

Ribonuclease Zymogen Induces Cytotoxicity upon HIV-1 Infection

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Table S1 Str2-EV kinetic parameters at pH 5.0

k_{cat}/K_M ($M^{-1}s^{-1}$)	k_{cat}/K_M ($M^{-1}s^{-1}$)
Inactive	Activated
$1.6 \pm 0.1 \times 10^2$	$3.9 \pm 0.2 \times 10^3$

Human RNase 1

KESRA KKFQR QHMDS DSSPS SSSTY CNQMM RRRNM TQGRG KPVNT FVHEP
 LVDVQ NVCFQ EKVTC KNGQG NCYKS NSSMH ITDCR LTNGS RYPNC AYRTS
 PKERH IIVAC EGSPY VPVHF DASVE DST

Str2-EV

{-----A KKFQR QHMDS DSSPS SSSTY CNQMM RRRNM TQRGC KPVNT FVHEP
 {LVDVQ NVCFQ EKVTC KRGQG NCYKS NSSMH ITDCR LTNRR RYPNC AYRTS
 {PKERH IIVAC EGSPY VPVHF DASGI FLETS}

Fig. S1 Amino acid sequences of human RNase 1 and the cyclic Str2-EV zymogen. In the zymogen, substitutions to evade RI are shown in red, the HIV-1 protease recognition site is shown in green, and the linkage created by the *Noctoc punctiforme* DnaE split intein is underlined.

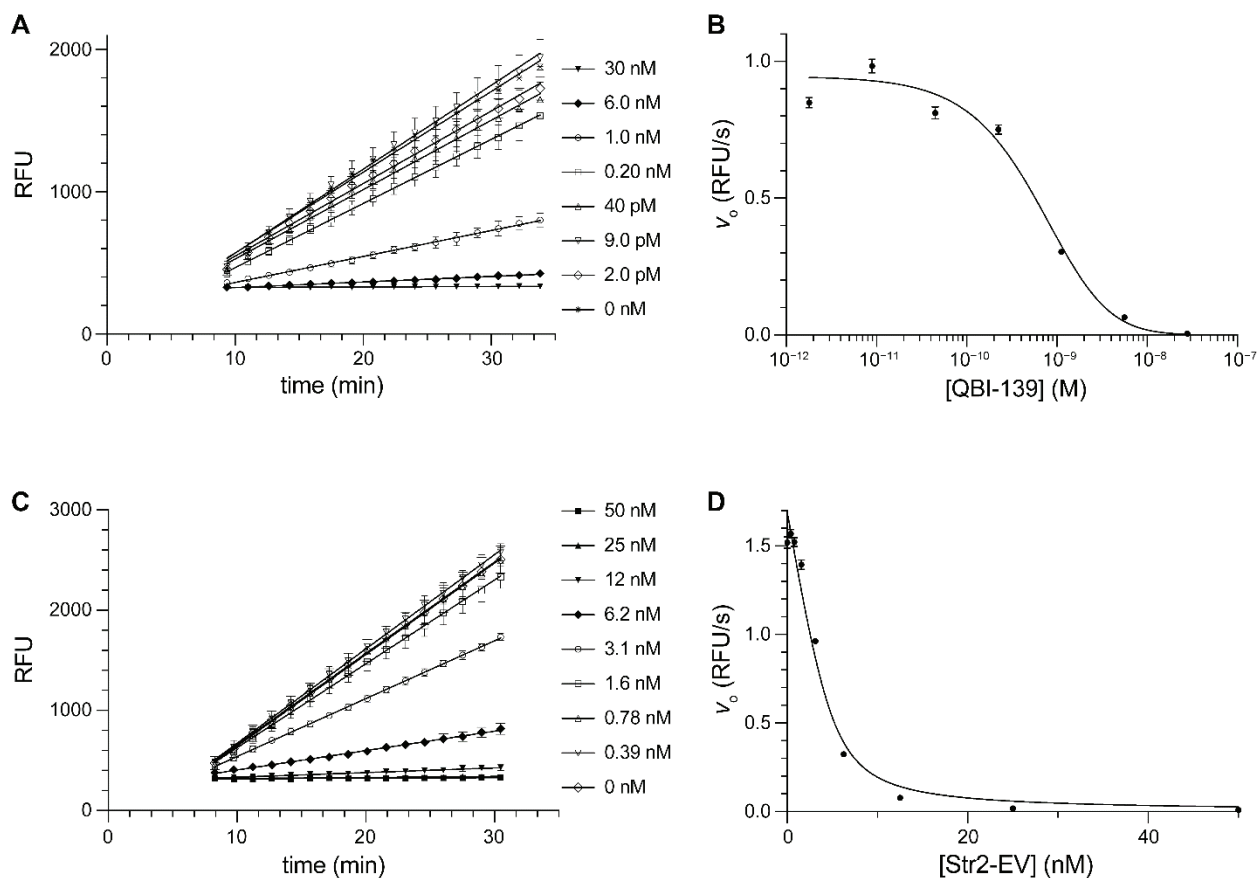


Fig. S2 Inhibition of QBI-139 and Str2-EV by human RI. A. QBI-139 (35 pM) was titrated with RI, and initial velocities were determined by linear regression. B. Initial velocities were plotted on a log scale, enabling the approximation of the K_i using the 5-parameter logistic equation to determine the value of IC_{50} . C. Str2-EV (5 nM) was titrated with RI, and initial velocities were determined by linear regression. D. The ensuing initial velocities were fitted with Morrison's equation for tight binding inhibitors to determine the value of K_i . Values of K_i for QBI-139 and Str2-EV are reported in Table 1.

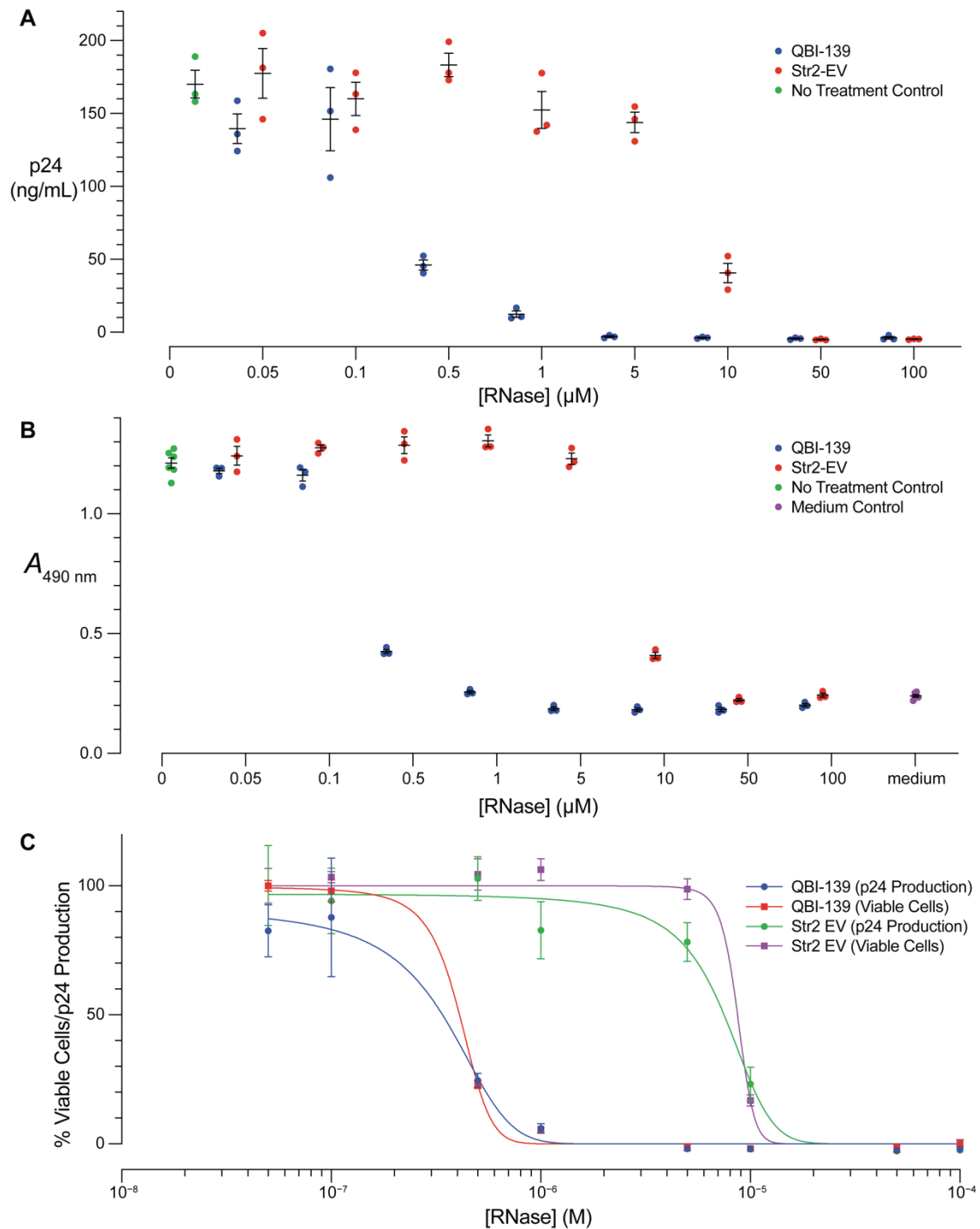


Fig. S3 Concentration-dependence of HIV-1 virus production (A) and cytotoxicity (B). MT-4 cells with (A) or without (B) virus were treated with QBI-139 and Str2-EV. (C) Data from these studies were normalized and fitted with the 5-parameter logistic equation to yield IC_{50} values, which are reported in Table 2.

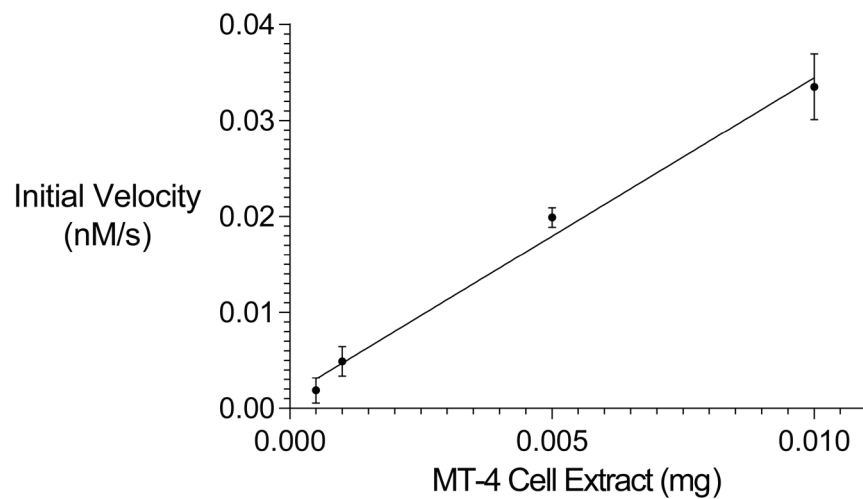


Fig. S4. Hydrolysis of SGIFLETS peptide by MT-4 cell extract. Initial velocities of 10 μ M RE(EDANS)SGIFLETSK(DABCYL)R substrate turnover are plotted against the mass of MT-4 cell extract. The slope of the linear least-squares fit of these data is 3.3 ± 0.2 (nM/s)/mg.