



**Figure SI.1** Chiral LC chromatogram at 520 nm upper and 225 nm below of the purified by column chromatography (CC) shikonin sample (IchimaruPharcos, Japan) tested to Huh7 cultures. Experimental conditions are provided in reference 62.

**Table S1.** Concentration ( $\mu\text{g/mL}$ ) of metabolites determined in the intracellular extracts of Huh-7 cells.

| Metabolite       | Concentration* ( $\mu\text{g/mL}$ ) |       |       |       |       |          |        |        |        |       |
|------------------|-------------------------------------|-------|-------|-------|-------|----------|--------|--------|--------|-------|
|                  | Control                             |       |       |       |       | Shikonin |        |        |        |       |
|                  | C1                                  | C2    | C3    | mean  | std   | S1       | S2     | S3     | mean   | std   |
| Pyruvic acid     | 28.50                               | 28.64 | 26.11 | 27.75 | 1.42  | 76.07    | 81.67  | 93.75  | 83.83  | 9.04  |
| Arginine         | 0                                   | 0     | 0     | 0     | 0     | 6.08     | 8.13   | 8.38   | 7.53   | 1.26  |
| Serine           | 2.04                                | 1.93  | 2.23  | 2.07  | 0.15  | 3.27     | 3.60   | 3.37   | 3.41   | 0.17  |
| Glucose          | 77.07                               | 80.83 | 57.52 | 71.81 | 12.51 | 140.29   | 137.62 | 170.78 | 149.56 | 18.42 |
| Methionine       | 1.44                                | 1.37  | 1.11  | 1.31  | 0.17  | 2.07     | 2.65   | 2.60   | 2.44   | 0.32  |
| Lysine           | 19.65                               | 21.36 | 19.59 | 20.20 | 1.01  | 22.86    | 23.74  | 25.76  | 24.12  | 1.49  |
| Glutamine        | 48.72                               | 53.15 | 48.85 | 50.24 | 2.52  | 57.93    | 62.52  | 69.71  | 63.39  | 5.94  |
| Tryptophan       | 1.64                                | 1.63  | 1.42  | 1.56  | 0.12  | 1.87     | 2.14   | 2.36   | 2.12   | 0.25  |
| Xanthine         | 0.00                                | 0.37  | 0.35  | 0.24  | 0.21  | 0.48     | 0.52   | 0.56   | 0.52   | 0.04  |
| Phenylalanine    | 38.17                               | 43.92 | 32.77 | 38.29 | 5.58  | 41.25    | 48.14  | 50.53  | 46.64  | 4.82  |
| Ornithine        | 1.57                                | 1.87  | 1.60  | 1.68  | 0.17  | 1.85     | 1.83   | 1.89   | 1.86   | 0.03  |
| Leucine          | 3.94                                | 4.15  | 3.71  | 3.93  | 0.22  | 4.04     | 4.36   | 4.88   | 4.43   | 0.42  |
| Tyrosine         | 5.31                                | 7.25  | 7.12  | 6.56  | 1.08  | 6.86     | 7.56   | 9.58   | 8.00   | 1.41  |
| Pyridoxine       | 0.29                                | 0.31  | 0.23  | 0.28  | 0.04  | 0.28     | 0.33   | 0.36   | 0.32   | 0.04  |
| Norvaline-Valine | 3.42                                | 3.37  | 2.87  | 3.22  | 0.30  | 3.25     | 3.44   | 3.90   | 3.53   | 0.33  |
| Benzoic acid     | 6.43                                | 2.84  | 3.04  | 4.10  | 2.02  | 3.74     | 8.33   | 5.81   | 5.96   | 2.30  |
| Pyroglutamic     | 37.21                               | 37.85 | 30.20 | 35.09 | 4.24  | 35.05    | 36.77  | 42.46  | 38.09  | 3.88  |
| Hypotaurine      | 1.80                                | 0.00  | 1.66  | 1.15  | 1.00  | 1.62     | 1.60   | 1.59   | 1.60   | 0.02  |
| Acetylcarnitine  | 0.32                                | 0.43  | 0.29  | 0.35  | 0.07  | 0.39     | 0.36   | 0.38   | 0.38   | 0.02  |
| Isoleucine       | 5.18                                | 5.81  | 5.41  | 5.47  | 0.32  | 4.62     | 5.93   | 6.71   | 5.75   | 1.06  |
| Choline          | 0.47                                | 0.74  | 0.31  | 0.51  | 0.22  | 0.55     | 0.54   | 0.55   | 0.55   | 0.01  |
| Inositol         | 55.55                               | 52.23 | 53.78 | 53.85 | 1.66  | 17.59    | 18.07  | 18.91  | 18.19  | 0.67  |

|               |        |        |        |        |       |        |        |        |        |       |
|---------------|--------|--------|--------|--------|-------|--------|--------|--------|--------|-------|
| Proline       | 24.56  | 22.39  | 25.89  | 24.28  | 1.77  | 9.19   | 9.29   | 8.52   | 9.00   | 0.42  |
| Alanine       | 53.04  | 56.05  | 54.70  | 54.60  | 1.51  | 32.90  | 37.84  | 36.25  | 35.66  | 2.52  |
| Threonine     | 16.95  | 16.63  | 17.21  | 16.93  | 0.29  | 10.75  | 12.03  | 12.48  | 11.75  | 0.90  |
| Glutamic acid | 48.23  | 52.25  | 63.92  | 54.80  | 8.15  | 13.74  | 13.92  | 16.32  | 14.66  | 1.44  |
| Pantothenate  | 1.78   | 1.75   | 1.56   | 1.70   | 0.12  | 1.08   | 1.11   | 1.13   | 1.11   | 0.03  |
| Spermidine    | 1.47   | 1.11   | 0.92   | 1.17   | 0.28  | 0      | 0      | 0      | 0      | 0     |
| Cadaverine    | 0.04   | 0.04   | 0.06   | 0.05   | 0.01  | 0      | 0      | 0      | 0      | 0     |
| Inosine       | 0.32   | 0.46   | 0.29   | 0.36   | 0.09  | 0      | 0      | 0      | 0      | 0     |
| Adenosine     | 0.34   | 0.25   | 0.38   | 0.32   | 0.07  | 0.09   | 0.07   | 0.09   | 0.08   | 0.01  |
| Nicotinamide  | 2.38   | 3.61   | 2.55   | 2.85   | 0.67  | 0.70   | 0.88   | 0.92   | 0.83   | 0.12  |
| Taurine       | 4.63   | 5.56   | 5.11   | 5.10   | 0.47  | 3.73   | 2.74   | 3.71   | 3.39   | 0.57  |
| Asparagine    | 2.74   | 2.12   | 2.98   | 2.61   | 0.44  | 1.49   | 1.68   | 1.58   | 1.58   | 0.10  |
| Aspartic acid | 2.13   | 2.11   | 3.27   | 2.50   | 0.66  | 1.25   | 1.16   | 1.19   | 1.20   | 0.05  |
| Galactose     | 11.22  | 12.99  | 7.89   | 10.70  | 2.59  | 7.94   | 8.40   | 7.88   | 8.07   | 0.28  |
| Creatinine    | 0.62   | 0.71   | 0.53   | 0.62   | 0.09  | 0.54   | 0.56   | 0.60   | 0.57   | 0.03  |
| Glycine       | 28.33  | 30.28  | 33.62  | 30.74  | 2.68  | 24.30  | 27.76  | 32.42  | 28.16  | 4.07  |
| Lactic acid   | 441.44 | 450.69 | 375.80 | 422.64 | 40.83 | 381.19 | 415.21 | 415.45 | 403.95 | 19.71 |
| Thiamine      | 0.48   | 0.48   | 0.41   | 0.46   | 0.04  | 0.42   | 0.45   | 0.47   | 0.45   | 0.03  |
| Creatine      | 0.06   | 5.81   | 5.40   | 3.76   | 3.21  | 3.52   | 3.23   | 3.28   | 3.34   | 0.16  |

\*The values correspond to 2 mL cell extracts which were diluted 1:3.

**Table S2.**Concentration ( $\mu\text{g/mL}$ ) of metabolites determined in the blank medium and the cell culture medium of Huh-7, Control and Shikonin treated.

| Metabolite   | Concentration* ( $\mu\text{g/mL}$ ) |         |         |        |         |        |          |        |        |        |       |
|--------------|-------------------------------------|---------|---------|--------|---------|--------|----------|--------|--------|--------|-------|
|              | Blank medium                        | Control |         |        |         |        | Shikonin |        |        |        |       |
|              |                                     | C1      | C2      | C3     | mean    | sd     | S1       | S2     | S3     | mean   | sd    |
| Arginine     | 21.29                               | 1.32    | 3.51    | 0.04   | 1.62    | 1.75   | 9.84     | 12.89  | 10.78  | 11.17  | 1.56  |
| Pyruvic acid | 27.63                               | 44.54   | 75.49   | 24.13  | 48.05   | 25.86  | 81.45    | 100.4  | 83.65  | 88.50  | 10.36 |
| Serine       | 11.74                               | 3.57    | 7.51    | 0.5    | 3.86    | 3.51   | 4.27     | 5.57   | 5.04   | 4.96   | 0.65  |
| Choline      | 1.18                                | 0       | 0       | 0      | 0.00    | 0.00   | 0.42     | 0.54   | 0.49   | 0.48   | 0.06  |
| Spermidine   | 5.43                                | 0       | 0       | 0      | 0.00    | 0.00   | 0        | 0      | 0      | 0.00   | 0.00  |
| Fructose     | 29.91                               | 0       | 0       | 0      | 0.00    | 0.00   | 0        | 0      | 0      | 0.00   | 0.00  |
| Pantothenate | 0                                   | 2.92    | 5.67    | 1.21   | 3.27    | 2.25   | 0        | 0      | 0      | 0.00   | 0.00  |
| Threonine    | 17.62                               | 51.44   | 117.16  | 13.87  | 60.82   | 52.28  | 10.47    | 14.27  | 11.66  | 12.13  | 1.94  |
| Galactose    | 5.67                                | 85.94   | 140.53  | 30.27  | 85.58   | 55.13  | 18.91    | 22.8   | 13.35  | 18.35  | 4.75  |
| Histidine    | 7.59                                | 16.63   | 33.17   | 4.29   | 18.03   | 14.49  | 3.5      | 5.8    | 3.99   | 4.43   | 1.21  |
| Alanine      | 13.87                               | 141.34  | 278.12  | 48.9   | 156.12  | 115.32 | 46.86    | 57.82  | 40.08  | 48.25  | 8.95  |
| Ornithine    | 1.26                                | 7.04    | 10.45   | 2      | 6.50    | 4.25   | 2.03     | 2.51   | 1.84   | 2.13   | 0.35  |
| Asparagine   | 0                                   | 4.29    | 8.2     | 1.49   | 4.66    | 3.37   | 1.54     | 1.79   | 1.35   | 1.56   | 0.22  |
| Proline      | 640.97                              | 26.54   | 28.6    | 17.01  | 24.05   | 6.18   | 8.13     | 10.13  | 7.67   | 8.64   | 1.31  |
| Nicotinamide | 0.64                                | 0.72    | 0.95    | 1.13   | 0.93    | 0.21   | 0.24     | 0.47   | 0.31   | 0.34   | 0.12  |
| Lysine       | 33.22                               | 68.51   | 155.09  | 26.61  | 83.40   | 65.52  | 29.28    | 34.36  | 27.93  | 30.52  | 3.39  |
| Lactic acid  | 75.57                               | 1276.79 | 1916.97 | 640.92 | 1278.23 | 638.03 | 490.33   | 575.49 | 439.08 | 501.63 | 68.90 |
| Glutamine    | 94.05                               | 164.14  | 401.45  | 48.02  | 204.54  | 180.14 | 76.78    | 100.81 | 75.35  | 84.31  | 14.30 |
| Pyroglutamic | 42.94                               | 123.93  | 189.65  | 58.71  | 124.10  | 65.47  | 45.58    | 60.99  | 49.44  | 52.00  | 8.02  |
| Glycine      | 15.39                               | 39.65   | 97.46   | 7.67   | 48.26   | 45.51  | 17.89    | 25.69  | 18.05  | 20.54  | 4.46  |
| Creatinine   | 1                                   | 1.88    | 2.79    | 1.03   | 1.90    | 0.88   | 0.81     | 1      | 0.71   | 0.84   | 0.15  |
| Inositol     | 9.98                                | 14.06   | 17.03   | 5.29   | 12.13   | 6.10   | 6.52     | 5.94   | 5.08   | 5.85   | 0.72  |

|                  |         |        |        |        |        |        |        |       |        |        |       |
|------------------|---------|--------|--------|--------|--------|--------|--------|-------|--------|--------|-------|
| Pyridoxine       | 0.66    | 0.98   | 1.33   | 0.57   | 0.96   | 0.38   | 0.48   | 0.61  | 0.45   | 0.51   | 0.09  |
| Glucose          | 1248.11 | 672.5  | 942.93 | 340.84 | 652.09 | 301.56 | 343.71 | 381   | 375.38 | 366.70 | 20.10 |
| Phenylalanine    | 108.7   | 126.63 | 187.26 | 63.55  | 125.81 | 61.86  | 72.36  | 96.89 | 69.83  | 79.69  | 14.95 |
| Tyrosine         | 13.89   | 18.99  | 28.31  | 10.43  | 19.24  | 8.94   | 11.85  | 15.05 | 11.11  | 12.67  | 2.09  |
| Thiamine         | 0       | 0.82   | 1.05   | 0.64   | 0.84   | 0.21   | 0.55   | 0.63  | 0.52   | 0.57   | 0.06  |
| Norvaline/Valine | 7.66    | 8.39   | 10.37  | 4.05   | 7.60   | 3.23   | 4.77   | 6.05  | 6.04   | 5.62   | 0.74  |
| Isoleucine       | 16.01   | 9.61   | 15.46  | 6.07   | 10.38  | 4.74   | 7.49   | 9.27  | 8.3    | 8.35   | 0.89  |
| Leucine          | 17.12   | 11.05  | 9.04   | 5.54   | 8.54   | 2.79   | 6.62   | 8.11  | 7.03   | 7.25   | 0.77  |
| Benzoic acid     | 3.48    | 6.38   | 0.19   | 2.18   | 2.92   | 3.16   | 3.13   | 3.94  | 1.36   | 2.81   | 1.32  |

\*The values refer to 2 mL of culture medium which was diluted 1:3.