



**Fig.S1.** Typical base peak chromatograms of plasma samples from control subjects (up) and HCC patients (down) obtained by UPLC-MS.

**Table S1.** Clinical characteristics of healthy humans and HCC patients.

| <b>Parameters</b>  | <b>Healthy Controls (n = 65)</b> | <b>HCC Patients (n =70)</b> |
|--------------------|----------------------------------|-----------------------------|
| Age                | 55.4 ± 8.5                       | 56.3 ± 6.7                  |
| Male/Female        | 36/29                            | 37/33                       |
| AFP value (ng/mL)  | 20 ± 1.3                         | 454.7 ± 187.4               |
| HBsAg (positive %) | -                                | 100                         |
| ALP (IU/L)         | 88.0 ± 16.7                      | 164.9 ± 31.4                |
| ALT (IU/L)         | 16.2 ± 5.3                       | 75.4 ± 15.1                 |
| AST (IU/L)         | 26.9± 13.7                       | 79.0± 12.6                  |
| D-BIL (µmol/L)     | 2.0 ± 0.8                        | 6.4 ± 1.3                   |
| T-BIL (µmol/L)     | 8.5 ± 2.1                        | 19.6 ± 5.3                  |

Note: AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; D-BIL, direct bilirubin; T-BIL, total bilirubin. Data are presented as mean ± SE.

**Table S2.** Information of plasma metabolites related to HCC detected by UPLC/MS.

| No | Rt (min) | m/z       | Compound ID | Adducts | Formula  | Mass Error (ppm) | Description                           | Anova (p) |
|----|----------|-----------|-------------|---------|--|------------------|---------------------------------------|-----------|
| 1  | 11.00    | 1010.9677 | HMDB01541   | M-H     | C <sub>20</sub> H <sub>30</sub> N <sub>10</sub> O <sub>26</sub> P <sub>6</sub> | -0.84            | Guanosine hexaphosphate adenosine     | 0.0076    |
| 2  | 0.50     | 952.6796  | HMDB12237   | M-H     | C <sub>50</sub> H <sub>100</sub> N <sub>13</sub> P                             | -0.67            | Inositol-P-ceramide                   | 0.0354    |
| 3  | 16.77    | 567.3154  | HMDB02596   | M-H     | C <sub>30</sub> H <sub>48</sub> O <sub>10</sub>                                | -0.57            | Deoxycholic acid 3-glucuronide        | 0.0322    |
| 4  | 10.20    | 477.1046  | HMDB33650   | M-H     | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>                                | 0.51             | Estragonoside                         | 0.0000    |
| 5  | 9.00     | 447.0942  | HMDB37359   | M-H     | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>                                | 0.06             | Azaleatin 3-arabinoside               | 0.0000    |
| 6  | 4.86     | 433.1149  | HMDB60772   | M-H     | C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>                                | 0.94             | 4'-Hydroxyfenoprofen glucuronide      | 0.0213    |
| 7  | 10.94    | 423.1360  | HMDB60786   | M-H     | C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>9</sub>                  | -0.51            | 6-Hydroxymelatonin glucuronide        | 0.0000    |
| 8  | 10.39    | 355.1205  | HMDB02040   | 2M-H    | C <sub>10</sub> H <sub>10</sub> O <sub>3</sub>                                 | 0.88             | 4-Methoxycinnamic acid                | 0.0000    |
| 9  | 6.70     | 116.0366  | HMDB00532   | M-H     | C <sub>4</sub> H <sub>7</sub> N <sub>3</sub> O                                 | 1.92             | Acetylglycine                         | 0.0100    |
| 10 | 0.91     | 88.0401   | HMDB00271   | M-H     | C <sub>3</sub> H <sub>7</sub> N <sub>2</sub> O                                 | -0.28            | Sarcosine                             | 0.0500    |
| 11 | 7.20     | 653.2793  | HMDB30286   | 2M-H    | C <sub>19</sub> H <sub>21</sub> N <sub>4</sub> O                               | -0.57            | Coreximine                            | 0.0100    |
| 12 | 12.86    | 349.2021  | HMDB02664   | M+H     | C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>                                 | 0.09             | Prostaglandin E3                      | 0.0000    |
| 13 | 5.87     | 338.0883  | HMDB10362   | M+H     | C <sub>15</sub> H <sub>19</sub> N <sub>3</sub> O                               | 0.51             | 6-Hydroxy-5-methoxyindole glucuronide | 0.0128    |
| 14 | 16.05    | 335.2236  | HMDB01085   | M+H     | C <sub>20</sub> H <sub>34</sub> O <sub>4</sub>                                 | 0.31             | Leukotriene B <sub>4</sub>            | 0.0014    |
| 15 | 14.64    | 331.1923  | HMDB04031   | M+H     | C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>                                 | 0.44             | 11b-Hydroxyprogesterone               | 0.0100    |
| 16 | 17.83    | 315.1968  | HMDB06254   | M+H     | C <sub>20</sub> H <sub>30</sub> O <sub>3</sub>                                 | 0.79             | 4-Hydroxyretinoic acid                | 0.0007    |
| 17 | 4.82     | 308.0983  | HMDB00230   | M+H     | C <sub>11</sub> H <sub>21</sub> N <sub>3</sub> O                               | -0.17            | N-Acetylneuraminic acid               | 0.0027    |
| 18 | 0.72     | 285.0633  | HMDB59998   | M+H     | C <sub>12</sub> H <sub>16</sub> O <sub>8</sub>                                 | 0.96             | Diphenol glucuronide                  | 0.0053    |
| 19 | 7.64     | 261.1340  | HMDB00064   | 2M+H    | C <sub>4</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>                   | 0.88             | Creatine                              | 0.0000    |
| 20 | 10.23    | 253.0505  | HMDB04195   | M+H     | C <sub>7</sub> H <sub>16</sub> N <sub>2</sub> O <sub>6</sub> S                 | 1.02             | 5-L-Glutamyl-aurine                   | 0.0074    |
| 21 | 7.67     | 245.0931  | HMDB13713   | M+H     | C <sub>13</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>                  | -0.45            | N-acetyltryptophan                    | 0.0261    |
| 22 | 2.72     | 229.1189  | HMDB00162   | 2M+H    | C <sub>5</sub> H <sub>11</sub> N <sub>2</sub> O                                | -0.96            | L-Proline                             | 0.0069    |
| 23 | 8.36     | 483.2075  | HMDB02224   | 2M+H    | C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>                  | 0.68             | 5-Methyldeoxycytidine                 | 0.0100    |

**Table S3** Summary of metabolic pathways of significantly changed metabolites with MetPA.

| <b>Pathways</b>                          | <b>Total</b> | <b>Expected</b> | <b>Hits</b> | <b>Raw p</b> | <b>Holm adjust</b> | <b>FDR</b> | <b>Impact</b> |
|--|--------------|-----------------|-------------|--------------|--------------------|------------|---------------|
| Arginine and proline metabolism          | 77           | 0.3519          | 3           | 0.0043       | 0.34535            | 0.34535    | 0.1403        |
| Glycine, serine and threonine metabolism | 48           | 0.2194          | 2           | 0.0191       | 1                  | 0.764      | 0.0504        |
| Taurine and hypotaurine metabolism       | 20           | 0.0914          | 1           | 0.0879       | 1                  | 1          | 0             |
| Retinol metabolism                       | 22           | 0.1005          | 1           | 0.0963       | 1                  | 1          | 0             |
| Starch and sucrose metabolism            | 50           | 0.2285          | 1           | 0.2066       | 1                  | 1          | 0.0127        |
| Pentose and glucuronate interconversions | 53           | 0.2422          | 1           | 0.2176       | 1                  | 1          | 0.0090        |
| Arachidonic acid metabolism              | 62           | 0.2833          | 1           | 0.2500       | 1                  | 1          | 0.0088        |
| Aminoacyl-tRNA biosynthesis              | 75           | 0.3428          | 1           | 0.2946       | 1                  | 1          | 0             |
| Steroid hormone biosynthesis             | 99           | 0.4524          | 1           | 0.3706       | 1                  | 1          | 0.0199        |

**Table S4.** Functional pathway category analysis

| Category  | p-value           | Molecules  |
|---|-------------------|--|
| Cellular Compromise                                   | 7.82E-06-3.66E-02 | L-proline,leukotriene B4,creatine  |
| Lipid Metabolism                                      | 7.82E-06-3.99E-02 | L-proline,leukotriene B4,11beta-hydroxyprogesterone,creatine<br>L-proline,sarcosine,leukotriene B4,11beta-hydroxyprogesterone, |
| Small Molecule Biochemistry                           | 7.82E-06-4.67E-02 | creatine   |
| Amino Acid Metabolism                                 | 3.1E-04-5.56E-03  | sarcosine,creatine   |
| Cell Morphology                                       | 3.1E-04-1.26E-02  | leukotriene B4,creatine  |
| Cell-mediated Immune Response                         | 3.1E-04-2.33E-02  | leukotriene B4   |
| Cellular Assembly and Organization                    | 3.1E-04-3.15E-02  | leukotriene B4,creatine  |
| Cellular Movement                                     | 3.1E-04-4.88E-02  | prostaglandin E3,sarcosine,leukotriene B4,creatine   |
| Endocrine System Disorders                            | 3.1E-04-6.18E-03  | prostaglandin E3,leukotriene B4,creatine   |
| Hematological System Development and Function         | 3.1E-04-4.88E-02  | leukotriene B4,creatine,N-acetylneuraminic acid  |
| Immune Cell Trafficking                               | 3.1E-04-4.88E-02  | leukotriene B4,creatine  |
| Inflammatory Response                                 | 3.1E-04-3.48E-02  | leukotriene B4,creatine<br>L-proline,leukotriene B4,creatine,  |
| Neurological Disease                                  | 3.1E-04-4.85E-02  | N-acetylneuraminic acid  |
| Organ Morphology                                      | 3.1E-04-2.12E-02  | leukotriene B4,creatine<br>prostaglandin E3,L-proline,leukotriene B4,  |
| Organismal Injury and Abnormalities                   | 3.1E-04-4.85E-02  | creatine,N-acetylneuraminic acid   |
| Skeletal and Muscular System Development and Function | 3.1E-04-4.26E-02  | creatine   |
| Tissue Morphology                                     | 3.1E-04-1.93E-02  | creatine   |
| Cell Death and Survival                               | 6.19E-04-1.66E-02 | L-proline,creatine   |
| Drug Metabolism                                       | 6.19E-04-4.08E-02 | creatine   |
| Gastrointestinal Disease                              | 6.19E-04-3.39E-02 | prostaglandin E3,creatine  |
| Hematopoiesis   | 6.19E-04-8.64E-03 | leukotriene B4,creatine,N-acetylneuraminic acid  |
| Hypersensitivity Response                             | 6.19E-04-2.12E-02 | leukotriene B4,creatine  |
| Nervous System Development and Function               | 6.19E-04-4.26E-02 | L-proline,sarcosine,creatine   |
| Skeletal and Muscular Disorders                       | 6.19E-04-4.05E-02 | creatine,N-acetylneuraminic acid   |
| Cellular Growth and Proliferation                     | 9.29E-04-1.32E-02 | L-proline,prostaglandin E3,sarcosine,leukotriene B4  |
| Inflammatory Disease                                  | 9.29E-04-7.41E-03 | leukotriene B4,creatine  |
| Organismal Development                                | 9.29E-04-3.09E-02 | L-proline,leukotriene B4,creatine  |
| Protein Synthesis                                     | 9.29E-04-1.57E-02 | L-proline,leukotriene B4,creatine  |
| Respiratory Disease                                   | 9.29E-04-3.74E-03 | leukotriene B4,creatine<br>L-proline,leukotriene B4,creatine,  |
| Cell-To-Cell Signaling and Interaction                | 1.24E-03-4.08E-02 | N-acetylneuraminic acid  |
| Metabolic Disease                                     | 1.24E-03-4.64E-03 | leukotriene B4,creatine  |
| Cancer  | 1.55E-03-2.03E-02 | prostaglandin E3,N-acetylneuraminic acid   |
| Cardiovascular System Development and Function        | 1.55E-03-1.55E-03 | creatine   |
| Hair and Skin Development and Function                | 1.55E-03-3.03E-02 | sarcosine,leukotriene B4   |
| Free Radical Scavenging                               | 1.59E-03-4.55E-02 | L-proline,leukotriene B4,creatine  |
| Carbohydrate Metabolism                               | 1.86E-03-4.67E-02 | leukotriene B4,creatine,N-acetylneuraminic acid  |
| Hereditary Disorder                                   | 1.86E-03-4.05E-02 | leukotriene B4,creatine,N-acetylneuraminic acid  |
| Molecular Transport                                   | 1.86E-03-4.67E-02 | L-proline,sarcosine,leukotriene B4,creatine  |
| Nutritional Disease                                   | 2.17E-03-2.17E-03 | creatine   |
| Cell Cycle  | 2.47E-03-3.09E-03 | L-proline  |
| Hepatic System Development and Function               | 2.47E-03-3.71E-03 | L-proline,leukotriene B4   |
| Hepatic System Disease                                | 2.47E-03-2.47E-03 | creatine   |
| Lymphoid Tissue Structure and Development             | 3.09E-03-3.09E-03 | leukotriene B4   |
| Developmental Disorder                                | 3.4E-03-4.05E-02  | leukotriene B4,creatine,N-acetylneuraminic acid  |
| Energy Production                                     | 3.4E-03-2E-02     | creatine   |
| Nucleic Acid Metabolism                               | 3.4E-03-3.24E-02  | leukotriene B4,creatine  |
| Psychological Disorders                               | 3.4E-03-1.63E-02  | leukotriene B4,creatine  |
| Behavior  | 3.71E-03-3.71E-03 | creatine   |
| Digestive System Development and Function             | 3.71E-03-3.71E-03 | leukotriene B4   |
| Organ Development                                     | 3.71E-03-3.71E-03 | leukotriene B4   |
| Organismal Functions                                  | 3.71E-03-3.78E-02 | leukotriene B4,creatine  |
| Reproductive System Development and Function          | 4.33E-03-4.33E-03 | leukotriene B4   |
| Endocrine System Development and Function             | 4.64E-03-4.64E-03 | L-proline  |
| Cellular Development                                  | 5.37E-03-1.32E-02 | prostaglandin E3,sarcosine,leukotriene B4,creatine   |
| Tumor Morphology                                      | 6.18E-03-6.18E-03 | prostaglandin E3   |
| Renal and Urological Disease                          | 7.41E-03-8.64E-03 | creatine   |
| Cellular Function and Maintenance                     | 8.02E-03-4.11E-02 | leukotriene B4,creatine  |
| Cardiovascular Disease                                | 8.64E-03-2.94E-02 | leukotriene B4,creatine  |

---

|  |                   |                         |
|--|-------------------|-------------------------|
| Connective Tissue Development and Function           | 8.64E-03-8.64E-03 | creatine                |
| DNA Replication, Recombination, and Repair           | 8.64E-03-3.15E-02 | creatine                |
| Tissue Development                                   | 8.64E-03-8.64E-03 | creatine                |
| Cell Signaling                                       | 1.63E-02-3.24E-02 | leukotriene B4,creatine |
| Vitamin and Mineral Metabolism                       | 1.63E-02-1.93E-02 | leukotriene B4,creatine |
| Embryonic Development                                | 2.97E-02-2.97E-02 | sarcosine               |
| Renal and Urological System Development and Function | 3.48E-02-3.48E-02 | sarcosine               |

---

**Table S5.** ROC curve analysis of potential biomarkers in HCC.

| <b>No</b> | <b>Metabolite</b>                     | <b>AUC</b> | <b>T-tests</b> | <b>Log2 FC</b> |
|-----------|---------------------------------------|------------|----------------|----------------|
| 1         | Deoxycholic acid 3-glucuronide        | 0.9362     | 5.34E-28       | -3.9683        |
| 2         | 6-Hydroxymelatonin glucuronide        | 0.9340     | 4.27E-28       | -4.1299        |
| 3         | 4-Methoxycinnamic acid                | 0.9327     | 3.47E-29       | -4.1143        |
| 4         | 11b-Hydroxyprogesterone               | 0.9319     | 4.62E-23       | -4.6511        |
| 5         | 4-Hydroxyretinoic acid                | 0.9316     | 1.68E-28       | -4.1871        |
| 6         | N-Acetylneuraminic acid               | 0.9303     | 1.28E-29       | -4.2756        |
| 7         | N-acetyltryptophan                    | 0.9301     | 7.39E-30       | -4.2299        |
| 8         | 6-Hydroxy-5-methoxyindole glucuronide | 0.9299     | 6.16E-19       | 4.3730         |
| 9         | 5-L-Glutamyl-aurine                   | 0.9298     | 3.68E-22       | 4.2142         |
| 10        | 5-Methyldeoxycytidine                 | 0.9252     | 1.69E-17       | 4.2020         |
| 11        | L-Proline                             | 0.9223     | 1.81E-18       | 4.2110         |
| 12        | Acetylglycine                         | 0.9138     | 4.08E-12       | 4.0813         |
| 13        | Leukotriene B4                        | 0.9077     | 1.62E-07       | 2.0671         |
| 14        | Diphenol glucuronide                  | 0.8986     | 1.37E-10       | 4.2491         |
| 15        | Creatine                              | 0.8866     | 1.49E-10       | 4.2471         |
| 16        | Sarcosine                             | 0.8615     | 3.19E-08       | 4.1774         |
| 17        | Estragonoside                         | 0.8477     | 2.00E-11       | 4.0628         |
| 18        | 4'-Hydroxyfenoprofen glucuronide      | 0.8154     | 5.19E-09       | 4.0231         |
| 19        | Azaleatin 3-arabinoside               | 0.8104     | 7.83E-07       | 4.0316         |
| 20        | Coreximine                            | 0.7668     | 4.09E-07       | 4.0219         |
| 21        | Guanosine hexaphosphate adenosine     | 0.7576     | 0.000812106    | 4.0378         |
| 22        | Prostaglandin E3                      | 0.7556     | 8.07E-07       | 3.9910         |
| 23        | Inositol-P-ceramide                   | 0.7022     | 3.09E-05       | 3.9865         |