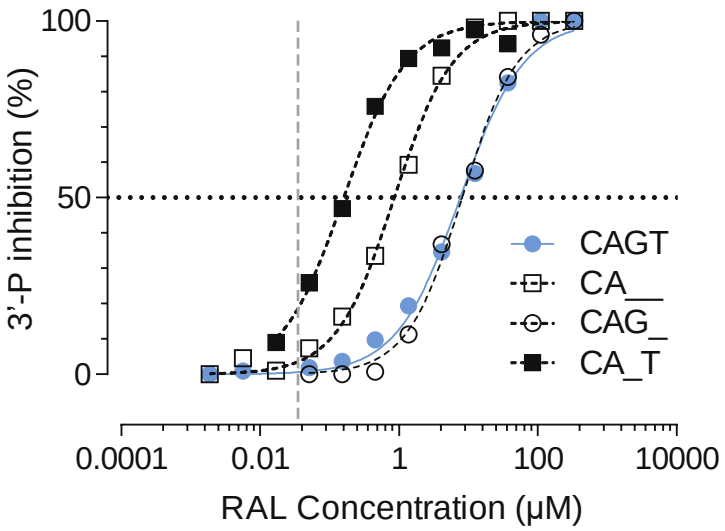
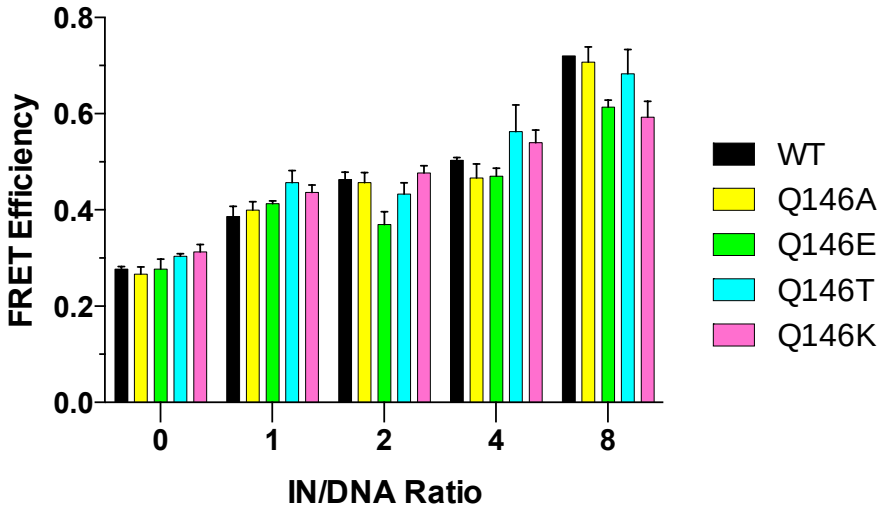
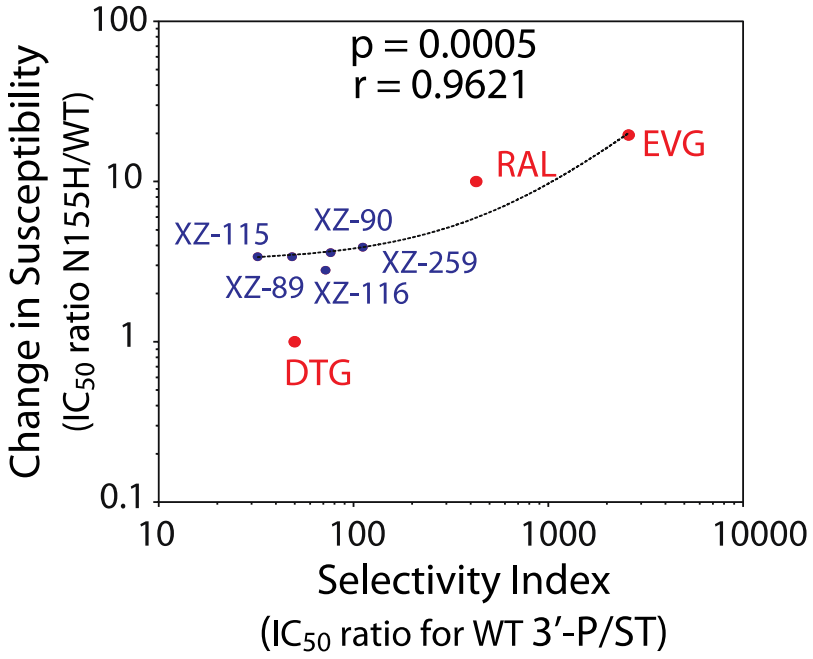


<u>Supplemental Figure 1.</u> Role of the +1 and +2 bases in RAL inhibition of 3'-P. The oligonucleotides with abasic sites at the indicated positions were used as substrates in 3'-P assays. Reactions were performed with recombinant WT IN for 120 minutes with increasing concentrations of RAL. For comparison, the vertical dashed line represents the IC_{50} value for ST inhibition by RAL. Data points and fitted curves are from a single representative experiment.

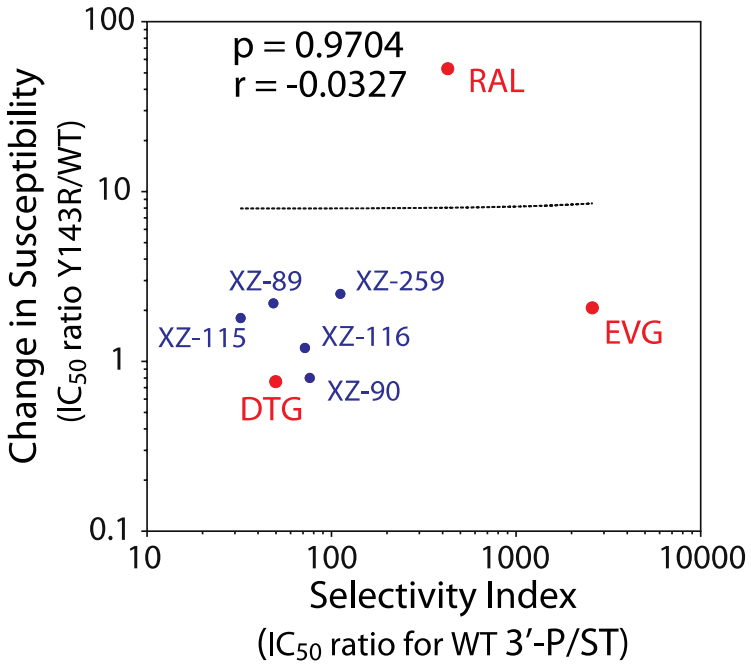


Supplemental Figure 2. FRET efficiency of the Cy3/Cy5 labeled dsDNA oligomer in presence of WT HIV IN and the Q146A, Q146E, Q146T, and Q146K mutants. The FRET efficiency was determined as $E_{FRET} = 1 - F_{DA}/F_D$ by measuring the donor (Cy3) fluorescence intensity in absence (F_D) and presence (F_{DA}) of the acceptor (Cy5) at different IN/DNA concentration ratios.

Interactions of HIV IN with the double stranded substrate were monitored by FRET. The substrate oligomer Cy5-22t/Cy3-22b (Cyanine5 (Cy5) dye 5´-end labeled top strand, 5'-Cy5-AGTGTGGAAAATCTCTAGCAGT was annealed to a fully complementary Cyanine3 (Cy3) dye 5´-end labeled bottom strand, 5'-Cy3-ACTGCTAGAGATTTTCCACACT). This dsDNA was incubated at a concentration of 63 nM with IN at 63, 125, 250 and 500 nM in buffer containing 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) pH 7.2, 7.5 mM $MnCl_2$, 14 mM 2-mercaptoethanol. The reaction mixtures (150 µL) were prepared on ice and, after incubation for 3 min, the fluorescence intensity was measured three times at 1 min intervals on EnVision 2104 Multilabel Reader (PerkinElmer) at 579 nm (Cy3 dye emission) and 685 (Cy5 dye emission) with excitation at 531 nm (Cy3). FRET efficiency was calculated as $E_{FRET} = 1 - F_{DA}/F_D$, where F_{DA} is the fluorescence of the donor in absence of the acceptor and F_D is measured in the absence of the acceptor.



Supplemental Figure 3. Correlation between the selectivity of a molecule for ST over 3'-P and the change in susceptibility induced by the N155H resistant mutant. The plot was generated as in Figure 7.



<u>Supplemental Figure 4.</u> Lack of correlation between the selectivity of a molecule for ST over 3'-P and the change in susceptibility induced by the RAL specific mutant Y143R. The plot was generated as in Figure 6.