Figure S1. Low concentration of EtOH does not affect the viability nor motility of lung cancer cells. The A549 cells were either UT or treated with 0.1% of EtOH (v/v). Cells were subjected to (A) viability assays or (B) migration assays, 48 h after treatment. The related viability or motility of EtOH-treated A549 cells were then normalized to that of UT cells. Scale bar, 40 μm. UT, untreated.

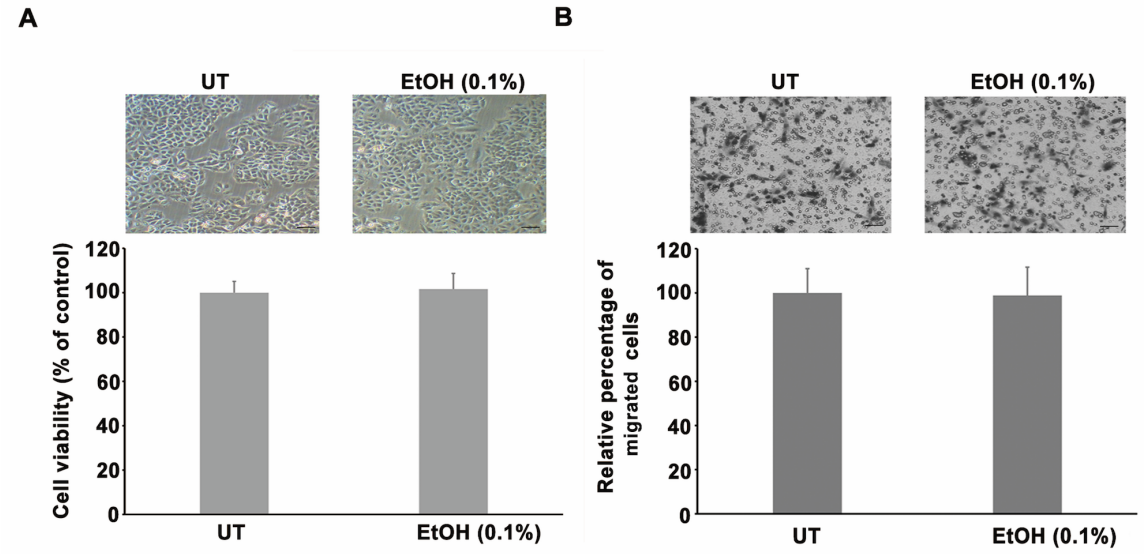

Figure S2. WA decreases the viability of both H460 and H1355 cell lines. (A) H460 and (B) H1355 cells were treated with different concentrations of WA (0.5, 1, 1.5, 2 and 3 μM) for 24 or 48 h, respectively. Cells were collected and subjected to MTT assay to analyze their viability. The MTT value of experimental set was then normalized to the UT cells as a percentage. (A) *P<0.05, **P<0.01 and ***P<0.001 were referred to the viability differences between 24 h WA treatments and UT in H460 cells; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 were referred to the viability differences between 48 h WA treatments and UT in H460 cells. (B) *P<0.05, **P<0.01 and ***P<0.001 were referred to the viability differences between 24 h WA treatments and UT in H1355 cells; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 were referred to the viability differences between 48 h WA treatments and UT in H1355 cells. UT, untreated cells; Veh, vehicle control 0.1% EtOH; WA, withaferin A.

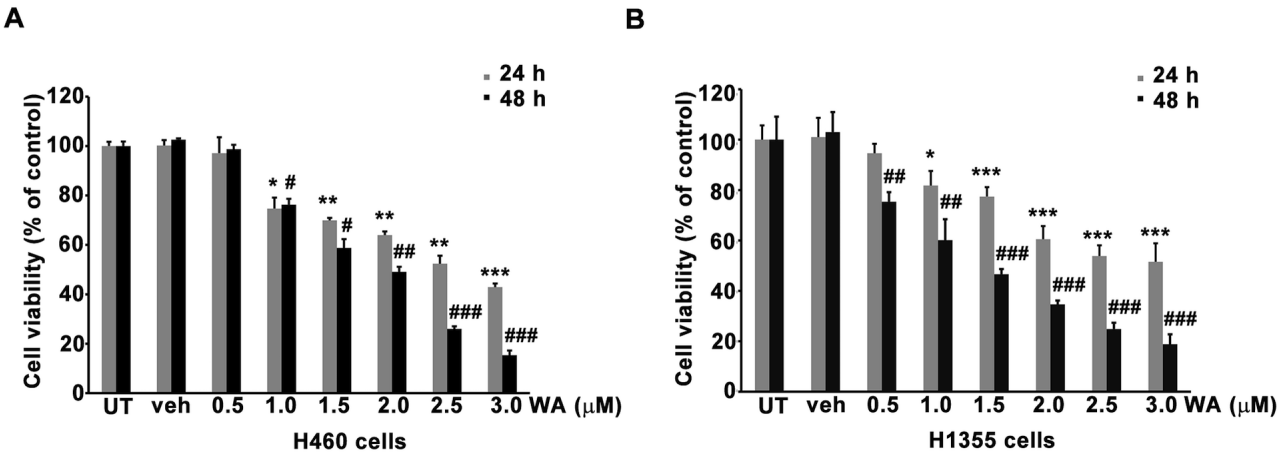

Figure S3. WA induces A549 cell death via an apoptosis manner. (A) The different caspase activities of various concentrations of WA-treated A549 cells were analyzed and quantified. (B) A549 cells were pretreated with inhibitor of caspase3 (Z-DEVD-FMK), caspase 8 (Z-IETD-FMK) or caspase 9 (Z-LEHD-FMK), respectively for 1 h followed by treated with 3 μM of WA for further 24 h, the cells were then collected and subjected to cellular viability assay. *P<0.05, **P<0.01, ***P<0.001 vs. UT. UT, untreated; WA, withaferin A; Z-DEVD-FMK, (benzoylcarbonyl-aspartyl-glutamyl-valyl-aspartyl-fluoromethyl ketone); Z-IETD-FMK, (benzyloxycarbonyl-isoleucyl-glutamyl-threonyl-aspartic acid fluoromethyl ketone); Z-LEHD-FMK (benzyloxycarbonyl-leucyl-glutamyl-histidyl-aspartic acid fluoromethyl ketone),

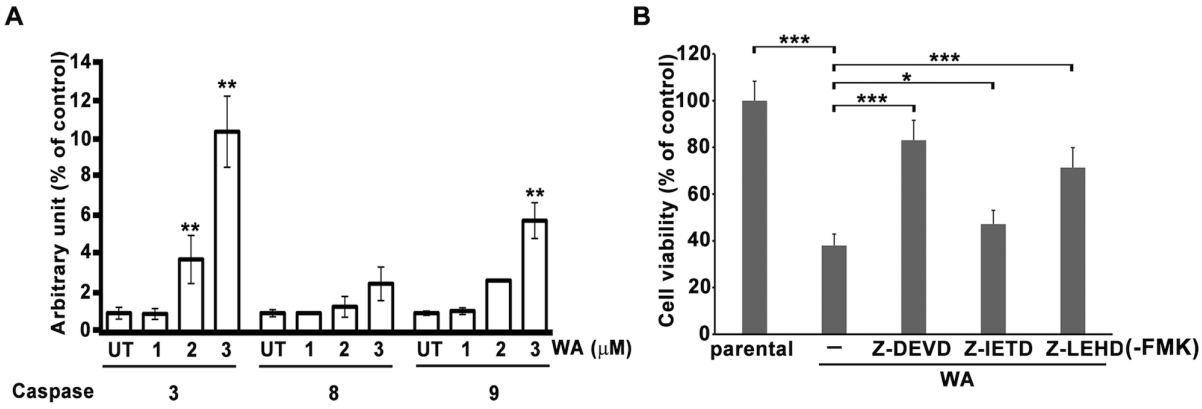

Figure S4. WA induces the generation of ROS, DNA damage and the disruption of mitochondrial membrane potential in A549 cells. (A) A549 cells were treated with different concentrations of WA for 24 h and were then collected and subjected to the comet assay. Scale bar, 40 μm. (B) A549 cells were treated with different concentrations of WA for 4 and 8 h to detect early events induced by WA. Cells were subsequently stained with JC-1 and subjected to flow cytometry to analyze the mitochondrial membrane potential. A549 cells were treated with different concentrations of WA and were stained with (C) DCFDA or (D) DHE to analyze the production of ROS. Scale bar, 40 μm. ***P<0.001 vs UT. UT, untreated; ROS, reactive oxygen species; WA, withaferin A; JC-1 (5, 5', 6, 6' -tetrachloro-1, 1', 3, 3'-tetraethylbenzimidazolylcarbocyanine iodide); FL1-H, (fluorescence parameter 1 height); FL2-H, (fluorescence parameter 2 height); DCFDA, (2',7'-Dichlorofluorescin Diacetate); DHE (dihydroethidium),

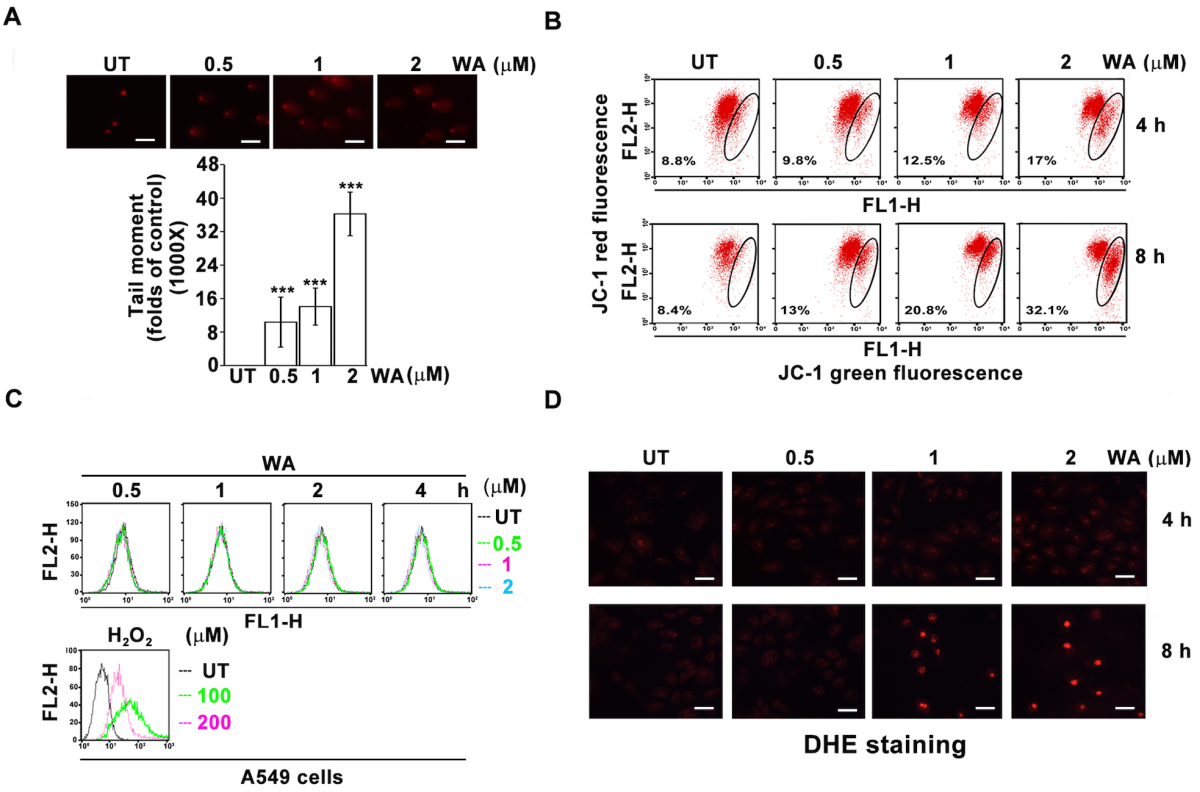

Figure S5. WA decreases the motility of both H460 and H1355 cells. 5,000 cells of (A) H460 cells and (B) H1355 cells were subjected to Transwell migration assay with different concentrations of WA. After 16 h, the migrated cells were fixed, stained with giemsa and counted under a phase-contrast light microscope. The number of migrated cells for each experimental set was then normalized to the UT cells as a percentage. Scale bar, 40 μm. ***P<0.001 vs UT. UT, untreated; WA, withaferin A.

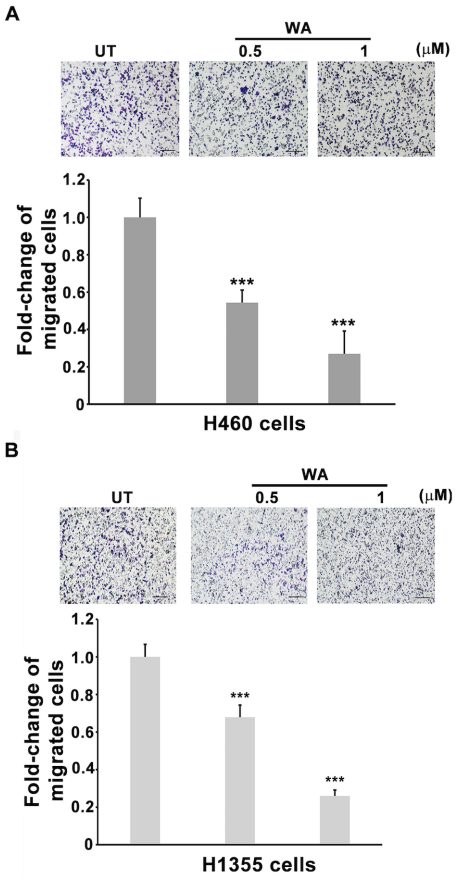

Figure S6. The transfection of miR-10b/miR-27a mimics/antagomiR in A549 or A549-p53shRNA cells regulates endogenous levels of miR-10b or miR-27a, respectively. (A) The SC or miR-10b or miR-27a mimics were transfected into A549 cells. After 24 h, the non-transfected and the transfected cells were collected and subjected to miRNA extraction and quantitative PCR to reveal the expressions of miR-10b and miR-27a. The values form SC or mimics transfected cells were then normalized to that of the parental cells as relative fold-change. SC, scramble control; miRNA/miR, microRNA. (B) The parental, SC, miR-10b or miR-27a antagomiR-transfected A549-p53 shRNA cells were collected and subjected to miRNA extraction and quantitative PCR to detect the expression levels of miR-10b and miR-27a. The values of SC or antagomir-transfected cells were normalized to that of the parental cells (con) as relative levels. ***P<0.001 vs con. SC, scramble control; miRNA/miR, microRNA.