Supplementary Information

Number of infection events per cell during HIV-1 cell-free infection

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Supplementary Note: Calculation of theoretical odds ratio

We derived the theoretical odds ratio, OR_M, by Eqs. (1–4) as follows:

$$OR_M = \frac{F_B(\boldsymbol{\theta})F_C(\boldsymbol{\theta})}{F_A(\boldsymbol{\theta})F_D(\boldsymbol{\theta})}$$

$$= 1 + \frac{\dfrac{1}{\left(1+\dfrac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p}} - \dfrac{1}{\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^{p}\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p}}}{\left\{\dfrac{1}{\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p}} - \dfrac{1}{\left(1+\dfrac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p}}\right\}\left\{\dfrac{1}{\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^{p}} - \dfrac{1}{\left(1+\dfrac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p}}\right\}}.$$

Because $\beta > 0,\ p > 0,\ 1/q > 0,\ \bar{V}_{\mathrm{HSA}} > 0,\ \bar{V}_{\mathrm{GFP}} > 0$ and

$$0 < 1 + \frac{\beta}{q}(\bar{V}_{\mathrm{HSA}} + \bar{V}_{\mathrm{GFP}}) < \left(1+\frac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right),$$

we obtained the following relationship of

$$0 < \left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p} < \left(1+\frac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^{p}\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p}.$$

Thus, it is shown that

$$\frac{1}{\left(1+\dfrac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p}} - \frac{1}{\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^{p}\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p}} > 0\,.$$

Hence, the numerator in the second term of OR_M is always positive. Similarly, since

$$0 < 1 + \frac{\beta}{q}\bar{V}_{\mathrm{GFP}} < 1 + \frac{\beta}{q}(\bar{V}_{\mathrm{HSA}} + \bar{V}_{\mathrm{GFP}}),$$

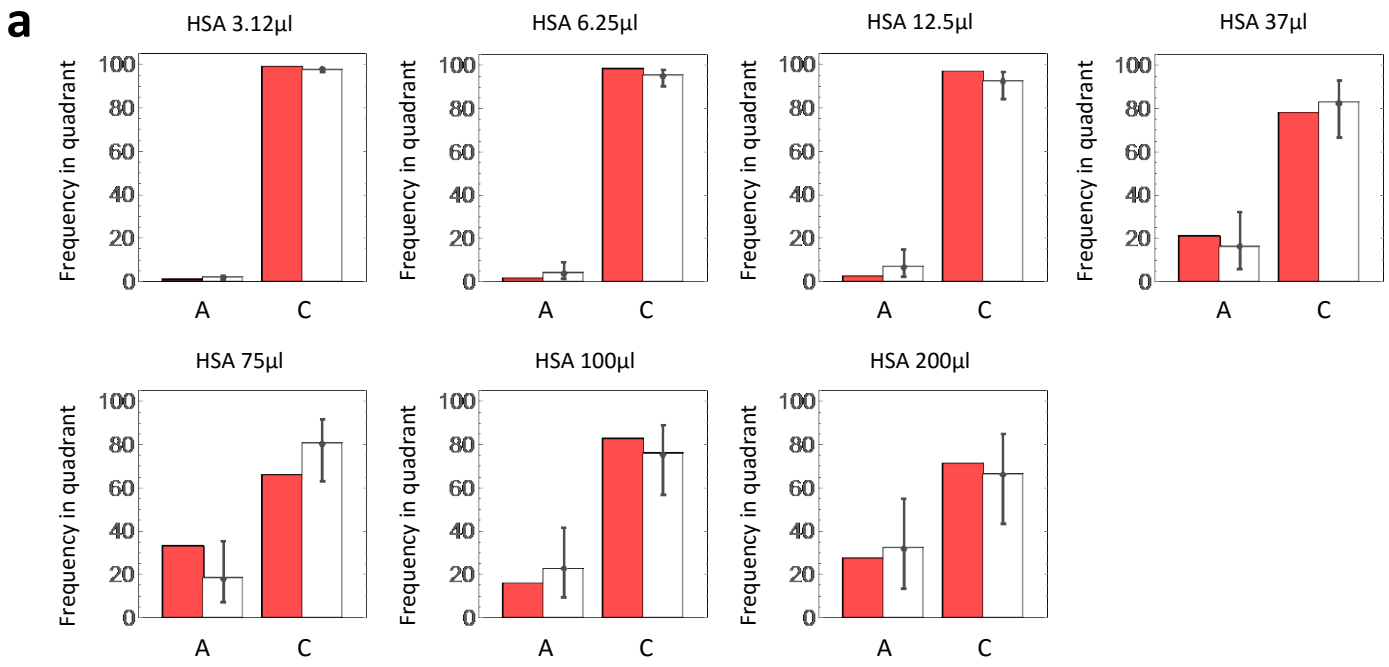
we obtained

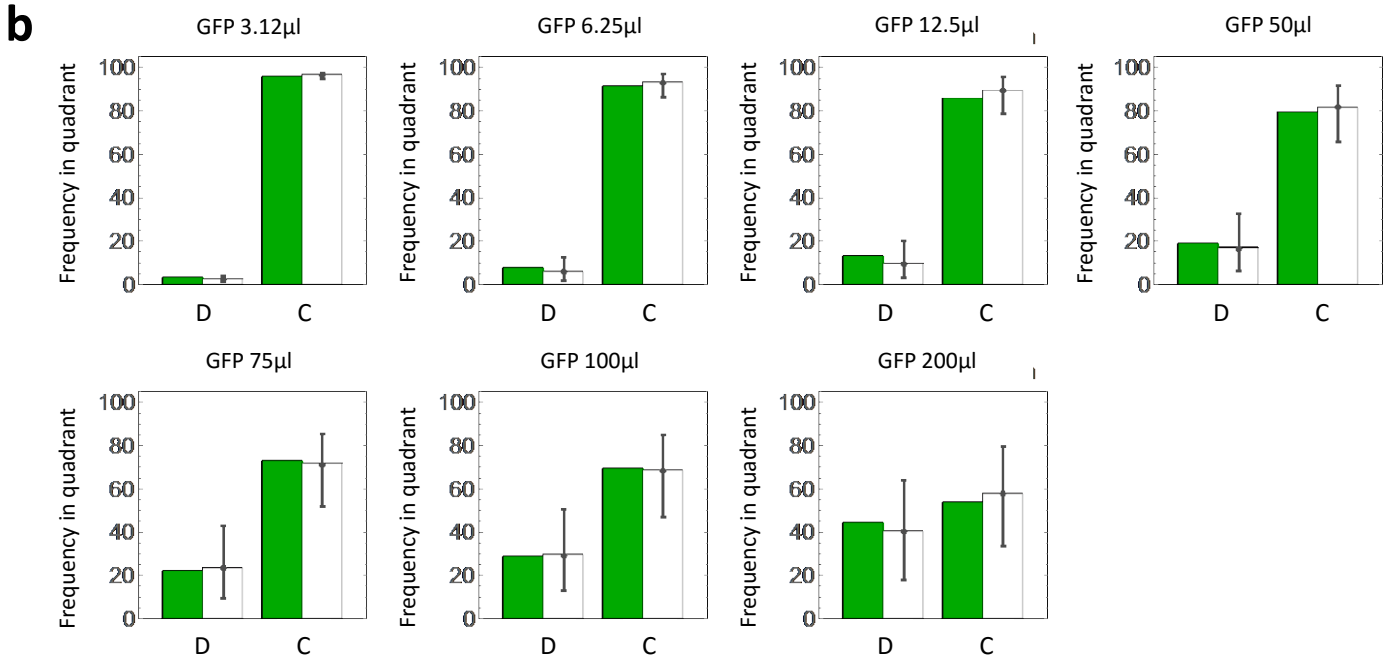
$$0 < \left(1+\frac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p} < \left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p},$$

this leads to

$$\frac{1}{\left(1+\dfrac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^{p}} - \frac{1}{\left(1+\dfrac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^{p}} > 0.$$

This inequality explains why the denominator in the second term of OR_M is always positive. Therefore, we were able to show that $OR_M > 1$ holds.


a
HSA 3.12µl
HSA 6.25µl
HSA 12.5µl
HSA 37µl
HSA 75µl
HSA 100µl
HSA 200µl
Frequency in quadrant
0
20
40
60
80
100
A
C


b
GFP 3.12µl
GFP 6.25µl
GFP 12.5µl
GFP 50µl
GFP 75µl
GFP 100µl
GFP 200µl
Frequency in quadrant
0
20
40
60
80
100
D
C

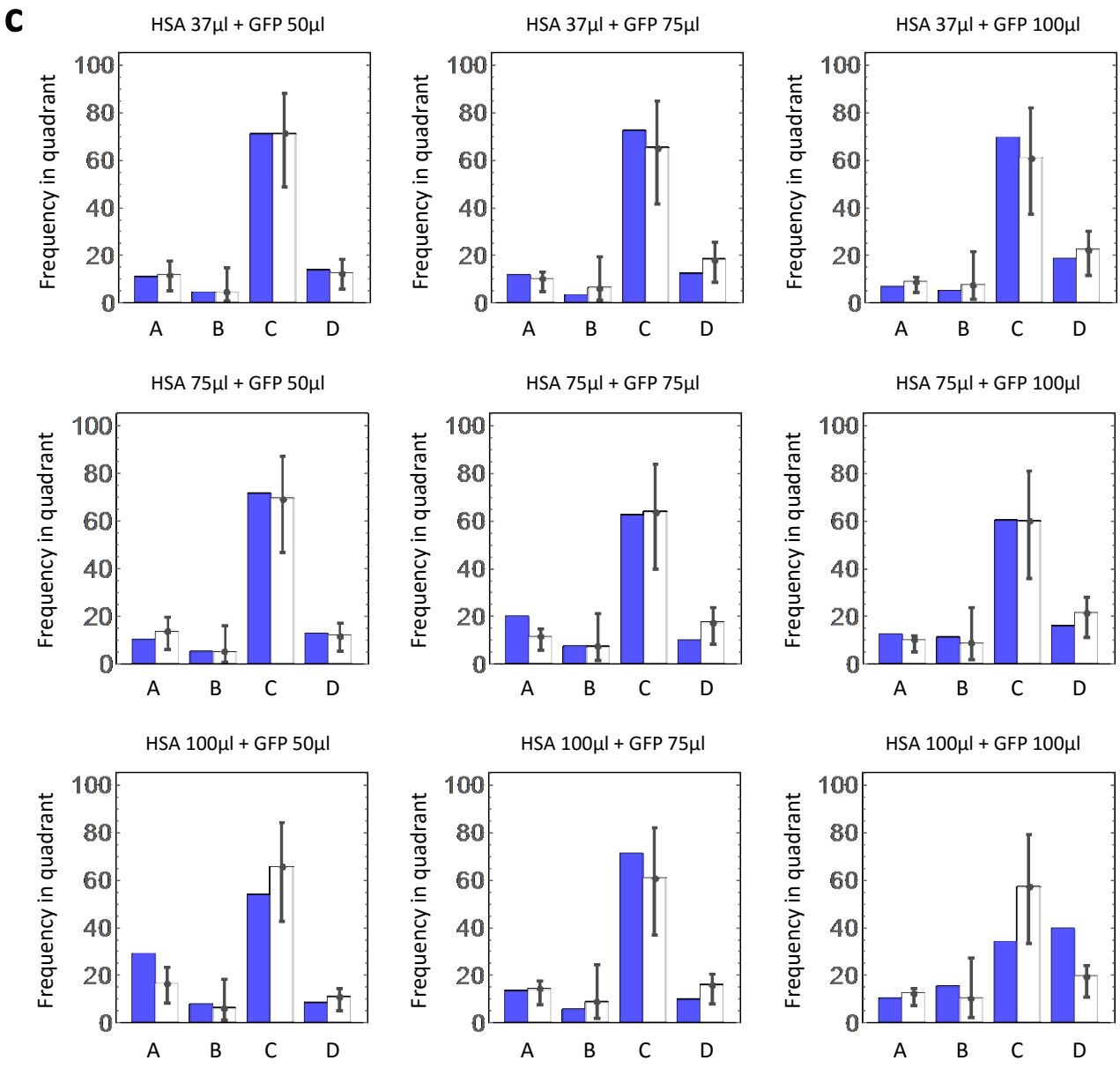


Figure S1 | Frequency of single infection and coinfection in other experiment: (a) The experimental and theoretical frequencies of quadrants A (i.e., HSA-positive) and C (i.e., HSA-negative) in seven independent experiments using only HSA HIV-1 are shown by red and white bars, respectively. **(b)** The experimental and theoretical frequencies of quadrants D (i.e., GFP-positive) and C (i.e., GFP-negative) in single GFP HIV-1 experiments are shown by green and white bars, respectively. **(c)** The experimental and theoretical frequencies of quadrants A, B (i.e., positive both for HSA and GFP), C (i.e., negative both for HSA and GFP), and D in double HIV-1 experiments are shown by blue and white bars, respectively, with different combinations of inoculated viral amount. Note that each error bar represents 95% credible interval obtained from Markov Chain Monte Carlo(MCMC) parameter inferences.

To examine how many HIV-1s infect T-cells in cell-free infection

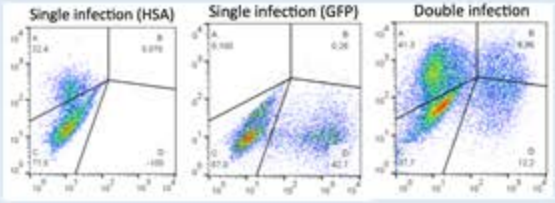
Data

F_A : frequency of cells infected by HSA$^+$ HIV-1 only in quadrant A

F_B : frequency of cells infected by HSA$^+$ & GFP$^+$ HIV-1 in quadrant B

F_C : frequency of non-infected cells in quadrant C

F_D : frequency of cells infected by GFP$^+$ HIV-1 only in quadrant D



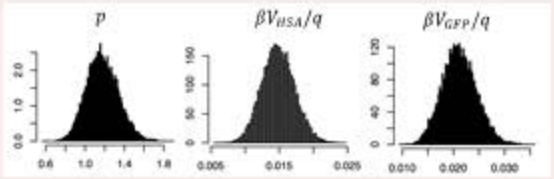
Details of the experiment

HSA	3.12μl	...	200μl	10 datasets
GFP	3.12μl	...	200μl	10 datasets
Double	25μl × 25μl	...	100μl × 100μl	18 datasets

Bayesian inference

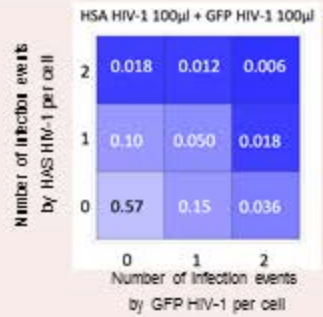
Result

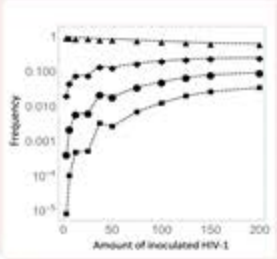
Distribution of the parameters

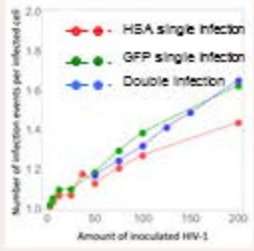


Prediction

Number of infection events







Predicted value

$$F_A = \frac{1}{\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^p} - \frac{1}{\left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{GFP}}+\bar{V}_{\mathrm{HSA}})\right)^p}$$

$$F_B = 1 - \frac{1}{\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^p} - \frac{1}{\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{GFP}}\right)^p} + \frac{1}{\left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^p}$$

$$F_C = \frac{1}{\left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^p}$$

$$F_D = \frac{1}{\left(1+\frac{\beta}{q}\bar{V}_{\mathrm{HSA}}\right)^p} - \frac{1}{\left(1+\frac{\beta}{q}(\bar{V}_{\mathrm{HSA}}+\bar{V}_{\mathrm{GFP}})\right)^p}$$

Parameters

p r_{37}

$\beta V_{HSA}/q$ r_{50}

$\beta V_{GFP}/q$ r_{75}

$r_{6.25}$ r_{100}

$r_{12.5}$ r_{200}

r_{25}

Unknown

Figure S2 | Outline of our study strategy: (**Blue**) The frequency of cells in each quadrant were taken by FACS analyses in 3 independent experiments. (**Green**) We calculated the predicted frequencies corresponding to the measurements, and the 11 parameters are unknown. **(Red)** To estimate the distribution of the all parameters, the Bayesian method are applied. Using the estimated distribution, the calculations on the number of infection events were carried out.