

# Supporting Information for

## Comparison of Two Different Astragali Radix by a $^1\text{H}$ NMR-Based Metabolomic Approach

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### This PDF file includes:

SI Figures S1-S10

SI Tables S1-S5

### Figures

**Figure S1.** The stack NMR spectra of all ARs (SX-AR-0001-0008 and GS-AR-0001-0008) in M1 and M2E; M1 (A), M2E (B).

**Figure S2.** Typical  $^1\text{H}$  NMR spectra of soluble fractions from AR in M2P (A), M2B (B) and M2W (C).

**Figure S3-S6.** The stack NMR spectra of mouse serum samples, lung, liver and spleen extracts in NS, GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H; n=8 in each group; serum (S3), lung (S4), liver (S5) and spleen (S6).

**Figure S7.** The OPLS-DA corresponding S-plot derived from  $^1\text{H}$  NMR spectra of AR treated groups compared with the control; serum (A), lung (B), liver (C) and spleen (D).

**Figure S8-S10.** Biplot obtained from PLS describing the correlation among all variables including chemical composition of AR (X variables, all major metabolites identified in M1 and M2E, nos. 1-38 in accord with Table 1), the grouping or cluster (observation) and the metabolites variation (Y variables, endogenous metabolites for quantitative data analysis) in serum samples, liver and spleen extracts induced by SX-AR and GS-AR; at low dose (A), at high dose (B); serum (S8), liver (S9) and spleen (S10).

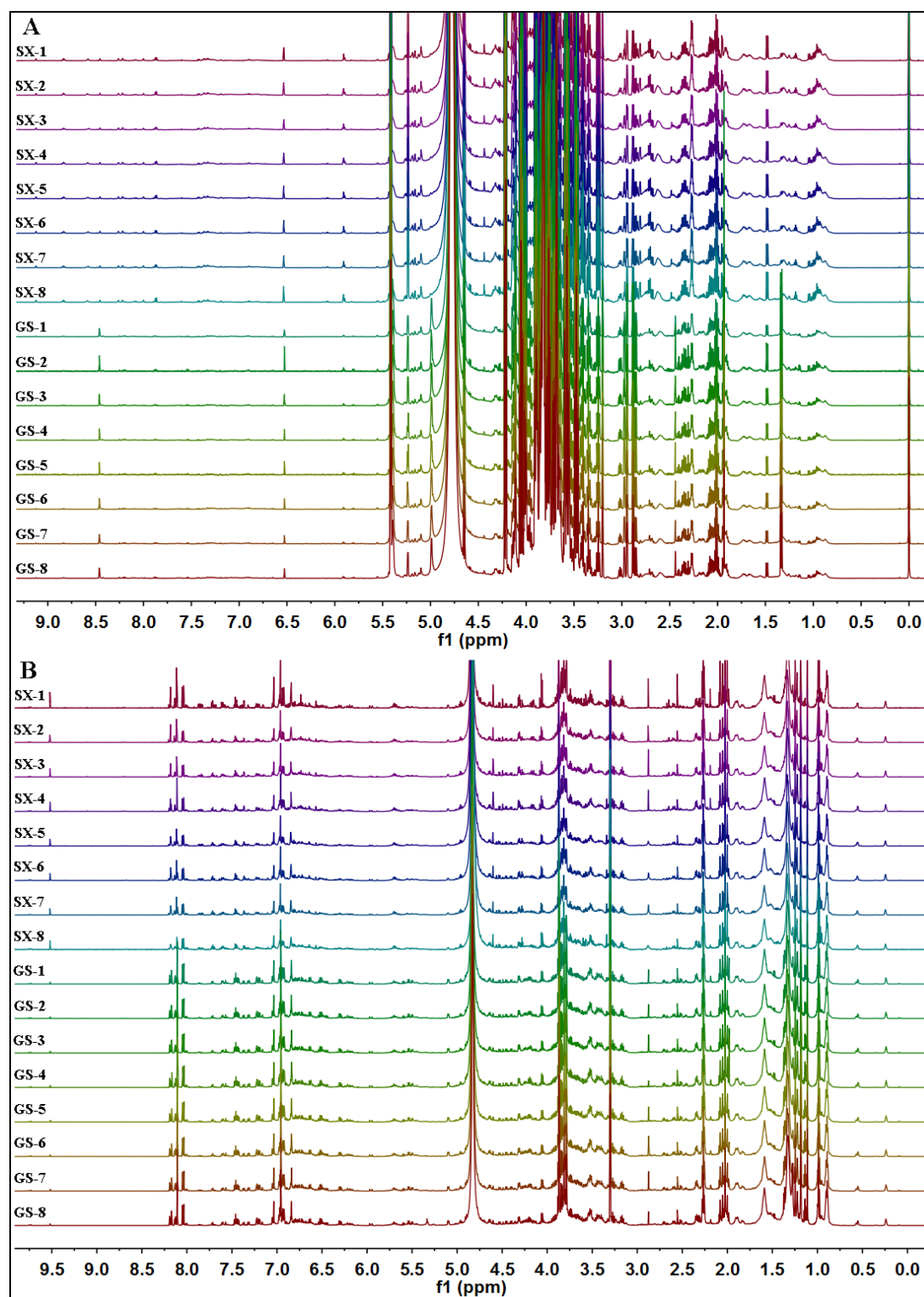
### Tables

**Table S1.**  $^1\text{H}$  NMR assignments of major metabolites from mouse serum, lung, liver and spleen.

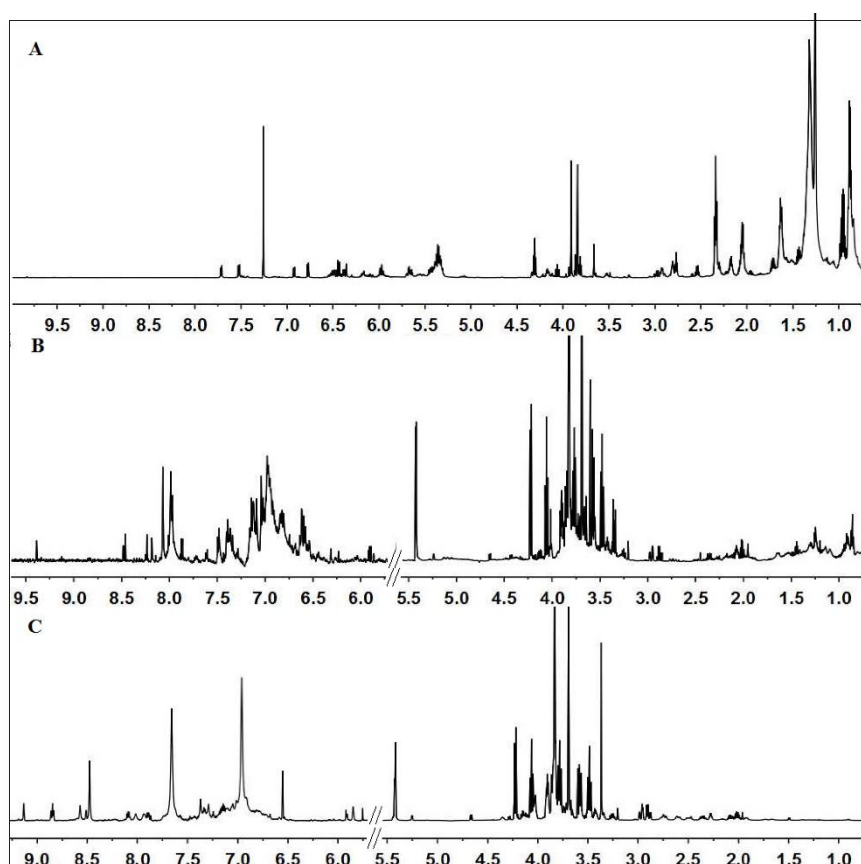
**Table S2.** Parameters indicating the model quality of PLS-DA.

**Table S3-S5.** A comparison of integral levels of metabolites in the control (NS) and AR-treated (GS-AR-L, GS-AR-H, SX-AR-L, SX-AR-H) mouse serum samples, liver and spleen extracts; serum (S3), liver (S4) and spleen (S5).

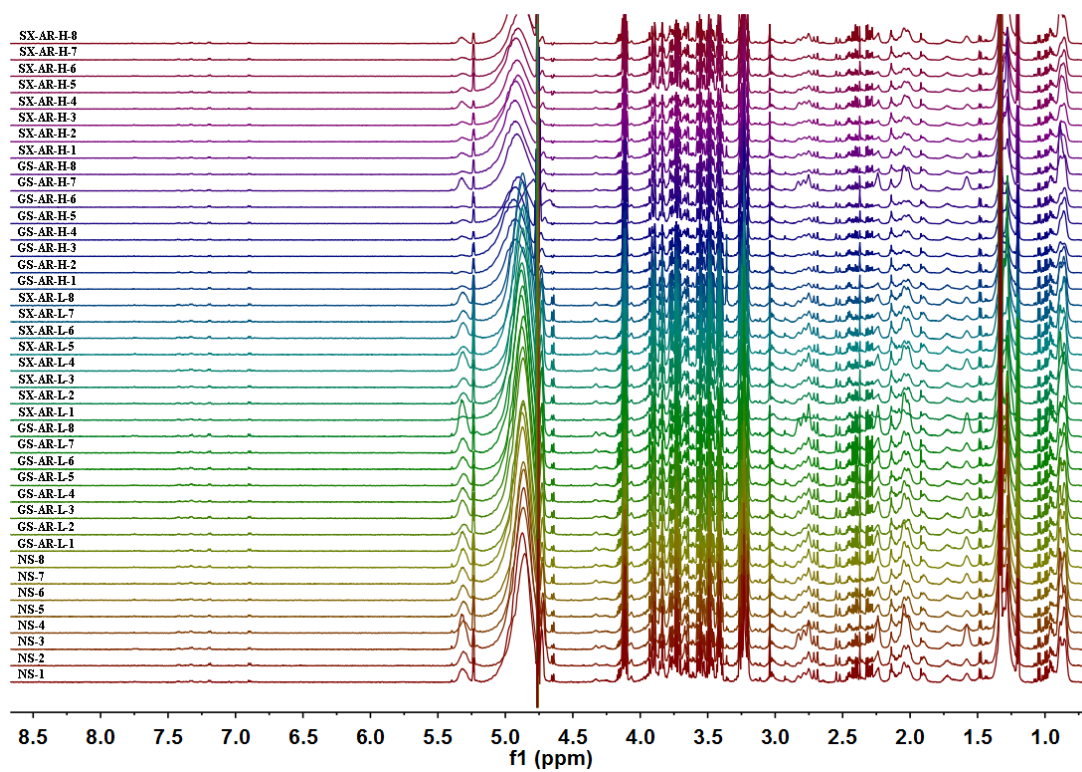
## Support Information



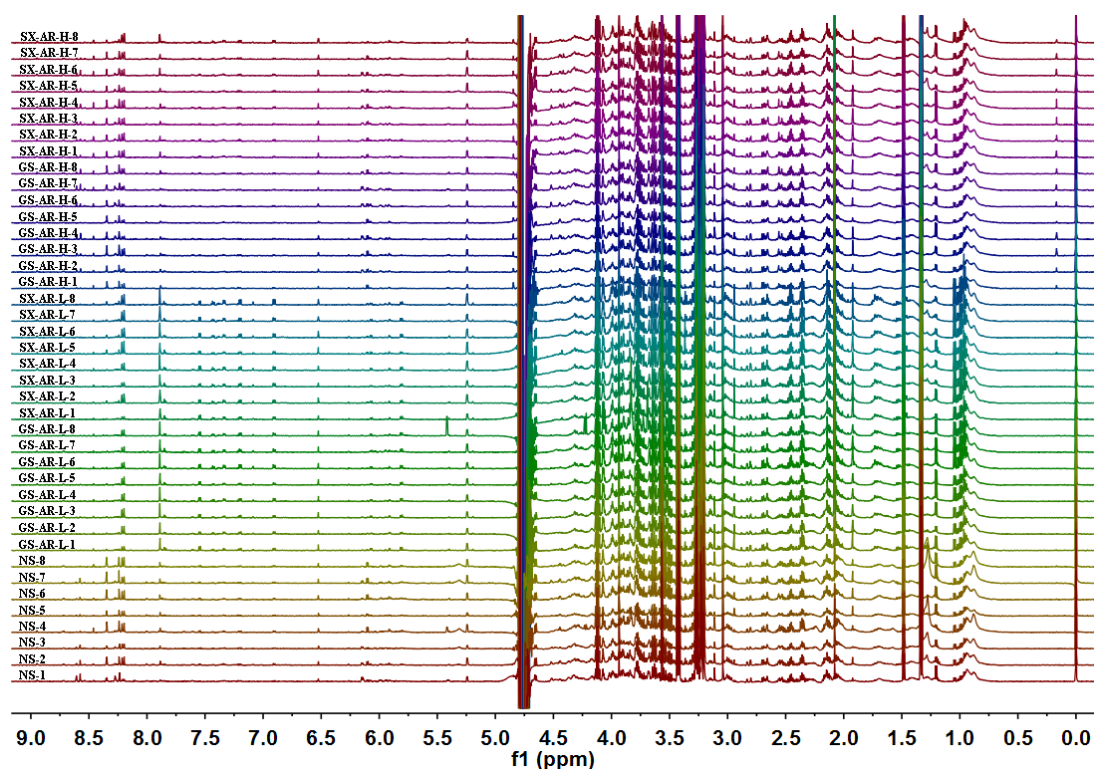
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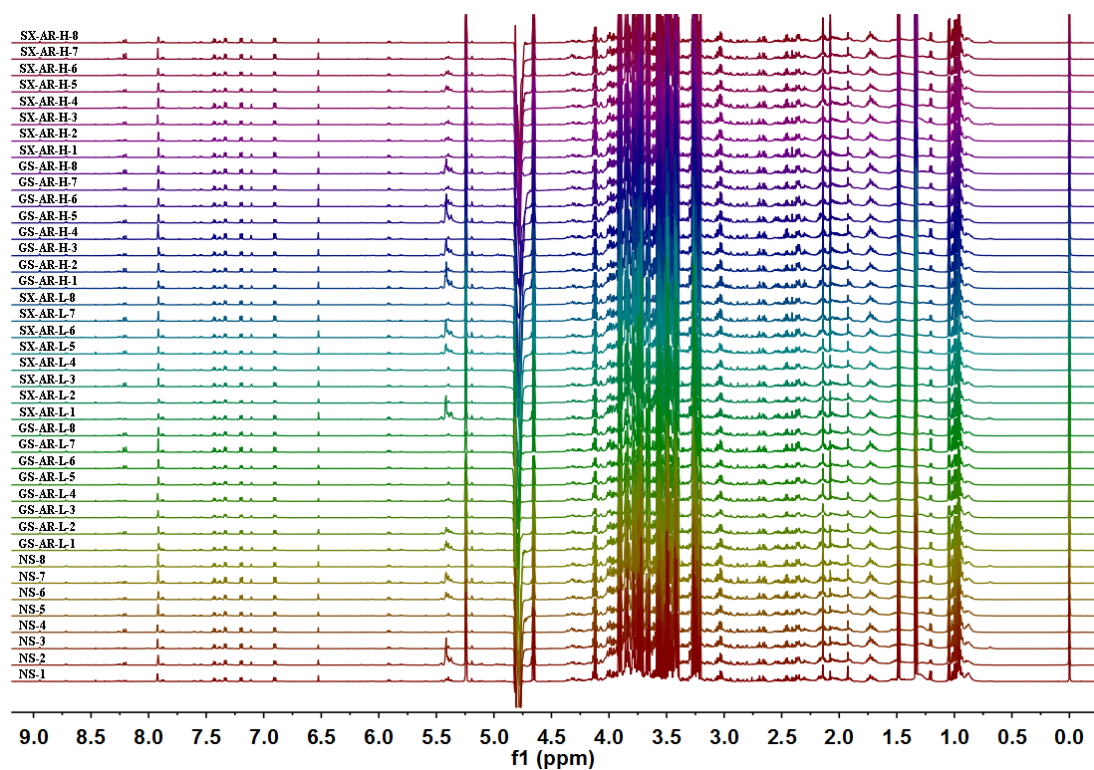
**Figure S2.** Typical  $^1\text{H}$  NMR spectra of soluble fractions from AR in M2P (A), M2B (B) and M2W (C).



**Figure S3.** The stack NMR spectra of mouse serum samples in NS, GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H;  $n=8$  in each group.

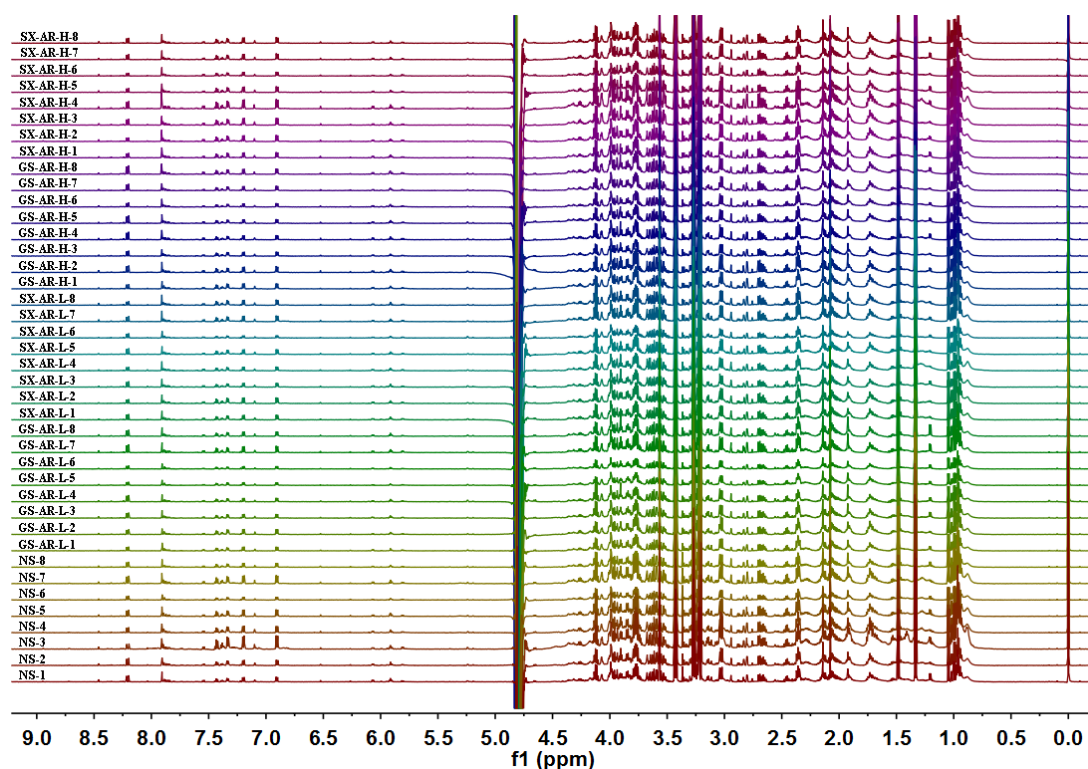


**Figure S4.** The stack NMR spectra of mouse lung extracts in NS, GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H; n=8 in each group.

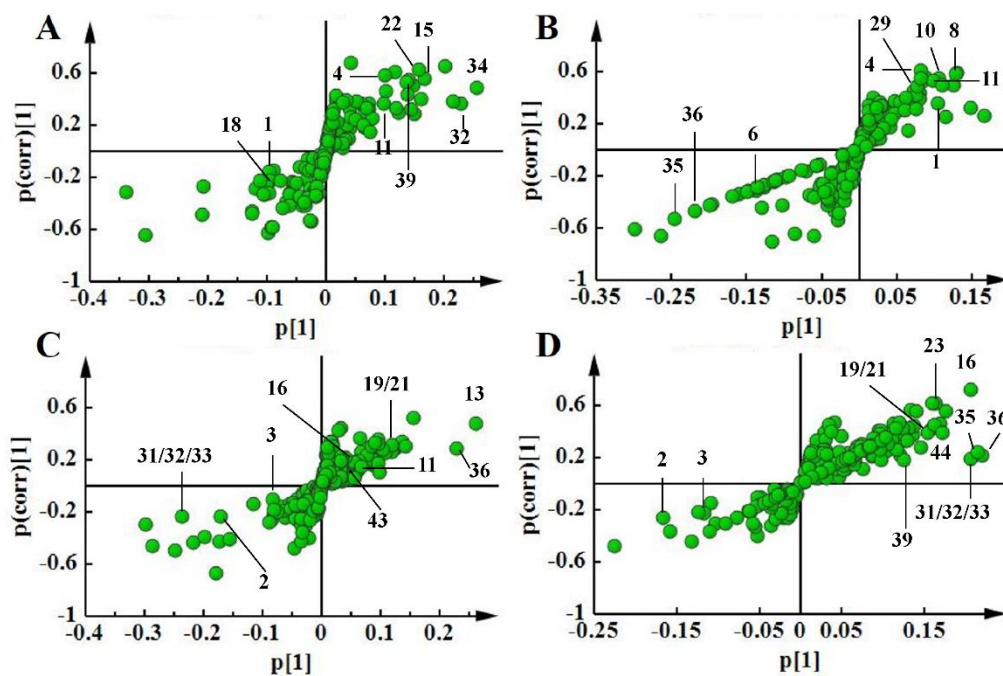


**Figure S5.** The stack NMR spectra of mouse liver extracts in NS, GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H; n=8 in each group.

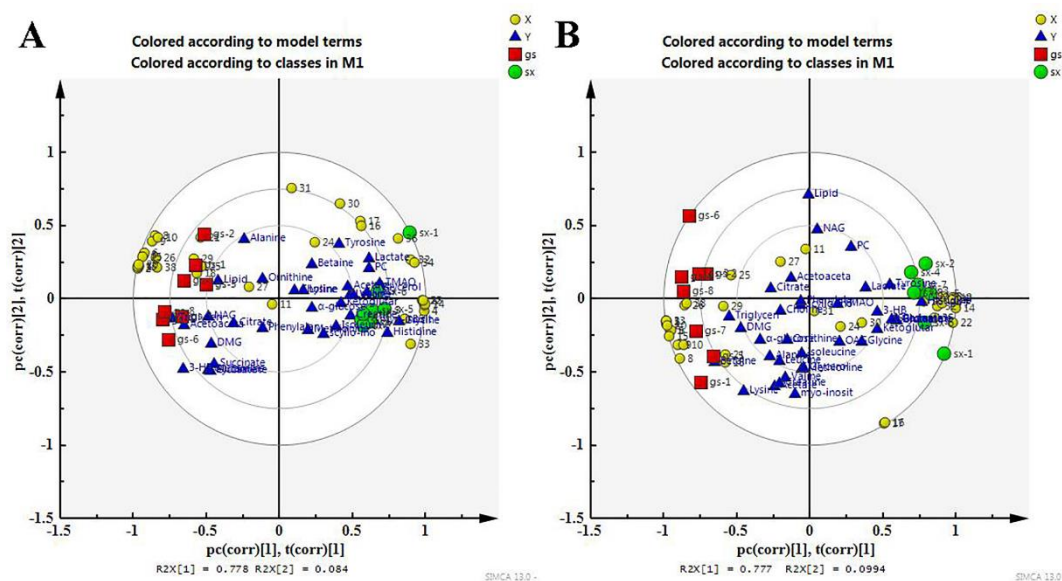




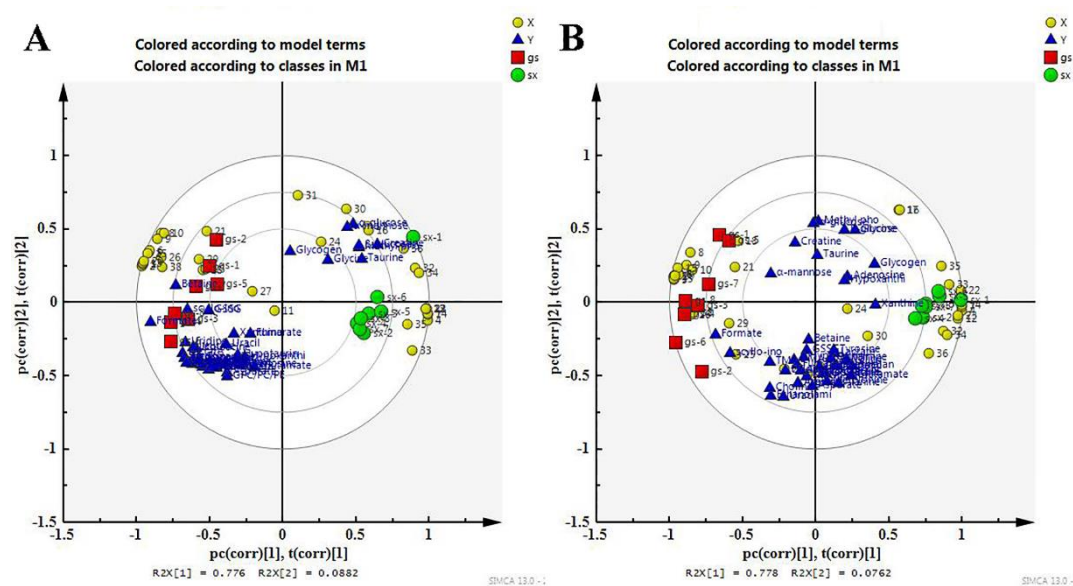
**Figure S6.** The stack NMR spectra of mouse spleen extracts in NS, GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H; n=8 in each group.



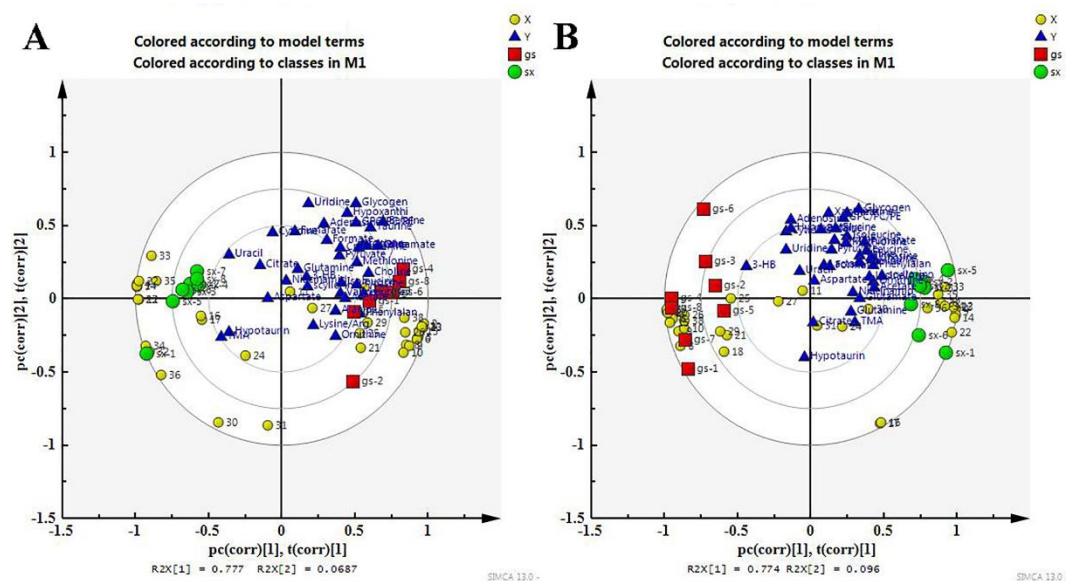
**Figure S7.** The OPLS-DA corresponding S-plot derived from  $^1\text{H}$  NMR spectra of AR treated groups compared with the control; serum (A), lung (B), liver (C) and spleen (D).



**Figure S8.** Biplot obtained from PLS describing the correlation among all variables including chemical composition of AR (X variables, all major metabolites identified in M1 and M2E, nos. 1-38 in accord with Table 1), the grouping or cluster (observation) and the metabolites variation (Y variables, endogenous metabolites for quantitative data analysis) in serum samples induced by SX-AR and GS-AR; at low dose (A); at high dose (B).



**Figure S9.** Biplot obtained from PLS describing the correlation among all variables including chemical composition of AR (X variables, all major metabolites identified in M1 and M2E, nos. 1-38 in accord with Table 1), the grouping or cluster (observation) and the metabolites variation (Y variables, endogenous metabolites for quantitative data analysis) in liver extracts induced by SX-AR and GS-AR; at low dose (A); at high dose (B).



**Figure S10.** Biplot obtained from PLS describing the correlation among all variables including chemical composition of AR (X variables, all major metabolites identified in M1 and M2E, nos. 1-38 in accord with Table 1), the grouping or cluster (observation) and the metabolites variation (Y variables, endogenous metabolites for quantitative data analysis) in spleen extracts induced by SX-AR and GS-AR; at low dose (A); at high dose (B).

**Table S1** <sup>1</sup>H NMR assignments of major metabolites from mouse serum, lung, liver and spleen.

| No. | Metabolites                    | <sup>1</sup> H chemical shift (multiplicity)                                            | Sample          |
|-----|--------------------------------|-----------------------------------------------------------------------------------------|-----------------|
| 1   | lipid                          | 0.87(m) <sup>a</sup> , 0.89(m), 1.28(m), 1.58(m),<br>2.03(m), 2.23(m), 2.78(m), 5.3(m), | Se <sup>b</sup> |
| 2   | isoleucine                     | 0.95(d), 1.01(d)                                                                        | Se, Lu, Li, Sp  |
| 3   | leucine                        | 0.97(t),                                                                                | Se, Lu, Li, Sp  |
| 4   | valine                         | 0.99(d), 1.05(d)                                                                        | Se, Lu, Li, Sp  |
| 5   | 3-HB <sup>c</sup>              | 1.20(d), 2.31(dd), 2.41(dd)                                                             | Se, Lu, Li, Sp  |
| 6   | lactate                        | 1.33(d), 4.12(q)                                                                        | Se, Lu, Li, Sp  |
| 7   | lysine                         | 1.49(m), 1.72(m), 1.92(m),                                                              | Se, Lu, Li, Sp  |
| 8   | alanine                        | 1.49(d)                                                                                 | Se, Lu, Li, Sp  |
| 9   | arginine                       | 1.70(m), 1.92(m)                                                                        | Lu, Li, Sp      |
| 10  | ornithine                      | 1.75(m), 1.93(m)                                                                        | Se, Lu, Li, Sp  |
| 11  | acetate                        | 1.93(s)                                                                                 | Se, Lu, Li, Sp  |
| 12  | <i>N</i> -acetyl-glycoproteins | 2.04(s)                                                                                 | Se              |
| 13  | glutamate                      | 2.05(m), 2.34(m), 3.75(m)                                                               | Se, Lu, Li, Sp  |
| 14  | methionine                     | 2.14(s), 2.14(m), 2.64(t), 3.85(m)                                                      | Lu, Li, Sp      |
| 15  | O-acetyl-glycoproteins         | 2.14(s)                                                                                 | Se              |
| 16  | glutamine                      | 2.15(m), 2.44(m), 3.77(m)                                                               | Se, Lu, Li, Sp  |
| 17  | GSSG                           | 2.17(m), 2.54(m), 2.95(m), 3.25(m)                                                      | Li, Lu          |
| 18  | acetoacetate                   | 2.28(s)                                                                                 | Se              |
| 19  | pyruvate                       | 2.37(s)                                                                                 | Se, Lu, Sp      |
| 20  | malate                         | 2.38(dd), 2.68(dd), 4.31(dd)                                                            | Li              |
| 21  | succinate                      | 2.40(s)                                                                                 | Se, Lu, Li, Sp  |
| 22  | 2- ketoglutarate               | 2.45(t), 3.03(t)                                                                        | Se              |
| 23  | citrate                        | 2.53(d), 2.70(d)                                                                        | Se              |
| 24  | hypotaurine                    | 2.64(t), 3.38(t)                                                                        | Li, Sp          |
| 25  | aspartate                      | 2.67(dd), 2.82(dd)                                                                      | Lu, Li, Sp      |
| 26  | DMA                            | 2.72(s)                                                                                 | Se, Lu, Li      |
| 27  | TMA                            | 2.88(s)                                                                                 | Lu, Li, Sp      |
| 28  | creatine                       | 3.04(s), 3.94(s)                                                                        | Se, Lu, Li, Sp  |
| 29  | ethanolamine                   | 3.15(t), 3.84(t)                                                                        | Lu, Sp          |
| 30  | choline                        | 3.2(s)                                                                                  | Se, Lu, Li, Sp  |
| 31  | GPC                            | 3.22(s), 3.63(m)                                                                        | Lu, Li, Sp      |
| 32  | PC                             | 3.23(s), 3.61(t)                                                                        | Se, Lu, Li, Sp  |
| 33  | PE                             | 3.23(t), 3.99(m)                                                                        | Se, Lu, Li, Sp  |
| 34  | TMAO                           | 3.27(s)                                                                                 | Se              |
| 35  | taurine                        | 3.27(t), 3.43(t)                                                                        | Lu, Li, Sp      |
| 36  | betaine                        | 3.27(s), 3.90(s)                                                                        | Se, Lu, Li, Sp  |
| 37  | <i>scyllo</i> -inositol        | 3.35(s)                                                                                 | Se, Lu, Li, Sp  |
| 38  | methyl phosphate               | 3.47(d)                                                                                 | Lu, Li          |
| 39  | glycine                        | 3.56(s)                                                                                 | Lu, Sp          |
| 40  | adenosine                      | 6.10(d), 8.24(s), 8.35(s)                                                               | Lu, Li, Sp      |
| 41  | β-glucose                      | 4.65(d)                                                                                 | Se, Lu, Li, Sp  |
| 42  | α-mannose                      | 5.19(d), 3.94(m)                                                                        | Li              |
| 43  | α-glucose                      | 5.24(d)                                                                                 | Se, Lu, Li      |
| 44  | glycogen                       | 5.38-5.45                                                                               | Se, Li          |
| 45  | uracil                         | 5.81(d), 7.55(d)                                                                        | Lu, Li, Sp      |
| 46  | cytidine                       | 5.91(d), 6.07(d), 7.85(d)                                                               | Lu, Sp          |
| 47  | fumarate                       | 6.53(s)                                                                                 | Lu, Li, Sp      |
| 48  | tyrosine                       | 6.91(d), 7.20(d)                                                                        | Se, Lu, Li, Sp  |
| 49  | histidine                      | 7.05(s), 7.75(s)                                                                        | Se              |
| 50  | phenylalanine                  | 7.33(d), 7.43(t)                                                                        | Se, Lu, Li, Sp  |
| 51  | niacinamide                    | 7.60(dd), 8.26(d), 8.72(d), 8.94(s)                                                     | Lu, Li, Sp      |
| 52  | uridine                        | 7.88(d), 5.92(d), 5.91(d)                                                               | Li, Sp          |



|    |              |                  |                |
|----|--------------|------------------|----------------|
| 53 | xanthine     | 7.92(s)          | Lu, Li, Sp     |
| 54 | hypoxanthine | 8.20(s), 8.22(s) | Lu, Li, Sp     |
| 55 | formate      | 8.46(s)          | Se, Lu, Li, Sp |
| 56 | DMG          | 2.92             | Se             |

<sup>a</sup>Multiplicity for <sup>1</sup>H resonances: s: singlet, d: doublet, t: triplet, m: multiplet, dd: doublet of doublet.

<sup>b</sup>Se represents serum, Lu represents lung, Li represents liver, and Sp represents spleen.

<sup>c</sup>TMA, trimethylamine; TMAO, trimethylamine N-oxide; PC, phosphocholine; GPC, glycerophosphocholine; PE, phosphoethanolamine; DMA, dimethylamine; GSSG, glutathione disulfide; 3-HB, 3-D-hydroxybutyrate; NAG, N-acetylated glycoproteins; OAG, O-acetylated glycoproteins; DMG, dimethylglycine

**Table S2.** Parameters indicating the model quality of PLS-DA.

|                     | PLS-DA model          |                      |                       |                      |                       |                      |                       |                      |
|---------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|
|                     | serum                 |                      | lung                  |                      | liver                 |                      | spleen                |                      |
|                     | R <sup>2</sup> X(cum) | Q <sup>2</sup> (cum) | R <sup>2</sup> X(cum) | Q <sup>2</sup> (cum) | R <sup>2</sup> X(cum) | Q <sup>2</sup> (cum) | R <sup>2</sup> X(cum) | Q <sup>2</sup> (cum) |
| GS-AR-L vs. SX-AR-L | 0.681                 | 0.58                 | 0.402                 | 0.515                | 0.79                  | 0.141                | 0.402                 | 0.515                |
| GS-AR-H vs. SX-AR-H | 0.653                 | -0.113               | 0.507                 | 0.362                | 0.872                 | 0.421                | 0.507                 | 0.362                |

**Table S3.** Comparison of integral levels of metabolites in control (NS) and AR-treated (GS-AR-L, GS-AR-H, SX-AR-L, SX-AR-H) mouse serum<sup>a</sup>.

| $\delta$ <sup>1</sup> H | Metabolites       | NS              | GS-AR-L                     | GS-AR-H                     | SX-AR-L                    | SX-AR-H                    | GS-AR-L/<br>SX-AR-L | GS-AR-H/<br>SX-AR-H |
|-------------------------|-------------------|-----------------|-----------------------------|-----------------------------|----------------------------|----------------------------|---------------------|---------------------|
| 0.88                    | lipid             | 420.85 ± 17.49  | 405.83 ± 13.99 <sup>d</sup> | 395.81 ± 23.21 <sup>d</sup> | 402.38 ± 32.28             | 404.73 ± 20.17             | 1.19 <sup>c</sup>   | 0.99                |
| 0.96                    | Isoleucine        | 114.29 ± 2.30   | 124.70 ± 2.84 <sup>d</sup>  | 129.95 ± 2.28 <sup>d</sup>  | 133.50 ± 9.39              | 131.13 ± 5.95 <sup>d</sup> | 0.95                | 1.00                |
| 1.04                    | Leucine           | 31.68 ± 1.70    | 37.38 ± 1.80 <sup>d</sup>   | 43.61 ± 2.74 <sup>d</sup>   | 40.28 ± 2.33 <sup>d</sup>  | 37.83 ± 1.69 <sup>d</sup>  | 0.96                | 1.18                |
| 1.08                    | Valine            | 21.42 ± 1.34    | 24.54 ± 1.43                | 30.20 ± 2.34                | 27.30 ± 2.01 <sup>d</sup>  | 25.60 ± 1.10 <sup>d</sup>  | 0.95                | 1.22                |
| 1.20                    | 3-HB <sup>b</sup> | 85.07 ± 12.67   | 124.35 ± 14.43              | 74.12 ± 10.43               | 71.87 ± 7.25               | 98.10 ± 11.70              | 1.86 <sup>c</sup>   | 0.81                |
| 1.36                    | Lactate           | 1552.00 ± 73.28 | 1500.13 ± 54.49             | 1843.57 ± 127.32            | 1447.24 ± 72.91            | 1676.01 ± 117.52           | 1.05                | 1.14                |
| 1.48                    | Alanine           | 37.82 ± 1.87    | 42.83 ± 2.29                | 38.81 ± 2.84                | 46.52 ± 2.78 <sup>d</sup>  | 41.80 ± 7.24               | 0.95                | 0.95                |
| 1.72                    | Lysine            | 32.26 ± 2.32    | 35.83 ± 2.04                | 37.82 ± 2.34                | 44.83 ± 5.38               | 32.38 ± 2.56               | 0.91                | 1.19                |
| 1.96                    | Ornithine         | 26.17 ± 1.71    | 28.10 ± 0.92                | 27.31 ± 1.37                | 31.70 ± 2.00               | 29.64 ± 2.17               | 0.92                | 0.97                |
| 1.92                    | Acetate           | 65.49 ± 3.24    | 65.88 ± 4.48                | 79.28 ± 5.73                | 73.72 ± 5.72               | 66.95 ± 2.75               | 0.94                | 1.21                |
| 2.04                    | NAG               | 171.85 ± 9.11   | 177.93 ± 9.21               | 146.75 ± 13.59              | 151.08 ± 8.29              | 155.83 ± 11.72             | 1.21 <sup>c</sup>   | 0.99                |
| 2.36                    | Glutamate         | 42.69 ± 2.56    | 49.98 ± 2.93                | 41.42 ± 4.29                | 41.47 ± 2.44               | 52.15 ± 3.10 <sup>d</sup>  | 1.24 <sup>c</sup>   | 0.79                |
| 2.16                    | OAG               | 81.81 ± 3.49    | 87.71 ± 3.13                | 104.03 ± 5.24 <sup>d</sup>  | 101.16 ± 6.50 <sup>d</sup> | 107.15 ± 5.49 <sup>d</sup> | 0.88                | 0.99                |
| 2.44                    | Glutamine         | 50.39 ± 5.88    | 69.25 ± 6.70                | 51.84 ± 7.12                | 49.25 ± 4.14               | 66.58 ± 5.16               | 1.48 <sup>c</sup>   | 0.79                |
| 2.28                    | Acetoacetate      | 80.21 ± 5.08    | 75.17 ± 4.58                | 62.11 ± 6.54 <sup>d</sup>   | 67.08 ± 1.76 <sup>d</sup>  | 65.20 ± 4.52 <sup>d</sup>  | 1.28 <sup>c</sup>   | 0.98                |
| 2.36                    | Pyruvate          | 42.69 ± 2.56    | 41.42 ± 2.93                | 49.98 ± 4.29                | 41.47 ± 2.44               | 52.15 ± 3.10 <sup>d</sup>  | 1.24 <sup>c</sup>   | 0.79                |
| 2.4                     | Succinate         | 54.93 ± 4.07    | 54.75 ± 3.48                | 62.66 ± 3.37                | 52.41 ± 2.77               | 63.55 ± 3.49               | 1.23 <sup>c</sup>   | 0.88                |
| 2.48                    | 2-ketoglutarate   | 43.37 ± 2.76    | 49.29 ± 1.87                | 58.02 ± 4.88 <sup>d</sup>   | 56.41 ± 3.37 <sup>d</sup>  | 64.42 ± 2.98 <sup>d</sup>  | 0.89                | 0.91                |
| 2.72                    | Citrate           | 55.34 ± 1.68    | 52.37 ± 1.45                | 47.62 ± 3.63                | 51.30 ± 1.59               | 48.70 ± 1.69 <sup>d</sup>  | 1.02                | 0.98                |
| 2.64                    | Methionine        | 2.30 ± 0.56     | 1.32 ± 0.30                 | 2.20 ± 0.92                 | 2.91 ± 0.58                | 2.74 ± 0.30                | 0.56 <sup>c</sup>   | 0.61                |
| 2.92                    | DMG               | 14.33 ± 1.16    | 12.61 ± 1.35                | 7.70 ± 2.38 <sup>d</sup>    | 13.54 ± 1.20               | 8.01 ± 2.17 <sup>d</sup>   | 1.00                | 0.35                |
| 3.04                    | Creatine          | 63.48 ± 2.87    | 61.55 ± 3.50                | 71.61 ± 3.56                | 69.82 ± 5.39               | 64.09 ± 2.44               | 0.91                | 1.14                |

|      |                 |                |                |                             |                             |                             |                   |      |
|------|-----------------|----------------|----------------|-----------------------------|-----------------------------|-----------------------------|-------------------|------|
| 3.2  | Choline         | 51.74 ± 2.37   | 48.14 ± 3.14   | 49.20 ± 3.02                | 48.27 ± 2.54                | 44.94 ± 2.62                | 1.00              | 1.14 |
| 3.24 | PC              | 484.33 ± 20.60 | 492.18 ± 22.36 | 581.71 ± 26.64 <sup>d</sup> | 505.87 ± 27.91              | 538.42 ± 13.49 <sup>d</sup> | 0.99              | 1.09 |
| 3.92 | Betaine         | 228.80 ± 16.78 | 205.89 ± 13.99 | 220.06 ± 14.47              | 290.12 ± 12.17 <sup>d</sup> | 219.31 ± 15.71              | 0.72 <sup>c</sup> | 1.04 |
| 3.28 | TMAO            | 251.00 ± 13.97 | 246.48 ± 12.37 | 324.32 ± 20.06 <sup>d</sup> | 295.62 ± 9.83 <sup>d</sup>  | 306.34 ± 14.92 <sup>d</sup> | 0.83 <sup>c</sup> | 1.08 |
| 3.36 | Scyllo-inositol | 15.41 ± 1.57   | 155.56 ± 2.47  | 19.30 ± 1.27                | 19.94 ± 5.25                | 18.83 ± 2.05                | 1.02              | 1.10 |
| 3.56 | Glycerol        | 156.99 ± 9.55  | 141.55 ± 9.04  | 185.69 ± 10.68              | 186.28 ± 6.29 <sup>d</sup>  | 182.23 ± 10.20              | 0.77 <sup>c</sup> | 1.05 |
| 3.6  | Glycine         | 93.66 ± 2.57   | 95.48 ± 1.61   | 107.44 ± 2.28 <sup>d</sup>  | 106.08 ± 4.99               | 114.29 ± 3.36 <sup>d</sup>  | 0.88 <sup>c</sup> | 0.95 |
| 3.64 | Myo-inositol    | 55.75 ± 2.10   | 53.73 ± 1.66   | 62.39 ± 2.19 <sup>d</sup>   | 62.55 ± 4.24                | 59.94 ± 2.19                | 0.88              | 1.05 |
| 4.28 | Triglyceride    | 13.94 ± 1.60   | 12.59 ± 1.56   | 6.23 ± 0.74 <sup>d</sup>    | 8.92 ± 1.23 <sup>d</sup>    | 5.61 ± 0.27 <sup>d</sup>    | 1.71              | 1.13 |
| 5.24 | α-glucose       | 66.08 ± 5.18   | 55.58 ± 4.57   | 62.58 ± 5.78                | 78.01 ± 2.94                | 65.67 ± 7.37                | 0.73 <sup>c</sup> | 1.12 |
| 6.92 | Tyrosine        | 5.50 ± 0.53    | 6.18 ± 0.41    | 7.34 ± 0.79                 | 6.28 ± 0.24                 | 7.29 ± 0.31 <sup>d</sup>    | 1.00              | 1.03 |
| 7.08 | Histidine       | 0.51 ± 0.12    | 0.81 ± 1.68    | 1.81 ± 0.15 <sup>d</sup>    | 0.90 ± 0.13 <sup>d</sup>    | 1.86 ± 0.16 <sup>d</sup>    | 1.44              | 1.02 |
| 7.36 | Phenylalanine   | 8.09 ± 0.53    | 8.10 ± 0.67    | 7.68 ± 0.50                 | 8.21 ± 0.68                 | 8.11 ± 0.52                 | 1.03              | 0.97 |

<sup>a</sup>Because some of the values are too small, all of the data were magnified 100 times. <sup>b</sup>TMA, trimethylamine; TMAO, trimethylamine N-oxide; PC, phosphocholine; GPC, glycerophosphocholine; PE, phosphoethanolamine; DMA, dimethylamine; GSSG, glutathione disulfide; 3-HB, 3-D-hydroxybutyrate; NAG, N-acetylated glycoproteins; OAG, O-acetylated glycoproteins. <sup>c</sup>Comparison between SX-AR and GS-AR at the same dose;  $p < 0.05$ . <sup>d</sup>Comparison between control (NS) and each AR-treated group (GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H), respectively;  $p < 0.05$ .

**Table S4.** Comparison of integral levels of metabolites in control (NS) and AR-treated (GS-AR-L, GS-AR-H, SX-AR-L, SX-AR-H) mouse liver<sup>a</sup>.

| $\delta$ <sup>1</sup> H | Metabolites        | NS             | GS-AR-L                    | GS-AR-H                     | SX-AR-L        | SX-AR-H                    | GS-AR-L/<br>SX-AR-L | GS-AR-H/<br>SX-AR-H |
|-------------------------|--------------------|----------------|----------------------------|-----------------------------|----------------|----------------------------|---------------------|---------------------|
| 0.96                    | Isoleucine         | 175.31 ± 14.09 | 171.64 ± 10.99             | 134.68 ± 11.91 <sup>d</sup> | 153.79 ± 14.31 | 162.38 ± 13.22             | 1.12 <sup>c</sup>   | 0.83                |
| 1                       | Leucine            | 220.93 ± 15.76 | 228.06 ± 13.09             | 179.24 ± 15.73              | 199.49 ± 17.65 | 215.75 ± 17.03             | 1.14                | 0.83                |
| 1.08                    | Valine             | 56.81 ± 4.44   | 56.89 ± 3.74               | 45.86 ± 5.55                | 49.60 ± 4.19   | 56.05 ± 5.09               | 1.15                | 0.82                |
| 1.2                     | 3-HB <sup>b</sup>  | 14.80 ± 2.26   | 23.87 ± 2.96 <sup>d</sup>  | 18.02 ± 2.80                | 17.14 ± 2.92   | 23.13 ± 4.26               | 1.39                | 0.78                |
| 1.36                    | Lactate            | 363.46 ± 28.59 | 347.02 ± 18.94             | 265.04 ± 20.83 <sup>d</sup> | 353.44 ± 29.61 | 372.12 ± 16.49             | 0.98                | 0.71 <sup>c</sup>   |
| 1.72                    | Lysine             | 77.49 ± 5.19   | 89.56 ± 7.56               | 71.94 ± 7.83                | 79.99 ± 6.93   | 83.36 ± 9.15               | 1.12                | 0.86                |
| 1.52                    | Alanine            | 147.54 ± 9.42  | 162.62 ± 10.02             | 133.45 ± 10.79              | 144.81 ± 8.33  | 153.30 ± 6.82              | 1.12                | 0.87                |
| 1.92                    | Arginine           | 57.33 ± 3.78   | 72.17 ± 6.29               | 61.42 ± 5.28                | 64.49 ± 6.86   | 62.11 ± 6.14               | 1.12                | 0.99                |
| 1.76                    | Ornithine          | 92.18 ± 6.33   | 104.10 ± 8.13              | 85.62 ± 9.39                | 94.60 ± 7.32   | 98.06 ± 9.89               | 1.10                | 0.87                |
| 1.96                    | Acetate            | 63.83 ± 4.01   | 76.48 ± 6.42               | 62.52 ± 6.35                | 67.83 ± 6.13   | 65.79 ± 6.14               | 1.13                | 0.95                |
| 2.08                    | Glutamate          | 80.15 ± 4.50   | 108.44 ± 9.80 <sup>d</sup> | 101.99 ± 6.61 <sup>d</sup>  | 95.67 ± 6.25   | 107.23 ± 8.13 <sup>d</sup> | 1.13                | 0.95                |
| 2.16                    | Methionine         | 110.14 ± 5.98  | 133.00 ± 8.31 <sup>d</sup> | 106.04 ± 7.66               | 114.25 ± 8.69  | 120.30 ± 8.98              | 1.16                | 0.88                |
| 2.44                    | Glutamine          | 31.32 ± 2.36   | 43.71 ± 4.28 <sup>d</sup>  | 31.37 ± 3.62                | 35.14 ± 3.12   | 37.86 ± 3.53               | 1.24                | 0.83                |
| 2.56                    | GSSG               | 28.11 ± 3.29   | 38.06 ± 2.71 <sup>d</sup>  | 30.46 ± 1.77                | 30.82 ± 3.04   | 30.23 ± 2.44               | 1.23                | 1.01                |
| 2.4                     | Pyruvate/Succinate | 45.30 ± 3.12   | 57.82 ± 5.00               | 48.09 ± 3.68                | 47.82 ± 3.60   | 49.59 ± 3.30               | 1.21                | 0.97                |
| 2.64                    | Hypotaurine        | 6.77 ± 1.72    | 12.68 ± 2.15               | 9.77 ± 1.84                 | 9.19 ± 2.42    | 8.85 ± 1.97                | 1.38                | 1.10                |
| 2.84                    | Asparate           | 18.59 ± 1.76   | 24.38 ± 2.61               | 20.24 ± 2.30                | 19.46 ± 3.13   | 19.97 ± 2.14               | 1.25                | 1.01                |
| 2.76                    | DMA                | 15.39 ± 1.91   | 18.93 ± 2.05               | 12.99 ± 2.04                | 15.83 ± 2.27   | 14.67 ± 2.34               | 1.20                | 0.89                |
| 2.92                    | TMA                | 12.59 ± 1.41   | 16.09 ± 2.03               | 11.39 ± 1.68                | 14.06 ± 2.31   | 11.38 ± 1.49               | 1.14                | 1.00                |
| 3.96                    | Creatine           | 120.21 ± 8.19  | 112.59 ± 4.99              | 136.43 ± 7.88               | 116.48 ± 6.92  | 110.69 ± 4.50              | 0.97                | 1.23 <sup>c</sup>   |
| 3.16                    | Ethanolamine       | 46.13 ± 2.87   | 48.96 ± 3.68               | 37.35 ± 3.19                | 46.11 ± 4.72   | 40.70 ± 2.50               | 1.06                | 0.92                |
| 3.2                     | Choline            | 47.46 ± 3.61   | 46.20 ± 3.94               | 37.57 ± 3.39                | 45.34 ± 4.65   | 40.17 ± 2.39               | 1.08                | 0.94                |
| 3.24                    | GPC/PC/PE          | 373.59 ± 32.24 | 338.76 ± 23.96             | 303.64 ± 20.97              | 337.27 ± 32.15 | 350.44 ± 15.67             | 1.00                | 0.87                |



|      |                 |                |                             |                           |                |                           |                   |      |
|------|-----------------|----------------|-----------------------------|---------------------------|----------------|---------------------------|-------------------|------|
| 3.44 | Taurine         | 540.08 ± 26.44 | 587.75 ± 37.69              | 583.06 ± 27.40            | 567.90 ± 30.10 | 557.82 ± 44.48            | 0.86              | 1.05 |
| 3.28 | Betaine         | 434.32 ± 15.30 | 492.00 ± 11.78 <sup>d</sup> | 428.60 ± 9.37             | 462.05 ± 16.38 | 456.71 ± 23.04            | 1.06              | 0.94 |
| 3.36 | Scyllo-inositol | 42.72 ± 1.10   | 44.10 ± 1.13                | 39.50 ± 0.96 <sup>d</sup> | 42.70 ± 1.32   | 38.09 ± 1.25 <sup>d</sup> | 1.03              | 1.04 |
| 3.48 | Methylphosphate | 303.60 ± 19.97 | 271.11 ± 27.89              | 337.66 ± 20.29            | 324.00 ± 25.76 | 318.52 ± 27.45            | 0.84              | 1.06 |
| 3.56 | Glycine         | 248.61 ± 5.00  | 238.96 ± 9.25               | 250.08 ± 4.38             | 250.29 ± 12.36 | 264.33 ± 10.16            | 0.95              | 0.95 |
| 8.24 | Adenosine       | 4.38 ± 0.55    | 5.76 ± 1.09                 | 4.83 ± 0.64               | 4.59 ± 0.82    | 5.41 ± 0.67               | 1.26              | 0.89 |
| 4.68 | β-glucose       | 108.89 ± 7.96  | 91.88 ± 11.85               | 120.65 ± 9.12             | 111.42 ± 11.56 | 120.80 ± 12.17            | 0.82              | 1.00 |
| 5.24 | α-glucose       | 56.85 ± 4.23   | 54.50 ± 6.32                | 67.67 ± 5.79              | 64.87 ± 4.46   | 63.20 ± 5.17              | 0.84              | 1.07 |
| 3.60 | Glycogen        | 310.75 ± 11.75 | 314.45 ± 12.29              | 313.05 ± 12.63            | 275.45 ± 11.82 | 300.42 ± 9.49             | 1.14 <sup>c</sup> | 1.04 |
| 5.84 | Uracil          | 3.88 ± 0.42    | 4.56 ± 0.44                 | 3.71 ± 0.40               | 4.15 ± 0.80    | 3.48 ± 0.36               | 1.10              | 1.07 |
| 6.56 | Fumarate        | 5.67 ± 0.42    | 6.32 ± 0.42                 | 5.93 ± 0.28               | 6.53 ± 0.42    | 6.25 ± 0.25               | 0.97              | 0.95 |
| 6.92 | Tyrosine        | 20.83 ± 1.40   | 22.09 ± 1.44                | 16.47 ± 1.54              | 18.42 ± 1.81   | 19.88 ± 1.65              | 1.20              | 0.83 |
| 7.44 | Phenylalanine   | 17.39 ± 1.16   | 18.26 ± 1.10                | 14.23 ± 1.23              | 15.08 ± 1.42   | 16.53 ± 1.56              | 1.21              | 0.86 |
| 7.6  | Niacinamide     | 1.95 ± 0.17    | 2.79 ± 0.31 <sup>d</sup>    | 1.84 ± 0.14               | 1.98 ± 0.28    | 1.93 ± 0.23               | 1.41              | 0.95 |
| 5.92 | Uridine         | 7.46 ± 0.50    | 8.93 ± 0.52                 | 6.63 ± 0.60               | 6.79 ± 0.64    | 6.30 ± 0.36               | 1.32 <sup>c</sup> | 1.05 |
| 8.2  | Hypoxanthine    | 4.17 ± 0.69    | 5.74 ± 1.66                 | 4.77 ± 0.68               | 4.31 ± 1.01    | 5.20 ± 0.74               | 1.33              | 0.92 |
| 8.48 | Formate         | 0.93 ± 0.12    | 1.07 ± 0.11                 | 0.31 ± 0.05               | 1.42 ± 0.29    | 0.32 ± 0.06               | 0.75              | 0.97 |
| 5.2  | α-mannose       | 11.60 ± 0.67   | 11.40 ± 0.87                | 12.96 ± 0.67              | 12.15 ± 0.72   | 10.61 ± 0.87              | 0.94              | 1.22 |

<sup>a</sup>Because some of the values are too small, all of the data were magnified 100 times. <sup>b</sup>TMA, trimethylamine; TMAO, trimethylamine N-oxide; PC, phosphocholine; GPC, glycerophosphocholine; PE, phosphoethanolamine; DMA, dimethylamine; GSSG, glutathione disulfide; 3-HB, 3-D-hydroxybutyrate; NAG, N-acetylated glycoproteins; OAG, O-acetylated glycoproteins. <sup>c</sup>Comparison between SX-AR and GS-AR at the same dose;  $p < 0.05$ . <sup>d</sup>Comparison between control (NS) and each AR-treated group (GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H), respectively;  $p < 0.05$ .

**Table S5.** Comparison of integral levels of metabolites in control (NS) and AR-treated (GS-AR-L, GS-AR-H, SX-AR-L, SX-AR-H) mouse spleen<sup>a</sup>.

| $\delta$ <sup>1</sup> H | Metabolites         | NS             | GS-AR-L                     | GS-AR-H                     | SX-AR-L        | SX-AR-H                   | GS-AR-L/<br>SX-AR-L | GS-AR-H/<br>SX-AR-H |
|-------------------------|---------------------|----------------|-----------------------------|-----------------------------|----------------|---------------------------|---------------------|---------------------|
| 0.96                    | Isoleucine          | 283.32 ± 11.52 | 268.84 ± 14.12 <sup>d</sup> | 239.07 ± 13.13 <sup>d</sup> | 271.64 ± 13.29 | 291.01 ± 21.64            | 0.99 <sup>c</sup>   | 0.82                |
| 1                       | Leucine             | 324.18 ± 12.11 | 327.89 ± 17.30              | 267.28 ± 17.79 <sup>d</sup> | 306.40 ± 15.97 | 352.01 ± 30.46            | 1.07                | 0.76 <sup>c</sup>   |
| 1.08                    | Valine              | 67.50 ± 3.27   | 69.85 ± 3.50                | 61.99 ± 3.28                | 67.68 ± 4.50   | 76.86 ± 6.79              | 1.03                | 0.81                |
| 1.2                     | 3-HB <sup>b</sup>   | 34.59 ± 4.26   | 27.55 ± 1.11                | 25.85 ± 2.58                | 41.62 ± 7.48   | 27.05 ± 2.72              | 0.66                | 0.96                |
| 1.36                    | Lactate             | 527.47 ± 43.30 | 545.71 ± 45.62              | 437.77 ± 23.83              | 569.58 ± 31.24 | 583.29 ± 36.11            | 0.96                | 0.75 <sup>c</sup>   |
| 1.72                    | Lysine/Arginine     | 147.52 ± 6.11  | 139.99 ± 6.53               | 130.69 ± 5.96               | 141.32 ± 7.84  | 161.62 ± 11.90            | 0.99                | 0.81                |
| 1.52                    | Alanine             | 188.79 ± 9.90  | 193.29 ± 11.39              | 169.72 ± 9.83               | 181.81 ± 8.72  | 202.98 ± 16.38            | 1.06                | 0.84                |
| 1.76                    | Ornithine           | 162.68 ± 7.77  | 158.08 ± 7.24               | 140.56 ± 7.14               | 152.24 ± 8.95  | 177.99 ± 14.12            | 1.04                | 0.79 <sup>c</sup>   |
| 1.96                    | Acetate             | 116.57 ± 6.65  | 121.011 ± 3.93              | 108.89 ± 4.79               | 113.33 ± 6.13  | 132.34 ± 9.86             | 1.07                | 0.82                |
| 2.08                    | Glutamate           | 269.24 ± 18.20 | 310.12 ± 26.13              | 233.59 ± 10.14              | 256.96 ± 12.22 | 285.70 ± 23.82            | 1.21                | 0.82                |
| 2.16                    | Methionine          | 202.70 ± 13.27 | 224.60 ± 11.02              | 196.10 ± 8.28               | 215.96 ± 9.33  | 230.20 ± 17.28            | 1.04                | 0.85                |
| 2.44                    | Glutamine           | 33.26 ± 2.91   | 43.89 ± 2.37 <sup>d</sup>   | 41.78 ± 3.88                | 39.19 ± 3.38   | 43.35 ± 2.81 <sup>d</sup> | 1.12                | 0.96                |
| 2.4                     | Pyruvate /succinate | 101.56 ± 7.45  | 114.65 ± 6.14               | 103.81 ± 3.59               | 110.45 ± 4.27  | 113.47 ± 7.79             | 1.04                | 0.91                |
| 2.56                    | Citrate             | 22.04 ± 1.36   | 27.16 ± 2.03                | 29.46 ± 3.94                | 25.07 ± 3.17   | 24.73 ± 1.52              | 1.08                | 1.19                |
| 2.64                    | Hypotaurine         | 10.32 ± 2.95   | 9.29 ± 1.14                 | 13.78 ± 4.00                | 12.12 ± 3.06   | 11.23 ± 1.65              | 0.77                | 1.23                |
| 2.84                    | Aspartate           | 49.24 ± 2.32   | 52.46 ± 2.32                | 53.43 ± 3.86                | 53.55 ± 2.79   | 53.61 ± 3.49              | 0.98                | 1.00                |
| 2.88                    | TMA                 | 15.99 ± 1.56   | 13.89 ± 0.98                | 18.71 ± 3.52                | 17.14 ± 2.35   | 20.03 ± 1.46              | 0.81                | 0.93                |
| 3.96                    | Creatine            | 151.97 ± 7.95  | 162.24 ± 8.96               | 156.82 ± 7.30               | 155.76 ± 10.10 | 169.61 ± 14.72            | 1.04                | 0.87                |
| 3.2                     | Choline             | 48.87 ± 3.36   | 54.72 ± 3.40                | 43.33 ± 2.80                | 44.25 ± 2.86   | 51.47 ± 3.43              | 1.24 <sup>c</sup>   | 0.84                |
| 3.24                    | GPC/PC/PE           | 575.47 ± 52.52 | 666.54 ± 41.09              | 572.19 ± 36.79              | 626.77 ± 41.69 | 674.27 ± 56.51            | 1.06                | 0.85                |
| 3.44                    | Taurine             | 399.36 ± 43.45 | 490.14 ± 32.66              | 398.65 ± 18.69              | 415.47 ± 20.74 | 433.77 ± 30.85            | 1.18                | 0.92                |

|      |                 |                |                             |                           |                |                |      |                   |
|------|-----------------|----------------|-----------------------------|---------------------------|----------------|----------------|------|-------------------|
| 3.28 | Betaine         | 524.71 ± 51.24 | 671.89 ± 44.54 <sup>d</sup> | 525.42 ± 28.34            | 569.77 ± 31.66 | 576.48 ± 42.52 | 1.18 | 0.91              |
| 3.36 | Scyllo-inositol | 38.38 ± 2.07   | 42.18 ± 1.96                | 39.71 ± 2.72              | 37.71 ± 1.70   | 45.37 ± 3.60   | 1.12 | 0.88              |
| 3.6  | Glycine         | 261.25 ± 27.78 | 312.04 ± 15.48              | 262.83 ± 15.46            | 279.82 ± 15.00 | 292.89 ± 23.82 | 1.12 | 0.90              |
| 8.24 | Adenosine       | 15.29 ± 2.12   | 18.16 ± 1.68                | 16.46 ± 1.49              | 14.60 ± 1.74   | 12.98 ± 1.49   | 1.24 | 1.27              |
| 3.56 | Glycogen        | 98.72 ± 8.02   | 122.90 ± 8.09               | 107.20 ± 4.98             | 106.34 ± 7.95  | 118.89 ± 7.89  | 1.16 | 0.90              |
| 5.84 | Uracil          | 6.94 ± 0.69    | 7.60 ± 0.91                 | 9.39 ± 0.74               | 8.33 ± 0.61    | 7.96 ± 0.43    | 0.91 | 1.18              |
| 6.08 | Cytidine        | 10.24 ± 1.26   | 10.52 ± 1.17                | 11.16 ± 0.47              | 11.99 ± 1.19   | 10.93 ± 0.98   | 0.88 | 1.02              |
| 6.56 | Fumarate        | 2.48 ± 0.27    | 2.65 ± 0.21                 | 2.62 ± 0.24               | 2.67 ± 0.24    | 3.14 ± 0.27    | 0.99 | 0.84              |
| 6.92 | Tyrosine        | 35.08 ± 1.26   | 36.14 ± 2.02                | 29.66 ± 2.30              | 34.03 ± 1.92   | 40.41 ± 3.78   | 1.06 | 0.73 <sup>c</sup> |
| 7.44 | Phenylalanine   | 23.30 ± 0.82   | 23.26 ± 1.47                | 18.31 ± 1.49 <sup>d</sup> | 22.07 ± 1.50   | 26.15 ± 2.20   | 1.05 | 0.70 <sup>c</sup> |
| 7.64 | Niacinamide     | 1.43 ± 0.50    | 1.71 ± 0.47                 | 1.66 ± 0.22               | 2.15 ± 0.17    | 2.43 ± 0.21    | 0.80 | 0.68 <sup>c</sup> |
| 5.92 | Uridine         | 17.10 ± 2.04   | 19.37 ± 1.41                | 18.93 ± 0.97              | 19.44 ± 1.42   | 17.63 ± 1.53   | 1.00 | 1.07              |
| 7.92 | Xanthine        | 34.63 ± 2.72   | 38.29 ± 2.62                | 32.01 ± 1.58              | 37.43 ± 1.95   | 38.36 ± 2.46   | 1.02 | 0.83              |
| 8.2  | Hypoxanthine    | 13.28 ± 1.60   | 15.87 ± 1.34                | 13.41 ± 1.34              | 12.09 ± 1.55   | 10.61 ± 1.24   | 1.31 | 1.26              |
| 8.48 | Formate         | 3.19 ± 0.36    | 3.02 ± 0.18                 | 2.74 ± 0.20               | 2.61 ± 0.28    | 2.91 ± 0.37    | 1.16 | 0.94              |

<sup>a</sup>Because some of the values are too small, all of the data were magnified 100 times. <sup>b</sup>TMA, trimethylamine; TMAO, trimethylamine N-oxide; PC, phosphocholine; GPC, glycerophosphocholine; PE, phosphoethanolamine; DMA, dimethylamine; GSSG, glutathione disulfide; 3-HB, 3-D-hydroxybutyrate; NAG, N-acetylated glycoproteins; OAG, O-acetylated glycoproteins. <sup>c</sup>Comparison between SX-AR and GS-AR at the same dose;  $p < 0.05$ . <sup>d</sup>Comparison between control (NS) and each AR-treated group (GS-AR-L, GS-AR-H, SX-AR-L and SX-AR-H), respectively;  $p < 0.05$ .

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