



Figures and figure supplements

Accelerated viral dynamics in bat cell lines, with implications for zoonotic emergence

Cara E Brook et al

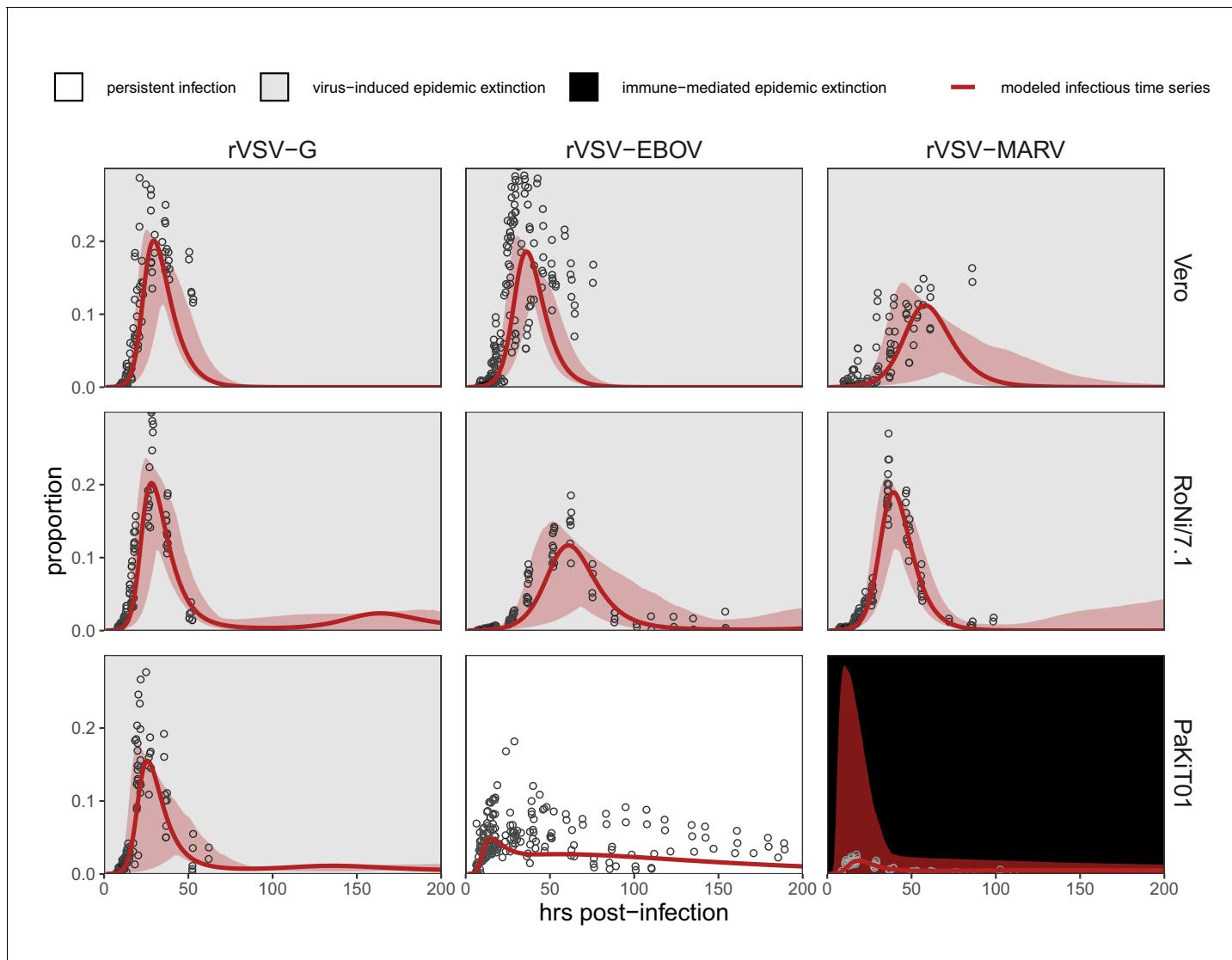


Figure 1. Fitted time series of infectious cell proportions from mean field model for rVSV-G, rVSV-EBOV, and rVSV-MARV infections (columns) on Vero, RoNi/7.1, and PaKiT01 cell lines (rows) at MOI = 0.001. Results are shown for the best fit immune absent model on Vero cells, induced immunity model on RoNi/7.1 cells, and constitutive (for rVSV-VSVG and rVSV-EBOV) and induced (for rVSV-MARV) immunity models on PaKiT01 cells. Raw data across all trials are shown as open circles (statistical smoothers from each trial used for fitting are available in **Figure 1—figure supplements 2–3**). Model output is shown as a solid crimson line (95% confidence intervals by standard error = red shading). Panel background corresponds to empirical outcome of the average stochastic cell culture trial (persistent infection = white; virus-induced epidemic extinction = gray; immune-mediated epidemic extinction = black). Parameter values are listed in **Table 1** and **Supplementary file 4**. Results for absent/induced/constitutive fitted models across all cell lines are shown in **Figure 1—figure supplement 4** (MOI = 0.001) and **Figure 1—figure supplement 5** (MOI = 0.0001).

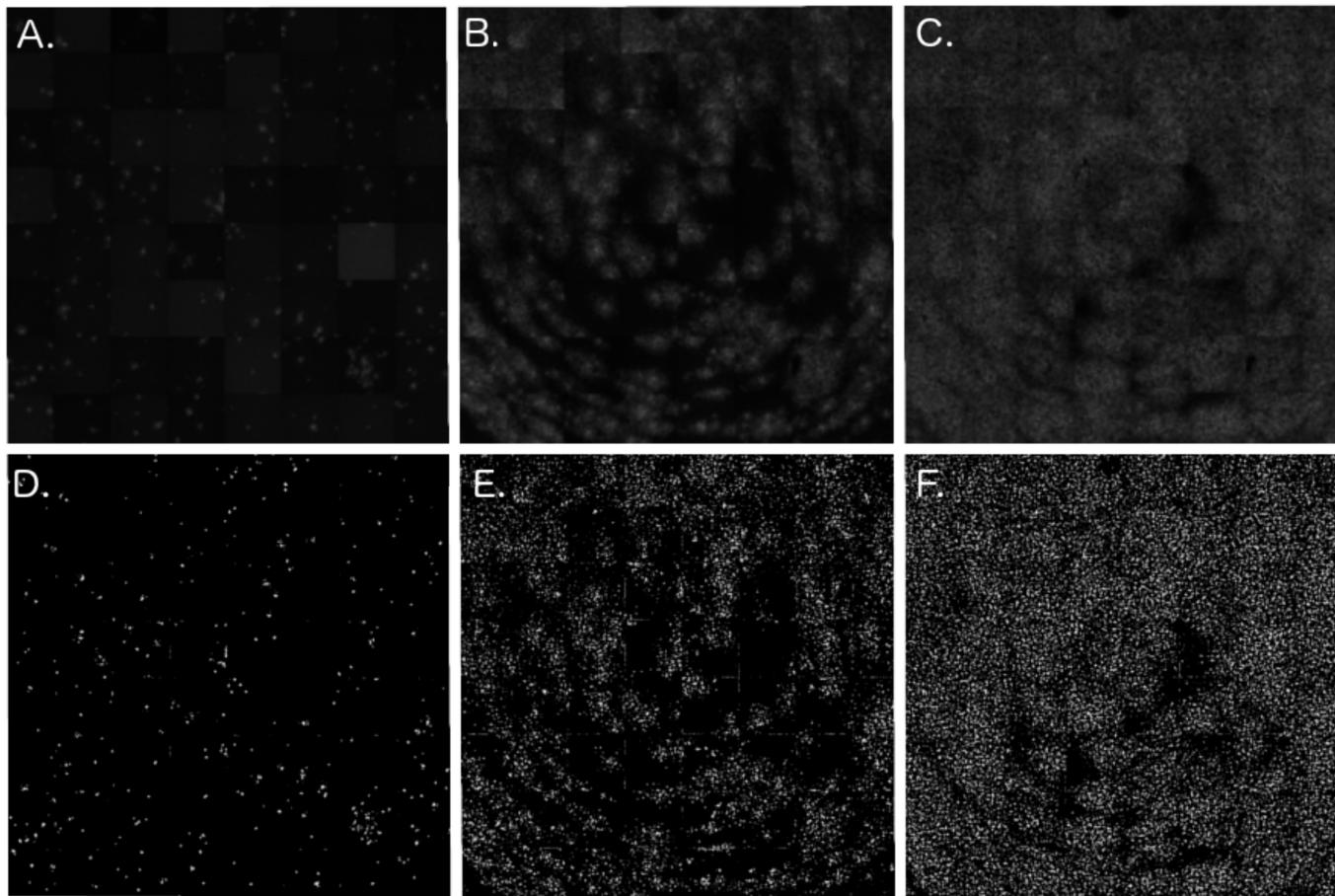


Figure 1—figure supplement 1. Cell culture models of viral propagation. (A), (B), and (C) show raw, original images of rVSV-EBOV propagation across Vero cell lines at, respectively, 17, 21, and 28 hr post-infection (timesteps 2, 3, and five from trial Ver6_B1). (D), (E), and (F) show corresponding, binary images processed in the R package, EBImage. Cells expressing viral eGFP are depicted in white and uninfected/dead cells in black.

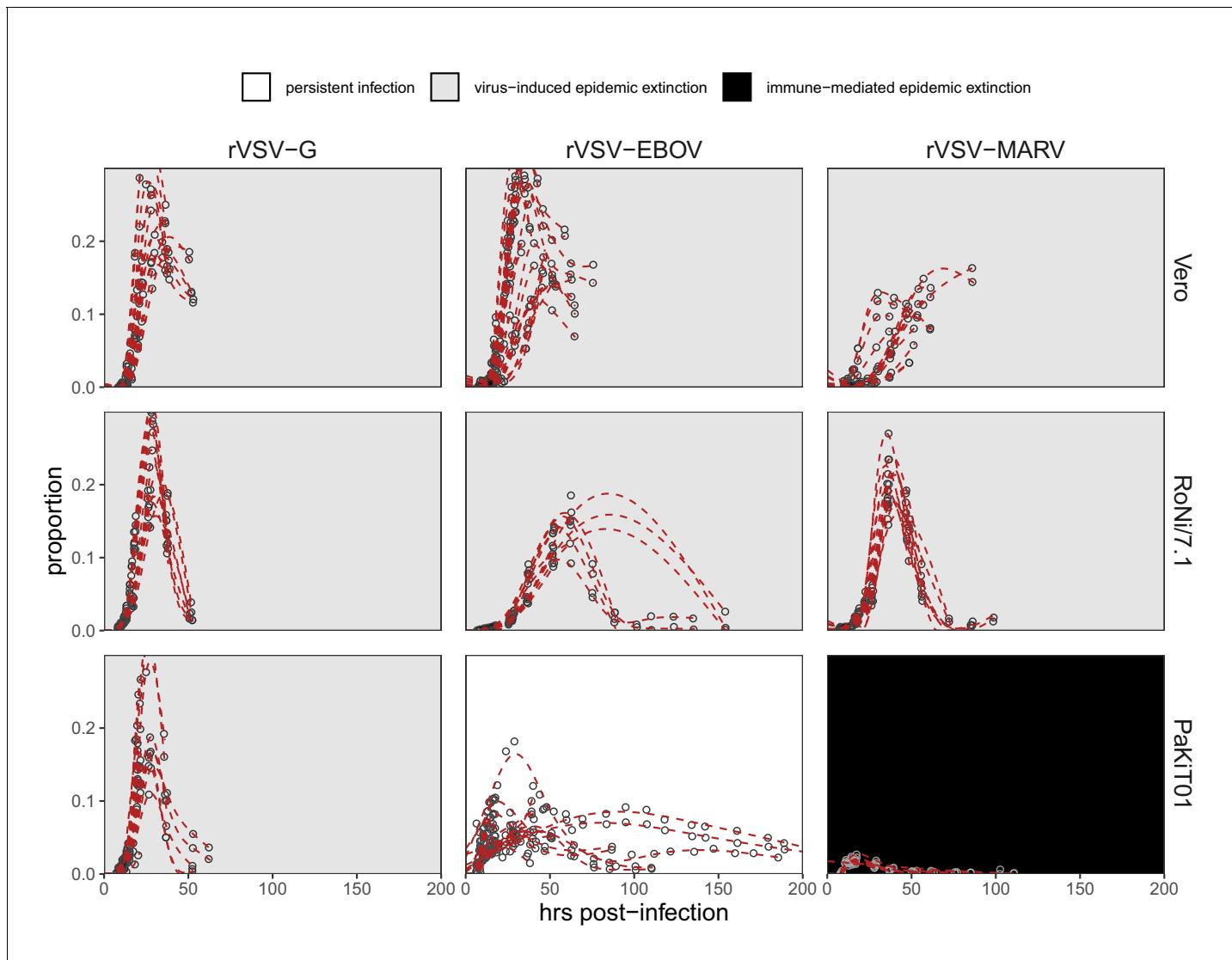


Figure 1—figure supplement 2. Time series data to which mean field mechanistic models were fit, across rVSV-G (left), rVSV-EBOV (middle), and rVSV-MARV (right) infections on Vero, RoNi/7.1, and PaKiT01 cell lines, at MOI = 0.001. Open circles show raw data across all trials, while red, dashed line gives the statistical mean of each trials, established from GAM model incorporating random effects per trial. Results for MOI = 0.0001 are shown in **Figure 1—figure supplement 3**.

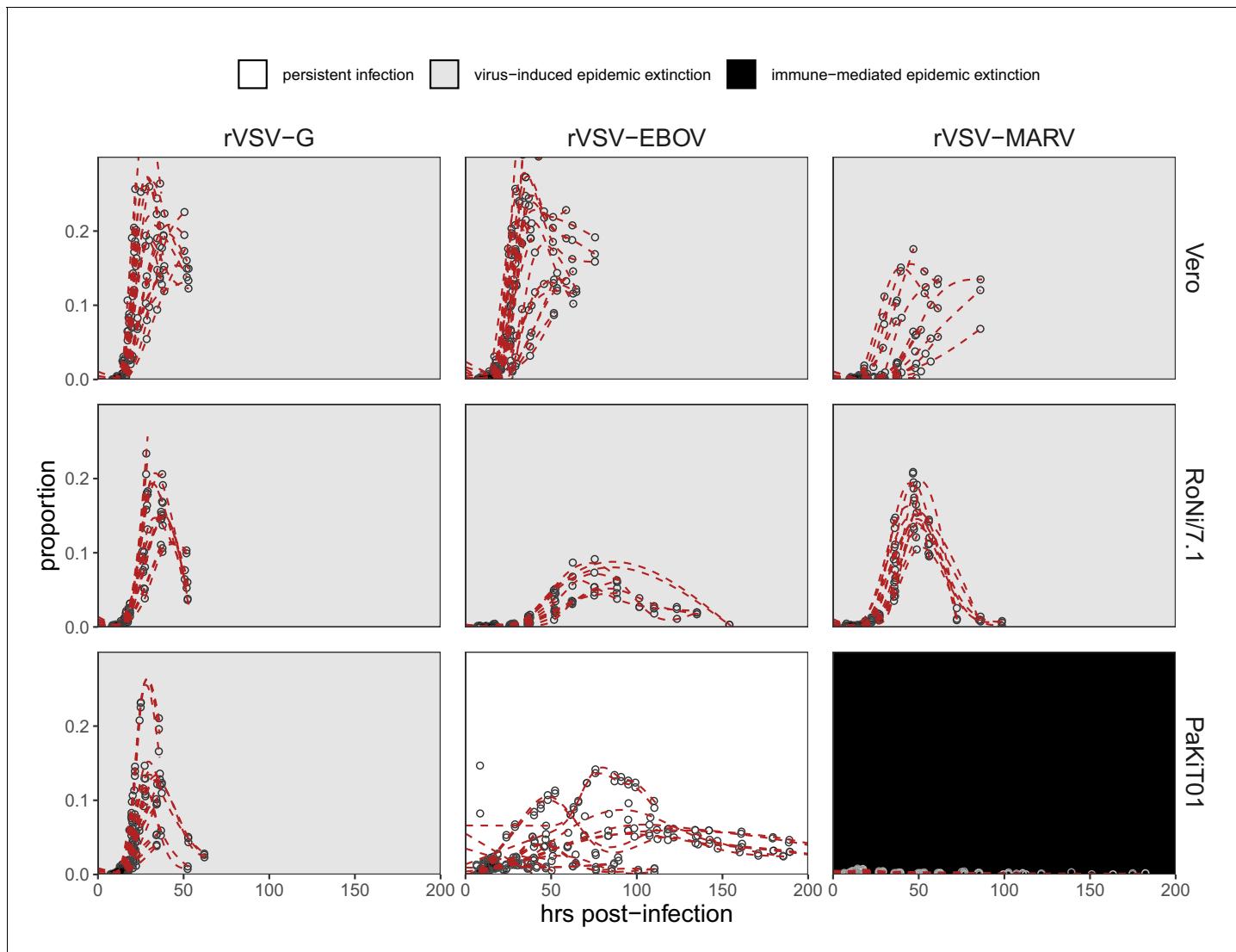


Figure 1—figure supplement 3. Time series data to which mean field mechanistic models were fit, across rVSV-G (left), rVSV-EBOV (middle), and rVSV-MARV (right) infections on Vero, RoNi/7.1, and PaKit01 cell lines, at MOI = 0.0001. Open circles show raw data across all trials, while red, dashed line gives the statistical mean of each trials, established from GAM model incorporating random effects per trial. Results for MOI = 0.001 are shown in **Figure 1—figure supplement 2**.

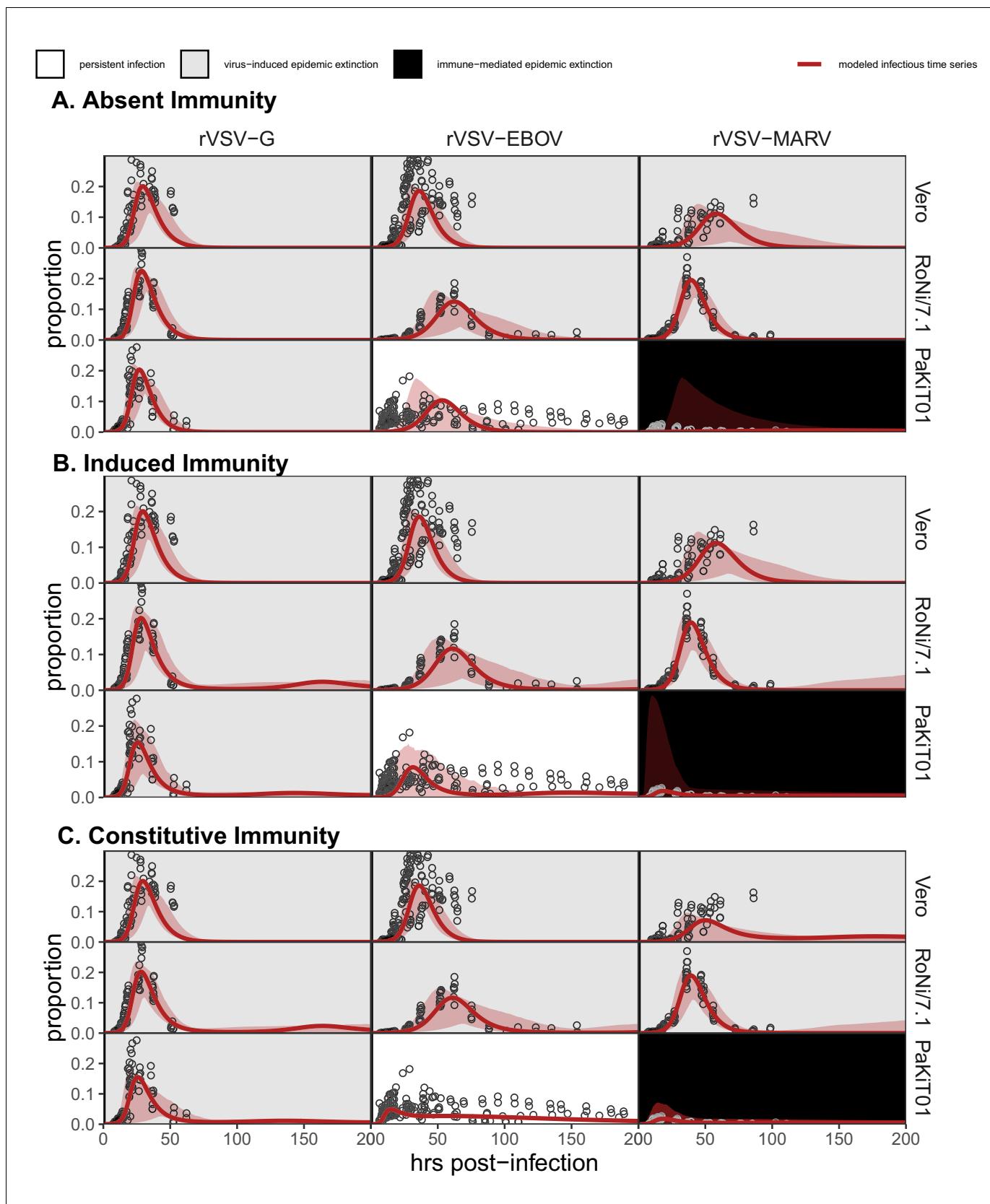


Figure 1—figure supplement 4. Figure replicates **Figure 1** (main text) but includes all output across mean field model fits assuming (A) absent immunity, (B) induced immunity, and (C) constitutive immunity. Figure shows fitted time series of infectious cell proportions for rVSV-G, rVSV-EBOV, and rVSV-MARV. Figure 1—figure supplement 4 continued on next page

Figure 1—figure supplement 4 continued

rVSV-MARV infections (columns) on Vero, RoNi/7.1, and PaKiT01 cell lines (rows) at MOI = 0.001. Raw data across all trials are shown as open circles and model output as the solid crimson line (95% confidence intervals by standard error = red shading). Panel background corresponds to empirical outcome of the average stochastic cell culture trial (persistent infection = white; virus-induced epidemic extinction = gray; immune-mediated epidemic extinction = black).

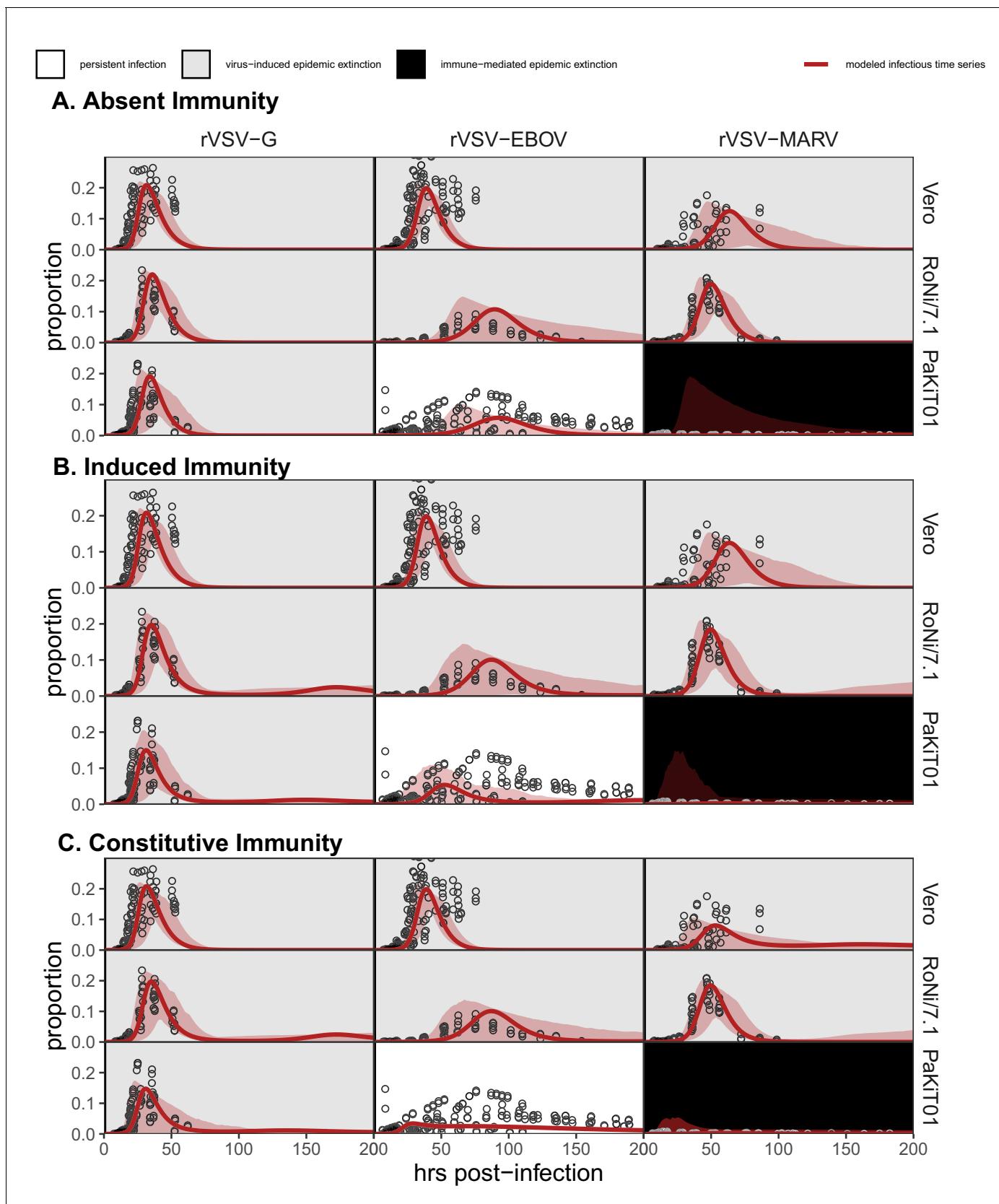


Figure 1—figure supplement 5. Figure replicates **Figure 1—figure supplement 4** exactly but shows model fits and data for all cell-virus combinations at MOI = 0.0001.

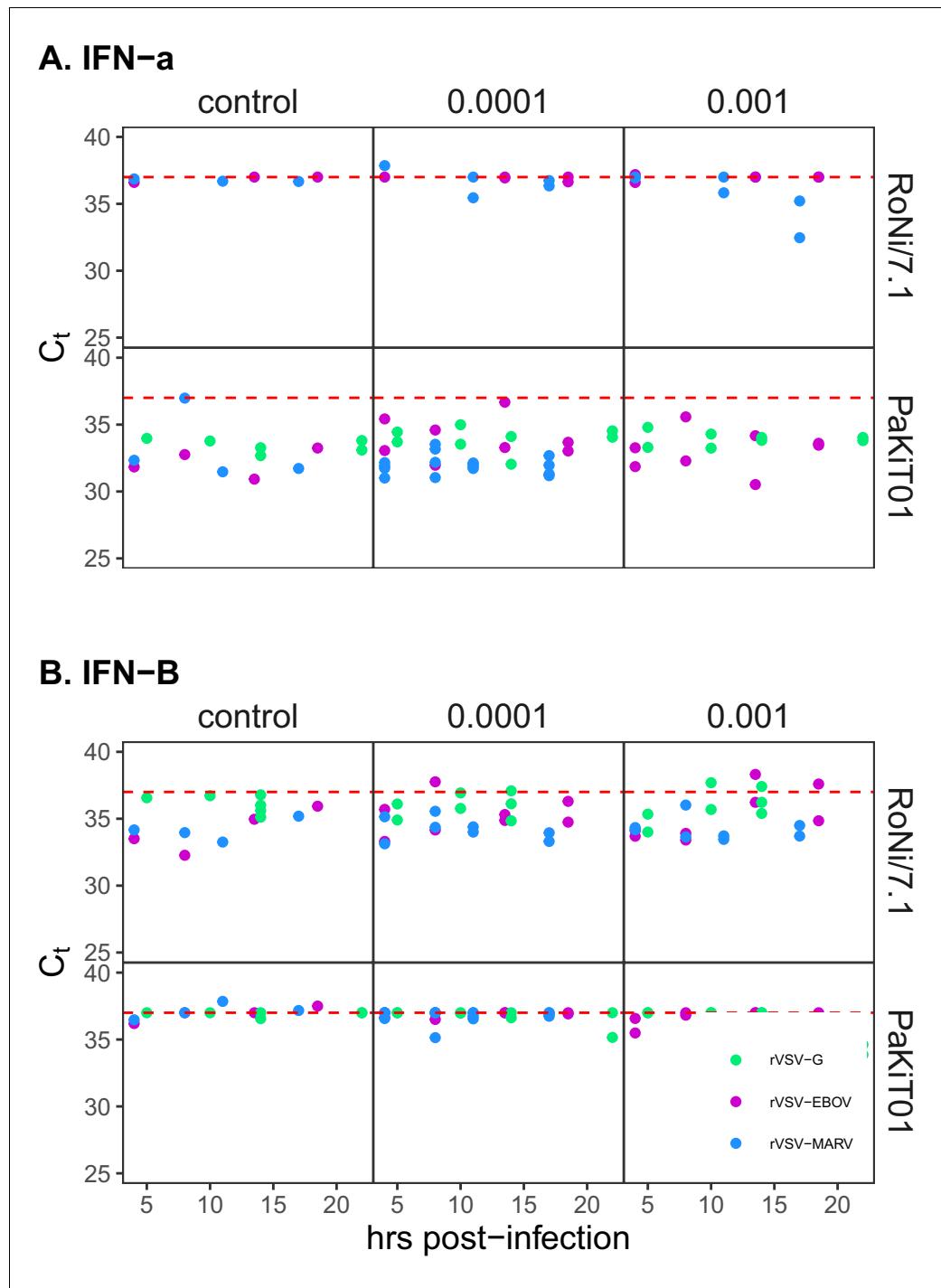


Figure 1—figure supplement 6. IFN gene expression in bat cells at baseline and upon viral stimulation. (A) IFN- α and (B) IFN- β gene expression profiles from qPCR for rVSV infections on RoNi/7.1 and PaKit01 cell lines. Panels show ΔCt (raw Ct of IFN gene assay subtracted from raw Ct of β -Actin housekeeping gene assay) across a time series for mock (left), MOI = 0.0001 (middle) and MOI = 0.001 (right) infections across a time series. Viruses are represented by color (rVSV-G = green, rVSV-EBOV = magenta, rVSV-MARV = blue). The red dashed line at ΔCt = 37 corresponds to no expression; higher expression is indicated at lower values for ΔCt . qPCR was carried out using primers summarized in **Supplementary file 6**.

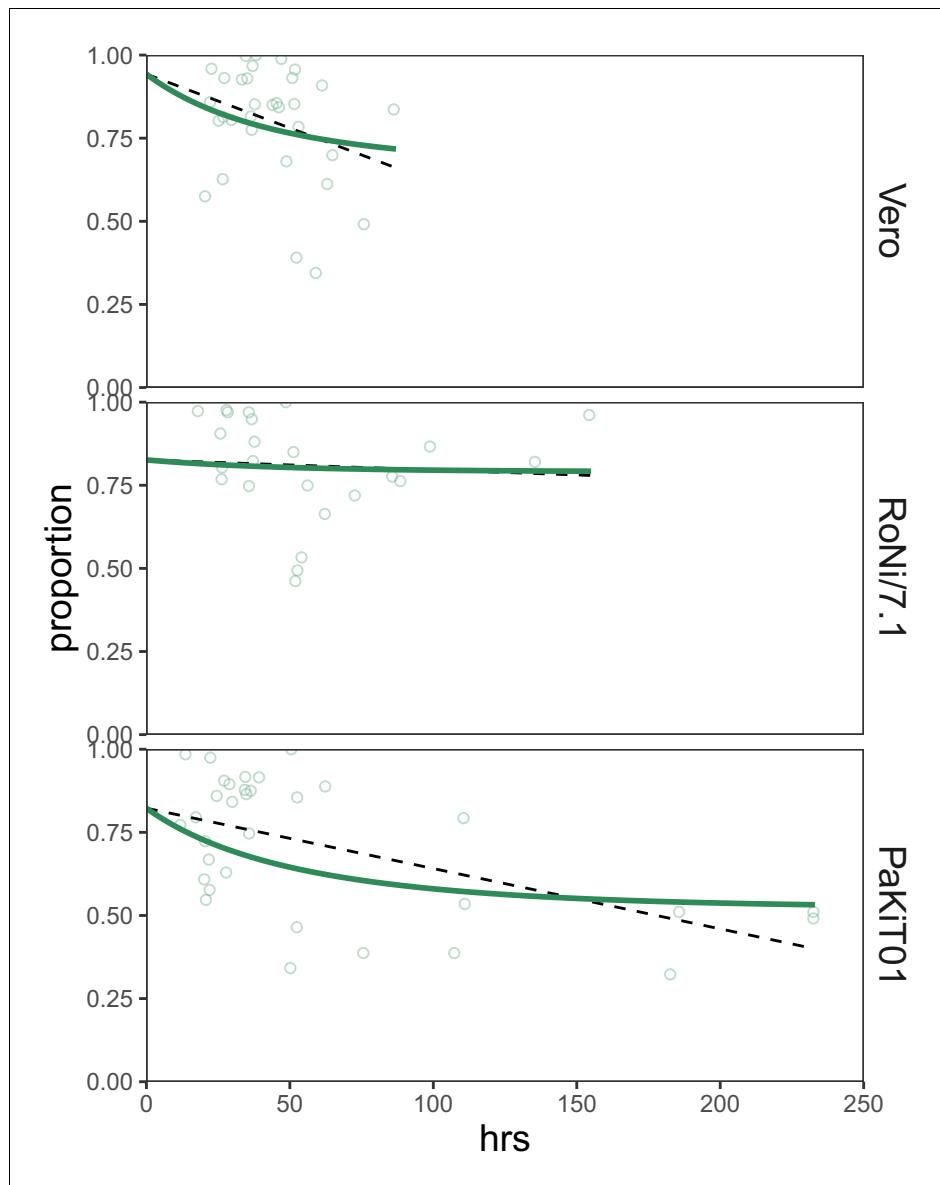


Figure 1—figure supplement 7. Curve fits to control data for standard birth ($b = .025$) and natural mortality ($\mu = \frac{1}{121}, \frac{1}{191}, \frac{1}{84}$ hours for, respectively, Vero, RoNi/7.1, and PaKiT01 cell lines) rates across all three cell lines. Raw data from multiple trials are shown as open circles, statistical means as dashed black lines, with the output from the mean field model, using the fixed birth rate and estimated mortality rate, in solid green.

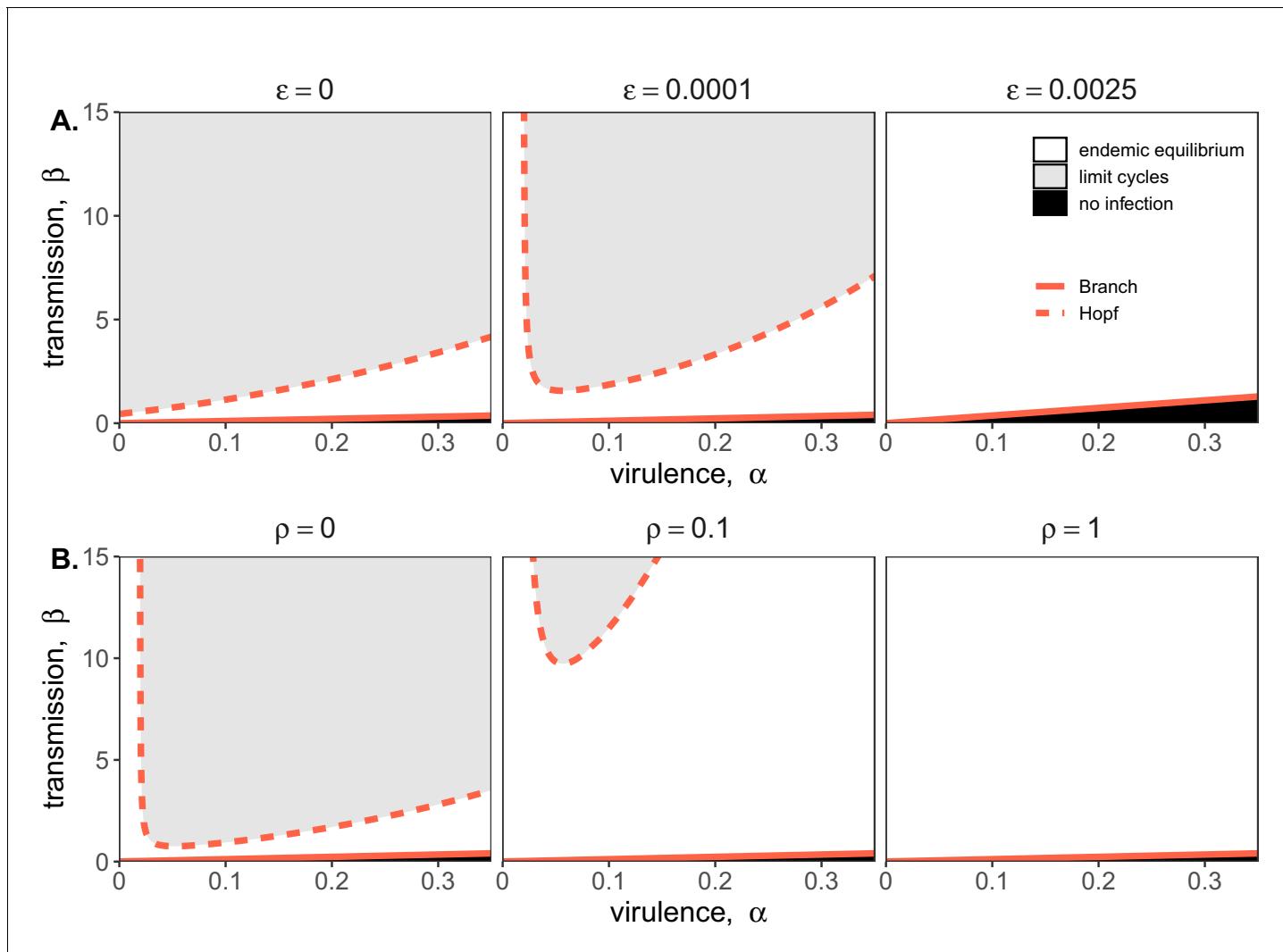


Figure 2. Two parameter bifurcations of the mean field model, showing variation in the transmission rate, β , against variation in the pathogen-induced mortality rate, α , under diverse immune assumptions. Panel (A) depicts dynamics under variably constitutive immunity, ranging from absent (left: $\varepsilon = 0$) to high (right: $\varepsilon = .0025$). In all panel (A) plots, the rate of induced immune antiviral acquisition (ρ) was fixed at 0.01. Panel (B) depicts dynamics under variably induced immunity, ranging from absent (left: $\rho=0$) to high (right: $\rho=1$). In all panel (B) plots, the rate of constitutive antiviral acquisition (ε) was fixed at 0.0001. Branch point curves are represented as solid lines and Hopf curves as dashed lines. White space indicates endemic equilibrium (persistence), gray space indicates limit cycles, and black space indicates no infection (extinction). Other parameter values for equilibrium analysis were fixed at: $b = .025$, $\mu = .001$, $\sigma = 1/6$, $c = 0$. Special points from bifurcations analyses are listed in *Supplementary file 3*.

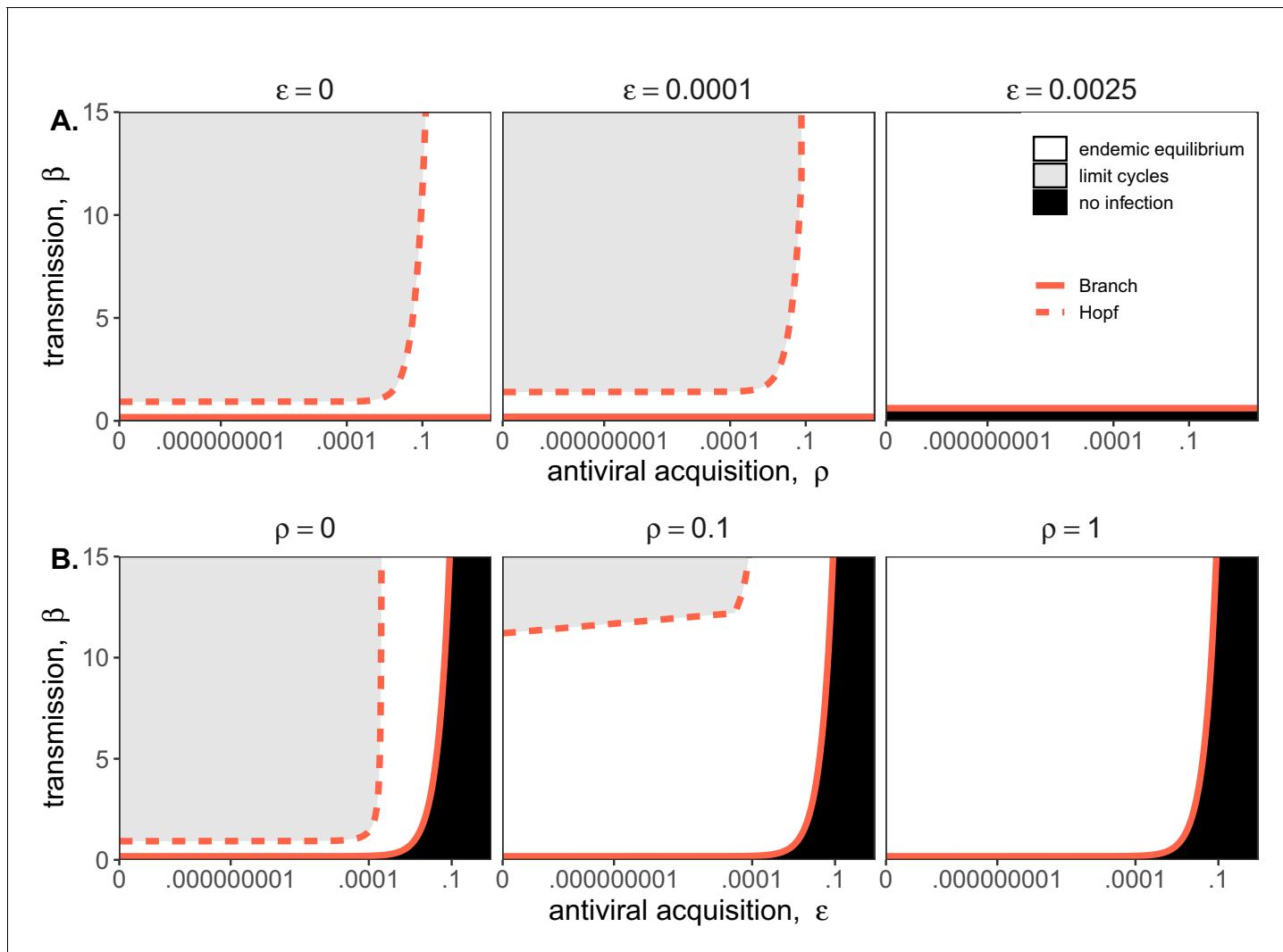


Figure 3. Two parameter bifurcations of the mean field model, showing variation in the transmission rate, β , against variation in: (A) the induced immunity rate of antiviral acquisition (ρ) and (B) the constitutive immunity rate of antiviral acquisition (ϵ). Panels show variation in the extent of immunity, from absent (left) to high (right). Branch point curves are represented as solid lines and Hopf curves as dashed lines. White space indicates endemic equilibrium (persistence), gray space indicates limit cycling, and black space indicates no infection (extinction). Other parameter values for equilibrium analysis were fixed at: $b = .025$, $\mu = .001$, $\sigma = 1/6$, $\alpha = 1/6$, $c = 0$. Special points from bifurcations analyses are listed in *Supplementary file 3*.

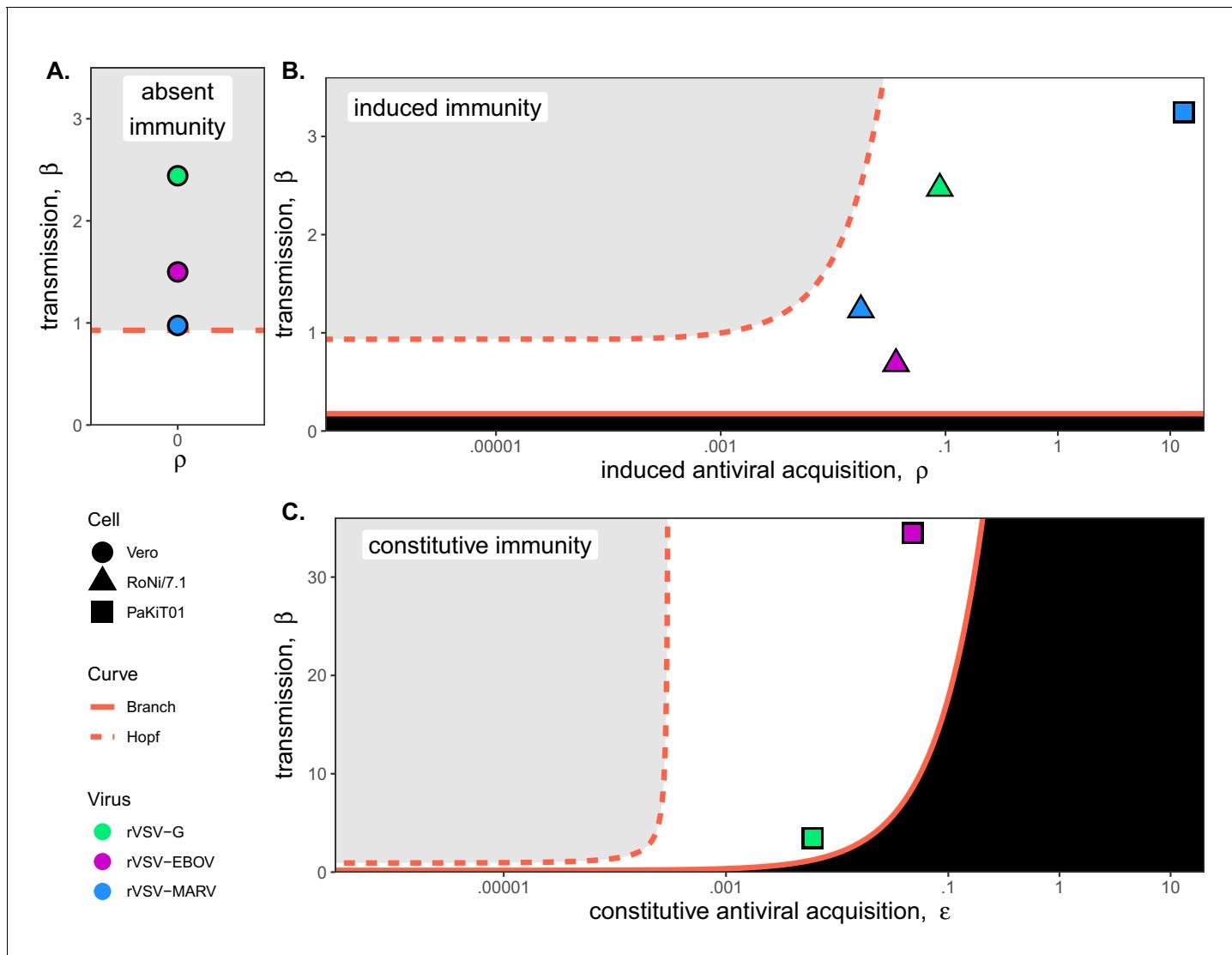


Figure 4. Best fit parameter estimates for β and ρ or ε from mean-field model fits to MOI=0.001 time series data, atop (A,B) $\beta - \rho$ and (C) $\beta - \varepsilon$ bifurcation. Fits and bifurcations are grouped by immune phenotype: (A) absent; (B) induced; (C) constitutive immunity, with cell lines differentiated by shape (Vero=circles; RoNi/7.1 = triangles; PaKiT01=squares) and viral infections by color (rVSV-G = green, rVSV-EBOV = magenta, rVSV-MARV = blue). Note that y-axis values are ten-fold higher in panel (C). Branch point curves (solid lines) and Hopf curves (dashed lines) are reproduced from **Figure 3**. White space indicates endemic equilibrium (pathogen persistence), gray space indicates limit cycling (virus-induced epidemic extinction), and black space indicates no infection (immune-mediated pathogen extinction). In panel (A) and (B), ε is fixed at 0; in panel (C), ρ is fixed at 5×10^{-8} for bifurcation curves and estimated at 4×10^{-8} and 8×10^{-8} for rVSV-EBOV and rVSV-G parameter points, respectively. Other parameter values were fixed at: $b = .025$, $\mu = 0.001$, $\sigma = 1/6$, $\alpha = 1/6$, and $c = 0$ across all panels. Raw fitted values and corresponding 95% confidence intervals for β , ρ , and ε , background parameter values, and AIC recovered from model fit, are reported in **Supplementary file 4**. Parameter fits at MOI=0.0001 are visualized in **Figure 4—figure supplement 1**.

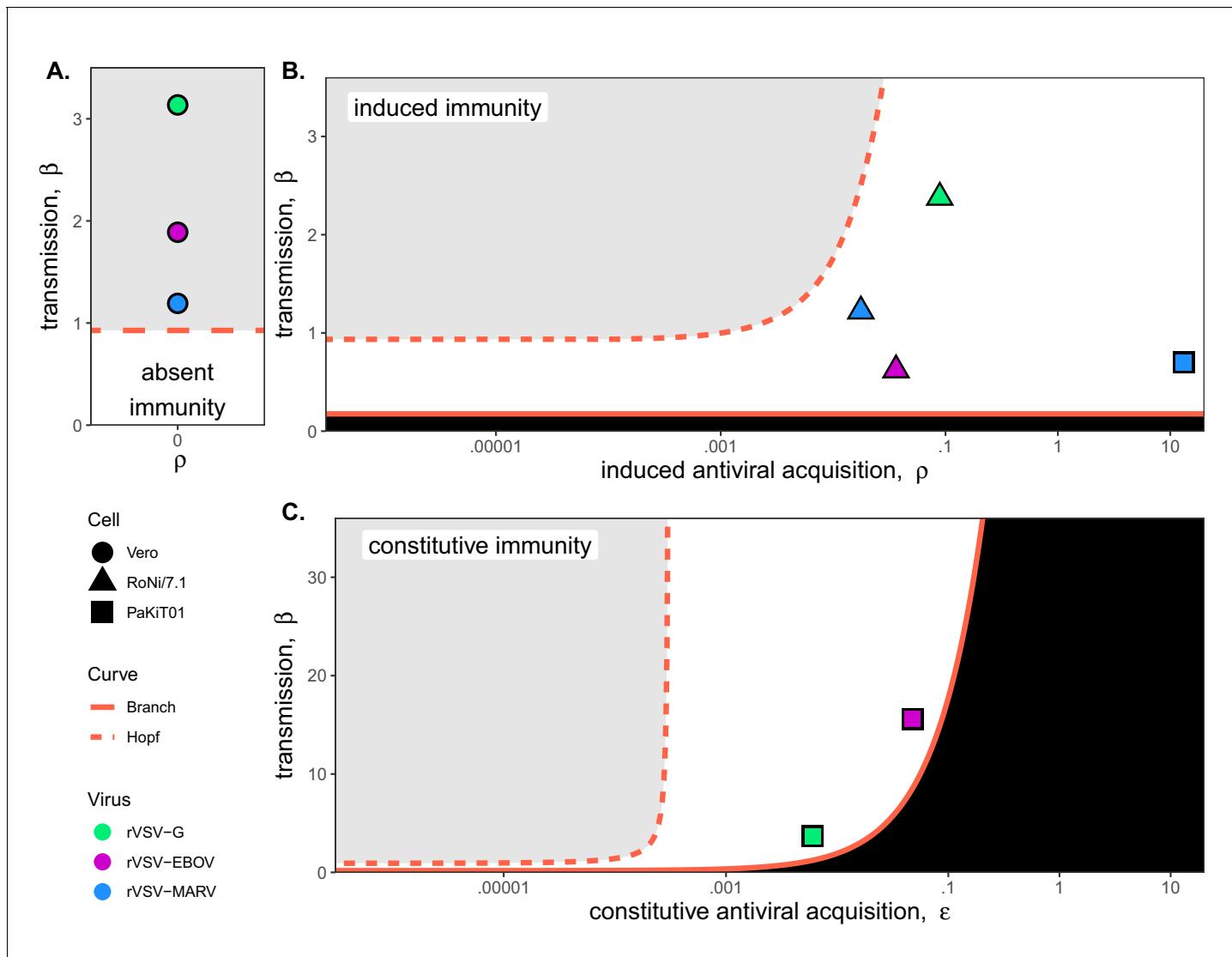


Figure 4—figure supplement 1. Best fit parameter estimates for β and p or ϵ from mean-field model fits to MOI=0.0001 time series data, atop (A,B) $\beta - p$ and (C) $\beta - \epsilon$ bifurcation. Fits and bifurcations are grouped by immune phenotype: (A) absent; (B) induced; (C) constitutive immunity, with cell lines differentiated by shape (Vero=circles; RoNi/7.1 = triangles; PaKit01=squares) and viral infections by color (rVSV-G = green, rVSV-EBOV = magenta, rVSV-MARV = blue). Note that y-axis values are ten-fold higher in panel (C). Branch point curves (solid lines) and Hopf curves (dashed lines) are reproduced from **Figure 3** (main text). White space indicates endemic equilibrium (pathogen persistence), gray space indicates limit cycling (virus-induced epidemic extinction), and black space indicates no infection (immune-mediated pathogen extinction). In panel (A) and (B), ϵ is fixed at 0; in panel (C), ϵ is fixed at 5×10^{-8} for bifurcation curves and estimated at 4×10^{-8} and 8×10^{-8} for rVSV-EBOV and rVSV-G parameter points, respectively. To construct bifurcation curves, other parameter values were fixed at: $b = 0.025$, $\mu = 0.001$, $\alpha = \frac{1}{\delta}$, and $c = 0$ across all panels. Raw fitted values and corresponding 95% confidence intervals for β , p , and ϵ , background parameter values, and AIC recovered from model fit, are reported in **Supplementary file 4**. Parameter fits at MOI=0.0001 are visualized in **Figure 4** of the main text.

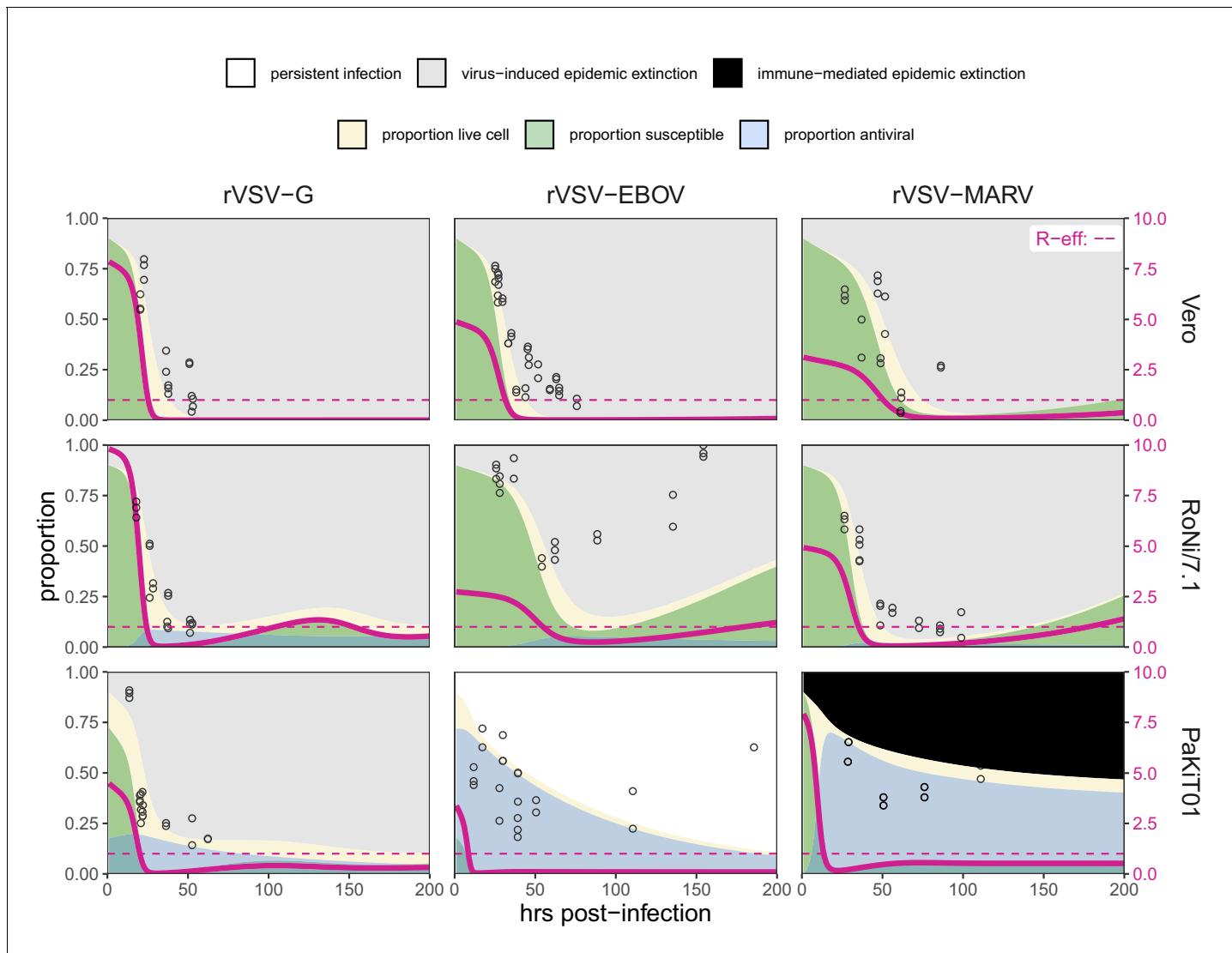
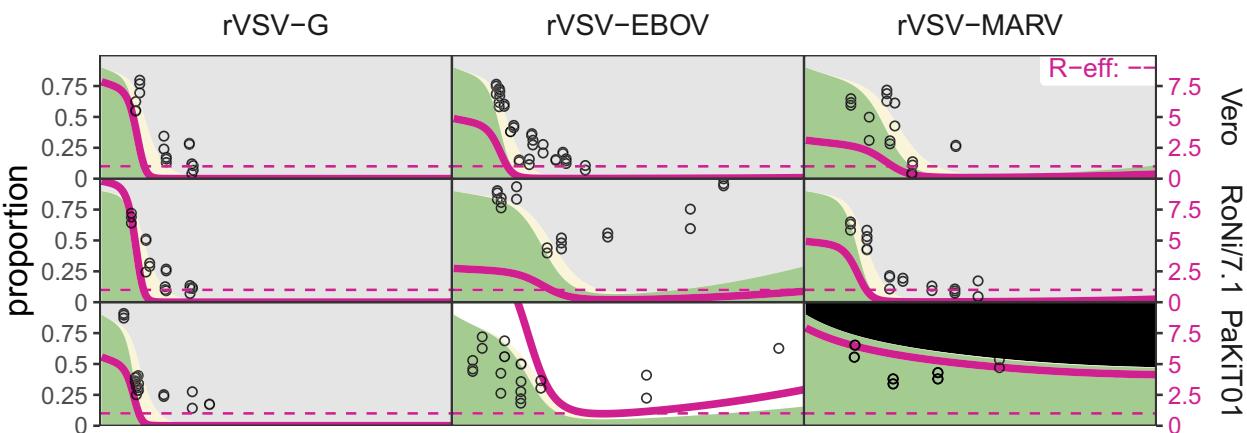


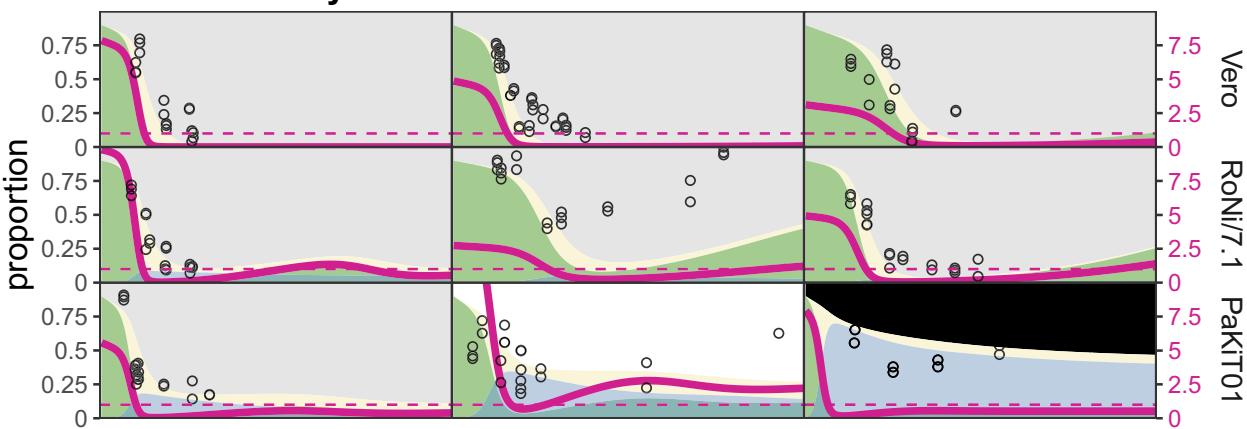
Figure 5. Fitted time series of susceptible (green shading) and antiviral (blue shading) cell proportions from the mean field model for rVSV-G, rVSV-EBOV, and rVSV-MARV infections (columns) on Vero, RoNi/7.1, and PaKiT01 cell lines (rows) at MOI = 0.001. Results are shown for the best fit immune absent model on Vero cells, induced immunity model on RoNi/7.1 cells and constitutive (rVSV-G and rVSV-EBOV) and induced (rVSV-MARV) immune models on PaKiT01 cells. Combined live, uninfected cell populations ($S + A + E$) are shown in tan shading, with raw live, uninfected cell data from Hoechst stains visualized as open circles. The right-hand y-axis corresponds to R-effective (pink solid line) across each time series; R-effective = 1 is a pink dashed, horizontal line. Panel background corresponds to empirical outcome of the average stochastic cell culture trial (persistent infection = white; virus-induced extinction = gray; immune-mediated extinction = black). Parameter values are listed in **Supplementary file 4** and results for absent/induced/constitutive fitted models across all cell lines in **Figure 5—figure supplement 1** (MOI = 0.001) and **Figure 5—figure supplement 2** (MOI = 0.0001).

persistent infection
 virus-induced epidemic extinction
 immune-mediated epidemic extinction
 proportion live cell
 proportion susceptible
 proportion antiviral

A. Absent Immunity



B. Induced Immunity



C. Constitutive Immunity

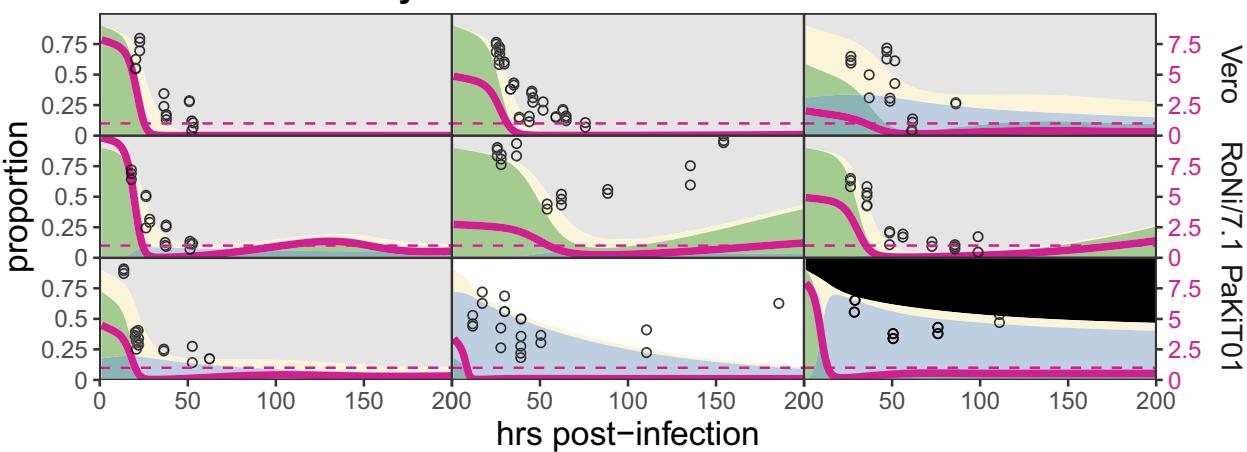
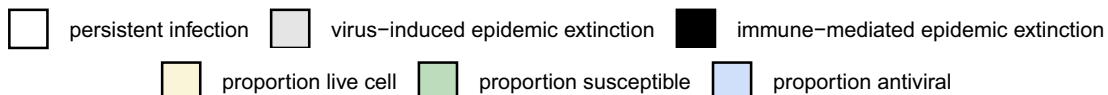


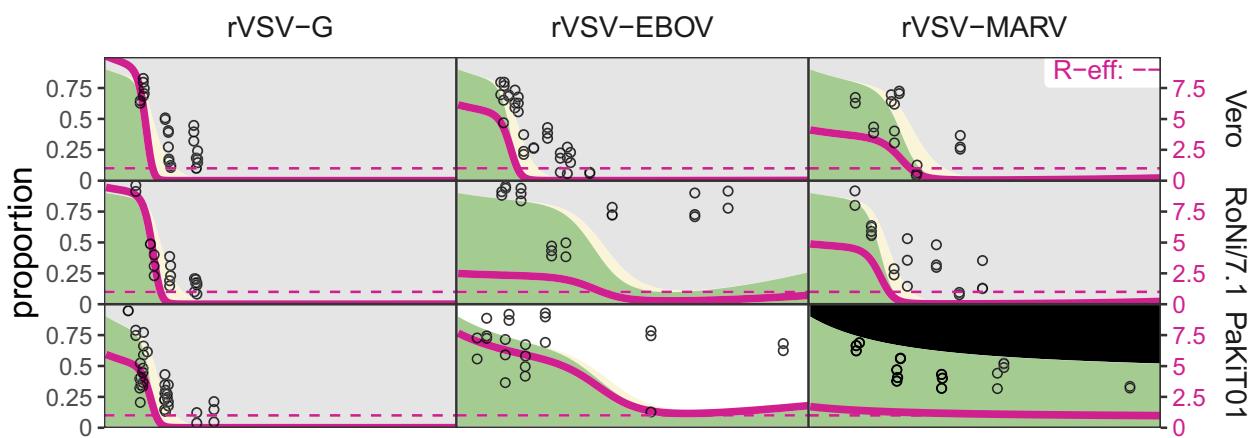
Figure 5—figure supplement 1. Figure replicates **Figure 5** (main text) but includes all output across mean field model fits assuming (A) absent immunity, (B) induced immunity, and (C) constitutive immunity. Figure shows fitted time series of susceptible (green shading) and antiviral (blue shading) proportions over time (hrs post-infection) for three different viruses (rVSV-G, rVSV-EBOV, and rVSV-MARV) across four different host cell lines (Vero, RoNi/7.1, PakIT01). The legend at the top indicates the color coding for different states: persistent infection (white), virus-induced epidemic extinction (grey), immune-mediated epidemic extinction (black), proportion live cell (yellow), proportion susceptible (green), and proportion antiviral (blue). The rightmost subplot in each panel includes an R_{eff} scale from - to 7.5.

Figure 5—figure supplement 1 continued

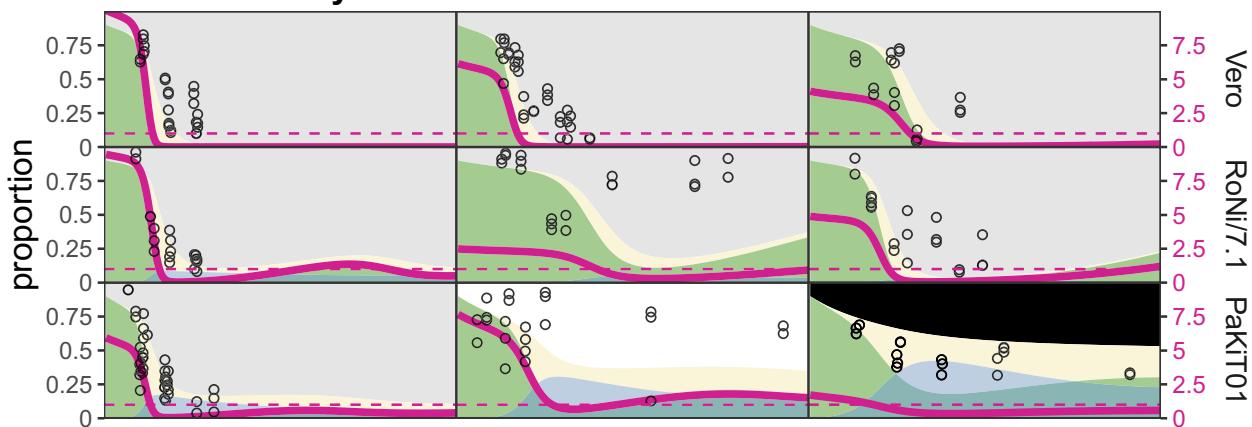
shading) cell proportions from the mean field model for rVSV-G, rVSV-EBOV, and rVSV-MARV infections (columns) on Vero, RoNi/7.1, and PaKiT01 cell lines (rows) at MOI = 0.001. Combined live, uninfected cell populations ($S + A + E$, summed across the time series) is shown in tan shading, with raw live, uninfected cell data from Hoechst stains of terminal time series visualized as open circles. The right-hand y-axis corresponds to R-effective (pink solid line) across each time series; R-effective = 1 is given as a pink dashed, horizontal line. Panel background corresponds to empirical outcome of the average stochastic cell culture trial (persistent infection = white; virus-induced epidemic extinction = gray; immune-mediated epidemic extinction = black).



A. Absent Immunity



B. Induced Immunity



C. Constitutive Immunity

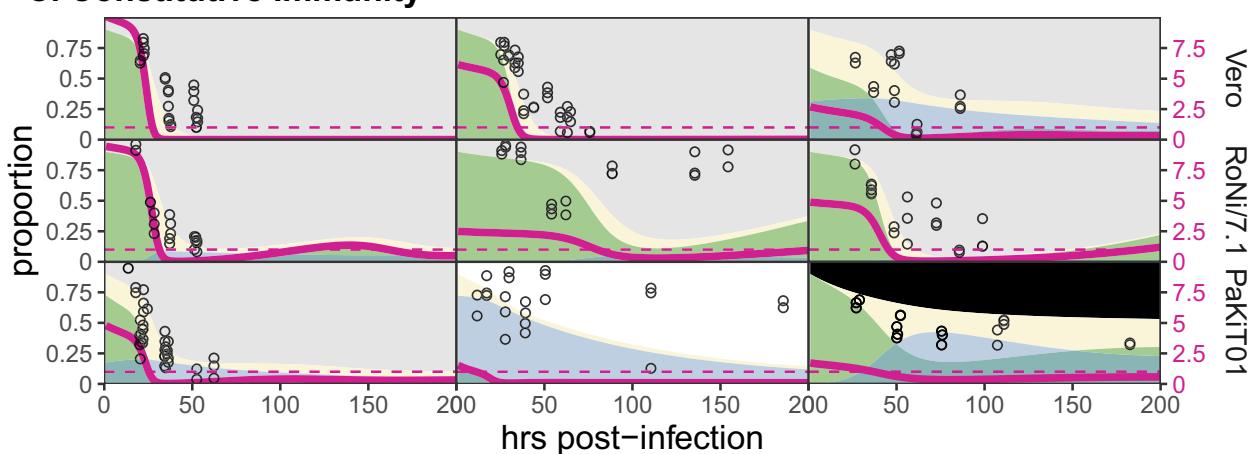
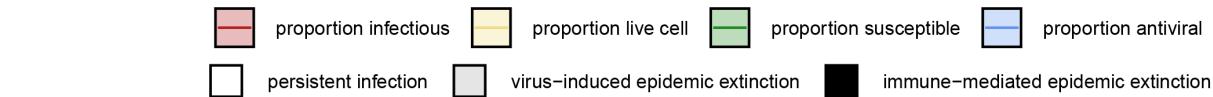
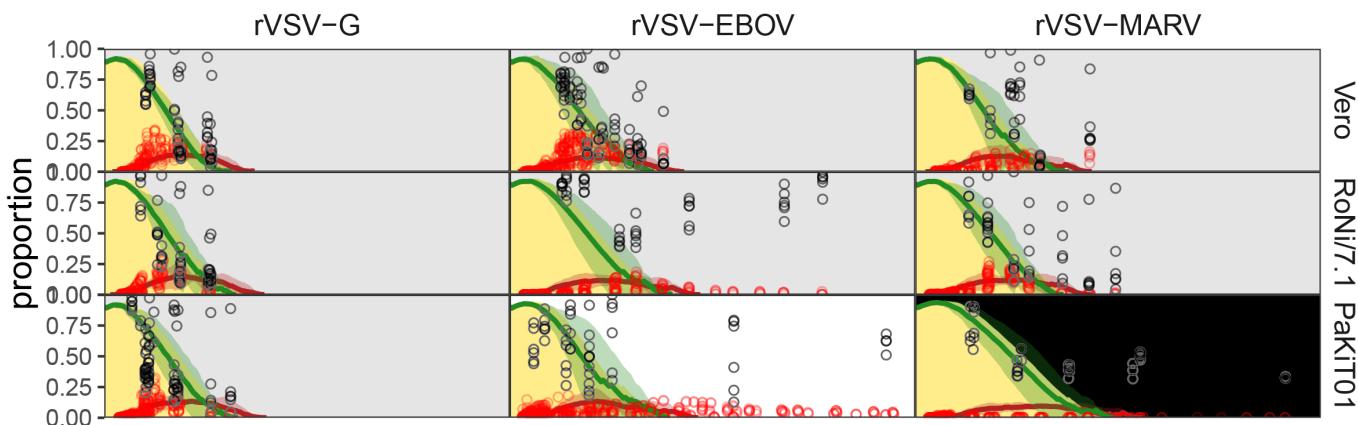


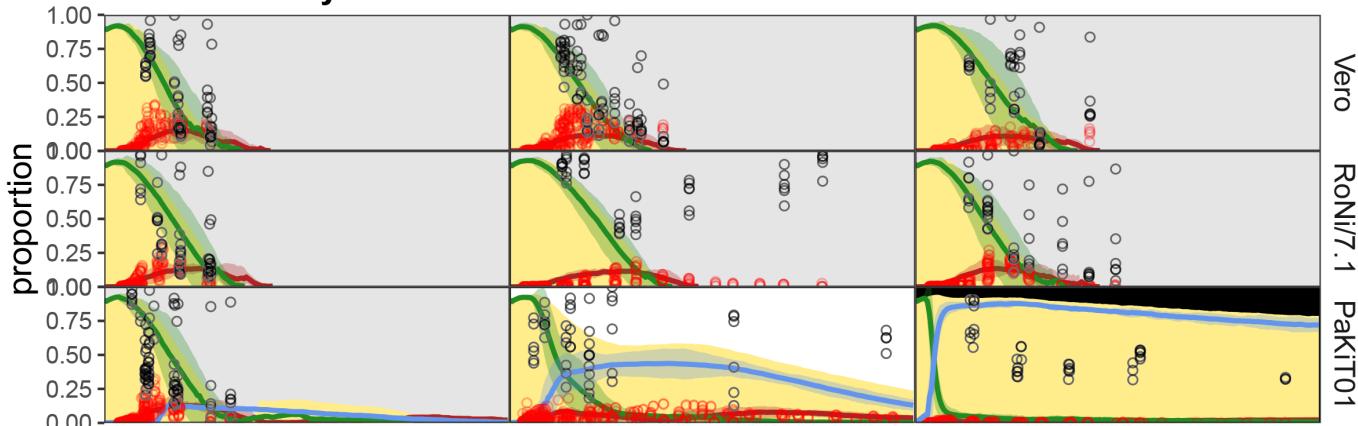
Figure 5—figure supplement 2. Figure replicates **Figure 5—figure supplement 1** exactly but shows model fits and data for all cell-virus combinations at MOI = 0.0001.



A. Absent Immunity



B. Induced Immunity



C. Constitutive Immunity

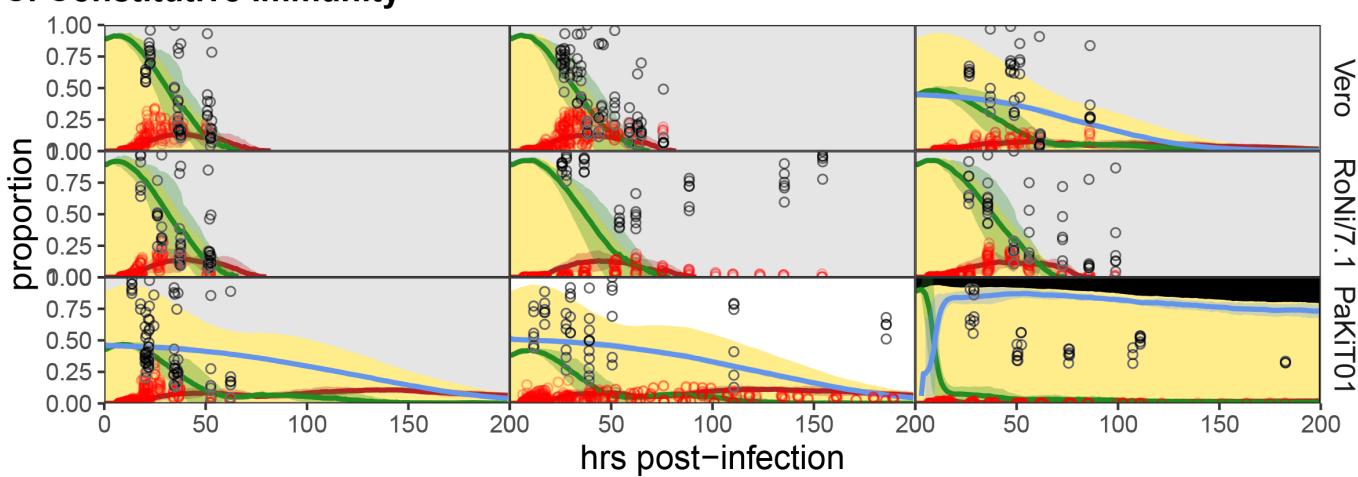


Figure 5—figure supplement 3. Spatial model state variable outputs, fit to MOI = 0.001 data only, for all 27 unique cell line - virus - immune assumption combinations: (A) absent immunity, (B) induced immunity, and (C) constitutive immunity. Values for ρ and ε were fixed at equivalent values. Figure 5—figure supplement 3 continued on next page

Figure 5—figure supplement 3 continued

to those optimized in mean field trials and β fixed at ten times the value estimated under mean field conditions. Figure shows mean output from 10 runs of the spatial stochastic model, on a 10,000 cell lattice for MOI = 0.001 infections of rVSV-G, rVSV-EBOV, and rVSV-MARV (columns) on Vero, RoNi/7.1, and PaKit01 (rows) cell lines. Mean state variable outputs are plotted as colored lines with 95% confidence intervals by standard error shown in corresponding shading (infectious = red; susceptible = green; antiviral = blue). Raw infectious cell data across all time trials are plotted as open red circles, with the Hoechst-stained live cell population as open black circles. Modeled live, uninfected cell populations (S+A+E) are shown in tan shading in the background. Panel background shading corresponds to the mean spatial model outcome for each cell line – virus combination (persistent infection = white; virus-induced epidemic extinction = gray; immune-mediated epidemic extinction = black). All parameter values are reported in **Supplementary file 4**.