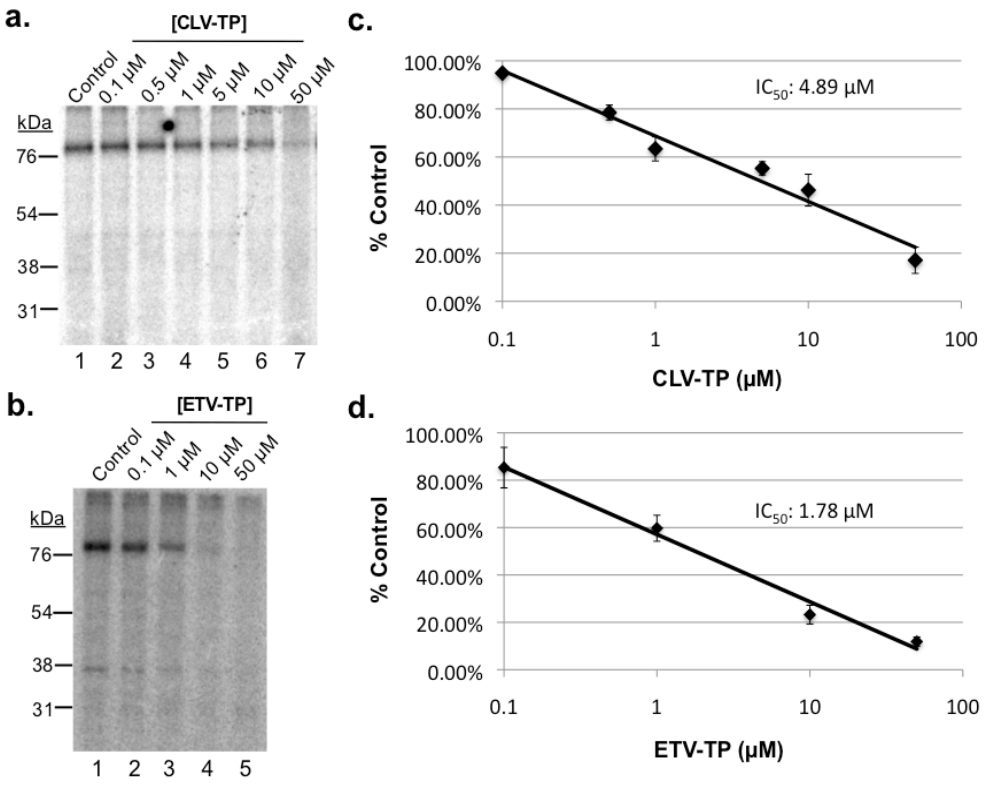
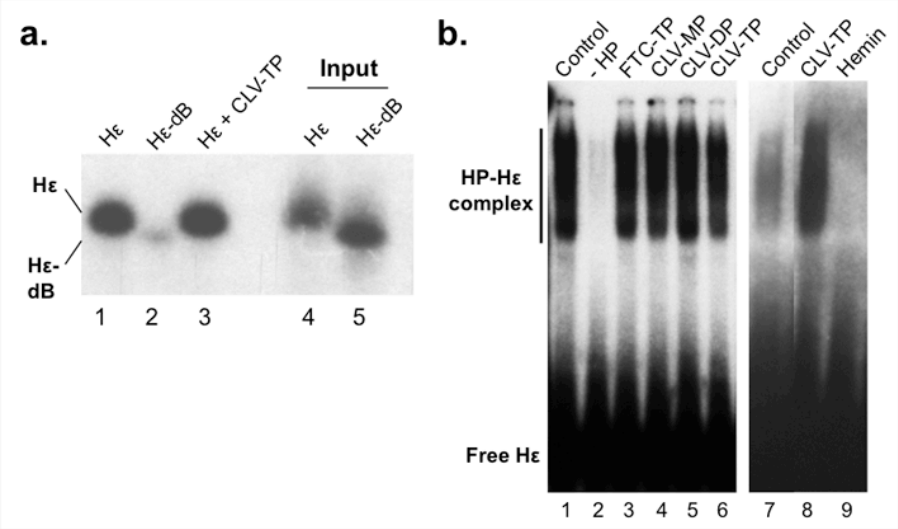
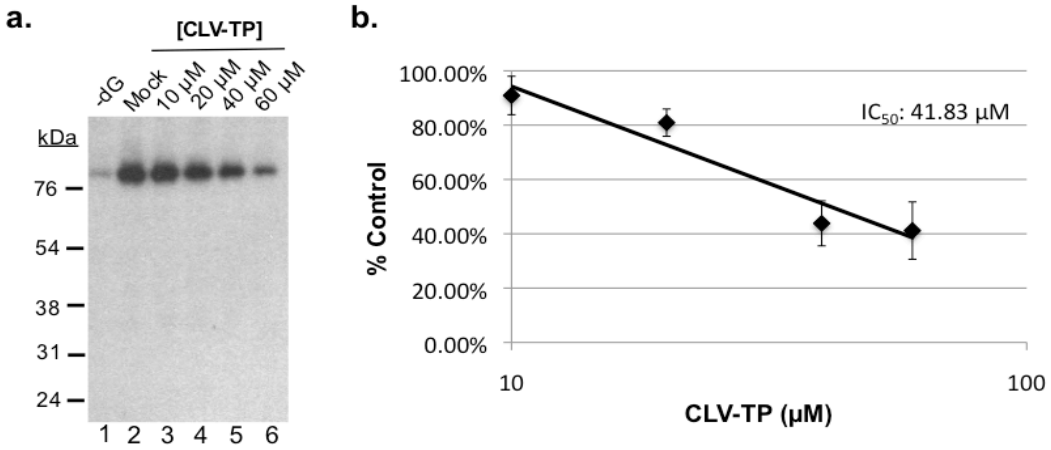
Supplemental Figures



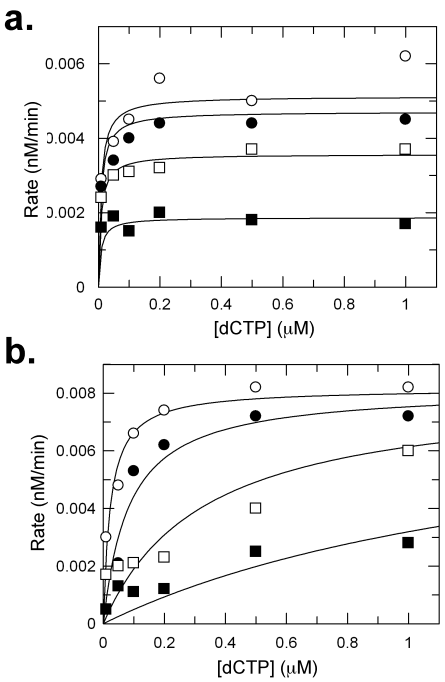
Supplemental Fig. 1. Dose response of clevudine- and entecavir-mediated inhibition of HBV protein priming initiation. In vitro priming reactions were performed as in Fig. 2 in the presence of dH_2O (control, lane 1) or the indicated concentrations of clevudine-TP (CLV-TP, a) or entecavir-TP (ETV-TP, b). The positions of the protein molecular mass markers (in kDa) are indicated. (c-d) Priming signals from three independent experiments were quantified by phosphor-imaging and expressed as percentages of controls. Error bars denote the SEM. IC_{50} values were calculated based on a logarithmic trend line plotted from the data using Microsoft Excel.



Supplemental Fig. 2. Clevudine did not inhibit HP-Hε interaction in vitro. (a) HP (purified without co-expression of Hε) was incubated with ^{32}P-labeled, in vitro transcribed wild type Hε (lanes 1, 3) or HP-binding defective mutant Hε-dB (lane 2) RNA in the presence (lane 3) or absence (lanes 1, 2) of 100 μM clevudine-TP (CLV-TP). After washing to remove unbound RNA, the bound RNA was resolved on an SDS–15% polyacrylamide gel. (b) Recombinant GST-tagged HP fusion protein was purified using bacteria expression (lanes 1, 3-9) and incubated with the activating chaperones and ^{32}P-labeled in vitro transcribed Hε, as outlined in the Materials and Methods. No protein was added to the reaction shown in lane 2. The binding reactions also contained dH_2O (control, lanes 1, 7), 200 μM emtricitabine-TP (FTC-TP, lane 3), clevudine-MP (CLV-MP, lane 4), -DP (CLV-DP, lane 5) or -TP (CLV-TP, lane 6), 100 μM clevudine-TP (CLV-TP, lane 8) or hemin (lane 9). The reactions were then resolved on a native polyacrylamide gel and subjected to autoradiography. The free Hε RNA and the HP-Hε complex are indicated.



Supplemental Fig. 3. Dose response of clevudine-mediated inhibition of the DNA polymerization stage of HP protein priming in vitro. In vitro priming reactions were performed as in Fig. 5. HP co-purified with Hε in TMgNK buffer was incubated with unlabeled dGTP to initiate protein priming (lanes 2-6). No dGTP was added in the reaction shown in lane 1 (-dG) to monitor dGTP-independent (background) dATP incorporation. Subsequently, samples were washed twice to remove unincorporated dGTP and fresh TMgNK buffer was added along with dH_2O (lanes 1, 2) or the indicated concentrations of CLV-TP (lanes 3-6). [α-^{32}P]dATP was then added to all reactions to allow for polymerization. (a) Priming products were resolved by SDS-PAGE and visualized by autoradiography. The positions of the protein molecular mass markers (in kDa) are indicated on the left. (b) Priming signals from three independent experiments were quantified by phosphor-imaging and after subtracting the background signal from lane 1 in panel a (-dG), expressed as percentages of mock treated control (lane 2). The error bars denote the SEM. IC_{50} values were calculated based on a logarithmic trend line produced from plotting the data in Microsoft Excel.



Supplemental Fig. 4. Mode of inhibition of the endogenous HBV polymerase by clevudine-TP or 3TC-TP in the presence of varying concentrations of dCTP. EPA was conducted using HBV nucleocapsids harvested from HepAD38 cells as described in Materials and Methods. The rate of product formation at each concentration was determined by measuring the radioactivity at 6 different time points and conducting a linear regression analysis. Dependence of the rate of radioactive product formation on dCTP concentration was examined in the presence of 0 (○), 0.2 (●), 1 (□), and 2 mM (■) clevudine-TP (a) or in the presence of 0 (○), 0.02 (●), 0.1 (□), and 0.5 mM (■) 3TC-TP (b) and all the data points were globally fit to a non-competitive or competitive inhibition equation, respectively. Since these experiments were performed once, apparent K_i values were reported.