Supplementary Material

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 | 1.6 ± 0.1 × 10$^2$             | 3.9 ± 0.2 × 10$^3$             |

Human RNase 1
KESRA KKFQR QHMDS DSSPS SSSTY CNQMM RRRNM TQGRC KPVNT FVHEP
LVDVQ NVCFQ EKVTC KNGQG NCYKS NSSMH ITDCR LTNGS RYPNC AYRTS
PKERH IIVAC EGSPY VPVHF DASVE DST

Str2-EV

\[ \begin{align*}
\text{ triangle } & A KKFQR QHMDS DSSPS SSSTY CNQMM RRRNM TQRGC KPVNT FVHEP \\
& \underline{LVDVQ \text{ NVCFQ} EKVTC \text{ KRQGG} NCYKS NSSMH ITDCR \text{ LTNRР RYPNC AYRTS} \\
& \text{ triangle } \text{ PKERH IIVAC EGSPY VPVHF DASGI FLETS} \\
\end{align*} \]

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 μM 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.