Supporting Information

White et al. 10.1073/pnas.1011921108

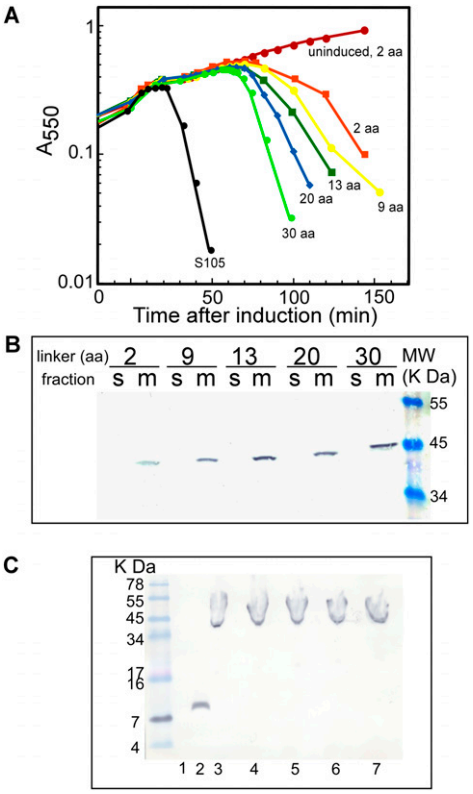


Fig. S1. (*A*) Lysis phenotypes of *S105–gfp* fusions. RY17303 cells carrying a λ*Sam7* prophage and pS105 plasmid derivatives were induced at time 0 and monitored for culture turbidity as A_{550}. The pS105 derivatives carry the parental *S105* gene (black circles) or *S105–gfp* fusions encoding short oligopeptide linkers of the indicated lengths (see text for sequences; colored curves). (*B* and *C*) Western blot analysis of inductions of *S105–gfp* fusions, using anti-S105 (N terminal) antibody, as described in *Materials and Methods*. In *A*, samples were taken 75 min after induction and fractionated into membrane (m) and soluble (s) samples. In *C*, whole cell samples were taken from isogenic inductions, with the empty vector (lane 1), parental *S105* (lane 2), and the five *S105–gfp* alleles with the different linkers (lanes 3–7: *S105–gfp* fusion allele encoding the 2-aa, 9-aa, 13-aa, 20-aa, and 30-aa linkers, respectively.) Note that no S105-sized degradation product is detectable in any of the fusion samples.

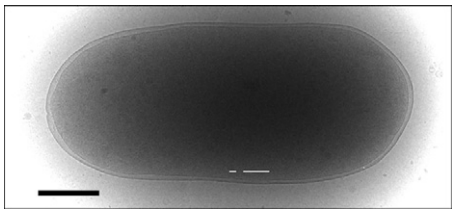


Fig. S2. S105–GFP triggers to form μm-scale holes in the cytoplasmic membrane. A culture of RY17303(λΔ(*SR*))pS105–GFP$R_{am}Rz_{am}Rz1_{am}$ was thermally induced, harvested at 105 min after induction, and examined by cryoelectron microscopy, as described in *Materials and Methods* and previously (1). White lines indicate the location and size of visible inner membrane gaps. (Scale bar, 500 nm.)

1. Dewey JS, et al. (2010) Micron-scale holes terminate the phage infection cycle. *Proc Natl Acad Sci USA* 107:2219–2223.

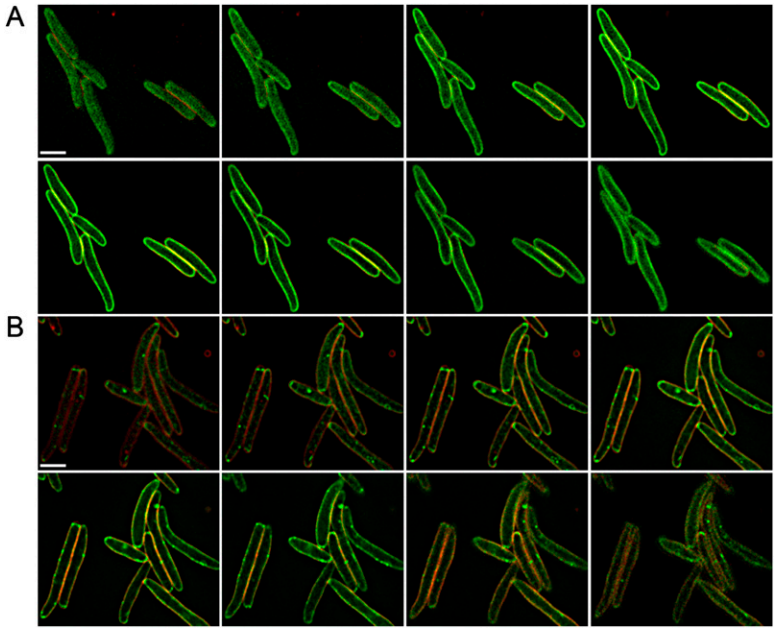
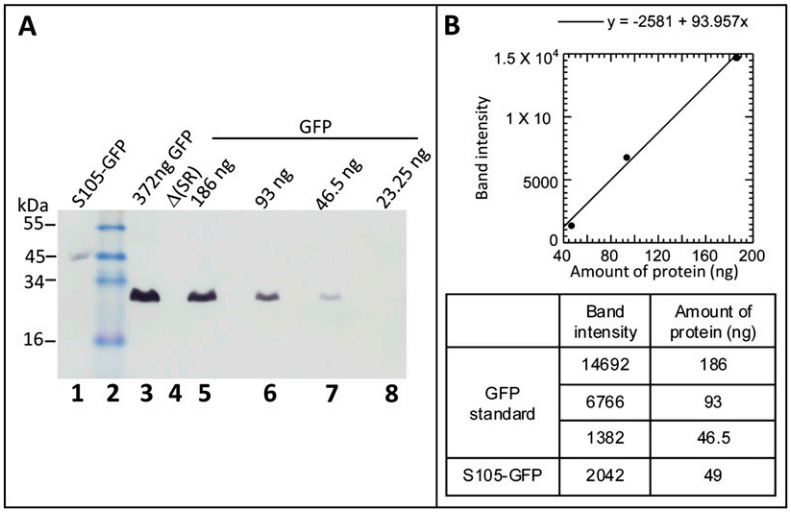


Fig. S3. Optical sections of cells induced for *S105–gfp* at 60 min (*A*) or 105 min (*B*). Each section is 0.2 μm apart. (Scale bar, 3 μm.)



	Band intensity	Amount of protein (ng)
GFP standard	14692	186
	6766	93
	1382	46.5
S105-GFP	2042	49

Fig. S4. Measurement of S105–GFP expression level at the time of triggering. (*A*) Logarithmic cultures were thermally induced, precipitated by trichloroacetic acid (TCA) immediately after the time of S105–GFP triggering, and resuspended in SDS/PAGE loading buffer. Samples containing 3.4×10^8 cells were subjected to SDS/PAGE and Western blotting using anti-GFP antibody. In lane 1, the cells were RY17303(λΔ(*SR*)) pS105–GFP, whereas in lanes 3–8, the cells were RY17303 (λΔ(*SR*)) without the plasmid. In lanes 3 and 5–8, the indicated amount of purified GFP was mixed with the sample before electrophoresis. In lane 4, no GFP was added. Lane 2 is a molecular mass standard. (*B*) The amount of S105–GFP present in the induced cultures was calculated using the standard curve generated from samples containing the purified GFP protein. Band intensities were measured using ImageJ software (1). On the basis of 49 ng of S105–GFP accumulated in 3.4×10^8 cells, ~3,000 S105–GFP molecules were present at the triggering time.

1. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophoton Int* 11:36–42.

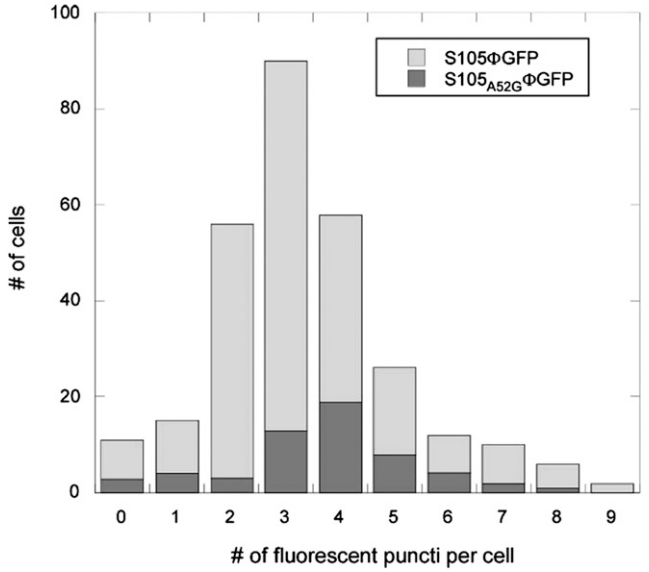


Fig. S5. Histogram of the number of puncti per cell for S105–GFP and $S105_{A52G}$–GFP. Only fluorescent puncti, or rafts, larger than 3 pixels in diameter were counted as "large" rafts.

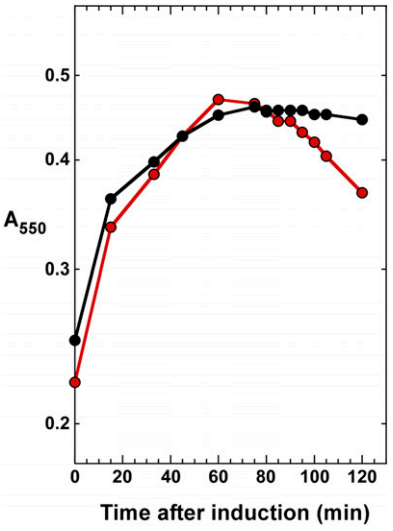


Fig. S6. $S105_{\Delta TMD1}$–mCherryFP blocks the triggering of S105–GFP. RY17303 cells carrying a λΔ(*SR*) prophage, pS105–GFP, and a compatible plasmid with either the null allele *Sam7* (red circles) or an allele encoding $S105_{\Delta TMD1}$–mCherryFP under the native λ-late promoter (black circles) were induced at time 0 and monitored for culture turbidity as A_{550}.

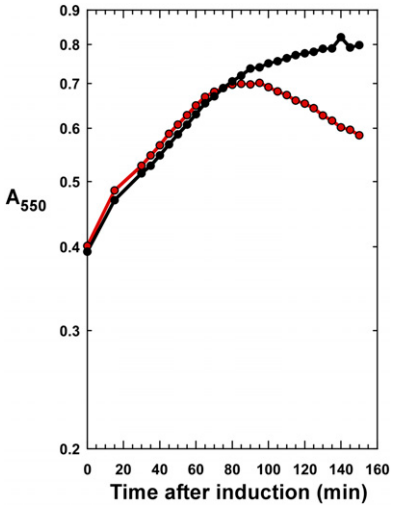


Fig. S7. S105–GFP triggers between 90 and 100 min in conditions used for fluorescence microscopy. RY17303 cells carrying a λΔ(*SR*) prophage and either pS105–GFP (red circles) or pR′–GFP (black circles) were grown in 0.25× LB, induced at time 0, and monitored for culture turbidity as A_{550}.

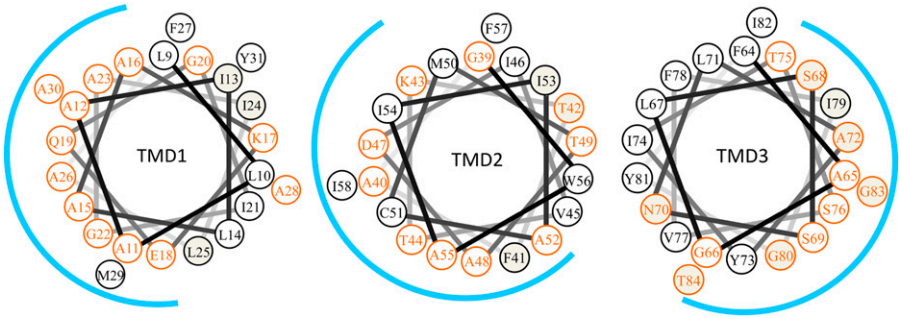
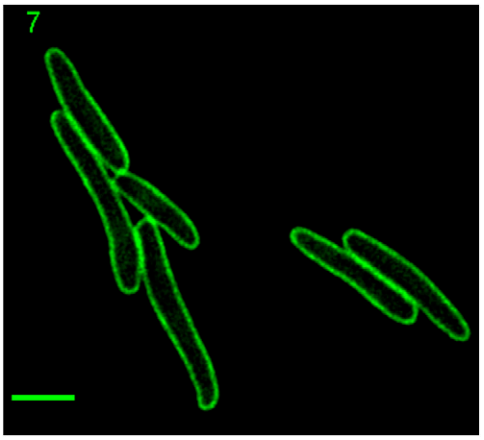
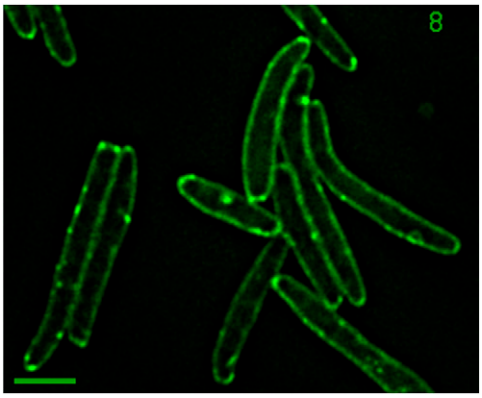


Fig. S8. The TMDs of S105 each have a relatively hydrophilic surface. Helical projections of TMDs I, II, and III (see underlined residues in Fig. 1) are shown, with the hydrophilic and weakly hydrophobic residues colored in orange. Arcs circumscribe the most hydrophilic face in each case.



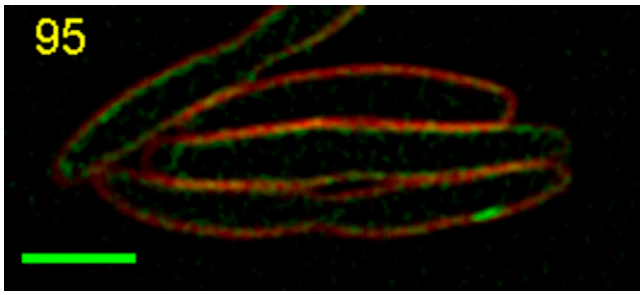
Movie S1. Optical sections (shown as number) of cells induced for S105–GFP for 60 min in Fig. S3*A*. Only the GFP channel is shown. (Scale bar, 3 μm.)

Movies S1



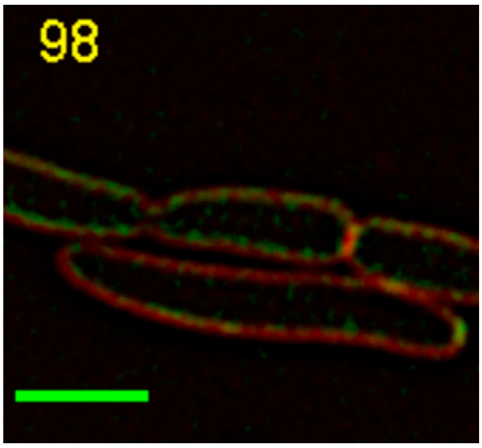
Movie S2. Optical sections (shown as number) of cells induced for S105–GFP for 105 min in Fig. S3*B*. Only the GFP channel is shown. (Scale bar, 3 μm.)

Movies S2



Movie S3. Time-lapse movie showing the sudden formation of S105–GFP raft in Fig. 4*A*, with the time (in minutes) after induction indicated. Shown is the overlay of the GFP and FM 4–64 channels. (Scale bar, 2 μm.)

Movies S3



Movie S4. Time-lapse movie showing the sudden formation of S105–GFP raft in Fig. 4*B*, with the time (in minutes) after induction indicated. Shown is the overlay of the GFP and FM 4–64 channels. (Scale bar, 2 μm.)

Movies S4