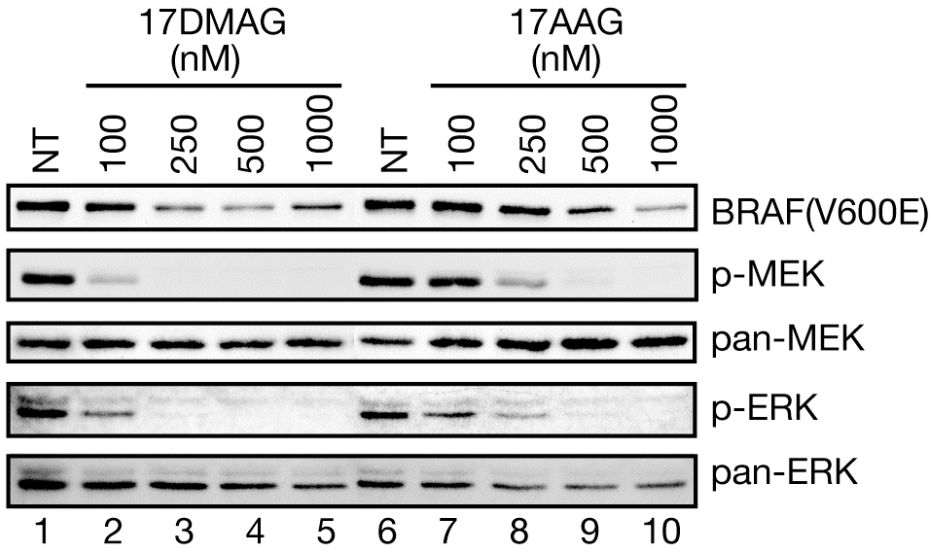
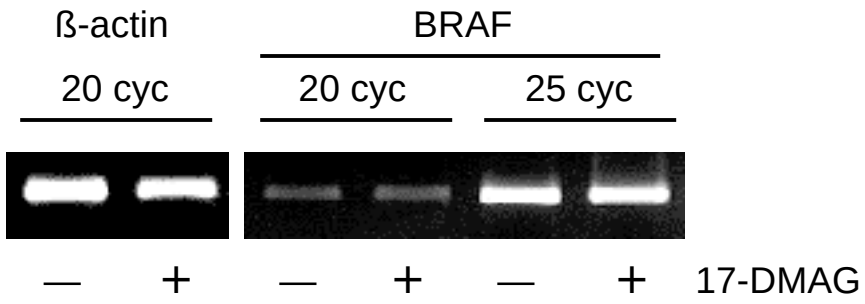
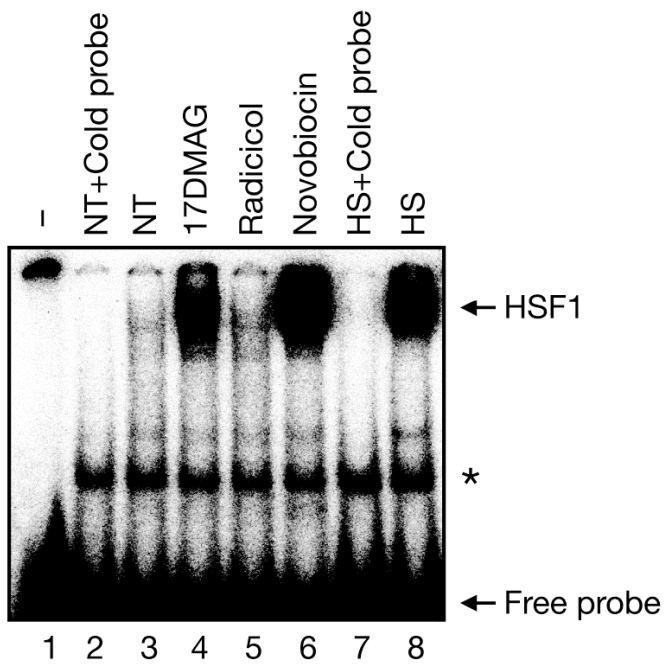


Supplemental Fig. s1



Supplemental Fig. s2



Supplemental Fig. s3

Supplemental Fig. s1: Inhibition of the MAP kinase pathway by17-AAG and 17-DMAG. HT29 cells were treated with increasing concentrations of 17-AAG or 17-DMAG for 16 h. Cell lysates were then prepared and analyzed by western blotting for cellular expression levels of BRAF, active MEK1/2 (p-MEK), total MEK1/2 (pan-MEK), active ERK1/2 (p-ERK), and total ERK1/2 (pan-ERK).

Supplemental Fig. s2: The effect of 17-DMAG treatment on cellular BRAF mRNA levels. HT29 cells were treated with or without 17-DMAG (1 μM) for 16 h and total cellular RNA was isolated. Semi-quantitive RT-PCR was performed to measure BRAF mRNA level utilizing ß-actin mRNA levels as a control. Reverse transcriptase (RT) reactions were carried out with SuperScript III (Invitrogen) and PCR reactions performed using KlenTaq LA (Sigma, MO) with the following primer combinations: BRAF-forward primer (5'-CAAGGGAAAGTGGCATGGTGATGT-3') and reverse primer (5'-AGCCCTCACACCACTGGGTAACAA-3'); ß-actin-forward primer (5'-AACTGGAACGGTGAAGGTGACAGC-3') and reverse primer (5'-TGGCTTTTAGGATGGCAAGGGACT-3').

Supplemental Fig. s3: HSF1-binding activities induced by the HSP90 inhibitors. HT29 cells were treated with 17-DMAG (1 μM), radicicol (3 μM) or novobiocin (0.8 mM) for 4 h. The heat shock (HS) was applied at 45˚C for 30 min followed by a 30 min recovery at 37˚C. Cell extract was analyzed by the gel mobility shift assay, using the ^{32}P-labeled HSF1-binding oligo DNA. Two hundred-fold molar excess of cold probe was added (lanes 2 and 7) to demonstrate binding specificity. Free ^{32}P-labeled probe migrated to the bottom of the gel. An arrow indicates the position of specific HSF1-HSE complex. Non-specific band is indicated by an asterisk. Electro Mobility Shift Assay (EMSA) for HSF-1 was performed as described previously (32) with DNA probe containing the heat shock element (HSE) sequence (5'-AGGAATCTTCCAGCAGTTTCGC-3'). The DNA binding reaction was performed with 0.8 μg of poly (dI-dC), 1.5 x 10^5 cpm of ^{32}P-labeled probe, and nuclear extract (6 μg). After incubation for 20 min at 30˚C, the protein-bound and free probes were resolved on 4% nondenaturing

polyacrylamide gel in 0.5 x Tris-glycine buffer (12.5 mM Tris base, 96 mM glycine). The gel was dried and scanned by PhosphorImager.