Numerous cellular factors belonging to the DNA repair machineries, including RAD18, RAD52, XPB and XPD, have been described to counteract human immunodeficiency virus type 1 (HIV-1) replication. Recently, Uracil DNA glycosylase 2 (UNG2), a major determinant of the uracil base excision repair pathway, was shown to undergo rapid proteasome-dependent degradation following HIV-1 infection. However, the specific role of intracellular UNG2 depletion during the course of HIV-1 infection is not clearly understood. Our study shows for the first time that overexpression of UNG2 inhibits HIV-1 replication. We demonstrate that this viral inhibition is correlated with a marked decrease in transcription efficiency as shown by monitoring HIV-1 LTR promoter activity and quantification of HIV-1 RNA levels. Interestingly, UNG2 inhibits LTR activity when stimulated by Tat trans-activator or TNFα, while barely affected using Phorbol ester activation. Mutational analysis of UNG2 indicates that antiviral activity may require the integrity of the UNG2 catalytic domain and Vpr-interacting domain. Altogether, our data indicate that UNG2 is likely to represent a new host defense factor specifically counteracted by HIV-1 Vpr. The molecular mechanisms involved in the UNG2 antiviral activity still remain elusive but may rely on the sequestration of specific cellular factor(s) critical for viral transcription.