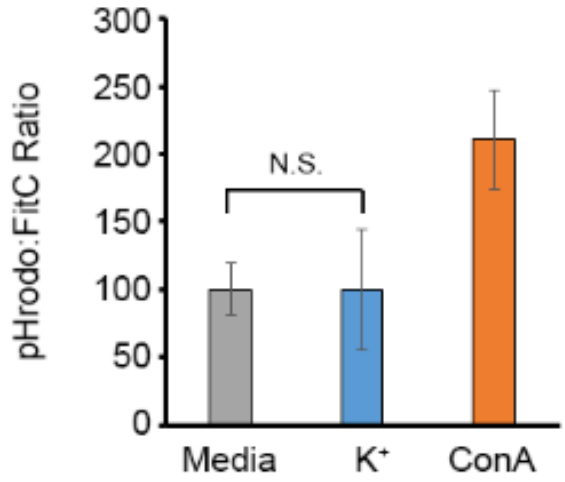

Supplemental Figure 1: Elevated extracellular K^+ does not alter endosomal pH. A549 cells were incubated for 24 hours in either regular media or media containing 50mM K^+. Cells were then incubated for 24 additional hours with dextran beads conjugated with the pH sensitive pHrodo dye (whose fluorescent intensity increases in acidic environments). To control for the differential phagocytosis caused by elevated K^+, control beads conjugated to FiTC were also included. Cells were then harvested and fluorescent intensity quantified using flow cytometry. Increased endosomal acidification is observed as an increase in the ratio of pHrodo:FitC intensity. Concanamycin A (which is known to cause endosomal acidification) is shown as a control). Data is normalized to media controls and represents the summation of two independent experiments. Significance was determined using Student's T-test (N.S. = not significant).

Supplemental Table 1: Observed diameter of primary myxoma lesions in rabbits

Rabbit ID	Days Post Injection										
	0	1	2	3	4	5	6	7	8	9	10
1511	0	0	1.39375	2.7875	5.575	11.15	17.5	21.5	24.5	24.5	23
1512	0	0	2.9375	5.875	11.75	23.5	25.5	27.5	33.5	34	
1513	0	0	3.375	6.75	13.5	27	24.5	29	31.5	28	37.5
1514	0	0	0.8125	1.625	3.25	6.5	13	22.5	20.5	22	23
1515	0	0	2.625	5.25	10.5	21	21	29.5	32	36	38.5
1516	0	0	1.4375	2.875	5.75	11.5	19	28	32.5	32.5	28

Supplemental Table 1: Observed diameters of primary myxoma lesions in rabbits. Six susceptible *Oryctolagus* rabbits intradermally with 1000 FFU of MYXV and the size of the resulting primary lesions were measured daily for 10 days.

Supplemental Information: Mathematical model and Numerical Method

In this study, we propose a novel mathematical model for MYXV infection regulated by extracellular K^+ ions released by the death of infected cells. Based on our *in vitro* results (Figures 1-5), the model places three variables under K^+ control: the amount of newly produced infectious virions, the efficiency at which virions can initiate infection of new cells, and the time it takes for infected cells to die. In this appendix we briefly describe our mathematical model, the parameter values, and the numerical method used to simulate the model.

Variables. Our model describes the spatial (x) and temporal (t) dynamics of the following quantities:

- $B_1(t,x)$ $(\mathrm{cell}\cdot\mathrm{mm}^{-3})$, the number density of normal (uninfected and living) cells.
- $B_2(t,x)$ $(\mathrm{cell}\cdot\mathrm{mm}^{-3})$, the number density of infected cells.
- $B_3(t,x)$ $(\mathrm{cell}\cdot\mathrm{mm}^{-3})$, the number density of dead cells.
- $V(t,x)$ $(\mathrm{virus}\cdot\mathrm{mm}^{-3})$, the number density of MYXV virions in the extracellular space.
- $P(t,x)$ $(\mathrm{nmol}\cdot\mathrm{mm}^{-3})$, the number of K^+ particles above the equilibrium value P_0 in the extracellular space.

Since the model involves both intracellular and extracellular concentrations of both K^+ and MYXV virions, we incorporate a conversion factor ρ between the intracellular and extracellular spaces.

Equations for modeling the infection state of cells. The dynamics of cell number densities are governed by the following assumptions:

- Normal cells are infected at a rate regulated by extracellular K^+ and viruses; once infected, a normal cell becomes an infected cell. The infection rate of normal cells, as a function of K^+ concentration, will be described by the function $\tilde{\theta}(P)$.

- Infected cells die at a rate that is also regulated by extracellular K^+ density; once dead, an infected cell becomes a dead cell. The cell death rate, as a function of K^+ concentration, will be described by the function $\tilde{\delta}(P)$.

Since the time scale at which normal cells die naturally is anticipated to be much longer than the time scale at which infected cell die, natural death of normal cells is omitted from this model. The dynamics of B_1, B_2 and B_3 are governed by the following system of equations (which can be viewed as either ordinary differential equations parameterized by the spatial coordinate or as partial differential equations):

$$\frac{\partial B_1}{\partial t} = -\tilde{\theta}(P)B_1V,$$

$$\frac{\partial B_2}{\partial t} = \tilde{\theta}(P)B_1V - \tilde{\delta}(P)B_2,$$

$$\frac{\partial B_3}{\partial t} = \tilde{\delta}(P)B_2,$$

Note that $B_1 + B_2 + B_3 = c$, a constant, so that cell incompressibility is preserved. In these equations, $\tilde{\theta}(P) = \theta\big(1 - \beta_1 H(P)\big)$ describes the rate of infection of normal cells as a function of K^+ concentration. The parameter θ is the constant infection rate in the absence of potassium. The function $H(P)$ is given by $H(P) = 1/(1 + e^{-2k(P-P_s)})$, a smoothed Heaviside function with parameter k governing the transition from 0 to 1. The parameter P_s is a threshold of extracellular K^+ above which virus infectivity is diminished. It is based on our empirical observations in this paper (Figures 1-5). The parameter β_1 captures the extent to which infectivity is reduced by high K^+ concentrations. Taken together, then, when K^+ concentration levels are below the threshold, $H(P) \approx 0$ and infection occurs at rate $\tilde{\theta}(P) \approx \theta$; when K^+ concentration levels are above the threshold, $H(P) \approx 1$ so that the infection rate drops to $\tilde{\theta}(P) \approx \theta(1 - \beta_1)$. The dynamics of cell death due to infection are modeled by the function $\tilde{\delta}(P) = \delta\big(1 - \beta_2 H(P)\big)$, a function of K^+ concentration. Here, δ is the death rate of infected cells in the absence of K^+ and β_2 captures the

extent to which the death rate of infected cells is reduced by high K^+ concentrations. Thus, when K^+ levels are below the threshold, infected cells die at rate $\tilde{\delta}(P) \approx \delta$ and when K^+ levels are above the threshold, infected cells die at rate $\tilde{\delta}(P) \approx \delta(1-\beta_2)$.

Equation for K^+ and virus. The dynamics of extracellular K^+ concentration are described by the following reaction-diffusion equation:

$$\frac{\partial P}{\partial t} = a\rho\tilde{\delta}(P)T_{\{1/\tilde{\delta}(P)\}}[B_2] + D_2\nabla^2 P.$$

The first term models the burst of intracellular K^+ released into the extracellular space when an infected cell dies. This term incorporates a delay between viral infection and cell death. The time delay operator is $T_\tau[f(t)] = f(t-\tau)$ for a given function $f(t)$ and delay τ. The average time until virally infected cells burst, as a function of the K^+ concentration, is $1/\tilde{\delta}(P)$, so $\tau = 1/\tilde{\delta}(P)$. The parameter a is the number of K^+ ions contained in one cell. It is scaled by the factor ρ, since K^+ ions from ρ units of intracellular space are released into 1 unit of extracellular space. The second term describes the diffusion of extracellular K^+ with diffusion coefficient D_2.

The dynamics of extracellular viruses are governed by the following reaction-diffusion equation:

$$\frac{\partial V}{\partial t} = \tilde{b}(P)\rho\tilde{\delta}(P)T_{\{1/\tilde{\delta}(P)\}}[B_2] - \tilde{\theta}(P)T_{\{1/\tilde{\delta}(P)\}}[B_1 V] + D_1\nabla^2 V.$$

The first term models the number of viruses released to the extracellular space when an infected cell dies, again incorporating the delay between viral infection and cell death. The function $\tilde{b}(P) = b\big(1-\beta_3 H(P)\big)$ describes the number of unconsumed viruses released into the extracellular space when a cell dies. Here, b is the burst size of the virus in the absence of K^+ and β_3 captures the extent to which virus production is reduced by high K^+ concentrations. When K^+ levels are below the threshold, the burst size is $\tilde{b}(P) \approx b$. When K^+ levels are above the threshold, the burst size is $\tilde{b}(P) \approx b(1-\beta_3)$. Here again, $\tilde{b}(P)$ is scaled by the factor ρ, since virus from ρ units of

intracellular space are released into 1 unit of extracellular space. The second term models the consumption of extracellular viruses through the infection of normal cells. (See the corresponding term $\tilde{\theta}(P)B_1V$ in the B_2 equation.) The last term describes the diffusion of extracellular viruses with diffusion coefficient D_1.

Parameters and estimated values

The parameters used for modeling are as follows:

1. θ = 0.5x10^{-9} mm^3· h^{-1}·virus^{-1}. This is the infection rate of the normal cells per virus density. It is estimated based on the empirically observed expansion of myxomatosis lesions in rabbits which were obtained from a re-analysis of our previously published studies (22). The specific lesion sizes used to calculate θ are included in the current manuscript as supplemental table 1.
2. δ = 0.08 h^{-1}. This is the death rate of infected cells without K^+ effects, whose reciprocal is the average time until virally infected cells burst. It is estimated based on the empirically observed time it takes to produce new infectious MYXV particles (based on data shown in Figure 3F).
3. $\beta_1 = 0.75$, $\beta_2 = 0.5$, $\beta_3 = 0.5$ represent reduction coefficients of high K^+ concentration on viral infectivity, infected cell death, and virus production, respectively. These are dimensionless and estimated by empirical analysis of the magnitude through which elevated K^+ impacts MYXV infection (based on data shown in Figure 1A, Figure 3F, and Figure 1C respectively).
4. D_2 = 0.1 mm^2·h^{-1}. This is the diffusion rate of extracellular K^+ in the interstitial fluid. It is estimated based on previously reported data (30, 31).
5. k = 4.0 nmol^{-1}·mm^3. This is a number that allows the smoothed Heaviside function to approximate the Heaviside function as close as possible while not creating numerical

issues. In particular, computing the smoothed Heaviside function requires evaluating $e^{-2k(P-P_S)}$, which at early stages equals e^{2kP_S} since $P \approx 0$. In a double-precision system, most widely adopted for scientific computing today, the largest real number that can be represented is approximately $10^{308} \approx e^{709.2}$; hence, to compute e^{2kP_S} properly one needs $k \lesssim 709.2/(2P_S) \approx 14$. Thus, the chosen value allows us to get a reasonable approximation to a Heaviside function while maintaining numerical feasibility when the number is involved in various numerical procedures such as Newton solves.

6. P_s = 25 nmol·mm^{-3}. This is the threshold value at which K^+ begins to impact viral infection. This corresponds to a threshold of 25-50 nmol·mm^{-3} which is estimated based on the empirically observed threshold at which K^+ begins to inhibit MYXV infection (based on data shown in Figure 1D)
7. α = 5.0625x10^{-4} nmol·cell^{-1}. This is the number of K^+ ions released per cell when a cell dies. It is based on previously reported literature indicating that the K^+ concentration inside a living cell is 150 nmol·mm^{-3} (10) and the volume of a single cell is 3.375×10^{-6}mm^3 (32).
8. D_1 = 1.0x10^{-2} mm^2·h^{-1}. This is the rate of spread of MYXV from cell to cell. It is estimated based on the empirically observed spread of MYXV in *vitro* (based on data shown in Figure 2E).
9. b = 300 virus·cell^{-1}. This is the burst size of MYXV from rabbit cells. It is estimated based on the empirically observed production of new infectious progeny from rabbit RK13 cells (based on data shown in Figure 3F in which ~3.1x10^7 FFU of MYXV can be recovered from 1x10^5 cells at 24 hours.
10. v_0 = 10$^5\rho$ virus·mm^{-1}. This is the number of initial viruses used to start the infection. It is based on the number of viruses typically used to induce myxomatosis in rabbits (22) and the extracellular-to-intracellular volume ratio $\rho = 10$.

We summarize these parameters and their estimated values in supplemental table 2.

Numerical method. We assume the lesion to be radially symmetric, as typically seen at early stage of infection *in vivo*. Thus, we cast the problem in the radial coordinate (r, t), where $r = \|x\|$, and the governing equations are as follows for all $r \in [0, R]$ and $t \geq 0$:

$$\frac{\partial B_1}{\partial t} = -\tilde{\theta}(P)B_1 V,$$

$$\frac{\partial B_2}{\partial t} = \tilde{\theta}(P)B_1 V - \tilde{\delta}(P)B_2,$$

$$\frac{\partial V}{\partial t} = \tilde{b}(P)\rho\tilde{\delta}(P)T_{1/\tilde{\delta}(P)}[B_2] - \tilde{\theta}(P)T_{1/\tilde{\delta}(P)}[B_1 V] + D_1 \frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial V}{\partial r}\right),$$

$$\frac{\partial P}{\partial t} = a\rho\tilde{\delta}(P)T_{1/\tilde{\delta}(P)}[B_2] + D_2 \frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial P}{\partial r}\right),$$

with the boundary conditions:

$$\frac{\partial V(0,t)}{\partial r} = \frac{\partial V(R,t)}{\partial r} = 0, \qquad \frac{\partial P(0,t)}{\partial r} = \frac{\partial P(R,t)}{\partial r} = 0.$$

Note that the homogeneous condition at $r = 0$ is due to radial symmetry.

Here $R = 100\ (mm)$ is chosen a constant radius that is much larger than any lesion size of interest. To avoid dealing with the singularity in the diffusion terms, we adopt a finite volume approach to discretize the equations. In particular, let the computational domain $[0, R]$ be divided into N_r uniform sub-intervals $[r_{i-1}, r_i], 1 \leq i \leq N_r$, where $r_i = i\Delta r$ and $\Delta r = R/N_r$ is the uniform interval size. Then our numerical method seeks approximations to averaged quantities on each interval. For example, the potassium concentration solutions are given by:

$$P_{i-1/2}^n \approx \frac{1}{r_{i-1/2}\Delta r}\int_{r_{i-1}}^{r_i} rP(r, t_n)dr\,, \qquad r_{i-1/2} = \left(i - \frac{1}{2}\right)\Delta r, \qquad t_n = n\Delta t,$$

where Δt is a fixed time step size.

The details of the discretization method except for the time-delayed terms can be found in (36). Overall, the major components of the method are summarized below:

- Central difference is used to discretize all spatial derivatives, which provide second-order accuracy in space.
- The backward Euler method is chosen to integrate the equations in time (i.e., updating solutions from t_{n-1} to t_n). Because the time-integrator is unconditionally stable, the chosen method is stable regardless of the choice of the size of Δt.
- To handle the time-delayed terms, numerical solutions at previous time steps are stored and they're used to interpolate the required data at an earlier time. For example, computing $T_{1/\tilde{\delta}(P)}\left[B^n_{2,i-1/2}\right]$ requires approximation to $B_2(r_{i-1/2,}t_n - 1/\tilde{\delta}(P))$, and it is computed by first finding m such that $(m-1)\Delta t \leq t_n - 1/\tilde{\delta}(P) < m\Delta t$, and then approximating $B_2(r_{i-1/2,}t_n - 1/\tilde{\delta}(P))$ by interpolation in time as a linear combination between $B^{m-1}_{2,i-1/2}$ and $B^m_{2,i-1/2}$.

All numerical simulations presented in this paper are conducted using $N_r = 160$ and $\Delta t = 0.125\ (hour)$. These numbers are chosen after careful convergence study so that the numerical solutions do not change significantly by further refining the grids either in space or in time.

Supplemental Table 2: Explanatin of parameters used in mathematical modelling

Parameters	Description	Values	Dimensions	Source
θ	Viral infection rate without K^+	0.5×10^{-9}	$mm3 \cdot h^{-1} \cdot virus^{-1}$	Our experiments (Supplemental Table 1)
θ_1	Blockage coefficient of high K^+ concentration on viral infectivity	0.75	dimensionless	Our experiments (Figure 1A)
δ	Infected cell death rate without effects of K^+	0.08	h^{-1}	Our experiments (Figure 3F)
θ_2	Reduction coefficient of high K^+ concentration on infected cell death	0.5	dimensionless	Our experiments (Figure 3F)
b	Burst size of myxoma viruses without effects of K^+	300	$viruses \cdot cell^{-1}$	Our experiments (Figure 3F)
θ_3	Effect factor of high K^+ level on virus production	0.5	dimensionless	Our experiments (Figure 1C)
P_s	K^+ threshold at which it starts affecting viral infection	25	$nmol \cdot mm^{-3}$	Our experiments (Figure 1D)
κ	Smoothed Heaviside variable	4	$nmol \cdot mm^{-3}$	Mathematical requirement
α	K^+ release rate by infected cells	5.0625×10^{-4}	$nmol \cdot mm^{-1}$	References 10, 32
ρ	Ratio between intracellular and extracellular space	10	dimensionless	Empirical value estimated from pathology in Figure 6A
D_1	Diffusion coefficient of myxoma viruses	1.0×10^{-2}	$mm^2 \cdot h^{-1}$	Reference 28
D_2	Diffusion coefficient of K^+ ions	0.1	$mm^2 \cdot h^{-1}$	References 30, 31
v_0	Virus initial value	$10^5\rho$	$virus \cdot mm^{-1}$	Reference 22

Supplemental Table 2: Explanation of parameters used in mathematical modeling.