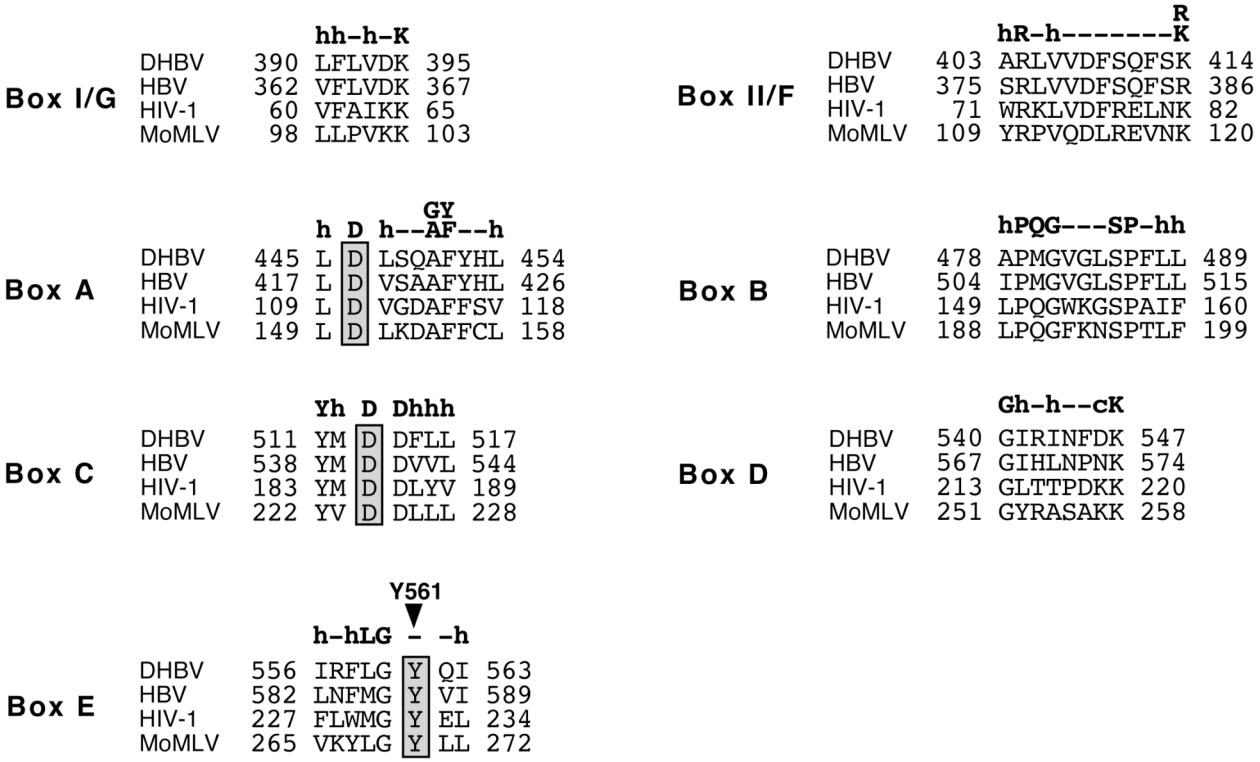
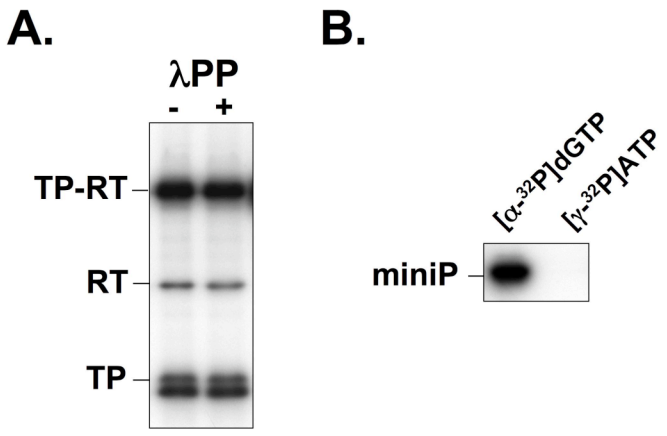
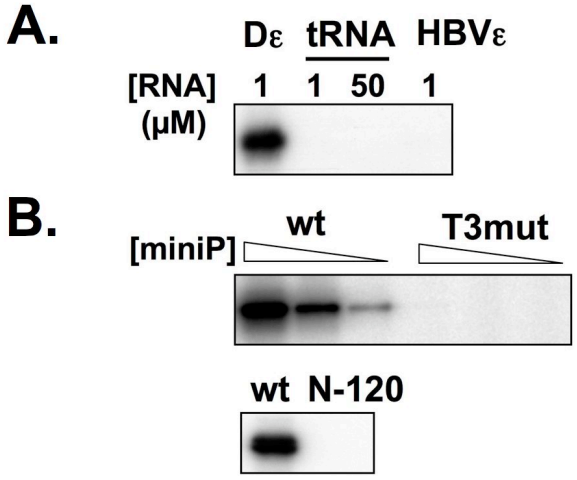


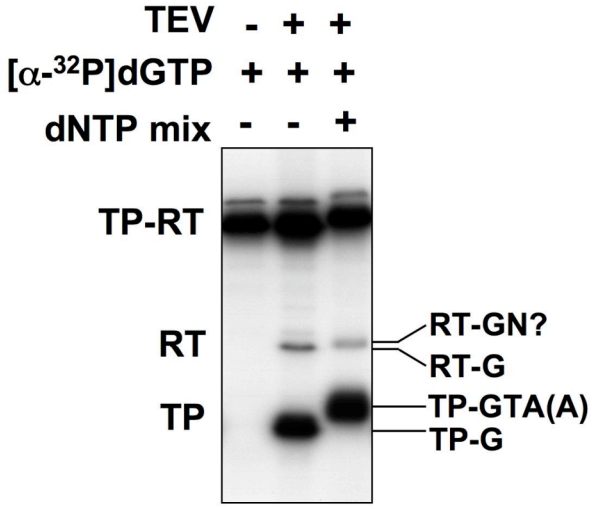
Supplementary FIG. S1. Alignment of the conserved reverse transcriptase motifs (boxes) in the RT domains of DHBV and HBV P protein with those of HIV-1 and MoMLV RT. Numbers are aa positions that for HBV correspond to subtype ayw, and for DHBV to isolate DHBV16. Individual sequences within the boxes are shown below the consensus motifs (Xiong, Y. and Eickbush, T.H. (1990) Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. 9:3353-3362). h, hydrophobic residues; c, charged residues. The Asp-residues known to coordinate Me^{2+} in HIV-1 and MoMLV RT and theTyr-residues at homologous positions to Tyr561 in DHBV P protein (arrowhead) are boxed.



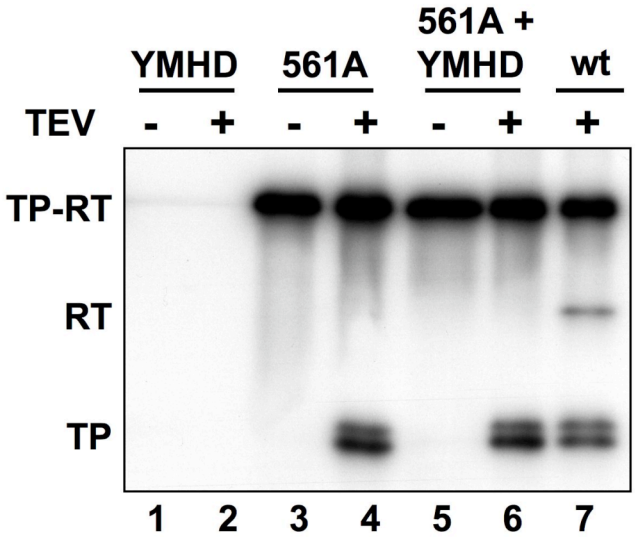
Supplementary FIG. S2. RT labeling is not due to phosphorylation. (A) The RT modification product is resistant to phosphatase treatment. MiniP_{TEV} was primed using wt Dε RNA and [α-^{32}P]dGTP, then treated with 200 U lambda protein phosphatase (λPP) at 30°C for 1 h as suggested by the supplier (NEB); Mn^{2+} essential as cofactor for λPP was provided by the priming buffer. Subsequently, EDTA was added to 3 mM final concentration. After TEV protease cleavage, the samples were analyzed by SDS-PAGE and autoradiography. Note that both the TP signal (as expected) and the RT signal remained unaffected. **(B) No detectable γ-phosphoryl transfer from ATP to miniP_{TEV} under priming conditions.** MiniP_{TEV} was primed as in (A), or [α-^{32}P]dGTP was replaced by [γ-^{32}P]ATP.



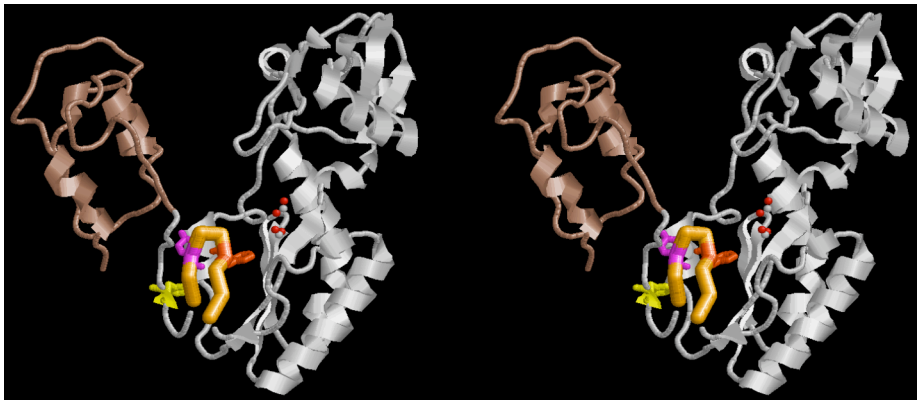
Supplementary FIG. S3. RT modification is dependent on the cognate Dε RNA and on RNA binding competence of TP. (A) Dε dependence. Priming assays were performed with miniP$_{TEV}$ in the presence of the indicated RNAs plus [α-^{32}P]dGTP and analyzed as in Supplementary FIG. S2. Note that only Dε RNA supported ^{32}P labeling of the protein. **(B) Dependence on RNA binding ability of TP.** Priming assays were performed with Dε RNA plus [α-^{32}P]dGTP using either wt miniP$_{TEV}$ or its non-RNA-binding variants T3mut or N-120 (see Table 1). Note that exclusively wt miniP$_{TEV}$ supported ^{32}P labeling. Wt miniP$_{TEV}$ and T3mut were tested using a three-fold dilution series.



Supplementary FIG. S4. Evidence for limited extension of the RT deoxynucleotidylation product. MiniP_{TEV} was primed using Dε RNA plus [α-^{32}P]dGTP, with or without a mix of unlabeled dNTPs (dATP, dCTP, dTTP; 10 μM each). Samples were digested with TEV protease as indicated and analyzed as in Supplementary FIG. S2. The additional dNTPs caused a significant shift of the labeled TP band compared to the dGTP only reaction (TP-G), as expected for synthesis of the 3 or 4 nt GTA(A) primer (TP-GTA(A)). A less pronounced yet reproducible upward shift was also seen for the RT band (RT-GN), suggesting either a less efficient extension of the RT-bound dGMP, or a smaller effect of the extended primer on electrophoretic mobility of the RT than the TP product.



Supplementary FIG. S5. RT nucleotidylation requires miniP DNA polymerase activity *in cis.* Priming assays using Dε RNA plus [α-^{32}P]dGTP were performed with the catalytically inactive miniP$_{TEV}$ variant YMHD and the RT dNMP acceptor deficient variant 561A, either separately, or in combination (561A+YMHD); wt miniP$_{TEV}$ served as control (wt). Samples were digested with TEV protease as indicated and analyzed as in Supplementary FIG. S2. Note the complete absence of a labeled RT band in the 561A+YMHD reaction, hence Tyr561 in the YMHD variant could not serve as a *trans*-substrate. This strongly suggests that in wt miniP$_{TEV}$ dNMP transfer to Tyr561 occurs by an intra-molecular mechanism.



Supplementary FIG. S6. Stereo view of the modeled RT domain of DHBV P protein. The labeling is exactly as in FIG. 10C, except the view is rotated by 90° around the x-axis.