Supplementary Information

S1. Lysis time determination of batch cultures using a plate reader

After sequence confirmation, the lysogens were first heat-induced in batch cultures to assess their lysis times. A 5-µL aliquot of overnight cultures was mixed with 1 mL LB in 24-well plates. Following growth at 30°C for 2 h, the plates were shifted to a 42°C water bath (time 0 for lysis time) for 15 min. After heat induction, the plates were shifted to a pre-warmed plate reader (Synergy™ HT, BioTek® Instruments, Inc., Vermont, USA) at 37°C, which measures A_{550} of the culture every 2 min. This protocol was repeated in triplicate for all lysogens. The complementary cumulative distribution function of the normal distribution was used to fit the A_{550} outputs generated by the plate reader. The estimated mean and standard deviation were defined as the lysis time and spread respectively. FPTs estimated using both batch culture and single-cell recordings were strongly correlated (Figure S1).

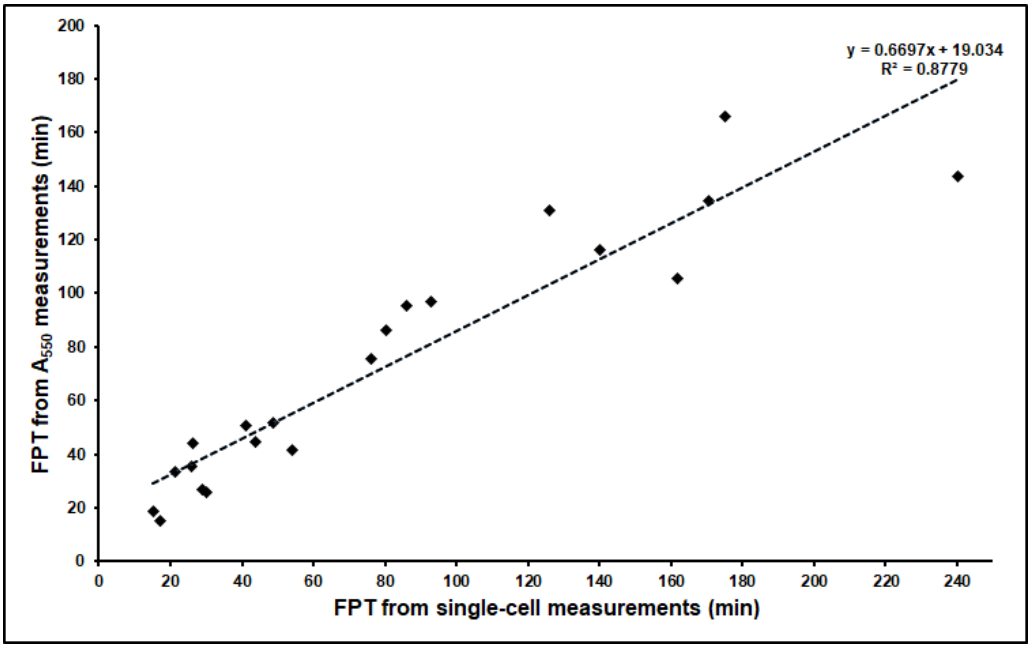

Figure S2. The FPT measurements using A_{550} and single-cell recordings were strongly correlated.

S2. Holin expression

We extracted holin from whole cells or from cell membranes to compare the relative holin levels in different mutant strains. An exponentially growing culture (A_{600} ~0.4) at 30°C was induced at 42°C for 20 min. A 5 mL aliquot of the culture was then immediately centrifuged to pellet the cells. The pellets were mixed with 2× SDS-PAGE sample buffer, heated at 100°C for 5 min, and loaded on a 4-20% TruPAGE™ precast gel (Sigma-Aldrich, St. Louis, MO, USA). Another 4 mL aliquot of the culture was heat induced for 30 min and then sonicated to disrupt the membranes. The membranes were collected by centrifugation at 100,000 × g for 1 h. The pelleted membranes

were mixed with 40 µl of ME buffer (10 mM Tris Cl [pH 8.0], 35 mM $MgCl_2$, 1% Triton X-100) by shaking on a platform shaker for 2 h at 25°C. The extracted samples were centrifuged at 100,000 × g for 30 min to pellet the insoluble fraction. The membrane extracts were mixed with 2× SDS-PAGE sample buffer, heated at 100°C for 3 min, and loaded on to precast gels. After electrophoresis, Western blotting was used to detect holin using a primary antibody (1:1000) raised in rabbits. A secondary antibody (donkey anti-rabbit polyclonal antibody conjugated to horseradish peroxidase [SA1-200, ThermoFisher Scientific, Waltham, MA, USA]) was used at a dilution of 1:1000 dilution, and the blot was developed as per the manufacturer's directions. An average of three preparations was used to estimate holin levels.

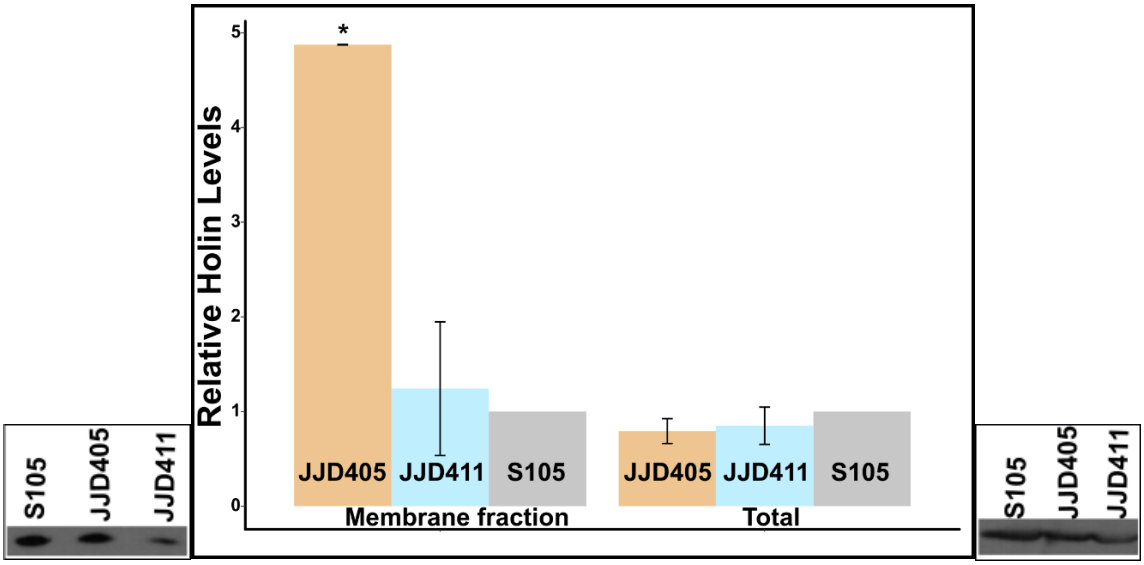

Figure S2. Total holin levels from whole-cell extracts and membrane fractions. The left and right panels show Western blots of membrane fractions and whole-cell extracts respectively. Bands represent holin extracted from strains S105 (mean LT = 58 min), JJD405 (32 min), and JJD411 (176 min). * = $p < 0.05$, t-test; error bars are SEM.

S3. Calculation of noise in the first passage time

We modeled the expression of holin occurring in intermittent bursts, with burst events arriving as a Poisson process with rate *k*. Whenever a burst occurs, the total cellular concentration of holin $x(t)$ at time *t* increases by a random amount b:

$$x(t) \mapsto x(t) + b, \qquad S1$$

where b is drawn from an arbitrary positively-valued probability distribution with the first and second moments $\langle b \rangle$ and $\langle b^2 \rangle$, respectively. The first moment $\langle b \rangle$ represents the mean burst size per unit volume. Between two consecutive bursts, the concentration diluters from cell growth as per the following the deterministic dynamics:

$$\dot{x}(t) = -\gamma x(t) \qquad S2$$

where γ is the cellular growth rate. For this hybrid system with stochastic bursts interspersed by first-order decay, the time evolution of the first and second-order moments of $x(t)$ are given by

$$\frac{d\langle x\rangle}{dt} = k\langle b\rangle - \gamma\langle x\rangle, \quad S3$$

$$\frac{d\langle x^2\rangle}{dt} = k\langle b^2\rangle + 2k\langle b\rangle\langle x\rangle - 2\gamma\langle x^2\rangle, \quad S4$$

(1,2,3). Solving the above differential equations, we get the mean $\langle x\rangle$ and variance $\langle x^2\rangle - \langle x\rangle^2$ of the holin concentration as a function of time t, assuming there is no holin at the onset of the protein synthesis;

$$\langle x\rangle = \frac{[1 - e^{-\gamma t}]\, k\, \langle b\rangle}{\gamma} \quad S5$$

$$\langle x^2\rangle - \langle x\rangle^2 = \frac{[1 - e^{-2\gamma t}]\, k\, \langle b^2\rangle}{2\,\gamma}. \quad S6$$

We formulate the lysis time as the first-passage time

$$FPT = \min\{t: x(t) \geq X | x(0) = 0\}, \quad S7$$

or the first time the holin concentration reaches a critical threshold level X, and quantify the noise in the first-passage time using the coefficient of variation squared,

$$CV^2_{FPT} = (\langle FPT^2\rangle - \langle FPT\rangle^2)/\langle FPT\rangle^2,$$

where $\langle FPT\rangle$ and $\langle FPT^2\rangle$ are the first two moments of *FPT*. CV^2_{FPT} is related to the fluctuations in the holin concentration as per

$$CV^2_{FPT} \approx \frac{\langle x^2\rangle - \langle x\rangle^2}{\langle FPT\rangle^2}\left(\frac{d\langle x\rangle}{dt}\right)^{-2}\Bigg|_{t=\langle FPT\rangle}, \quad S8$$

(4). From eq. S5 the mean first-passage time is obtained as

$$\langle FPT\rangle = -\frac{1}{\gamma}\log(1-\alpha), \text{ with } \alpha = \frac{X}{x_s}. \quad S9$$

Here x_s denotes the steady-state mean holin concentration and is given by,

$$x_s = \langle x(t\to\infty)\rangle = \frac{k\,\langle b\rangle}{\gamma},$$

with the underlying assumption in eq. S9 being that the threshold for lysis *X* is less than x_s. Using equations (S5), (S6), and (S8), we write down the formula for the noise in *FPT*,

$$CV^2_{FPT} = CV_x^2\,\frac{\alpha\,(2-\alpha)}{[(1-\alpha)\ln(1-\alpha)]^2}, \quad S10$$

where, CV_x^2 is the coefficient of variation squared for the holin concentration at steady state

$$CV_x^2 = \lim_{t\to\infty} \frac{\langle x^2\rangle - \langle x\rangle^2}{\langle x\rangle^2} = \frac{\langle b^2\rangle}{2\langle b\rangle x_s}$$

and quantifies the extent of stochasticity in holin expression. The above formula can be rewritten in terms of $\langle FPT\rangle$ as,

$$CV_{FPT}^2 = CV_x^2 \frac{\left[e^{2\gamma\langle FPT\rangle} - 1\right]}{(\gamma\langle FPT\rangle)^2} \qquad S11$$

and varies nonmonotonically with the mean FPT consistent with experimental data. The optimal value of the mean FPT (in the unit of γ^{-1}), where noise is minimum is $\gamma\langle FPT\rangle \approx 0.8$. The corresponding value of the threshold (in the unit of steady state concentration) is $\alpha = \frac{X}{x_s} \approx 0.55$.

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