

Supplement 1. Cell viability of shikonin on HCT-116 cells

| Concentration (μM) | Shikonin treatment (Mean \pm SD) | | | |
|---------------------------------|------------------------------------|---------------------|---------------------|----------------------|
| | 3 h | 6 h | 12 h | 24 h |
| Control | 100.13 \pm 3.30 | 93.88 \pm 1.74 | 93.92 \pm 1.84 | 92.24 \pm 1.43 |
| 0.25 | 99.9 \pm 6.07 | 77.39 \pm 2.85*** | 79.00 \pm 2.33*** | 82.24 \pm 6.64 *** |
| 0.5 | 81.28 \pm 8.27*** | 65.74 \pm 3.22*** | 49.29 \pm 5.80*** | 28.32 \pm 3.75*** |
| 1 | 63.15 \pm 2.91*** | 47.33 \pm 2.68*** | 36.66 \pm 1.22*** | 25.73 \pm 0.95*** |
| 1.5 | 54.34 \pm 2.29*** | 42.22 \pm 2.75*** | 32.17 \pm 3.05*** | 20.68 \pm 2.26*** |
| 2 | 40.51 \pm 2.02*** | 16.11 \pm 1.51*** | 8.26 \pm 0.88*** | 4.01 \pm 0.43*** |
| 2.5 | 37.7 \pm 1.87*** | 8.73 \pm 0.51*** | 5.95 \pm 0.39*** | 4.26 \pm 0.20*** |
| 3 | 37.01 \pm 2.90*** | 6.97 \pm 0.50*** | 7.06 \pm 0.61*** | 6.16 \pm 0.31*** |

Supplement 2. Cell viability of shikonin on HCT-15 cells

| Concentration (μM) | Shikonin treatment (Mean \pm SD) | | | |
|---------------------------------|------------------------------------|---------------------|---------------------|---------------------|
| | 3 h | 6 h | 12 h | 24 h |
| Control | 101.49 \pm 3.31 | 94.04 \pm 2.10 | 97.87 \pm 2.52 | 94.57 \pm 2.68 |
| 0.25 | 95.82 \pm 1.69* | 83.20 \pm 3.57*** | 82.67 \pm 3.12*** | 77.56 \pm 3.24*** |
| 0.5 | 93.20 \pm 4.77*** | 76.72 \pm 3.76*** | 69.93 \pm 3.88*** | 37.90 \pm 2.83*** |
| 1 | 51.94 \pm 4.63*** | 48.96 \pm 3.17*** | 45.09 \pm 4.05*** | 20.53 \pm 0.70*** |
| 1.5 | 11.72 \pm 1.13*** | 13.76 \pm 1.66*** | 11.71 \pm 2.01*** | 6.80 \pm 1.14*** |
| 2 | 3.90 \pm 0.57*** | 3.44 \pm 0.51*** | 3.21 \pm 0.16*** | 2.12 \pm 0.11*** |
| 2.5 | 3.21 \pm 0.18*** | 2.35 \pm 0.23*** | 2.46 \pm 0.13*** | 1.64 \pm 0.09*** |
| 3 | 3.23 \pm 0.13*** | 2.50 \pm 0.17*** | 2.64 \pm 0.09*** | 1.68 \pm 0.10*** |

Supplement 3. *In vivo* efficacy studies of shikonin in colorectal cancer xenograft models. HCT-116 and HCT-15 cells were implanted subcutaneously in BALB/c nude mice. The tumor-bearing mice were oral administered with shikonin (20 mg/kg) or vehicle for two or three weeks. **(a)** The tumor growth curve and the body weight change in HCT-116 xenograft model. **(b)** The tumor growth curve and the body weight change in HCT-15 xenograft model.

