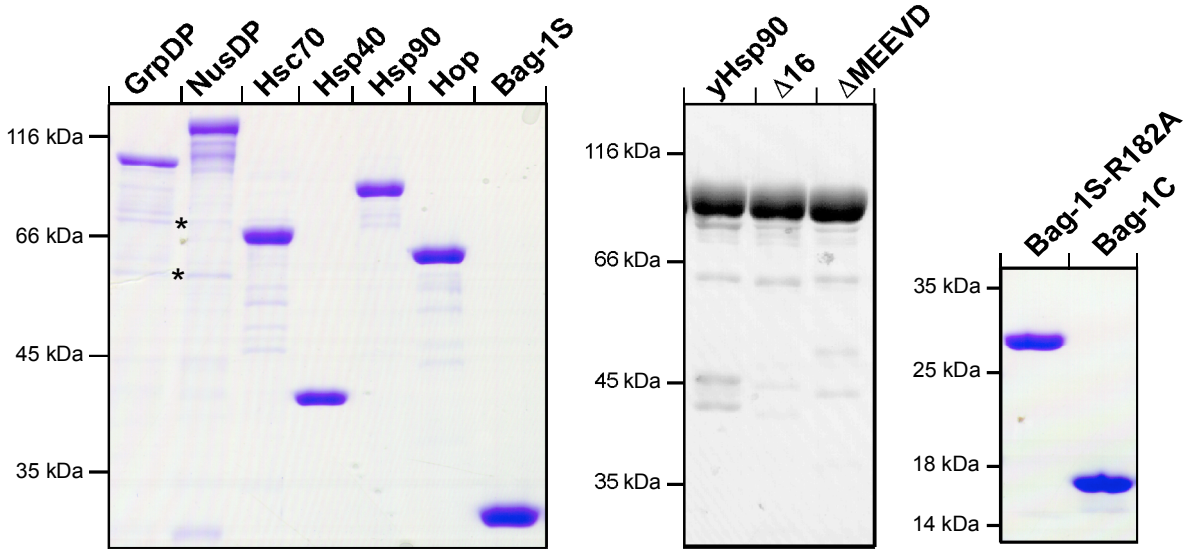
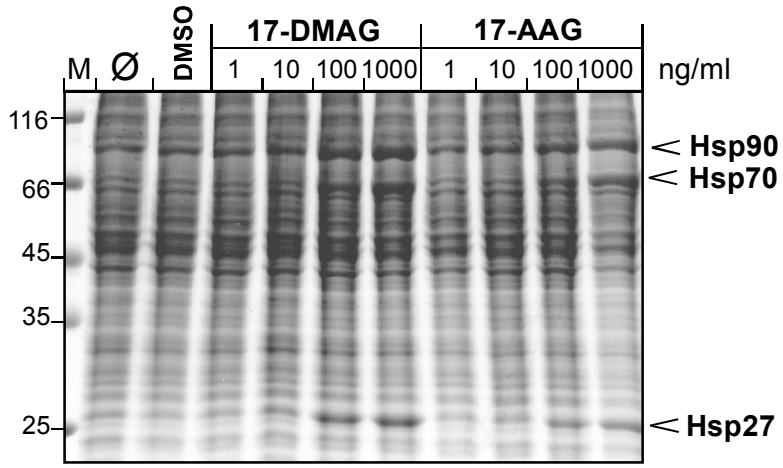
Supplementary Figure S1



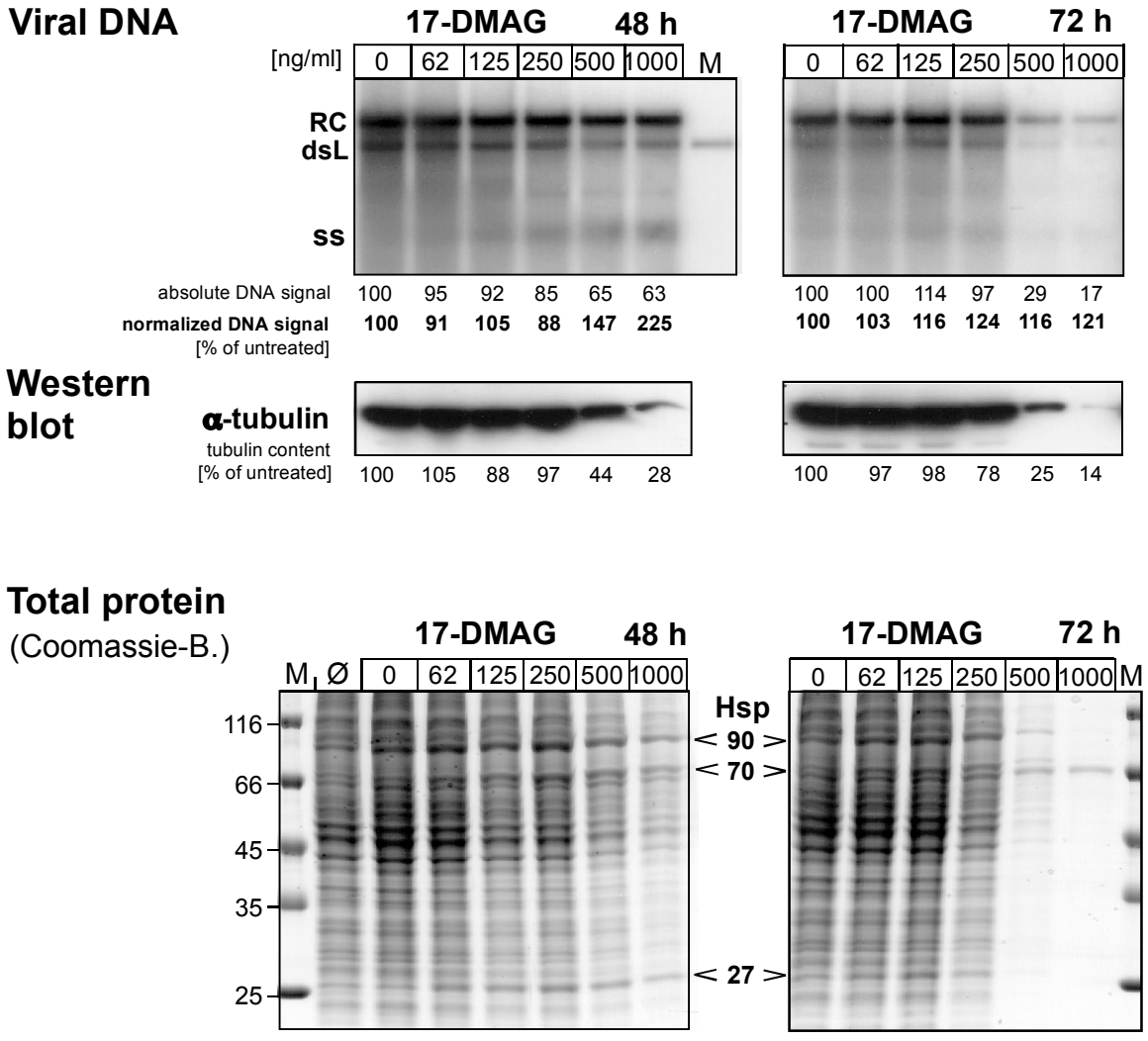
S1. Purity of recombinant proteins used. About 2-3 µg of each protein preparation were subjected to SDS-PAGE analysis and proteins were stained using Coomassie-Blue. All proteins were produced in E. coli, except human Hsp90β (Hsp90 on the left panel) which was purified from baculovirus infected insect cells; see Materials and Methods section for further details.The asterisks on the left panel indicate proteins that, according to Western blotting, correspond to bacterial GroEL (lower position) and DnaK (upper position). Densitometric scanning suggests they are present at between 10% and maximally 20% of the amount of the full-length RT fusion proteins GrpDP and NusDP.

Supplementary Figure S2



S2. Geldanamycin (GA) analogs 17-DMAG and 17-AAG induce heat-shock response in the chicken hepatoma cell line LMH. LMH cells were cultured for 24 h in the presence of the indicated concentrations of the two drugs. Cytoplasmic cell lysates were prepared as outlined in Materials and Methods, and 10 µl of each lysate was analyzed by SDS-PAGE (12.5% polyacrylamide gel) and Coomassie-Blue staining. Note the strong relative increase in the intensity of bands migrating at positions corresponding to apparent molecular masses of about 90 kDa, 70 kDa, and 25 kDa. The two large proteins were identified by Western blotting as Hsp90 and Hsc70/Hsp70; the small protein was identified by mass spectroscopy after tryptic digest as the chicken ortholog of the small heat-shock protein Hsp27.

Supplementary Figure S3

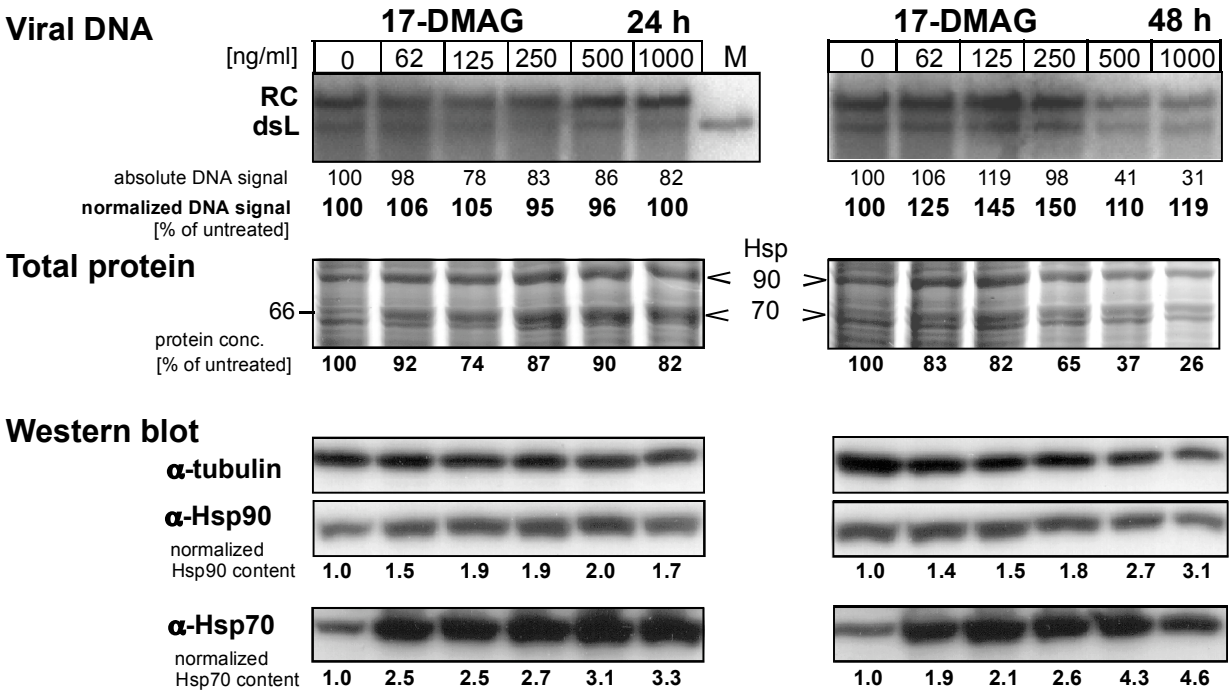


S3. Long-term high-dose 17-DMAG treatment started after transfection does not induce specific inhibition of virus replication. LMH cells were transfected with a DHBV expression vector. Therafter, cells were cultured in the presence of the indicated concentrations of 17-DMAG and harvested 48 h (left panels) or 72 h later (rigth panels). Viral DNA was isolated from cytoplasmic lysates as outlined in Materials and Methods and detected by Southern blotting and autoradiography (top). In all samples, the typical relaxed circular (RC), double-stranded linear (dsL) and some single-stranded (ss) DNA forms of the 3 kb DHBV genome were present; M denotes a 3 kb linear DNA marker fragment. RC-DNA signals were quantified by phosphorimaging and are given relative to the untreated sample set at 100% ("absolute DNA signal"). These values suggested a modest (48 h) to strong (72 h) decrease in viral replication at the highest drug concentrations.

However, the source lysates for the DNA samples contained fewer viable cells. To account for this loss equal aliquots from all lysates were analyzed by Western blotting for tubulin using a chemiluminescent substrate. Band intensities were quantified using a Fuji LAS3000 imaging instrument, and normalized to the untreated sample set at 100%. DNA signal intensities normalized for tubulin content did not show any decrease. The nominal increase at the highest inhibitor concentrations is likely to reflect the difficulty in accurately quantifying the weak tubulin Western blot signals. However, SDS-PAGE analysis of the lysates, plus of an additional untransfected control lysate (Ø), and subsequent Coomassie-Blue staining confirmed a strong loss of total protein in these samples (lower panels), particularly after 72 h of treatment.

Thus no specific inhibition of viral replication by 17-DMAG occurred under these conditions.

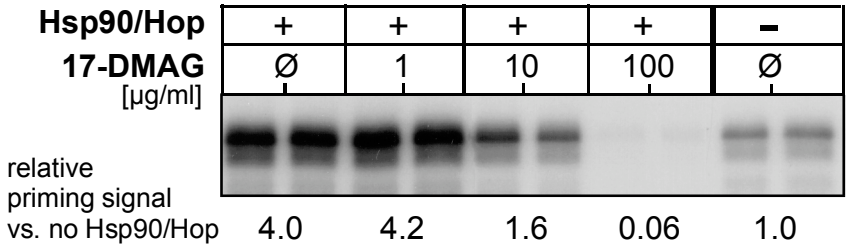
Supplementary Figure S4



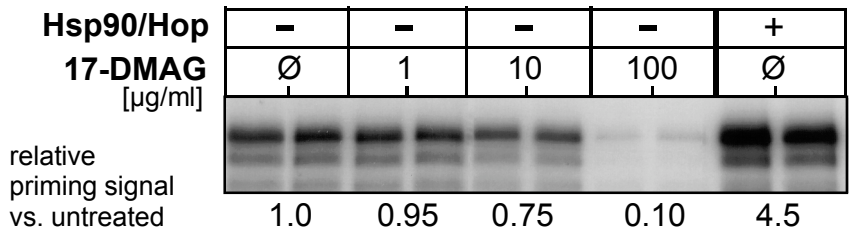
S4. High-dose 17-DMAG treatment started 1 h prior to transfection does not induce specific inhibition of virus replication. LMH cells were transfected with a DHBV expression vector as describted in figure S3, except 17-DMAG was added 1 h prior to transfection and maintained at the indicated concentrations troughout. Cells were harvested 24 h (left panels) or 48 h later (right panels). Viral DNA was analyzed by Southern blotting (top). Total protein was analyzed by SDS-PAGE and Coomassie-Blue staining; in addition, total protein concentration was determined using the BCA assay as recommended by the manufacturer (Pierce); values were normalized to that of the untreated sample set as 100%.
DNA signals normalized to total protein content of the source lysates showed no inhibition by the drug.
Lysates were also tested for Hsp90 and Hsc/Hsp70 by Western blotting; tubulin served as a loading control approximately reflecting total protein content. Hsp90 and Hsp70 signal intensities were monitored using an LAS3000 instrument, then normalized for the total protein content in the corresponding lysate. Hsp90 concentration was increased in all treated samples by 1.4- to 3.1-fold, Hsc70/Hsp70 by 1.9- to 4.6-fold.

Supplementary Figure S5

(A) Effect of 17-DMAG on Hsp90/Hop stimulated in vitro RT activation



(B) Effect of 17-DMAG on Hsc70/Hsp40 only in vitro RT activation



S5. Stronger relative inhibition by 17-DMAG of Hsp90/Hop stimulated vs. Hsc70/Hsp40 only RT activation in vitro. (A) GrpDP was in vitro reconstituted under standard conditions (see Materials and Methods for details) for 3.5 h in the presence of Hsp90/Hop and the indicated concentrations of 17-DMAG (all in duplicates). Note that 17-DMAG concentrations are in µg/ml not ng/ml as in the in vivo experiments. A reaction without Hsp90/Hop served as control, and the corresponding priming signal intensity was set at 1.0. At 10 µg/ml of drug the priming signal was reduced by about 60%, at 100 µg/ml by more than 95%.
(B) GrpDP was reconstituted in the presence of 17-DMAG as in (A) but without Hsp90/Hop; a reaction with Hsp90/Hop but without inhibitor served as control (right panel). The priming signal from the Hsc70/Hsp40 only reaction without drug was set at 1.0. At 10 µg/ml of drug priming was only slightly reduced (by about 25%) but at 100 µg/ml an about 90% inhibition was observed.
These data are compatible with specific inhibition of the stimulatory Hscp90/Hop activity at intermediate 17-DMAG concentrations; however, no Hsp90-specific statements are possible at the high drug concentration.