

## **Supplementary Material**

### ***M1: Plasma sample preparation***

A volume of 100  $\mu\text{L}$  of thawed plasma was added with 400  $\mu\text{L}$  of sodium phosphate buffer (0.045 M  $\text{K}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$ , dissolved in  $\text{D}_2\text{O}$ , 0.05% TSP, pH = 7.4). Following vortexing (5 min) and centrifugation (14 000 rpm, 10 min, 4°C), 450  $\mu\text{L}$  of supernatant was transferred to a 5 mm NMR tube for NMR measurement.

After NMR experiment, the plasma samples in the NMR tube were transferred to centrifuge tube. Rinse the NMR tube with an appropriate amount of water to ensure complete sample transfer. Subsequently, the polar and nonpolar extracts were prepared by using the biphasic extraction method. Briefly, 750  $\mu\text{L}$  of ice-cold chloroform/methanol (1:2, *v:v*) was added to 100  $\mu\text{L}$  of plasma. Samples were vortexed and incubated at  $-20^\circ\text{C}$  for 1 h. Subsequently, cold chloroform (250  $\mu\text{L}$ ) and cold water (350  $\mu\text{L}$ ) were added to form a biphasic system. Samples were mixed (2 min), left on ice (5 min), and centrifuged (14 000 rpm, 10 min, 4°C), resulting in a biphasic mixture. The lower nonpolar layer was firstly collected into a new centrifuge tube. Then, another 450  $\mu\text{L}$  of ice-cold chloroform was added to the remaining methanol/water phase. Samples were vortexed for 2 min, put on ice for 5 min, and then centrifuged at 14 000 rpm for 10 min at 4°C. The lower organic phase was obtained and combined together with the first round of extraction, and then the nonpolar fraction was dried. The remaining polar phase was then centrifuged at 14 000 rpm for 10 min at 4°C to obtain a clean supernatant containing polar metabolites. The sample was dried under  $\text{N}_2$ . The dried polar extract was resuspended in 100  $\mu\text{L}$  of methanol/water (1:1, *v:v*) for MS-based metabolomics analysis.

### ***M2: Liver tissue preparation***

50 mg of the liver tissue was accurately weighed and homogenized with 250  $\mu\text{L}$  of water using a multi-sample tissue grinder (Shanghai Jingxin Industrial

Development Co., Ltd., China). Then, 500  $\mu$ L of ice-cold ( $-20^{\circ}\text{C}$ ) MeOH was added into homogeneous solution, and the final ratio of methanol to water in the system was 2:1 (*v:v*). The mixture was vortexed for 10 min, placed on ice for 5 min, and subjected to centrifuge at 14 000 rpm for 10 min at  $4^{\circ}\text{C}$ . The entire supernatant was transferred to a clean centrifuge tube (the precipitate should be preserved) and dried with  $\text{N}_2$ . The residue was re-dissolved in 600  $\mu$ L of sodium phosphate buffer (0.15 M  $\text{K}_2\text{HPO}_4$ - $\text{NaH}_2\text{PO}_4$ , dissolved in  $\text{D}_2\text{O}$ , 0.001% TSP, pH = 7.4). After vortexing and centrifugation, 550  $\mu$ L of supernatant was transferred into a 5 mm NMR tube for NMR measurement.

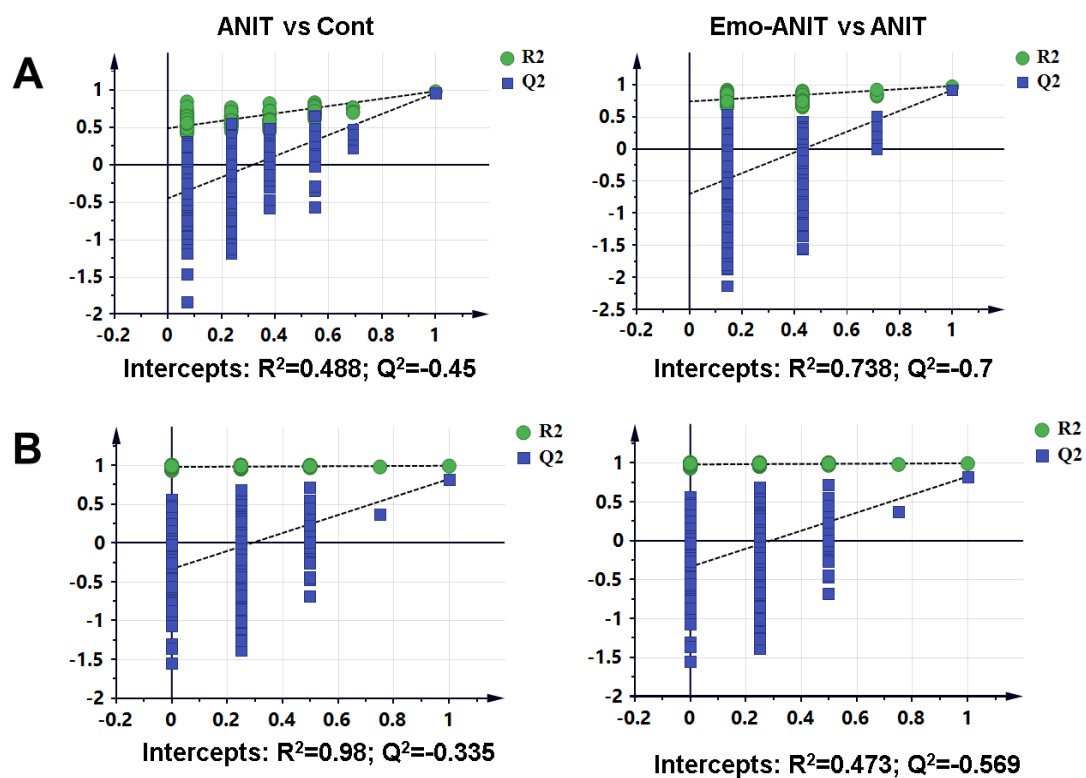
After NMR detection, the samples were transferred to centrifuge tube, dried, re-dissolved in 50  $\mu$ L methanol/water (2:1, *v:v*), vortexed, and centrifuged. The supernatant was collected for MS-based metabolomics analysis.

### ***M3: NMR analysis***

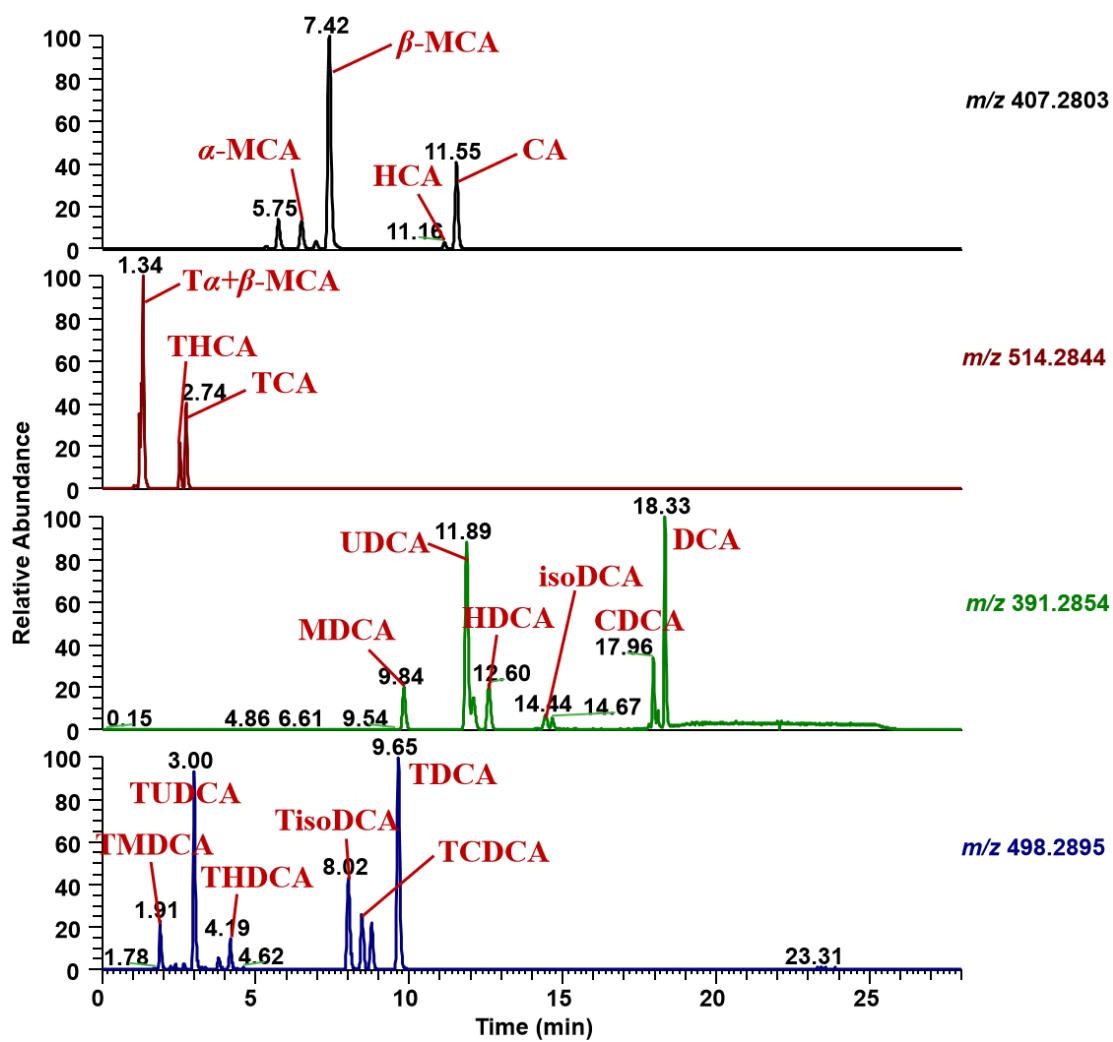
All NMR spectra were obtained at 298 K on a Bruker Ascend 600 MHz NMR spectrometer (Bruker Biospin, Karlsruhe, Germany) equipped with a TXI cryogenic probe. TSP- $\text{d}_4$  was used as the internal standard for the chemical shifts. The plasma sample was analyzed using the standard one-dimensional cpmgpr1d pulse sequence by the suppression of water resonance through presaturation (22.78 Hz). The acquisition parameters were as follows: spectral width (SW), 10330 Hz; the transmitter frequency offset (O1P), 4.70 ppm; the number of scans (NS), 128; relaxation delay (RD), 2 s; data points (DP), 32 k. The one-dimensional NMR spectra of the liver tissue extract was acquired utilizing the noesygppr1d pulse sequence. The sequential parameters employed were as follows: SW, 12019.230 Hz; RD, 4 s; NS, 128; DP, 60 k. Additionally, two-dimensional NMR experiments including *J*-resolved spectroscopy (*J*-Res),  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC, and HSQC were recorded for the signal assignment and confirmation of metabolites.

### ***M4: UHPLC/Q-Orbitrap-MS analysis***

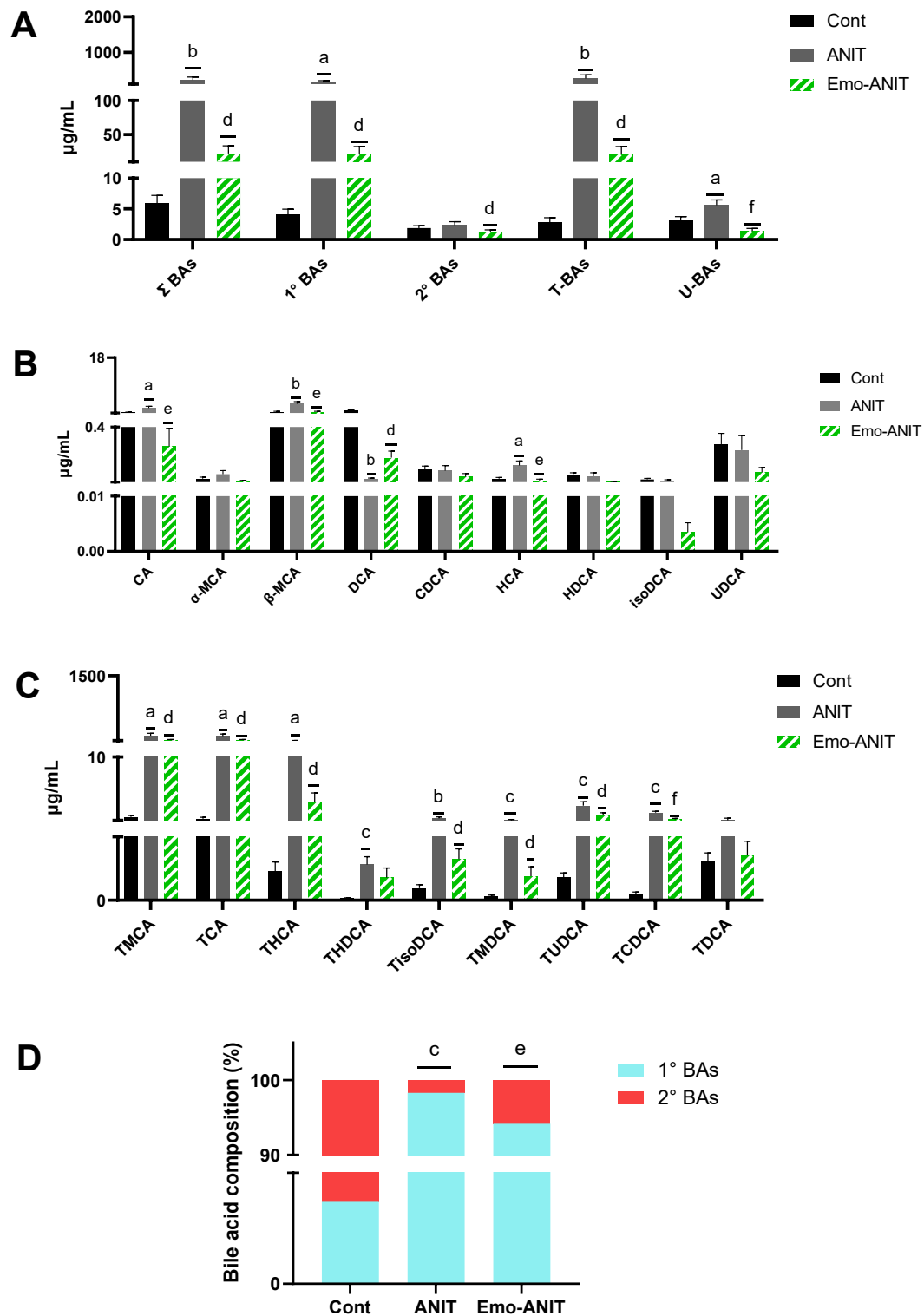
Untargeted metabolomics analysis was performed on an Ultimate 3000 UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation was achieved on a Waters ACQUITY UPLC HSS T<sub>3</sub> column (2.1 × 100 mm, 1.8 μm). The mobile phase was composed of water with 0.1% formic acid (A) and acetonitrile (D). The elution gradient was as follows: 0–7 min, 5%–100% D; 7–10 min, 100% D. The column temperature was maintained at 30°C, and the flow rate was 0.3 mL/min. The sample injection volume was 2 μL. MS data were acquired through Full MS/dd-MS<sup>2</sup> (TopN) scan method operated in positive and negative ion modes. The heated electrospray ionization (HESI) source conditions were as follows: spray voltage, -3.0 kV/+3.5 kV; capillary temperature, 320°C; and auxiliary gas heater temperature, 350°C. The mass range of full MS was *m/z* 60–900. The acquisition resolution of MS<sup>1</sup> and MS<sup>2</sup> was set as 70,000 and 17,500, respectively. The normalized collision energy (NCE) was 10/30/50 V.



Supplementary Figure 1 The permutation test results for the OPLS-DA model using plasma (A) and liver (B) NMR-based metabolomics data.

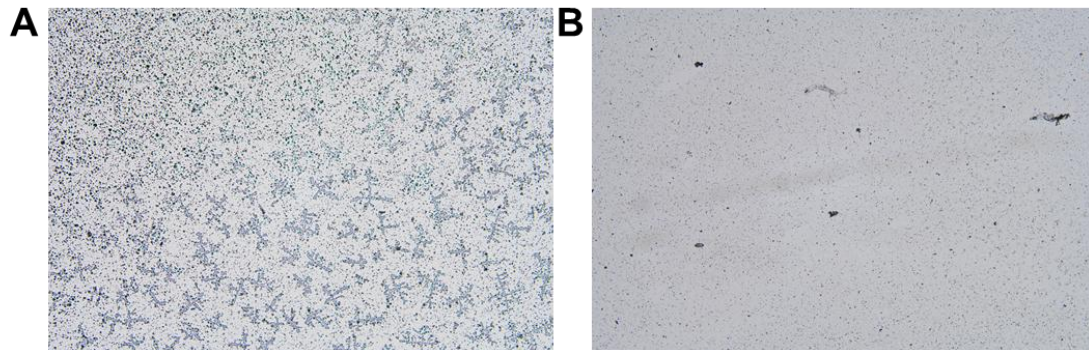


Supplementary Figure 2 The extracted ion chromatograms of 20 BAs.

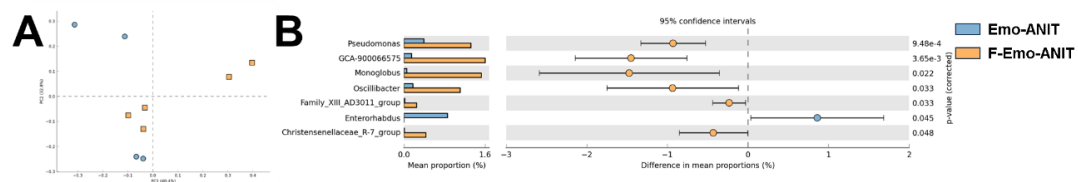


**Supplementary Figure 3 Effect of Emo on serum BAs in mice with ANIT-induced CLI. A: Changes of  $\Sigma$  BAs, 1° BAs, 2° BAs, T-BAs and U-BAs; B: Changes of U-BAs; C: Changes of T-BAs; D: Ratio of 1° BAs to 2° BAs in the serum. <sup>a</sup> $P < 0.05$  vs Cont group; <sup>b</sup> $P < 0.01$  vs Cont group; <sup>c</sup> $P < 0.001$  vs Cont**

group; <sup>d</sup> $P < 0.05$  *vs* ANIT group; <sup>e</sup> $P < 0.01$  *vs* ANIT group; <sup>f</sup> $P < 0.01$  *vs* ANIT group,  $n = 8$ .



**Supplementary Figure 4** Smear validation of ABX-induced depletion of intestinal flora. A: Before ABX administration; B: After ABX administration.



**Supplementary Figure 5** FMT revealed the similar microbiota profile to Emo treatment. A: PCA analysis; B: STAMP analysis between Emo-ANIT and F-Emo-ANIT groups. Genera with a significance of  $P < 0.05$  are shown.

**Supplementary Table 1 Differential plasma metabolites between Cont and ANIT group identified from <sup>1</sup>H-NMR-based metabolomics study**

| No. | Metabolites                     | Moieties                         | $\delta_H$ | Multiplicity | ANIT <i>vs</i> Cont |
|-----|---------------------------------|----------------------------------|------------|--------------|---------------------|
| 1   | Leucine                         | $\delta$ -CH <sub>3</sub>        | 0.97       | t            | ↑                   |
| 2   | Valine                          | CH <sub>3</sub>                  | 0.99       | d            | ↑                   |
|     |                                 | CH <sub>3</sub>                  | 1.06       | d            |                     |
| 3   | Isoleucine                      | $\beta$ -CH <sub>3</sub>         | 1.01       | d            | ↑                   |
| 4   | Lactate                         | CH <sub>3</sub>                  | 1.35       | d            | ↑                   |
|     |                                 | CH                               | 4.12       | q            |                     |
| 5   | 3-Hydroxybutyrate               | CH <sub>3</sub>                  | 1.21       | d            | ↑                   |
| 6   | Alanine                         | CH <sub>3</sub>                  | 1.48       | d            | ↑                   |
| 7   | Acetate                         | CH <sub>3</sub>                  | 1.94       | s            | ↑                   |
| 8   | Proline                         | $\beta$ -CH <sub>2</sub>         | 2.07       | m            | ↑                   |
| 9   | Acetone                         | CH <sub>3</sub>                  | 2.23       | s            | ↑                   |
| 10  | Acetoacetate                    | CH <sub>3</sub>                  | 2.29       | s            | ↑                   |
|     |                                 | $\beta$ -CH <sub>2</sub>         | 2.16       | m            |                     |
| 11  | Glutamine                       | $\gamma$ -CH <sub>2</sub>        | 2.47       | m            | ↑                   |
|     |                                 | CH                               | 3.79       | t            |                     |
| 12  | Creatine                        | CH <sub>3</sub>                  | 3.06       | s            | ↑                   |
| 13  | Choline                         | N(CH <sub>3</sub> ) <sub>3</sub> | 3.22       | s            | ↑                   |
| 14  | Taurine                         | CH <sub>2</sub> SO <sub>3</sub>  | 3.25       | t            | ↑                   |
|     |                                 | NCH <sub>2</sub>                 | 3.43       | t            |                     |
| 15  | Trimethylamine- <i>N</i> -oxide | N(CH <sub>3</sub> ) <sub>3</sub> | 3.28       | s            | ↑                   |
| 16  | Glycerophosphorylcholine        | N(CH <sub>3</sub> ) <sub>3</sub> | 3.38       | s            | ↑                   |
| 17  | Glycine                         | CH <sub>2</sub>                  | 3.57       | s            | ↑                   |
| 18  | Phosphocreatine                 | CH <sub>2</sub>                  | 3.95       | s            | ↑                   |

Note: s, singlet; d: doublet; t: triplet; q: quartet; m: multiplet; dd: doublet of doublets; ddd: doublet of double doublets.

**Supplementary Table 2 Differential plasma metabolites between ANIT and Emo-ANIT group identified from <sup>1</sup>H-NMR-based metabolomics study**

| No. | Metabolites       | Moieties        | $\delta_H$ | Multiplicity | Emo-ANIT <i>vs</i> ANIT |
|-----|-------------------|-----------------|------------|--------------|-------------------------|
| 1   | Lactate           | CH <sub>3</sub> | 1.34       | d            | ↓                       |
|     |                   | CH              | 4.12       | q            |                         |
|     |                   | C-H4            | 3.42       | t            |                         |
| 2   | $\alpha$ -Glucose | C-H2            | 3.55       | dd           | ↑                       |
|     |                   | C-H6'           | 3.74       | m            |                         |
|     |                   | C-H6            | 3.85       | m            |                         |
|     |                   | C-H5            | 3.47       | ddd          |                         |
| 3   | $\beta$ -Glucose  | C-H3            | 3.49       | t            | ↑                       |
|     |                   | C-H6            | 3.90       | dd           |                         |

**Supplementary Table 3 Differential liver metabolites between Cont and ANIT group identified from <sup>1</sup>H-NMR-based metabolomics study**

| No. | Metabolites               | Moieties                         | $\delta_H$ | Multiplicity | ANIT<br>Cont | <i>vs</i> |
|-----|---------------------------|----------------------------------|------------|--------------|--------------|-----------|
| 1   | Bile acids (mixed)        | CH <sub>3</sub>                  | 0.72       | s            | ↑            |           |
| 2   | 3-Hydroxybutyrate         | CH <sub>3</sub>                  | 1.21       | d            | ↑            |           |
| 3   | Lysine                    | $\delta$ -CH <sub>2</sub>        | 1.73       | m            | ↑            |           |
| 4   | Proline                   | $\beta$ -CH <sub>2</sub>         | 2.07       | m            | ↑            |           |
| 5   | Glutathione               | $\alpha$ -CH <sub>2</sub>        | 2.17       | m            | ↑            |           |
| 6   | Choline                   | N(CH <sub>3</sub> ) <sub>3</sub> | 3.21       | s            | ↑            |           |
|     |                           | OCH <sub>2</sub>                 | 4.07       | m            |              |           |
| 7   | O-<br>Phosphoethanolamine | NCH <sub>2</sub>                 | 3.23       | t            | ↑            |           |
| 8   | $\beta$ -Glucose          | C-H6'                            | 3.72       | dd           | ↓            |           |
|     |                           | C-H6                             | 3.90       | dd           |              |           |
|     |                           | C-H1                             | 4.64       | d            |              |           |
| 9   | $\alpha$ -Glucose         | C-H1                             | 5.23       | d            | ↓            |           |

**Supplementary Table 4 Differential liver metabolites between ANIT and Emo-ANIT group identified from <sup>1</sup>H-NMR-based metabolomics study**

| No. | Metabolites        | Moieties                        | $\delta_H$ | Multiplicity | Emo-ANIT<br><i>vs</i> ANIT |
|-----|--------------------|---------------------------------|------------|--------------|----------------------------|
| 1   | Bile acids (mixed) | CH <sub>3</sub>                 | 0.72       | s            | ↓                          |
| 2   | Lysine             | $\delta$ -CH <sub>2</sub>       | 1.73       | m            | ↓                          |
| 3   | Proline            | $\beta$ -CH <sub>2</sub>        | 2.07       | m            | ↓                          |
|     |                    | $\alpha$ -CH <sub>2</sub>       | 2.35       | m            |                            |
| 4   | Taurine            | CH <sub>2</sub> SO <sub>3</sub> | 3.27       | t            | ↓                          |
| 5   | Choline            | CH                              | 4.12       | q            | ↓                          |

**Supplementary Table 5 Differential plasma metabolites between Cont and ANIT group identified from MS-based metabolomics study**

| No. | Name                    | Rt   | <i>m/z</i> | Formula                                           | Adduct             | VIP  | <i>P</i> | ANIT<br>Cont | <i>vs</i> |
|-----|-------------------------|------|------------|---------------------------------------------------|--------------------|------|----------|--------------|-----------|
| 1   | 2-Hydroxyphenethylamine | 2.34 | 138.0914   | C <sub>8</sub> H <sub>11</sub> NO                 | [M+H] <sup>+</sup> | 1.57 | 5.24E-06 | ↑            |           |
| 2   | Lactate                 | 1.37 | 89.0231    | C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>      | [M-H] <sup>-</sup> | 2.86 | 6.88E-03 | ↑            |           |
| 3   | LysoPC (18:2)           | 6.76 | 520.3397   | C <sub>26</sub> H <sub>51</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 3.21 | 2.09E-03 | ↑            |           |
| 4   | Cholic acid             | 5.72 | 407.2806   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] <sup>-</sup> | 1.97 | 1.63E-02 | ↑            |           |
| 5   | L-Acetylcarnitine       | 1.02 | 204.1231   | C <sub>9</sub> H <sub>17</sub> NO <sub>4</sub>    | [M+H] <sup>+</sup> | 4.25 | 1.08E-03 | ↑            |           |
| 6   | Oxoglutaric acid        | 1.07 | 145.0132   | C <sub>5</sub> H <sub>6</sub> O <sub>5</sub>      | [M-H] <sup>-</sup> | 4.59 | 2.17E-04 | ↑            |           |
| 7   | Citric acid             | 1.07 | 191.0192   | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>      | [M-H] <sup>-</sup> | 4.50 | 5.59E-04 | ↑            |           |
| 8   | LysoPC (18:1)           | 7.29 | 522.3552   | C <sub>26</sub> H <sub>53</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 1.29 | 4.51E-03 | ↑            |           |
| 9   | LysoPC (20:4)           | 6.76 | 544.3394   | C <sub>28</sub> H <sub>51</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 1.44 | 5.70E-03 | ↑            |           |
| 10  | LysoPC (22:6)           | 6.72 | 568.3394   | C <sub>30</sub> H <sub>51</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 1.60 | 1.90E-03 | ↑            |           |
| 11  | LysoPC (18:0)           | 8.13 | 524.3709   | C <sub>26</sub> H <sub>55</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 1.26 | 2.28E-05 | ↑            |           |
| 12  | β-Muricholic acid       | 5.31 | 407.2804   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] <sup>-</sup> | 1.12 | 1.49E-02 | ↑            |           |
| 13  | 4-Oxo-L-proline         | 1.14 | 128.0341   | C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>     | [M-H] <sup>-</sup> | 1.02 | 3.26E-07 | ↑            |           |

|    |                                                       |      |          |                                                                |                    |      |          |   |
|----|-------------------------------------------------------|------|----------|----------------------------------------------------------------|--------------------|------|----------|---|
| 14 | Taurochenodeoxycholic acid                            | 4.96 | 498.2905 | C <sub>26</sub> H <sub>45</sub> NO <sub>6</sub> S              | [M-H] <sup>-</sup> | 1.21 | 1.48E-02 | ↑ |
| 15 | Tauro-α+β-muricholic acid                             | 4.36 | 514.2848 | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S              | [M-H] <sup>-</sup> | 1.07 | 2.95E-03 | ↑ |
| 16 | Phytosphingosine                                      | 5.74 | 318.3000 | C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub>                | [M+H] <sup>+</sup> | 1.16 | 5.03E-06 | ↑ |
| 17 | L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid | 3.10 | 217.0972 | C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>  | [M+H] <sup>+</sup> | 1.05 | 1.61E-08 | ↑ |
| 18 | Taurocholic acid                                      | 5.04 | 514.2849 | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S              | [M-H] <sup>-</sup> | 1.48 | 1.86E-03 | ↑ |
| 19 | Sphinganine                                           | 5.68 | 302.3050 | C <sub>18</sub> H <sub>39</sub> NO <sub>2</sub>                | [M+H] <sup>+</sup> | 4.15 | 7.18E-04 | ↑ |
| 20 | Pantothenic acid                                      | 2.50 | 218.1030 | C <sub>9</sub> H <sub>17</sub> NO <sub>5</sub>                 | [M-H] <sup>-</sup> | 1.76 | 2.60E-04 | ↑ |
| 21 | Alanine                                               | 1.06 | 90.0554  | C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>                  | [M+H] <sup>+</sup> | 1.16 | 6.62E-03 | ↑ |
| 22 | Bilirubin glucuronide                                 | 6.28 | 761.3030 | C <sub>39</sub> H <sub>44</sub> N <sub>4</sub> O <sub>12</sub> | [M+H] <sup>+</sup> | 1.16 | 5.38E-04 | ↑ |
| 23 | Biliverdin                                            | 5.68 | 581.2399 | C <sub>33</sub> H <sub>34</sub> N <sub>4</sub> O <sub>6</sub>  | [M-H] <sup>-</sup> | 1.03 | 8.75E-03 | ↑ |
| 24 | Glutamate                                             | 1.00 | 146.0448 | C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>                  | [M-H] <sup>-</sup> | 1.16 | 3.86E-03 | ↑ |
| 25 | 2-C-Methyl-D-erythritol 4-phosphate                   | 0.97 | 215.0325 | C <sub>5</sub> H <sub>13</sub> O <sub>7</sub> P                | [M-H] <sup>-</sup> | 3.51 | 3.86E-03 | ↓ |
| 26 | Creatine                                              | 0.95 | 132.0769 | C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>    | [M+H] <sup>+</sup> | 2.99 | 4.82E-02 | ↓ |
| 27 | Phenol sulphate                                       | 3.53 | 172.9906 | C <sub>6</sub> H <sub>6</sub> O <sub>4</sub> S                 | [M-H] <sup>-</sup> | 3.13 | 7.70E-04 | ↓ |
| 28 | Uric acid                                             | 1.35 | 167.0201 | C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O <sub>3</sub>    | [M-H] <sup>-</sup> | 3.73 | 9.14E-04 | ↓ |
| 29 | Methacholine                                          | 1.02 | 160.1331 | C <sub>8</sub> H <sub>17</sub> NO <sub>2</sub>                 | [M+H] <sup>+</sup> | 2.39 | 5.41E-04 | ↓ |

|    |                    |      |           |                                                              |                    |      |          |   |
|----|--------------------|------|-----------|--------------------------------------------------------------|--------------------|------|----------|---|
| 30 | Methylmalonic acid | 1.57 | 117.0181  | C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>                 | [M-H] <sup>-</sup> | 1.25 | 9.04E-05 | ↓ |
| 31 | Glucose            | 0.90 | 179.0554  | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                | [M-H] <sup>-</sup> | 1.28 | 3.16E-03 | ↓ |
| 32 | Arginine           | 0.88 | 175.119   | C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> | [M+H] <sup>+</sup> | 1.71 | 2.79E-05 | ↓ |
| 33 | Hexanoylglycine    | 3.88 | 172.0972  | C <sub>8</sub> H <sub>15</sub> NO <sub>3</sub>               | [M-H] <sup>-</sup> | 1.11 | 3.70E-03 | ↓ |
| 34 | Indoxyl sulfate    | 3.52 | 212.0018  | C <sub>8</sub> H <sub>7</sub> NO <sub>4</sub> S              | [M-H] <sup>-</sup> | 2.07 | 1.26E-03 | ↓ |
| 35 | Betaine            | 0.94 | 118.08649 | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>               | [M+H] <sup>+</sup> | 3.64 | 4.95E-02 | ↓ |
| 36 | Pyruvic acid       | 1.06 | 87.0074   | C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>                 | [M-H] <sup>-</sup> | 1.17 | 2.36E-02 | ↓ |

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**Supplementary Table 6 Differential plasma metabolites between ANIT and Emo-ANIT group identified from MS-based metabolomics study**

| No. | Name                       | Rt   | <i>m/z</i> | Formula                                           | Adduct     | VIP   | <i>P</i> | Emo-ANIT<br><i>vs</i> ANIT |
|-----|----------------------------|------|------------|---------------------------------------------------|------------|-------|----------|----------------------------|
| 1   | Lactate                    | 1.37 | 89.0231    | C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>      | [M-H] -    | 13.41 | 4.14E-02 | ↓                          |
| 2   | Cholic acid                | 5.72 | 407.2806   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] -    | 4.25  | 2.73E-02 | ↓                          |
| 3   | LysoPC (18:1)              | 7.29 | 522.3552   | C <sub>26</sub> H <sub>53</sub> NO <sub>7</sub> P | [M+H]<br>+ | 2.41  | 2.06E-02 | ↓                          |
| 4   | LysoPC (20:4)              | 6.76 | 544.3394   | C <sub>28</sub> H <sub>51</sub> NO <sub>7</sub> P | [M+H]<br>+ | 1.08  | 2.56E-02 | ↓                          |
| 5   | LysoPC (22:6)              | 6.72 | 568.3394   | C <sub>30</sub> H <sub>51</sub> NO <sub>7</sub> P | [M+H]<br>+ | 2.83  | 1.58E-02 | ↓                          |
| 6   | β-Muricholic acid          | 5.31 | 407.2804   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] -    | 1.34  | 2.17E-02 | ↓                          |
| 7   | Taurochenodeoxycholic acid | 4.96 | 498.2905   | C <sub>26</sub> H <sub>45</sub> NO <sub>6</sub> S | [M-H] -    | 1.10  | 1.26E-02 | ↓                          |
| 8   | Tauro-α+β-muricholic acid  | 4.36 | 514.2848   | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S | [M-H] -    | 1.05  | 4.10E-02 | ↓                          |
| 9   | Phytosphingosine           | 5.74 | 318.2999   | C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub>   | [M+H]<br>+ | 1.24  | 1.40E-02 | ↓                          |

|    |                                                       |      |          |                                                               |            |      |          |   |
|----|-------------------------------------------------------|------|----------|---------------------------------------------------------------|------------|------|----------|---|
| 10 | L-1,2,3,4-Tetrahydro-beta-carboline-3-carboxylic acid | 3.12 | 217.0973 | C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> | [M+H]<br>+ | 1.17 | 1.55E-03 | ↓ |
| 11 | Taurocholic acid                                      | 5.04 | 514.2849 | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S             | [M-H] -    | 1.14 | 1.86E-02 | ↓ |
| 12 | 16-Hydroxyhexadecanoic acid                           | 7.13 | 271.2281 | C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>                | [M-H] -    | 1.21 | 5.66E-05 | ↓ |
| 13 | Xanthosine                                            | 2.17 | 283.0687 | C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>6</sub> | [M-H] -    | 1.46 | 1.33E-02 | ↓ |
| 14 | 11,12-Epoxyeicosatrienoic acid                        | 7.47 | 319.2282 | C <sub>20</sub> H <sub>32</sub> O <sub>3</sub>                | [M-H] -    | 1.59 | 9.33E-03 | ↓ |
| 15 | Palmitic acid                                         | 9.37 | 255.2332 | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>                | [M-H] -    | 1.03 | 2.81E-03 | ↓ |
| 16 | 2-Methylbenzoic acid                                  | 4.94 | 135.0440 | C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>                  | [M-H] -    | 1.57 | 4.52E-04 | ↓ |
| 17 | Glutamate                                             | 1.00 | 146.0448 | C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>                 | [M-H] -    | 1.14 | 3.33E-02 | ↓ |
| 18 | 2-C-Methyl-D-erythritol 4-phosphate                   | 0.96 | 215.0325 | C <sub>5</sub> H <sub>13</sub> O <sub>7</sub> P               | [M-H] -    | 4.18 | 2.46E-02 | ↑ |
| 19 | Phenol sulphate                                       | 3.46 | 172.9906 | C <sub>6</sub> H <sub>6</sub> O <sub>4</sub> S                | [M-H] -    | 2.38 | 3.72E-02 | ↑ |
| 20 | Pyruvic acid                                          | 1.06 | 87.0074  | C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>                  | [M-H] -    | 1.22 | 3.12E-02 | ↑ |
| 21 | Methacholine                                          | 1.04 | 160.1332 | C <sub>8</sub> H <sub>17</sub> NO <sub>2</sub>                | [M+H]<br>+ | 2.47 | 3.14E-02 | ↑ |
| 22 | Glucose                                               | 0.93 | 179.0554 | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                 | [M-H] -    | 1.40 | 2.12E-02 | ↑ |
| 23 | Hexanoylglycine                                       | 3.86 | 172.0971 | C <sub>8</sub> H <sub>15</sub> NO <sub>3</sub>                | [M-H] -    | 1.09 | 3.11E-02 | ↑ |
| 24 | Indoxyl sulfate                                       | 3.83 | 212.0019 | C <sub>8</sub> H <sub>7</sub> NO <sub>4</sub> S               | [M-H] -    | 1.27 | 2.54E-02 | ↑ |

|    |                        |      |          |                                                   |            |      |          |   |
|----|------------------------|------|----------|---------------------------------------------------|------------|------|----------|---|
| 25 | Betaine                | 0.94 | 118.0865 | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>    | [M+H]<br>+ | 4.12 | 3.00E-02 | ↑ |
| 26 | 2-Hydroxycinnamic acid | 1.54 | 165.0546 | C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>      | [M+H]<br>+ | 3.74 | 1.22E-02 | ↑ |
| 27 | <i>L</i> -Tyrosine     | 1.55 | 182.0812 | C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>    | [M+H]<br>+ | 3.20 | 2.43E-02 | ↑ |
| 28 | LysoPE (18:2)          | 6.73 | 478.2924 | C <sub>23</sub> H <sub>44</sub> NO <sub>7</sub> P | [M+H]<br>+ | 1.54 | 3.88E-02 | ↑ |
| 29 | Niacinamide            | 1.35 | 123.0555 | C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O    | [M+H]<br>+ | 1.11 | 1.38E-02 | ↑ |

**Supplementary Table 7 Differential liver metabolites between Cont and ANIT group identified from MS-based metabolomics study**

| No. | Name                          | Rt   | <i>m/z</i> | Formula                                                                       | Adduct             | VIP  | <i>P</i> | ANIT<br>Cont | <i>vs</i> |
|-----|-------------------------------|------|------------|-------------------------------------------------------------------------------|--------------------|------|----------|--------------|-----------|
| 1   | β-Muricholic Acid             | 5.20 | 407.2799   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>                                | [M-H] <sup>-</sup> | 7.68 | 3.27E-02 | ↑            |           |
| 2   | Pyroglutamic acid             | 1.31 | 128.0341   | C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>                                 | [M-H] <sup>-</sup> | 4.97 | 2.21E-04 | ↑            |           |
| 3   | Taurochenodeoxycholic acid    | 5.25 | 498.2896   | C <sub>26</sub> H <sub>45</sub> NO <sub>6</sub> S                             | [M-H] <sup>-</sup> | 3.37 | 4.27E-04 | ↑            |           |
| 4   | Glutathione                   | 1.24 | 611.1453   | C <sub>20</sub> H <sub>32</sub> N <sub>6</sub> O <sub>12</sub> S <sub>2</sub> | [M-H] <sup>-</sup> | 3.22 | 4.84E-02 | ↑            |           |
| 5   | Fumaric acid                  | 1.01 | 115.0025   | C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>                                  | [M-H] <sup>-</sup> | 2.59 | 1.91E-02 | ↑            |           |
| 6   | N-Acetyl-L-glutamic acid      | 1.43 | 188.0558   | C <sub>7</sub> H <sub>11</sub> NO <sub>5</sub>                                | [M-H] <sup>-</sup> | 2.21 | 4.89E-02 | ↑            |           |
| 7   | Taurocholic acid              | 4.57 | 514.2849   | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S                             | [M-H] <sup>-</sup> | 1.14 | 1.74E-02 | ↑            |           |
| 8   | 1-Phenylethylamine            | 2.91 | 122.0970   | C <sub>8</sub> H <sub>11</sub> N                                              | [M+H] <sup>+</sup> | 1.95 | 1.04E-03 | ↑            |           |
| 9   | UDP-N-acetylglucosamine       | 1.09 | 606.0747   | C <sub>17</sub> H <sub>27</sub> N <sub>3</sub> O <sub>17</sub> P <sub>2</sub> | [M-H] <sup>-</sup> | 2.02 | 1.27E-03 | ↑            |           |
| 10  | L-Glutamic acid 5-phosphate   | 1.33 | 226.0119   | C <sub>5</sub> H <sub>10</sub> NO <sub>7</sub> P                              | [M-H] <sup>-</sup> | 1.45 | 8.88E-03 | ↑            |           |
| 11  | 1-Pyrroline-5-carboxylic acid | 1.02 | 114.0554   | C <sub>5</sub> H <sub>7</sub> NO <sub>2</sub>                                 | [M+H] <sup>+</sup> | 1.26 | 3.44E-02 | ↑            |           |
| 12  | Citric acid                   | 1.27 | 191.0190   | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>                                  | [M-H] <sup>-</sup> | 1.72 | 6.24E-05 | ↑            |           |
| 13  | Choline phosphate             | 0.95 | 184.0736   | C <sub>5</sub> H <sub>14</sub> NO <sub>4</sub> P                              | [M+H] <sup>+</sup> | 1.36 | 5.21E-04 | ↑            |           |

|    |                           |      |          |                                                               |                    |       |          |   |
|----|---------------------------|------|----------|---------------------------------------------------------------|--------------------|-------|----------|---|
| 14 | DOPA sulfate              | 1.57 | 276.0178 | C <sub>9</sub> H <sub>11</sub> NO <sub>7</sub> S              | [M-H] <sup>-</sup> | 1.17  | 1.33E-03 | ↑ |
| 15 | gamma-Glutamylleucine     | 1.37 | 261.1447 | C <sub>11</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> | [M+H] <sup>+</sup> | 1.00  | 1.31E-02 | ↑ |
| 16 | Spermidine                | 0.81 | 146.1654 | C <sub>7</sub> H <sub>19</sub> N <sub>3</sub>                 | [M+H] <sup>+</sup> | 1.02  | 1.88E-07 | ↑ |
| 17 | Aspartic acid             | 0.95 | 132.029  | C <sub>4</sub> H <sub>7</sub> NO <sub>4</sub>                 | [M-H] <sup>-</sup> | 1.05  | 6.11E-03 | ↑ |
| 18 | Tauro-α+β-muricholic acid | 4.11 | 514.2848 | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S             | [M-H] <sup>-</sup> | 1.26  | 3.14E-02 | ↑ |
| 19 | Deoxycholic acid          | 6.67 | 391.2854 | C <sub>24</sub> H <sub>40</sub> O <sub>4</sub>                | [M-H] <sup>-</sup> | 1.15  | 1.09E-06 | ↓ |
| 20 | Ornithine                 | 0.75 | 133.0972 | C <sub>5</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>  | [M+H] <sup>+</sup> | 1.22  | 4.07E-03 | ↓ |
| 21 | L-Leucine                 | 1.61 | 130.0861 | C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>                | [M-H] <sup>-</sup> | 13.65 | 3.64E-05 | ↓ |
| 22 | L-Phenylalanine           | 2.26 | 166.0863 | C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>                | [M+H] <sup>+</sup> | 9.89  | 3.21E-05 | ↓ |
| 23 | Nicotinamide              | 1.23 | 123.0556 | C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O                | [M+H] <sup>+</sup> | 8.32  | 1.15E-03 | ↓ |
| 24 | L-Tyrosine                | 1.42 | 182.0815 | C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>                | [M+H] <sup>+</sup> | 2.51  | 1.31E-03 | ↓ |
| 25 | Tryptophan                | 2.69 | 205.0974 | C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> | [M+H] <sup>+</sup> | 4.94  | 1.26E-04 | ↓ |
| 26 | Xanthine                  | 1.27 | 151.0251 | C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O <sub>2</sub>   | [M-H] <sup>-</sup> | 3.33  | 3.76E-04 | ↓ |
| 27 | Hypoxanthine              | 1.24 | 135.0300 | C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O                | [M-H] <sup>-</sup> | 2.87  | 1.05E-02 | ↓ |
| 28 | Indoleacrylic acid        | 2.69 | 188.0708 | C <sub>11</sub> H <sub>9</sub> NO <sub>2</sub>                | [M+H] <sup>+</sup> | 3.86  | 1.45E-04 | ↓ |
| 29 | Leucylproline             | 2.59 | 229.1550 | C <sub>11</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> | [M+H] <sup>+</sup> | 1.72  | 1.83E-07 | ↓ |
| 30 | L-Valine                  | 0.95 | 118.0866 | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>                | [M+H] <sup>+</sup> | 1.22  | 5.18E-03 | ↓ |

|    |                                     |      |          |                                                                 |                    |      |          |   |
|----|-------------------------------------|------|----------|-----------------------------------------------------------------|--------------------|------|----------|---|
| 31 | N-Benzylformamide                   | 1.42 | 136.0758 | C <sub>8</sub> H <sub>9</sub> NO                                | [M+H] <sup>+</sup> | 1.15 | 2.48E-03 | ↓ |
| 32 | Inosine                             | 1.59 | 269.0881 | C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>5</sub>   | [M+H] <sup>+</sup> | 3.21 | 3.47E-07 | ↓ |
| 33 | 2-C-Methyl-D-erythritol 4-phosphate | 0.97 | 215.0325 | C <sub>5</sub> H <sub>13</sub> O <sub>7</sub> P                 | [M-H] <sup>-</sup> | 1.38 | 4.22E-02 | ↓ |
| 34 | 2-Phenylacetamide                   | 1.42 | 136.0759 | C <sub>8</sub> H <sub>9</sub> NO                                | [M+H] <sup>+</sup> | 1.06 | 2.12E-03 | ↓ |
| 35 | L-Histidine                         | 0.87 | 154.0611 | C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>     | [M-H] <sup>-</sup> | 1.05 | 6.06E-04 | ↓ |
| 36 | Adenosine monophosphate             | 1.36 | 348.0707 | C <sub>10</sub> H <sub>14</sub> N <sub>5</sub> O <sub>7</sub> P | [M+H] <sup>+</sup> | 1.24 | 2.19E-02 | ↓ |
| 37 | cis-4-Octenedioic acid              | 3.74 | 171.0654 | C <sub>8</sub> H <sub>12</sub> O <sub>4</sub>                   | [M-H] <sup>-</sup> | 1.70 | 1.44E-07 | ↓ |
| 38 | D-4'-Phosphopantothenate            | 1.52 | 300.0844 | C <sub>9</sub> H <sub>18</sub> NO <sub>8</sub> P                | [M+H] <sup>+</sup> | 2.38 | 3.05E-04 | ↓ |
| 39 | 3-Hydroxymethylglutaric acid        | 1.65 | 161.0445 | C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>                   | [M-H] <sup>-</sup> | 1.34 | 1.23E-03 | ↓ |
| 40 | 4-Pyridoxic acid                    | 1.84 | 182.0452 | C <sub>8</sub> H <sub>9</sub> NO <sub>4</sub>                   | [M-H] <sup>-</sup> | 1.55 | 1.47E-04 | ↓ |
| 41 | Pentanamide                         | 0.92 | 102.0919 | C <sub>5</sub> H <sub>11</sub> NO                               | [M+H] <sup>+</sup> | 1.10 | 9.82E-04 | ↓ |
| 42 | Deoxyadenosine monophosphate        | 1.24 | 330.0612 | C <sub>10</sub> H <sub>14</sub> N <sub>5</sub> O <sub>6</sub> P | [M-H] <sup>-</sup> | 1.11 | 1.53E-05 | ↓ |
| 43 | 5-Thymidylic acid                   | 1.46 | 321.0495 | C <sub>10</sub> H <sub>15</sub> N <sub>2</sub> O <sub>8</sub> P | [M-H] <sup>-</sup> | 1.31 | 5.23E-04 | ↓ |
| 44 | Phosphoribosyl formamidocarboxamide | 1.58 | 365.0506 | C <sub>10</sub> H <sub>15</sub> N <sub>4</sub> O <sub>9</sub> P | [M-H] <sup>-</sup> | 1.18 | 6.73E-08 | ↓ |
| 45 | N-(4-Aminobenzoyl)-L-glutamic acid  | 2.27 | 265.0832 | C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub>   | [M-H] <sup>-</sup> | 1.36 | 2.69E-05 | ↓ |
| 46 | Glucose                             | 0.90 | 179.0554 | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                   | [M-H] <sup>-</sup> | 1.57 | 6.59E-04 | ↓ |
| 47 | Glutaryl carnitine                  | 1.42 | 276.1444 | C <sub>12</sub> H <sub>21</sub> NO <sub>6</sub>                 | [M+H] <sup>+</sup> | 1.10 | 1.73E-05 | ↓ |

|    |          |      |          |                                                              |                    |      |          |   |
|----|----------|------|----------|--------------------------------------------------------------|--------------------|------|----------|---|
| 48 | Arginine | 0.87 | 175.1159 | C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> | [M+H] <sup>+</sup> | 1.12 | 1.22E-03 | ↓ |
|----|----------|------|----------|--------------------------------------------------------------|--------------------|------|----------|---|

**Supplementary Table 8 Differential liver metabolites between ANIT and Emo-ANIT group identified from MS-based metabolomics study**

| No. | Name                       | Rt   | <i>m/z</i> | Formula                                           | Adduct             | VIP  | <i>P</i> | Emo-ANIT<br>ANIT | <i>vs</i> |
|-----|----------------------------|------|------------|---------------------------------------------------|--------------------|------|----------|------------------|-----------|
| 1   | Choline phosphate          | 0.95 | 184.0736   | C <sub>5</sub> H <sub>14</sub> NO <sub>4</sub> P  | [M+H] <sup>+</sup> | 1.13 | 4.23E-02 | ↓                |           |
| 2   | β-Muricholic Acid          | 5.19 | 407.2799   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] <sup>-</sup> | 8.98 | 7.44E-03 | ↓                |           |
| 3   | Tauroursodeoxycholic acid  | 4.49 | 498.2899   | C <sub>26</sub> H <sub>45</sub> NO <sub>6</sub> S | [M-H] <sup>-</sup> | 2.33 | 2.02E-02 | ↓                |           |
| 4   | α-Muricholic acid          | 5.04 | 407.2804   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] <sup>-</sup> | 1.72 | 3.30E-02 | ↓                |           |
| 5   | Tauro-α+β-muricholic acid  | 4.11 | 514.2848   | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S | [M-H] <sup>-</sup> | 1.08 | 4.41E-02 | ↓                |           |
| 6   | Taurocholic acid           | 4.57 | 514.2849   | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S | [M-H] <sup>-</sup> | 1.12 | 3.92E-03 | ↓                |           |
| 7   | Cholic acid                | 5.64 | 407.2806   | C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>    | [M-H] <sup>-</sup> | 3.71 | 9.99E-03 | ↓                |           |
| 8   | Taurochenodeoxycholic acid | 5.30 | 498.2896   | C <sub>26</sub> H <sub>45</sub> NO <sub>6</sub> S | [M-H] <sup>-</sup> | 2.97 | 6.82E-02 | ↓                |           |
| 9   | N-Acetyl-L-glutamic acid   | 1.43 | 188.0558   | C <sub>7</sub> H <sub>11</sub> NO <sub>5</sub>    | [M-H] <sup>-</sup> | 1.95 | 3.58E-02 | ↓                |           |
| 10  | L-Valine                   | 0.95 | 118.0866   | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>    | [M+H] <sup>+</sup> | 2.67 | 4.03E-02 | ↑                |           |
| 11  | LysoPC (18:2)              | 6.81 | 520.3395   | C <sub>26</sub> H <sub>50</sub> NO <sub>7</sub> P | [M+H] <sup>+</sup> | 2.48 | 4.87E-02 | ↑                |           |

|    |                              |      |          |                                                                 |                    |      |          |   |
|----|------------------------------|------|----------|-----------------------------------------------------------------|--------------------|------|----------|---|
| 12 | N,N-Dimethylarginine         | 0.89 | 203.1505 | C <sub>8</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>    | [M+H] <sup>+</sup> | 1.16 | 3.80E-02 | ↑ |
| 13 | LysoPC (20:4)                | 6.71 | 544.3401 | C <sub>28</sub> H <sub>51</sub> NO <sub>7</sub> P               | [M+H] <sup>+</sup> | 1.51 | 4.97E-02 | ↑ |
| 14 | LysoPC (22:6)                | 6.74 | 568.3394 | C <sub>30</sub> H <sub>51</sub> NO <sub>7</sub> P               | [M+H] <sup>+</sup> | 1.15 | 4.73E-02 | ↑ |
| 15 | Threonine                    | 0.87 | 118.0497 | C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>                   | [M-H] <sup>-</sup> | 1.01 | 3.96E-02 | ↑ |
| 16 | Fexofenadine                 | 6.6  | 502.2932 | C <sub>32</sub> H <sub>39</sub> NO <sub>4</sub>                 | [M+H] <sup>+</sup> | 1.23 | 2.72E-02 | ↑ |
| 17 | Deoxyadenosine monophosphate | 1.24 | 330.0612 | C <sub>10</sub> H <sub>14</sub> N <sub>5</sub> O <sub>6</sub> P | [M-H] <sup>-</sup> | 1.02 | 3.60E-03 | ↑ |

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