A conservation law for virus infection kinetics *in vitro*

Yusuke Kakizoe ¹, Satoru Morita ², Shinji Nakaoka ³, Yasuhiro Takeuchi ⁴, Kei Sato ^{5,6},
Tomoyuki Miura ⁵, Catherine A. A. Beauchemin ⁷, and Shingo Iwami ^{1,6,8,†}

¹Department of Biology, Kyushu University, Fukuoka, Japan

²Department of Mathematical and Systems Engineering, Shizuoka University, Shizuoka, Japan

³Graduate School of Medicine, University of Tokyo, Tokyo, Japan

⁴College of Science and Engineering, Aoyama Gakuin University, Kanagawa, Japan

⁵Institute for Virus Research, Kyoto University, Kyoto, Japan

⁶CREST, JST, Saitama, Japan

⁷Department of Physics, Ryerson University, Toronto, Canada

⁸PRESTO, JST, Saitama, Japan

[†]E-mail: siwami@kyushu-u.org

Abstract. Conservation laws are among the most important properties of a physical system, but are not commonplace in biology. We derived a conservation law from the basic model for viral infections which consists in a small set of ordinary differential equations. We challenged the conservation law experimentally for the case of a virus infection in a cell culture. We found that the derived, conserved quantity remained almost constant throughout the infection period, implying that the derived conservation law holds in this biological system. We also suggest a potential use for the conservation law in evaluating the accuracy of experimental measurements.

Keywords: Mathematical model, Conservation law, Population dynamics, Virus infection

27 1. Introduction

28 In physics, a conserved quantity of a dynamical system is a quantity expressed as a
 29 function of the systems' dynamical, dependent variables, but which remains constant along each
 30 trajectory of the system. In the natural sciences, various conservation laws, such as the
 31 conservation of mass-energy and of linear momentum, have been identified and experimentally
 32 validated (Blanchard et al., 2005). Conservation laws for generalized symmetric 2n-dimensional
 33 nonlinear systems of differential equations were also more recently derived and analyzed (Uechi
 34 and Akutsu, 2012; Uechi and Akutsu, 2013), but with limited or no experimental validation. In
 35 theoretical biology, such equation systems, as well as larger sets of differential equations, are
 36 often used to describe complex biological phenomena. Two good examples are the
 37 Lotka-Volterra model and the Kermack-McKendrick models, which are almost identical sets of
 38 equations but are used to describe two different dynamical systems. The Lotka-Volterra model is
 39 well-studied in ecology (Lotka, 1956), and describes the interactions and resulting dynamics of
 40 a population of prey, $X(t)$, and predators, $Y(t)$ over time, t , as

$$\frac{dX(t)}{dt} = rX(t) - aX(t)Y(t), \quad \frac{dY(t)}{dt} = bX(t)Y(t) - cY(t).$$

41 From these two equations, we obtain the relation

$$\frac{dX(t)}{dt} \left(b - \frac{c}{X(t)} \right) + \frac{dY(t)}{dt} \left(a - \frac{r}{Y(t)} \right) = 0.$$

42 Integrating both sides yields the conserved quantity $V(X(t), Y(t)) = bX(t) - c \ln X(t) +$
 43 $aY(t) - r \ln Y(t)$, which is known as a Lyapunov function of the model. Similarly, the
 44 Kermack-McKendrick model (Kermack and McKendrick, 1927), which is almost identical to
 45 the Lotka-Volterra model, is used in epidemiology to describe the transmission of an infectious
 46 disease by infected individuals, $I(t)$, to susceptible individuals, $S(t)$, over time, t , as

$$\frac{dS(t)}{dt} = -\beta S(t)I(t) - \mu S(t), \quad \frac{dI(t)}{dt} = \beta S(t)I(t) - aI(t).$$

47 Using a calculation similar to that shown above for the Lotka-Volterra model, one finds the
 48 following conserved quantity for the Kermack-McKendrick model: $W(S(t), I(t)) = \beta S(t) -$
 49 $a \ln S(t) + \beta I(t) + \mu \ln I(t)$. From this particular conserved quantity, an important concept in
 50 theoretical epidemiology called “final size equation” was derived (Anderson, 1991). This
 51 suggests that conservation laws likely exist in quantitative descriptions of the population
 52 dynamics of real biological systems. However, although the concept of conservation laws and
 53 the conserved quantities themselves can provide important insights in biology, direct evidence
 54 of such laws in biological systems is currently lacking, and no method has been established to
 55 prove otherwise (Hagiwara et al., 2011).

56 The recent rapid development of experimental techniques in molecular biology and

57 cell biology has provided many new insights into the complex interactions between cells,
58 proteins, and genes. Similarly, in virology, this knowledge enables us to track the kinetics of
59 virus and target cells in experiments, especially in cell cultures, thus generating a time-course of
60 experimental data. To better understand the quantitative features of virus replication, we have
61 developed an experimental-mathematical system that quantifies the kinetics of virus infection,
62 combining time-course experimental data with mathematical models (Fukuhara et al., 2013;
63 Iwami et al., 2012a; Iwami et al., 2012b). Here, for the standard, basic model of viral infection
64 kinetics widely used to analyze viral kinetics in vivo and in vitro (Nowak and May, 2000), we
65 mathematically derive a conserved quantity and provide experimental confirmation of its
66 conservation in our *in vitro* virus infection assays. To our knowledge, this is the first time that
67 the existence of a conservation law for population dynamics in a real biological system has been
68 experimentally confirmed.

69 2. Results and Discussion

70 2.1. Derivation of a conservation law for viral infections

71 We introduce the well-known, extensively used basic model to describe virus infection
72 kinetics (Nowak and May, 2000; Perelson, 2002):

$$\frac{dT(t)}{dt} = -\beta T(t)V(t), \quad \frac{dI(t)}{dt} = \beta T(t)V(t) - \delta I(t), \quad \frac{dV(t)}{dt} = pI(t) - cV(t), \quad (1)$$

73 where $T(t)$ and $I(t)$ are the numbers of target (susceptible) and infected (virus-producing)
74 cells per ml of medium, respectively, and $V(t)$ is the viral load per ml of medium. Parameter
75 δ represents the death rate of infected cells, c is the rate of virus loss, β is the rate constant
76 for infection of target cells by virus, and p is the virus production rate per cell. In our
77 experiments discussed below, the viral load was measured either as a total count of extracellular
78 virions, expressed in units of RNA copies/ml (two RNA copy equals one virion) and measured
79 via quantitative PCR, or as a relative concentration of extracellular infectious virions, expressed
80 in units of TCID₅₀/ml (proportional to the number of infectious virions) and measured via virus
81 titration on cell cultures (see **Materials and methods** for details). For each of the daily
82 measurements of the virus concentration, the medium in our experiments was harvested,
83 reducing the viral concentration by 85.4%. This removal can be captured using Eq. (1) by
84 approximating the punctual removal of virus at each sampling time as a continuous, exponential
85 decay of the viral load over the period between samples (one day). In such a case, parameter c
86 corresponds to the sum of the rate of virus loss due to harvesting of the medium plus the rate of
87 loss due to degradation of either the total extracellular virus (when $V(t)$ is measured in units of
88 RNA copies/ml) or of infectivity (when $V(t)$ is measured in units of TCID₅₀/ml). In our earlier
89 work, we have shown that approximating punctual removal as a continuous exponential decay
90 has minimal impact on the model parameters and provides an appropriate fit to the experimental
91 data (Iwami et al., 2012a; Iwami et al., 2012b), and we will be using this approximation here.
92 For completeness, in **Appendix 5.1.**, we also consider a model analogous to (1) wherein
93 punctual removal has been included by using impulsive differential equations, and to which we
94 will refer as the punctual removal model. Note that the measurement units of virus (RNA copies
95 or TCID₅₀) used in our analysis changes both the units and physical meaning of the virus
96 production (p) and cell infection (β) rates, changes the meaning and value of the virus loss (c),
97 and the value of the infectious cell lifespan estimated from the experimental data (δ).

98 Let us define a new variable, $L(t) \equiv \ln[T(t)/N]$, where N is the total cell
99 concentration at time $t = 0$, i.e., $N = T(0) + I(0)$, and is used to make the argument of the $\ln$
100 function unitless. It follows from $dT(t)/dt$ in Eq.(1) that $dL(t)/dt = -\beta V(t)$. Then from
101 Eq.(1) we have

$$\frac{dT(t)}{dt} + \frac{dI(t)}{dt} + \frac{\delta}{p} \frac{dV(t)}{dt} - \frac{\delta c}{p\beta} \frac{dL(t)}{dt} = 0, \quad (2)$$

102 and integrating both sides of Eq.(2), we obtain

$$H = T(t) + I(t) + \frac{\delta}{p} V(t) - \frac{\delta c}{p\beta} L(t). \quad (3)$$

103 This equation holds for all t , indicating that H is a conserved quantity of the dynamical
 104 system in Eq.(1). Note that it has been shown in prior work that the more biologically realistic
 105 inclusion of an eclipse phase into Eq.(1) is required to correctly capture infection kinetics, and
 106 that the duration of this phase is best captured by a Gaussian-like distribution (normal,
 107 log-normal, gamma, Erlang, etc.), but is incorrectly captured by an exponential or Dirac-delta
 108 distribution (Holder and Beauchemin, 2011; Petravic et al., 2014; Pinilla et al., 2012). In
 109 **Appendix 5.2.**, we derive quantity H for a model analogous to (1) wherein an
 110 Erlang-distributed eclipse phase has been included. We show that one arrives at what is
 111 essentially the same expression for H with an additional term for eclipse cells, $+E(t)$, such
 112 that expression (3) also holds under this more realistic model. However, the conservation law
 113 does not hold if cell regeneration and cell division is non-negligible or if the lifespan of
 114 infectious cells follows a Gaussian-like distribution rather than the exponential distribution used
 115 here (not shown).

116

117 2.2. Biological interpretation of the conservation law for viral infection

118 Through simple re-arrangement of conserved quantity H , we can arrive at another
 119 conserved quantity, Q , with a clearer and more useful biological interpretation. Because Eq.(3)
 120 holds at $t = 0$, we have the following relation

$$T(t) + I(t) + \frac{\delta}{p} V(t) - \frac{\delta c}{p\beta} L(t) = T(0) + I(0) + \frac{\delta}{p} V(0) - \frac{\delta c}{p\beta} L(0). \quad (4)$$

121 In typical cell culture experiments, infection is initiated from a virus inoculum in a fully
 122 susceptible cell population such that $I(0) \approx 0$. Furthermore, since $L(t) \equiv \ln[T(t)/N]$, we
 123 have $L(0) = 0$ such that Eq.(4) becomes

$$T(0) = T(t) + I(t) + \frac{\delta}{p} [V(t) - V(0)] - \frac{\delta c}{p\beta} L(t). \quad (5)$$

124 We remark that $L(t) = -\beta \int_0^t V(s) ds$, and re-write Eq.(5) as

$$T(0) = T(t) + I(t) + \frac{V(t) - V(0)}{(p/\delta)} + \frac{c \int_0^t V(s) ds}{(p/\delta)}. \quad (6)$$

125 Note that the term p/δ corresponds to the viral burst size which, depending on the
 126 measurement units of the viral load, corresponds to either the number virus RNA copies or
 127 TCID₅₀ of virus produced by an infected cell over its lifetime $1/\delta$ (Nowak and May, 2000). As

such, the term $[V(t) - V(0)]/(p/\delta)$ essentially converts the observed total or infectious virus concentration in the medium at time t into the concentration of cells that would have had to die to produce them. The term $c \int_0^t V(s) ds/(p/\delta)$ effects the same conversion of virus into a cell count, but does so for the sum total of all virus (RNA copies) or only the infectious virus (TCID₅₀) which are no longer present in the medium because they have been harvested and degraded at rate c from time $t = 0$ up to and including time t . In other words, the terms $[V(t) - V(0)]/(p/\delta) + c \int_0^t V(s) ds/(p/\delta)$ correspond to the total concentration of cells which have died due to infection by time t , i.e., $D(t) = \int_0^t \delta I(s) ds$. In the case of a non-lytic virus (i.e., $\delta = 0$), the burst size becomes infinity, which implies these two terms become 0 and Eq. (6) becomes simply $T(0) = T(t) + I(t)$. Thus, for all t , the total mass (or energy) of the system, which is contained in the initial target cells, $T(0)$, remains nearly constant over time in an isolated system even as cells change state ($T \rightarrow I$) or are converted to total or infectious virions ($I \rightarrow V$). For convenience, from here on, we will instead make use of the following normalized, conserved quantity

$$Q = 1 = \frac{1}{T(0)} \left\{ T(t) + I(t) + \frac{V(t) - V(0)}{(p/\delta)} + \frac{c \int_0^t V(s) ds}{(p/\delta)} \right\}. \quad (7)$$

142

143 **2.3. Experimental confirmation of the conservation law for viral infection**

144 We can verify whether this theoretical conservation law holds experimentally by
 145 calculating the normalized quantity Q in Eq.(7) over the course of an experimental viral
 146 infection in a cell culture. We infected HSC-F cell cultures with two different strains of the
 147 simian-human immunodeficiency virus (SHIV-KS661 and SHIV-#64 (Kozyrev et al., 2001): the
 148 intravenous infection of rhesus macaques with SHIV-KS661 consistently results in high viremia
 149 and CD4+ T cell depletion, whereas infection with SHIV-#64 causes more subdued
 150 pathogenesis), as previously described (Iwami et al., 2012a; Iwami et al., 2012b), with
 151 additional details in **Materials and methods**. Briefly, cultures of HSC-F cells were infected
 152 with SHIV-KS661 (or SHIV-#64) at 24 h prior to the first measurement (i.e., $t = -24$ h),
 153 incubated with the virus for 4 h (i.e., the cell were placed in fresh, virus-free medium at time
 154 $t = -20$ h), and sampled daily over 10 consecutive days (i.e., $t = 0, 1, \dots, 9$ days). Each day,
 155 most of the virus (85.4 %) and a small percentage of the cells (5.5 %) were removed from the
 156 culture supernatant for measurement, and fresh medium was added. Each sample was assayed to
 157 determine the concentration of cells (cells/ml) and the fraction of these cells which had either
 158 remained uninfected (Nef-negative = Target, $T(t)$) or had been infected (Nef-positive =
 159 Infected, $I(t)$) by SHIV, and to determine the total viral load (RNA copies/ml) and infectious
 160 viral titer (TCID₅₀/ml) of SHIV-KS661 (or SHIV-#64) in the extracellular medium. The
 161 infection experiments were performed at two different values of the initial viral inoculum

162 (multiplicity of infection (MOI) of 2.0×10^{-4} and 2.0×10^{-5}) so as to provide sufficient
163 data for robust parameter estimation and for calculation of Q .

164 To determine the parameter values and initial conditions in Eq.(1) for SHIV-KS661
165 and SHIV-#64 infections, we fitted Eq.(1) to the time-course data listed under "For parameter
166 estimation (2.0×10^{-4} MOI)" in **Table 1** (see **Materials and methods**). The parameter and
167 initial values estimated by fitting Eq.(1) to datasets using either the total viral load (RNA
168 count/ml) or the infectious viral titer (TCID₅₀/ml) concentrations are listed in **Tables 2** and **3**,
169 with the model's predicted kinetics for these best-fit parameter estimates presented alongside the
170 data in **Figures 1(A)(B)** and **2(A)(B)**, respectively. For the alternate model with punctual virus
171 removal at each sampling time (**Appendix 5.1.**), the estimated parameters and initial values are
172 listed in **Tables 4** and **5**, with their predicted kinetics presented alongside the data in **Figures**
173 **3(E)(F)** and **4(E)(F)**, respectively.

174 The degradation rate of total virus (RNA copies) is inferior (slower) than that of
175 infectious virus (TCID₅₀). For example, for SHIV-KS661, we have determined in previous work
176 that the degradation rate of total extracellular virus (RNA copies) is 0.039/day, whereas that of
177 infectious virus (TCID₅₀) is 0.91/day (Iwami et al., 2012a; Iwami et al., 2012b). Since
178 infectious virus degrades at a higher rate than total virus (which includes both infectious and
179 non-infectious virus), we expect that Eq.(1) should be appropriate when $V(t)$ is proportional to
180 infectious virus (TCID₅₀). But when $V(t)$ represents the total virus (RNA copies), a more
181 accurate model might require that, at the very least, the infection rate, β , decrease over time,
182 $\beta(t)$, to reflect that for an equivalent number of total virions, one has a decreasing number of
183 infectious virions (or decreasing infectivity) as time passes. By using the same model, Eq. (1),
184 to fit both the total (RNA copies) and infectious (TCID₅₀) viral load, we are assuming that the
185 ratio of infectious to total viral load is relatively constant over the course of our infection. This
186 can be justified in part because, during the viral load growth phase (virus upslope), when the
187 majority of cell infection takes place, the rate of virus production exceeds the rate of virus
188 clearance such that the ratio of infectious to total virus is roughly constant. Additionally, in our
189 experiments, the rate of virus loss due to harvesting ($-\ln(1 - 0.854)/(1 \text{ day}) = 1.92/\text{day}$)
190 exceeds the rate of degradation of either extracellular viral RNA copies (e.g., 0.91/day) or
191 infectious, TCID₅₀ viral titer (e.g., 0.039/day), such that it dominates the rate of viral loss, and
192 further helps maintain the ratio of infectious to total virus relatively constant over the course of
193 the infection. While it is clear from **Table 1** that the ratio of TCID₅₀/RNA in our experiments
194 varies significantly for infection with SHIV-KS661 at an MOI of 2.0×10^{-4} , it does not
195 decrease too significantly up to viral titer peak (day 6) in the other three infection time courses.
196 This suggests that the large deviation in this one infection time course is more likely a stochastic
197 deviation due to experimental error rather than a significant trend, and the use of Eq. (1) to

198 capture our infection experiments measuring either total total (RNA copies) or infectious
199 (TCID₅₀) viral load is appropriate, though it will contribute some error.

200 As evident from these figures, Eq.(1) accurately models these cell culture experiments,
201 although there is some difference between our prediction and experimental data caused by the
202 property of Eq.(1) that target cells are partially depleted, and will not ultimately approach “0”.

203 Using the experimental data, the theoretically conserved quantity Q was determined
204 by substituting the parameter values in **Tables 2** and **3** into Eq.(7). In this calculation, the
205 time-course data listed under "For calculation of Q (2.0×10^{-5} MOI)" in **Table 1** were used.
206 The data at day 0 were assumed as $T(0)$ and $\int_0^t V(s) ds$ was calculated from the viral load
207 data using the trapezoidal rule. In **Materials and methods**, we provide an example for the
208 calculation of Q at one time point. The calculated Q s, based on datasets including the viral
209 RNA and infectivity, at each time point are plotted in **Figure 1(C)(D)** and **2(C)(D)**, respectively.
210 For the punctual removal model, the calculated Q s are also plotted in **Figures 3(G)(H)** and
211 **4(G)(H)**, respectively.

212 Theoretically, Q should remain around 1 throughout the virus infection if the model
213 is a valid representation of the infection kinetics and if the conservation law holds.
214 Interestingly, the calculated Q values remain around 1, with slight fluctuations, throughout the
215 infection period for both virus strains. This provides compelling evidence that the mathematical
216 model used is an appropriate description of the system and that, as such, its associated
217 conservation law does hold over the course of experimental viral infections in cell cultures, at
218 least for the SHIV-KS661 and SHIV-#64 infection of HSC-F cells. Conversely, this means that
219 the conservation law can be used as a measure of the degree of uncertainty and variability in
220 experimental measurements, namely by comparing the standard deviations (SDs) of our
221 computed experimental Q values from the true, theoretical value of $Q = 1$. In our experiments,
222 we measured either the total viral load via quantitative RT-PCR or the infectious viral titer via
223 TCID₅₀ assays performed in HSC-F cell cultures, and obtained higher SDs for Q values based
224 on the TCID₅₀ than for those based on RT-PCR (**Tables 6** and **7**). This is consistent with the
225 known, greater experimental measurement errors and poorer sensitivity (larger limit of
226 detection) of TCID₅₀ assays compared to RT-PCR assays (see the numerous d.l. entries in **Table**
227 **1**). While the computed Q values depend on both the experimental measurements and the
228 estimated parameters, we have determined through a parameter sensitivity analysis (not shown)
229 that in our experiments, deviations from $Q = 1$ are largely attributable to experimental
230 measurement errors rather than error in parameter estimates. As discussed in (Fukuhara et al.,
231 2013; Iwami et al., 2012a; Iwami et al., 2012b), our experimental-mathematical strategy can
232 robustly estimate parameter values from time-course data, even if there are relatively greater
233 errors in viral load, because Eq.(1) is fitted simultaneously to the concentrations of target cells,

234 infected cells and the viral load, which complement one another, minimizing the impact of
235 stochastic deviations present in any one particular data set. Thus, the higher SDs in the Q
236 values estimated from the viral load measured via the TCID₅₀ assay reflects larger measurement
237 errors in this assay, rather than errors in parameter estimation.

238 3. Conclusion

239 Although nonlinear phenomena in virus infection experiments are usually difficult to
240 understand and explain intuitively, the dynamical behavior of the system can be exposed
241 through well-constructed mathematical models. The experimental-mathematical strategy is a
242 powerful methodology used to quantitatively investigate the dynamics of virus infections that is
243 not accessible by conventional experimental strategies (Fukuhara et al., 2013; Iwami et al.,
244 2012a; Iwami et al., 2012b). To date, the basic model has been empirically used as a de facto
245 standard model for quantitatively understanding nonlinear phenomena, especially in cell
246 cultures experimentally infected with virus, which are considered as closed systems
247 (Beauchemin et al., 2008; Fukuhara et al., 2013; Iwami et al., 2012a; Iwami et al., 2012b;
248 Mohler et al., 2005; Pinilla et al., 2012). In this work, we have derived a conserved quantity
249 with a clear biological interpretation and have shown that our conservation law holds in our cell
250 culture experiments. Furthermore, we showed that computation of our conserved quantity from
251 experimental data can serve as a valuable measure of the degree of experimental error, and
252 could be used as a data validation tool to identify aberrant or erroneous measurements. Although
253 the conservation law was shown here to hold only for two different strains of SHIV, it should
254 hold for all other lytic virus in cultures where cell division is negligible over the time scale of
255 the infection experiment.

256 4. Materials and methods

257 4.1. SHIV infection in HSC-F cell culture

258 The virus solution of SHIV-KS661 (Shinohara et al., 1999) (or SHIV-#64 (Kozyrev et
259 al., 2001)) was prepared in a CD4⁺ human T lymphoid cell line, M8166 (a subclone of C8166)
260 (Clapham et al., 1987), and was stored in liquid nitrogen until use. The HSC-F cell line (Akari
261 et al., 1996) was cultured in a culture medium (RPMI-1640 supplemented with 10% fetal calf
262 serum) at 37°C and 5% CO₂ in humidified conditions.

263 Each experiment was performed using 2 wells of a 24-well plate with a total
264 suspension volume of 2 ml (1 ml per well) and an initial cell concentration of $T_0 = 6.46 \times 10^6$
265 cells/ml in each well. Because the initial cell concentration is close to the carrying capacity of
266 24-well plates, and HSC-F cells replicate slowly, and in the absence of SHIV-KS661 (or
267 SHIV-#64) infection, the population of target cells changes very little on the timescale of our
268 experiment (data not shown). We therefore neglected the effects of potential regeneration of
269 HSC-F cells in our analysis and in constructing the mathematical model.

270 For virus infection, cultures of HSC-F cells were inoculated 24 h prior to the first
271 infection sampling ($t = -24$ h) at two different multiplicity of infections (MOIs) of $2.0 \times$
272 10^{-4} or 2.0×10^{-5} TCID₅₀ (50% tissue culture infectious dose) per cell of SHIV-KS661 (or
273 SHIV-#64), and were incubated at 37°C. Four hours after inoculation ($t = -20$ h), the cells
274 were washed to remove the remaining viruses and were replaced into a fresh culture medium.
275 The culture supernatant was harvested daily for 10 days ($t = 0, 1, \dots, 9$ d), and was replaced
276 with fresh medium. On a daily basis, 5.5% of the cells in the culture were harvested to measure
277 the number of target cells and infected cells. Cells were counted by staining them with an
278 anti-SIV Nef monoclonal antibody (04-001, Santa Cruz Biotechnology, Santa Cruz, CA) labeled
279 by Zenon Alexa Fluor 488 (Invitrogen, Carlsbad, CA), as previously described (Iwami et al.,
280 2012a; Iwami et al., 2012b). Each harvested supernatant, including 85.4% of the culture virus
281 was stored at -80°C, and the amount of viral RNA was quantified by RT-PCR, as previously
282 described (Iwami et al., 2012a; Iwami et al., 2012b; Motohara et al., 2006). The infectious viral
283 load was measured by TCID₅₀ assay in HFC-S cell cultures using 96-well flat bottom plates at
284 cell concentrations of 1.0×10^6 cells/ml. The titer of the virus was determined as described by
285 Reed and Muench (Reed and Muench, 1938). The time-course data of this experiment are
286 summarized in **Table 1**.

287

288 4.2. Extraction of parameters from the experimental infection data

289 In relating our daily experimental measurements at time $t = 0, 1, \dots, 9$ day to our
290 mathematical models, we define $t = 0$ as the time of our first experimental measurements, i.e.
291 when $T(0)$, $I(0)$, and $V(0)$ are measured. As such, by time $t = 0$, some cells (T) have

become infected (I), and while the inoculum virus has been rinsed, some new virus (V) will have been produced by the newly infected cells. Parameter c in Eq.(1), or c_{deg} in the punctual removal model, were held fixed at their value estimated in previous work (Iwami et al., 2012a; Iwami et al., 2012b). The concentration of uninfected target cells at measurement time $t = 0$, $T(0)$, was fixed to its experimentally measured quantity. The remaining two concentrations, $I(0)$, and $V(0)$, had to be estimated through fitting because they were below detection levels at that measurement time. Note that while $V(0)$ was above the detection limit when measured in units of RNA copies/ml, we still estimated its value through fitting so as to use the same analysis performed when virus was in units of TCID₅₀/ml.

Eq.(1) (or the punctual removal model) was fitted simultaneously to the log₁₀ of the concentrations of target cells, infected cells, and of either total (RNA) or infectious (TCID₅₀) virus by nonlinear least-squares regression using the *FindMinimum* package of *Mathematica 9.0* which minimizes the sum of squared residuals (SSR). Experimental measurements below the detection limit were excluded from the SSR calculation.

306

307 **4.3. Computation of conserved quantity Q from experimental measures**

308 As an example, we calculate the quantity Q for SHIV-KS661 at 5 days post
309 inoculation using the experimental viral RNA data (the shaded values in **Table 1**) as follows

$$Q = \frac{1}{T(0)} \left\{ T(5) + I(5) + \frac{V(5) - V(0)}{(p/\delta)} + \frac{c \int_0^5 V(s) ds}{(p/\delta)} \right\}.$$

310 From fitting Eq.(1) to the experimental data, we obtained estimated values for $p = 3.2 \times 10^4$
311 (RNA copies/cell) $\cdot$ day⁻¹, $\delta = 1.71$ day⁻¹ and $c = 2.02$ day⁻¹ (see **Table 2**), which we use in
312 the above equation to compute Q . Note that both the degradation rate of viral RNA and the
313 85.4 % removal virus due to experimental sampling are included in the value for the clearance
314 rate, c , by approximating the decrease of virus as a continuous exponential decay. The loss of
315 5.5 % of cell at each sampling point due to the harvesting of the sample is assumed to be
316 negligible. The value of the integral $\int_0^5 V(s) ds$ was approximated using the trapezoidal rule in
317 log-scale, namely

$$\begin{aligned} \int_0^5 V(s) ds \approx & [10^{(\log_{10} V(0) + \log_{10} V(1)) \times [1 \text{ day}] / 2} + 10^{(\log_{10} V(1) + \log_{10} V(2)) \times [1 \text{ day}] / 2} \\ & + 10^{(\log_{10} V(2) + \log_{10} V(3)) \times [1 \text{ day}] / 2} + 10^{(\log_{10} V(3) + \log_{10} V(4)) \times [1 \text{ day}] / 2} \\ & + 10^{(\log_{10} V(4) + \log_{10} V(5)) \times [1 \text{ day}] / 2}] = 1.47 \times 10^9 \text{ RNA copies/ml} \cdot \text{day}. \end{aligned}$$

318 We also considered the use of an interpolation function running through the viral load data,

319 instead of using the actual data points, to estimate this integral and obtained instead 1.16×10^9
 320 RNA copies/ml·day. From **Table 1**, since $T(0) = 6.31 \times 10^6$ cells/ml, $V(0) = 1.64 \times 10^4$
 321 RNA copies/ml, $T(5) = 5.91 \times 10^6$ cells/ml, $I(5) = 4.12 \times 10^5$ cells/ml, and $V(5) =$
 322 2.18×10^9 RNA copies/ml, quantity Q on day 5 is given by

$$\frac{1}{6.31 \times 10^6} \left\{ 5.91 \times 10^6 + 4.12 \times 10^5 + \frac{2.18 \times 10^9 - 1.64 \times 10^4}{(3.2 \times 10^4/1.71)} + \frac{2.02 \times 1.47 \times 10^9}{(3.2 \times 10^4/1.71)} \right\} = 1.043.$$

323 For the estimated integration value using the interpolation function, quantity $Q = 1.038$, which
 324 shows that the trapezoidal rule provides a reasonable approximation for the integration.

325 5. Appendix

326 5.1. A punctual removal model for virus infections

327 We consider punctual removal of virus due to experimental sampling by adding Dirac
328 delta function to Eq.(1) as follows:

$$\begin{aligned}\frac{dT(t)}{dt} &= -\beta T(t)V(t), \\ \frac{dI(t)}{dt} &= \beta T(t)V(t) - \delta I(t), \\ \frac{dV(t)}{dt} &= pI(t) - c_{\text{deg}}V(t) - \sum_{i=0}^9 \delta_{\text{Dirac}}(t - t_i)\varepsilon V(t),\end{aligned}$$

329 where parameter ε denotes the fraction of virus removed by the sampling (i.e., $\varepsilon = 0.854$),
330 and the rate of virus degradation, c_{deg} , is only that due to degradation of either total
331 extracellular virus (for virus quantified in RNA copies) or virus infectivity (for virus quantified
332 in TCID₅₀). In the above equation, the removal of virus due to sampling is captured explicitly by
333 an impulsive term, unlike in Eq.(1) where c also includes harvesting of the medium. As such,
334 the value for c in Eq.(1) (reported in **Tables 2 and 3**) is equal to the value of c_{deg} in the above
335 model with punctual virus removal (reported in **Tables 4 and 5**) plus the rate of virus loss due to
336 harvesting, namely $1.92/\text{day}$ ($-\ln(1 - 0.854)/(1 \text{ day})$). Both the values of c and c_{deg} were
337 determined in earlier work (Iwami et al., 2012a; Iwami et al., 2012b), and fixed to their
338 previously determined value in the present work. We define $t_i = i$ days for $i = 0, 1, 2, \dots, 9$
339 because the experiments last from day 0 to 9, and δ_{Dirac} is the Dirac delta such that

$$\int_0^t \delta_{\text{Dirac}}(s - t_i)\varepsilon V(s) ds = \sum_{i=0}^{i \leq t} \varepsilon V(t_i).$$

340 Similar to the derivation of Eq.(2) and Eq.(3), we have

$$\frac{dT(t)}{dt} + \frac{dI(t)}{dt} + \frac{\delta}{p} \frac{dV(t)}{dt} - \frac{\delta c_{\text{deg}}}{p\beta} \frac{dL(t)}{dt} + \frac{\delta}{p} \left(\sum_{i=0}^9 \delta_{\text{Dirac}}(t - t_i)\varepsilon V(t) \right) = 0,$$

341 and integrating both side, we obtain

$$H = T(t) + I(t) + \frac{\delta}{p} V(t) - \frac{\delta c_{\text{deg}}}{p\beta} L(t) + \frac{\delta}{p} \left(\sum_{i=0}^{i \leq t} \varepsilon V(t_i) \right).$$

342 Therefore, we derive the following normalized, conserved quantity for the punctual removal
343 model:

$$Q = 1 = \frac{1}{T(0)} \left\{ T(t) + I(t) + \frac{V(t) - V(0)}{(p/\delta)} + \frac{\delta c_{\text{deg}} \int_0^t V(s) ds}{(p/\delta)} + \frac{\sum_{i=0}^{i \leq t} \varepsilon V(t_i)}{(p/\delta)} \right\}.$$

344

345 **5.2. Derivation of a general conservation law for viral infections**

346 The eclipse phase is the time elapsed between the successful infection of a cell and the
 347 release of the first virion released by that newly infected cell (Petravic et al., 2014; Pinilla et al.,
 348 2012). Interestingly, Eq.(3) can be generalized for models which include an eclipse phase. By
 349 modifying Eq.(1) to incorporate an exponentially ($n_E = 1$) or Erlang-distributed ($n_E > 1$)
 350 eclipse phase with mean of τ_E , where n_E is the number of exponentially distributed eclipse
 351 compartments in the model, and modifying $dI(t)/dt$ accordingly, one obtains the more
 352 general model for $i = 2 \dots n_E$

$$\begin{aligned} \frac{dT(t)}{dt} &= -\beta T(t)V(t), \\ \frac{dE_1(t)}{dt} &= \beta T(t)V(t) - \frac{n_E}{\tau_E} E_1(t), \\ \frac{dE_i(t)}{dt} &= \frac{n_E}{\tau_E} E_{i-1}(t) - \frac{n_E}{\tau_E} E_i(t), \\ \frac{dI(t)}{dt} &= \frac{n_E}{\tau_E} E_{n_E}(t) - \delta I(t), \\ \frac{dV(t)}{dt} &= pI(t) - cV. \end{aligned}$$

353 It is easy to show that the corrected quantity H for this model is simply

$$H = T(t) + \sum_{i=1}^{n_E} E_i(t) + I(t) + \frac{\delta}{p} V(t) - \frac{\delta c}{p\beta} L(t),$$

354 which simplifies to Eq.(3) when $\sum_{i=1}^{n_E} E_i(t) = 0$, i.e. when there is no eclipse phase. If one
 355 wanted to compute H experimentally, cells in the eclipse phase would appear either as
 356 uninfected or infected cells and so the experimental calculation of H would follow Eq.(1),
 357 independently of the choice to use a model with or without an eclipse phase.

358

359 **Acknowledgments**

360 This research was supported in part by the Kyushu University Fund (Y.K.), JST CREST
361 program (K.S. and S.I.), the Aihara Innovative Mathematical Modeling Project, JSPS, through
362 the “Funding Program for World-Leading Innovative R & D on Science and Technology (FIRST
363 Program)”, initiated by Council for Science and Technology Policy (to K.S. and S.I.), Takeda
364 Science Foundation (to K.S.), Sumitomo Foundation Research Grant (to K.S.), Senshin Medical
365 Research Foundation (to K.S.), Imai Memorial Trust for AIDS Research (to K.S.), Ichiro
366 Kanehara Foundation (to K.S.), Kanae Foundation for the Promotion of Medical Science (to
367 K.S.), Suzuken Memorial Foundation (to K.S.), Kyushu University Short-term Young Scholar
368 Exchange Program, which is based on Japanese Ministry of Education, Culture, Sports, Science
369 and Technology’s grant The Program for Promoting the Enhancement of Research Universities
370 (C.A.A.B. and S.I.), JST PRESTO program (S.I.), Grants-in-Aid for Young Scientists
371 B25800092 from the Japan Society for the Promotion of Science or JSPS (S.I.), with additional
372 funding from the Inamori Foundation (S.I.).

373

374

375

376

377 **Authors contributions**

378 Conceived and designed the experiments: TM KS SI. Performed the experiments: TM.
379 Analyzed the data: YK SM SN YT CAAB SI. Contributed reagents/materials/ analysis tools:
380 TM. Wrote the paper: YK SM SN YT KS TM CAAB SI.

381

382

383 **References**

- 384 Akari, H., Mori, K., Terao, K., Otani, I., Fukasawa, M., Mukai, R., Yoshikawa, Y., 1996. In
385 vitro immortalization of Old World monkey T lymphocytes with Herpesvirus saimiri:
386 its susceptibility to infection with simian immunodeficiency viruses. *Virology* 218,
387 382-8, doi:10.1006/viro.1996.0207.
- 388 Anderson, R. M., 1991. Discussion: the Kermack-McKendrick epidemic threshold theorem.
389 *Bull Math Biol* 53, 3-32.
- 390 Beauchemin, C. A., McSharry, J. J., Drusano, G. L., Nguyen, J. T., Went, G. T., Ribeiro, R. M.,
391 Perelson, A. S., 2008. Modeling amantadine treatment of influenza A virus in vitro. *J*
392 *Theor Biol* 254, 439-51, doi:10.1016/j.jtbi.2008.05.031.
- 393 Blanchard, P., Devaney, R., Hall, G., 2005. *Differential Equations*. Thomson Brooks/Cole,
394 Belmont.
- 395 Clapham, P. R., Weiss, R. A., Dalglish, A. G., Exley, M., Whitby, D., Hogg, N., 1987. Human
396 immunodeficiency virus infection of monocytic and T-lymphocytic cells: receptor
397 modulation and differentiation induced by phorbol ester. *Virology* 158, 44-51.
- 398 Fukuhara, M., Iwami, S., Sato, K., Nishimura, Y., Shimizu, H., Aihara, K., Koyanagi, Y.,
399 2013. Quantification of the dynamics of enterovirus 71 infection by
400 experimental-mathematical investigation. *J Virol* 87, 701-5,
401 doi:10.1128/jvi.01453-12.
- 402 Hagiwara, T., Ushimaru, T., Tainaka, K., Kurachi, H., Yoshimura, J., 2011. Apoptosis at
403 inflection point in liquid culture of budding yeasts. *PLoS One* 6, e19224,
404 doi:10.1371/journal.pone.0019224.
- 405 Holder, B. P., Beauchemin, C. A., 2011. Exploring the effect of biological delays in kinetic
406 models of influenza within a host or cell culture. *BMC Public Health* 11 Suppl 1, S10,
407 doi:10.1186/1471-2458-11-s1-s10.
- 408 Iwami, S., Sato, K., De Boer, R. J., Aihara, K., Miura, T., Koyanagi, Y., 2012a. Identifying
409 viral parameters from in vitro cell cultures. *Front Microbiol* 3, 319,
410 doi:10.3389/fmicb.2012.00319.
- 411 Iwami, S., Holder, B. P., Beauchemin, C. A., Morita, S., Tada, T., Sato, K., Igarashi, T., Miura,
412 T., 2012b. Quantification system for the viral dynamics of a highly pathogenic
413 simian/human immunodeficiency virus based on an in vitro experiment and a
414 mathematical model. *Retrovirology* 9, 18, doi:10.1186/1742-4690-9-18.
- 415 Kermack, W., McKendrick, A., 1927. A contribution to the mathematical theory of epidemics.
416 *Proc R Soc A* 115, 700-21.
- 417 Kozyrev, I. L., Ibuki, K., Shimada, T., Kuwata, T., Takemura, T., Hayami, M., Miura, T., 2001.
418 Characterization of less pathogenic infectious molecular clones derived from

419 acute-pathogenic SHIV-89.6p stock virus. *Virology* 282, 6-13,
 420 doi:10.1006/viro.2000.0839.

421 Lotka, A., 1956. *Elements of Physical Biology* (Williams and Wilkins, Baltimore, 1925).
 422 *Elements of Mathematical Biology*.

423 Mohler, L., Flockerzi, D., Sann, H., Reichl, U., 2005. Mathematical model of influenza A
 424 virus production in large-scale microcarrier culture. *Biotechnol Bioeng* 90, 46-58,
 425 doi:10.1002/bit.20363.

426 Motohara, M., Ibuki, K., Miyake, A., Fukazawa, Y., Inaba, K., Suzuki, H., Masuda, K.,
 427 Minato, N., Kawamoto, H., Nakasone, T., Honda, M., Hayami, M., Miura, T., 2006.
 428 Impaired T-cell differentiation in the thymus at the early stages of acute pathogenic
 429 chimeric simian-human immunodeficiency virus (SHIV) infection in contrast to less
 430 pathogenic SHIV infection. *Microbes Infect* 8, 1539-49,
 431 doi:10.1016/j.micinf.2006.01.011.

432 Nowak, M., May, R. M., 2000. *Virus Dynamics: Mathematical Principles of Immunology and*
 433 *Virology: Mathematical Principles of Immunology and Virology*. Oxford university
 434 press.

435 Perelson, A. S., 2002. Modelling viral and immune system dynamics. *Nat Rev Immunol* 2,
 436 28-36, doi:10.1038/nri700.

437 Petravic, J., Ellenberg, P., Chan, M. L., Paukovics, G., Smyth, R. P., Mak, J., Davenport, M.
 438 P., 2014. Intracellular dynamics of HIV infection. *J Virol* 88, 1113-24,
 439 doi:10.1128/jvi.02038-13.

440 Pinilla, L. T., Holder, B. P., Abed, Y., Boivin, G., Beauchemin, C. A., 2012. The H275Y
 441 neuraminidase mutation of the pandemic A/H1N1 influenza virus lengthens the
 442 eclipse phase and reduces viral output of infected cells, potentially compromising
 443 fitness in ferrets. *J Virol* 86, 10651-60, doi:10.1128/jvi.07244-11.

444 Reed, L., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. *Am J*
 445 *Hyg* 27, 493-7.

446 Shinohara, K., Sakai, K., Ando, S., Ami, Y., Yoshino, N., Takahashi, E., Someya, K., Suzaki,
 447 Y., Nakasone, T., Sasaki, Y., Kaizu, M., Lu, Y., Honda, M., 1999. A highly pathogenic
 448 simian/human immunodeficiency virus with genetic changes in cynomolgus monkey.
 449 *J Gen Virol* 80 (Pt 5), 1231-40.

450 Uechi, L., Akutsu, T., 2012. Conservation laws and symmetries in competitive systems.
 451 *Progress of Theoretical Physics Supplement* 194, 210-222.

452 Uechi, L., Akutsu, T., 2013. Stability and restoration phenomena in competitive systems.
 453 *Progress of Theoretical and Experimental Physics* 2013, 103J01.

454

Figure legends

Figure 1. Model against experimental data based on the total viral load (RNA copies).

HSC-F cells were inoculated with SHIV-KS661 or SHIV-#64 at time $t = -1$ day and each *in vitro* experimental quantity was measured daily from $t = 0$ d to 9 d. The solid curves in (A) and (B) show the best-fit of Eq.(1) to the experimental data (points) for the target cells, infected cells, and the total viral load (RNA copies/ml) for infection with an MOI of 2.0×10^{-4} of SHIV-KS661 and SHIV-#64, respectively. The black circles in (C) and (D) are values of conserved quantity Q , computed throughout the viral infection, using parameters extracted from the fit presented in (A)(B) and experimental data points from a separate infection experiment conducted at an MOI of 2.0×10^{-5} with SHIV-KS661 and SHIV-#64, respectively. The dotted line represents the theoretical value of $Q = 1$ assuming that the conservation law holds and that no experimental errors exist in this isolated system. Panels (E)(F) and (G)(H) are results obtained with the model with punctual removal (Appendix 5.1.) and are analogous to panels (A)(B) and (C)(D), respectively.

Figure 2. Model against experimental data based on the infectious viral titer (TCID₅₀).

HSC-F cells were inoculated with SHIV-KS661 or SHIV-#64 at time $t = -1$ day and each *in vitro* experimental quantity was measured daily from $t = 0$ d to 9 d. The solid curves in (A) and (B) show the best-fit of Eq.(1) to the experimental data (points) for the target cells, infected cells, and the infectious viral titer (TCID₅₀/ml) for infection with an MOI of 2.0×10^{-4} of SHIV-KS661 and SHIV-#64, respectively. The grey circles in (C) and (D) are values of conserved quantity Q , computed throughout the viral infection, using parameters extracted from the fit presented in (A)(B) and experimental data points from a separate infection experiment conducted at an MOI of 2.0×10^{-5} with SHIV-KS661 and SHIV-#64, respectively, throughout the viral infection. The dotted line represents the theoretical value of $Q = 1$ assuming that the conservation law holds and that no experimental errors exist in this isolated system. Panels (E)(F) and (G)(H) are results obtained with the model with punctual removal (Appendix 5.1.) and are analogous to panels (A)(B) and (C)(D), respectively.

Table 1. Cell culture experimental data on the infection of HSC-F cells with SHIV-KS661 and SHIV-#64.

SHIV-KS661	Unit	day0	day1	day2	day3	day4	day5	day6	day7	day8	day9
For parameter estimation (2.0×10^{-4} MOI)											
Target cells	10^4 cells/ml	631.5	640.5	620.1	476.3	468.5	112.9	28.4	14.3	3.66	1.08
Infected cells	10^4 cells/ml	d.l. ^a	d.l.	d.l.	1.88	54.4	184.3	87.2	22.8	7.06	1.44
Viral loads	10^6 RNA copies/ml	0.15	2.11	12.0	322.0	7090	26000	23400	8430	1560	511.0
Viral infectivity	10^3 TCID ₅₀ /ml	d.l.	d.l.	d.l.	1.11	124.2	81.9	41.0	25.8	14.5	2.56
For calculation of Q (2.0×10^{-5} MOI)											
Target cells	10^4 cells/ml	631.0 ^b	710.0	761.9	576.6	716.7	591.1	207.9	49.8	22.1	6.84
Infected cells	10^4 cells/ml	d.l.	d.l.	d.l.	d.l.	4.80	41.2	318.8	136.7	44.2	4.75
Viral loads	10^6 RNA copies/ml	0.016	0.16	0.62	17.7	362.0	2180	21600	21300	9000	2360
Viral infectivity	10^3 TCID ₅₀ /ml	d.l.	d.l.	d.l.	0.16	2.56	23.5	163.8	94.1	65.0	10.2
SHIV-#64	Unit	day0	day1	day2	day3	day4	day5	day6	day7	day8	day9
For parameter estimation (2.0×10^{-4} MOI)											
Target cells	10^4 cells/ml	623.5	676.4	621.0	632.8	501.0	364.8	88.1	29.1	8.45	4.74
Infected cells	10^4 cells/ml	d.l.	d.l.	d.l.	1.34	11.6	83.2	159.8	42.3	11.1	1.18
Viral loads	10^6 RNA copies/ml	3.24	65.1	29.6	159.0	1460	7380	17700	10600	2620	917.0
Viral infectivity	10^3 TCID ₅₀ /ml	d.l.	d.l.	d.l.	d.l.	1.94	7.24	4.46	2.56	1.47	0.64
For calculation of Q (2.0×10^{-5} MOI)											
Target cells	10^4 cells/ml	628.5	816.7	729.8	721.9	721.4	506.6	121.3	24.3	13.7	5.28
Infected cells	10^4 cells/ml	d.l.	d.l.	d.l.	d.l.	3.34	31.7	249.8	67.0	18.3	1.37
Viral loads	10^6 RNA copies/ml	0.35	4.78	2.78	190.0	234.0	2280	30100	31900	11200	3200
Viral infectivity	10^3 TCID ₅₀ /ml	d.l.	d.l.	d.l.	d.l.	0.32	2.56	14.5	4.06	10.2	1.16

^a "d.l." designates samples in which the concentration is below the detection limit and this value is set to be 0 in the calculation of Q .

^b The shadowed values are used for the calculation of Q in **Materials and methods** as an example.

Table 2. Fitted parameter and initial values in Eq.(1) for the cell culture SHIV-KS661 and SHIV-#64 with viral RNA data.

Estimated values	Unit	SHIV-KS661	SHIV-#64
β	(RNA copies/ml $\cdot$ day) ⁻¹	9.46×10^{-11}	7.11×10^{-11}
p	(RNA copies/cell) $\cdot$ day ⁻¹	3.20×10^4	3.63×10^4
δ	day ⁻¹	1.71	2.00
c [held fixed [†]]	day ⁻¹	2.02	2.09
$T(0)$ [held fixed ^{††}]	cells/ml	6.31×10^6	6.24×10^6
$I(0)$	cells/ml	20.88	0.006
$V(0)$	RNA copies/ml	1.50×10^5	1.94×10^6

[†] The rate of RNA viral loss due to harvesting plus degradation was estimated in separate experiments (Iwami et al., 2012a; Iwami et al., 2012b).

^{††} This initial condition was fixed to the experimentally measured concentration of target cell on day 0 listed in **Table 1**.

Table 3. Fitted parameter and initial values for the cell culture SHIV-KS661 and SHIV-#64 with viral infectivity data.

Estimated values	Unit	SHIV-KS661	SHIV-#64
β	$(\text{TCID}_{50}/\text{ml} \cdot \text{day})^{-1}$	1.69×10^{-5}	14.7×10^{-5}
p	$(\text{TCID}_{50}/\text{cell}) \cdot \text{day}^{-1}$	0.219	0.024
δ	day^{-1}	1.53	1.74
c [held fixed [†]]	day^{-1}	2.89	3.08
$T(0)$ [held fixed ^{††}]	cells/ml	6.31×10^6	6.24×10^6
$I(0)$	cells/ml	18.94	0.015
$V(0)$	$\text{TCID}_{50}/\text{ml}$	0.37	0.25

[†] The rate of TCID_{50} viral loss due to harvesting plus degradation was estimated in separate experiments (Iwami et al., 2012a; Iwami et al., 2012b).

^{††} This initial condition was fixed to the experimentally measured concentration of target cell on day 0 listed in **Table 1**.

Table 4. Fitted parameter and initial values in the punctual removal model for the cell culture SHIV-KS661 and SHIV-#64 with viral RNA data.

Estimated values	Unit	SHIV-KS661	SHIV-#64
β	(RNA copies/ml $\cdot$ day) ⁻¹	1.74×10^{-10}	1.36×10^{-10}
p	(RNA copies/cell) $\cdot$ day ⁻¹	1.4×10^4	1.6×10^4
δ	day ⁻¹	1.84	2.18
c_{deg} [held fixed [†]]	day ⁻¹	0.039	0.109
$T(0)$ [held fixed ^{††}]	cells/ml	6.31×10^6	6.24×10^6
$I(0)$	cells/ml	46.33	39.08
$V(0)$	RNA copies/ml	1.5×10^5	3.3×10^6

[†] The rate of RNA viral degradation was estimated in separate experiments (Iwami et al., 2012a; Iwami et al., 2012b).

^{††} This initial condition was fixed to the experimentally measured concentration of target cell on day 0 listed in **Table 1**.

Table 5. Fitted parameter and initial values in the punctual removal model for the cell culture SHIV-KS661 and SHIV-#64 with viral infectivity data.

Estimated values	Unit	SHIV-KS661	SHIV-#64
β	$(\text{TCID}_{50}/\text{ml} \cdot \text{day})^{-1}$	2.79×10^{-5}	2.53×10^{-4}
p	$(\text{TCID}_{50}/\text{cell}) \cdot \text{day}^{-1}$	0.11	0.013
δ	day^{-1}	1.61	1.93
c_{deg} [held fixed [†]]	day^{-1}	0.91	1.10
$T(0)$ [held fixed ^{††}]	cells/ml	6.31×10^6	6.24×10^6
$I(0)$	cells/ml	7.82	0.042
$V(0)$	$\text{TCID}_{50}/\text{ml}$	0.206	0.361

[†] The rate of degradation of infectivity (TCID_{50}) was estimated in separate experiments (Iwami et al., 2012a; Iwami et al., 2012b).

^{††} This initial condition was fixed to the experimentally measured concentration of target cell on day 0 listed in **Table 1**.

Table 6. Statistics of the conserved quantities of SHIV-KS661 and SHIV-#64 for Eq.(1).

Summary statistics	The conserved quantities of SHIV-KS661		The conserved quantities of SHIV-#64	
	with viral RNA	with viral infectivity	with viral RNA	with viral infectivity
Average	1.033	1.075	1.073	1.022
Variance	0.014	0.011	0.041	0.029
Standard Deviation	0.120	0.104	0.203	0.171

Table 7. Statistics of the conserved quantities of SHIV-KS661 and SHIV-#64 for the punctual removal model.

Summary statistics	The conserved quantities of SHIV-KS661		The conserved quantities of SHIV-#64	
	with viral RNA	with viral infectivity	with viral RNA	with viral infectivity
Average	1.117	1.142	1.213	1.154
Variance	0.014	0.015	0.067	0.019
Standard Deviation	0.117	0.123	0.258	0.137

Figure 2