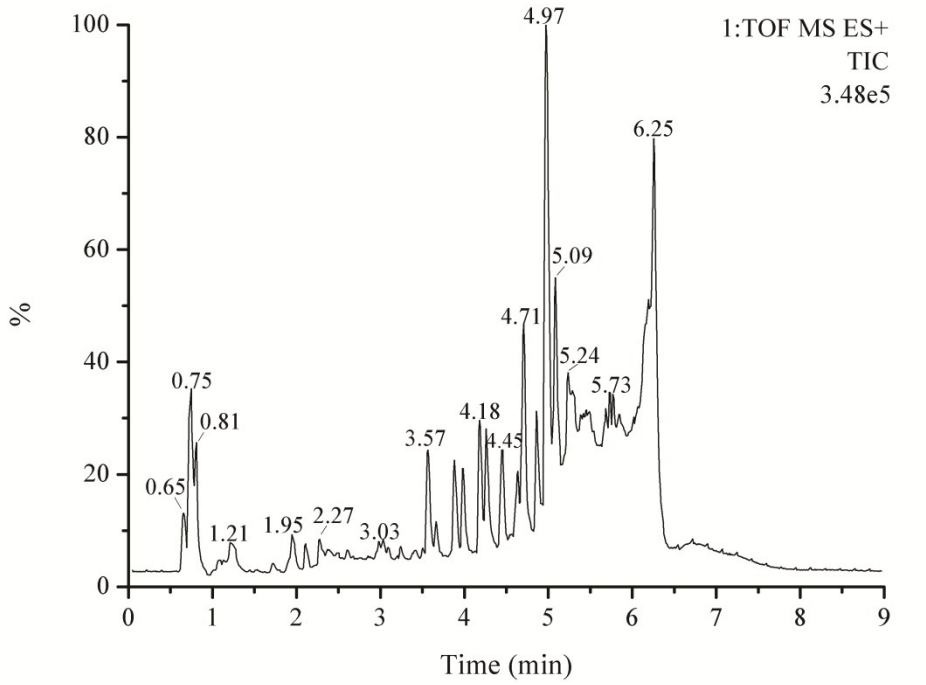
0.75
0.80
1.22
1.95
2.27
3.17
3.50
3.56
3.99
4.26
4.63
4.71
4.97
5.09
5.29
5.77
6.19
6.25

1

(B)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%
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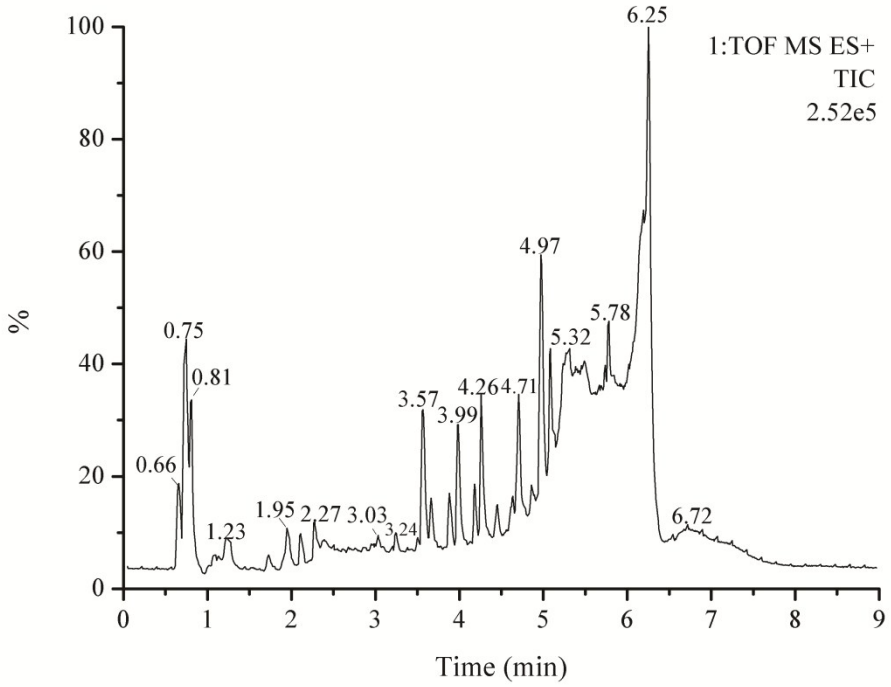
0.75
0.81
1.21
1.95
2.27
3.03
3.57
4.18
4.45
4.71
4.97
5.09
5.24
5.73
6.25

2

(C)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%
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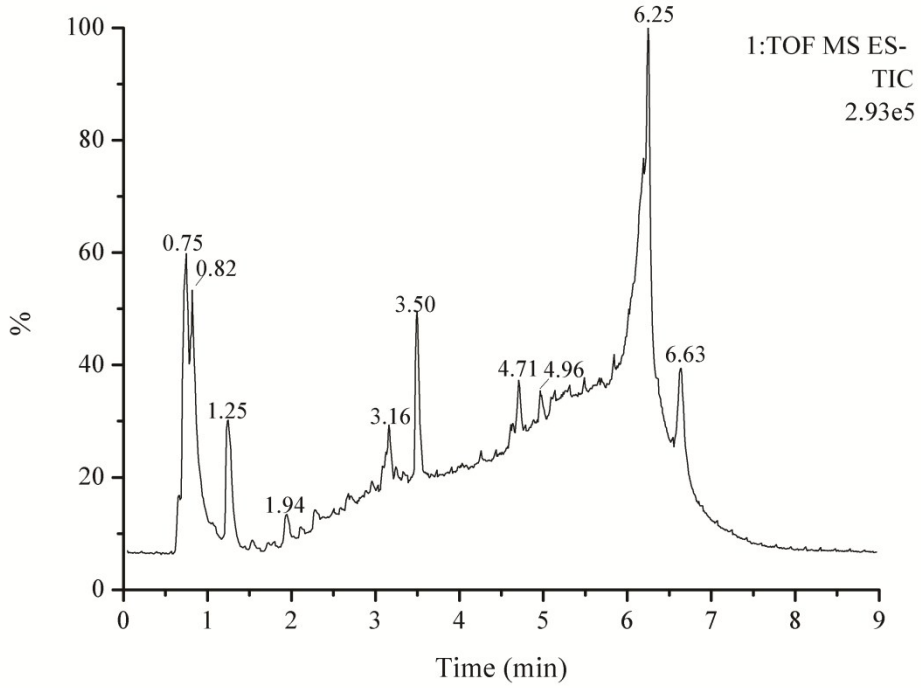
0.81
1.23
1.95
2.27
3.03
3.24
3.57
3.99
4.26
4.71
4.97
5.32
5.78
6.25
6.72

1

(D)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TIC
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%
Time (min)
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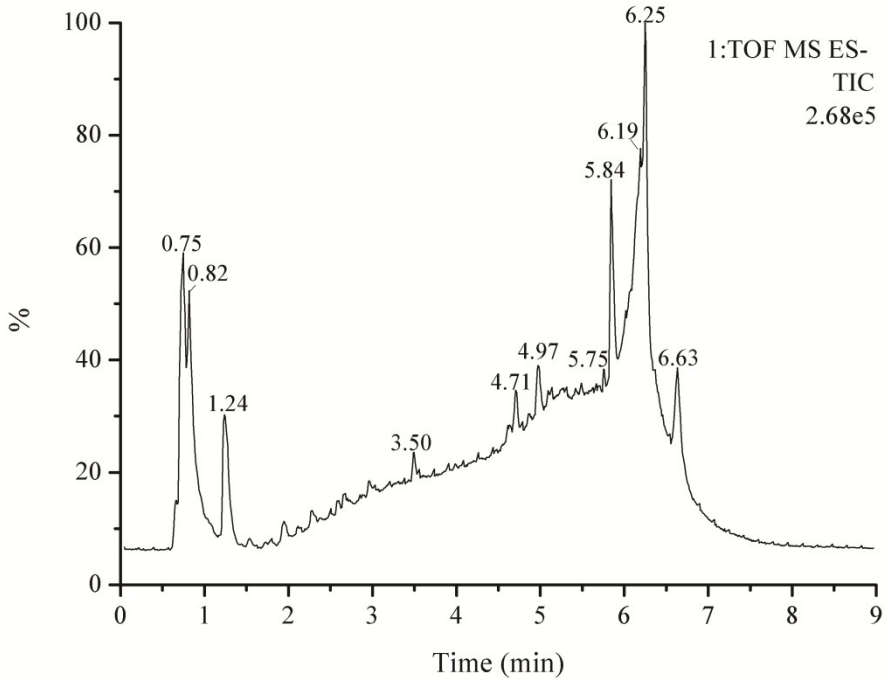
1.25
1.94
3.16
3.50
4.71
4.96
6.25
6.63

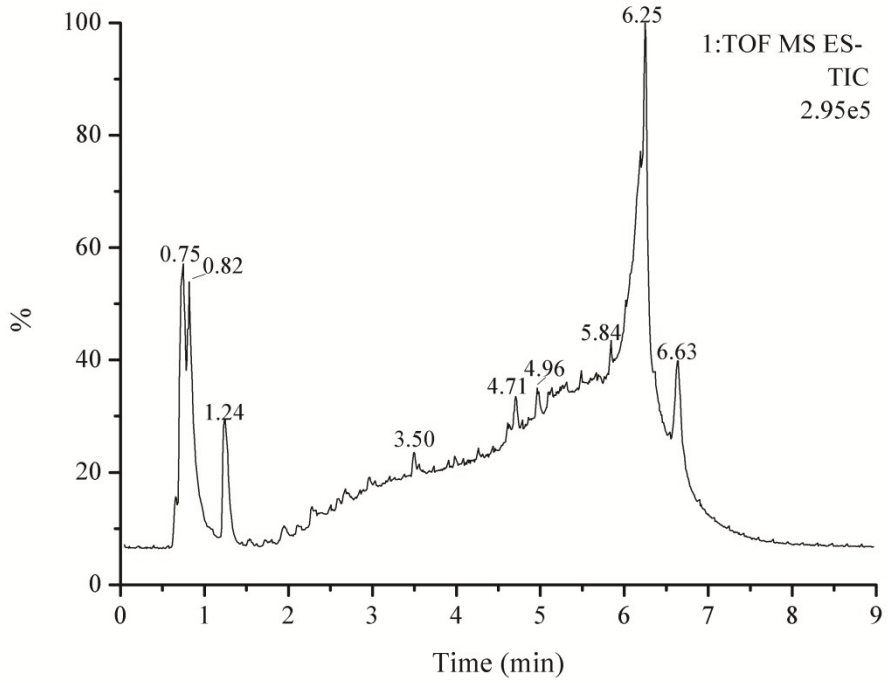
2

(E)



1

(F)



2

3 **Fig. S2** Typical LC−ESI−TOF total ion chromatograms of serum.

4 Metabolites were extracted from serum from (A) mutant control, (B) 0.1 mg BaP-

5 treated mice, (C) 2.0 mg BaP-treated mice for positive ion mode, (D) mutant control,

1 (E) 0.1 mg BaP-treated mice ,and (F) 2.0 mg BaP-treated mice for negative ion mode.

2

Table S1A The list of significantly changed metabolites ($p < 0.05$) in serum of vehicle groups, 0.1 mg/mouse BaP and 2 mg/mouse BaP for 16 weeks using positive ion modes.

Retention time (min)	Detected mass $[M+H]^+$	Name[a]	Database	VIP value	Fold change (over control)
0.73	123.0927	2-Ethyl-3-methylpyrazine	HMDB41254	2.34	2.02
0.77	104.1044	Choline	C00114	1.41	2.18
0.79	151.1445	(E)-4,8-Dimethyl-1,3,7-nonatriene	HMDB35792	2.00	0.34
0.8	114.0692	Creatinine	HMDB00562	1.29	1.64
0.8	120.0696	L-Threonine	HMDB00167	1.09	0.81
0.82	126.0258	Taurine	HMDB00251	1.26	2.00
0.85	132.0803	3-Methylindole	HMDB00466	1.21	1.55
1.07	146.0987	4-Guanidinobutanoic acid	HMDB03464	1.80	0.34
1.93	132.0941	L-Leucine	HMDB00687	1.38	0.76
2.11	166.0822	L-Phenylalanine	HMDB00159	1.40	0.58
4.09	325.3254	6,8-Heneicosanedione	HMDB35570	2.19	0.03
5.43	100.0751	2,5-Dihydro-2,4-dimethyloxazole	HMDB40518	1.13	1.63
5.48	675.5012	PA(15:0/19:1(9Z))	LMGP10010147	2.09	3.10
6.15	150.0599	L-Methionine	HMDB00696	0.94	0.86

[a] these putative metabolites were identified by comparing with METLIN (http://metlin.scripps.edu) and HMDB (http://www.hmdb.ca/) databases.

Table S1B The list of significantly changed metabolites ($p < 0.05$) in serum of vehicle groups, 0.1 mg/mouse BaP and 2 mg/mouse BaP for 16 weeks using negative ion modes.

Retention time (min)	Detected mass $[M-H]^-$	Name[a]	Database	VIP value	Fold change (over control)
0.82	132.0428	L-Aspartic Acid	HMDB00191	1.34	0.64
0.83	174.1018	Argininic acid	HMDB03148	2.03	0.68
1.72	180.0782	L-Tyrosine	HMDB00158	1.92	0.65
1.93	59.0244	Urea	HMDB00294	1.75	0.56
1.95	188.0265	Kynurenic acid	HMDB00715	1.72	0.56
2.11	164.0847	L-Phenylalanine	HMDB00159	1.83	0.72
2.28	203.0943	L-Tryptophan	HMDB00929	1.89	0.67
2.96	187.1103	N-Alpha-acetyllysine	HMDB00446	1.95	0.71

[a] these putative metabolites were identified by comparing with METLIN (http://metlin.scripps.edu) and HMDB (http://www.hmdb.ca/) databases.

Table S2 Amino acid concentrations in the sera of EGFR mutant control and wild-type control mice at 16 and 32 weeks old.

	16 weeks old						32 weeks old					
	Mutant control (n = 14)			Wild Type (n = 20)			Mutant control (n = 11)			Wild Type (n = 8)		
	Conc. (mM)	±	SD[a]	Conc. (mM)	±	SD[a]	Conc. (mM)	±	SD[a]	Conc. (mM)	±	SD[a]
Amino acids												
Phe	0.057	±	0.022	0.055	±	0.028	0.064	±	0.018[c]	0.089	±	0.013
Tyr	0.070	±	0.019	0.063	±	0.016	0.105	±	0.028[c]	0.163	±	0.046
Ala	0.194	±	0.056	0.206	±	0.092	0.177	±	0.056[c]	0.286	±	0.067
Pro	0.081	±	0.019	0.073	±	0.03	0.041	±	0.012[d]	0.062	±	0.01
Val	0.166	±	0.034	0.144	±	0.042	0.109	±	0.025[b]	0.128	±	0.014
Leu	0.113	±	0.029	0.098	±	0.045	0.169	±	0.066	0.189	±	0.033
Met	0.040	±	0.012	0.038	±	0.016	0.031	±	0.007	0.032	±	0.003
Trp	0.060	±	0.019	0.054	±	0.022	0.106	±	0.026[d]	0.149	±	0.022
Thr	0.079	±	0.027	0.071	±	0.024	0.033	±	0.009[b]	0.04	±	0.004
Gln	0.294	±	0.070	0.34	±	0.087	0.237	±	0.029	0.225	±	0.020
Arg	0.057	±	0.027	0.058	±	0.021	0.033	±	0.007	0.034	±	0.002
His	0.029	±	0.012	0.035	±	0.013	0.014	±	0.004[b]	0.018	±	0.003

[a] Concentration (mM) ± standard deviation

[b] *$p<0.05$, [c]**$p<0.01$, [d] ***$p<0.001$