**Additional file**

**Arenobufagin induces MCF-7 cell apoptosis by promoting JNK-mediated multisite phosphorylation of Yes-associated protein**

**Running title: ABF induces apoptosis through the phosphorylation of YAP**

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Table S1. The sequence-specific oligonucleotide primers

|  |  |
| --- | --- |
| Target genes | Primers |
| *BAX* | forward: 5’-AGGATGCGTCCACCAAGAAG-3’; |
|  | reverse: 5’-AGCTGCCACTCGGAAAAAGA-3’. |
| *p53AIPI* | forward: 5’-CCGGGCCAGGAGTAAGTAAC-3’; |
|  | reverse: 5’-ACAACGCCGAAGAGGATCAG-3’. |
| *GAPDH* | forward: 5’-CCACCCCAATGTCTCTGTT-3’; |
|  | reverse: 5’-CAACCTGGTGCTCCGTGTAT-3’. |

Table S2. The target siRNA duplexes

|  |  |
| --- | --- |
| Target genes | siRNA duplex sequences |
| *YAP* | forward: 5’-UCUGCAGUUGGGAGCUGUUTT-3’; |
|  | reverse: 5’-AACAGCUCCCAACUGCCAGATT-3’. |
| *LATS1* | forward: 5’-GGAGUGUUACUCCUCCACCTT-3’; |
|  | reverse: 5’-GGUGGAGGAGUAACACUCCTT-3’. |
| Negative siRNA duplex | forward: 5’-UUCUCCGAACGUGUCACGUTT-3’; |
|  | reverse: 5’-ACGUGACACGUUCGGAGAATT-3’. |

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**Figure S1** Representative data from the Annexin V/PI apoptosis assay. Following ABF treatment, MCF-7 cells were subjected to Annexin V/PI staining and analyzed by flow cytometry, n = 3.

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**Figure S2** Distribution of YAP in the nuclei and cytoplasm of ABF-treated MCF-7 cells. Nuclear and cytoplasmic fractions were collected and evaluated by Western blotting. Lamin B1 was used as the nuclear protein control, while GAPDH was used as the cytoplasmic protein control.