Supporting Information

Dewey et al. 10.1073/pnas.0914030107

SI Methods

Geometry of the Lesions and Probability of Observation. A lesion in the inner membrane can be observed in the transmission electron microscope if part of the lesion is located at the edge of the cell and perpendicular to the optical axis in a given cell orientation. Assuming a circular hole with a diameter of 340 nm, the probability of its visualization can be approximately calculated from the angle formed by the edges of the hole and the center of mass of the cell (Fig. S1). This calculation results in a 40° angle for an average cell width of 1,000 nm. Thus, the probability of observing such a hole is ~22%. (40°/180°, where 180° is the image projection range on one side of the cell). Because holes are observed in 48% of the cells, these data suggest that approximately two holes are created, on average, in a cell induced for *S105* expression (Fig. 3*A*).

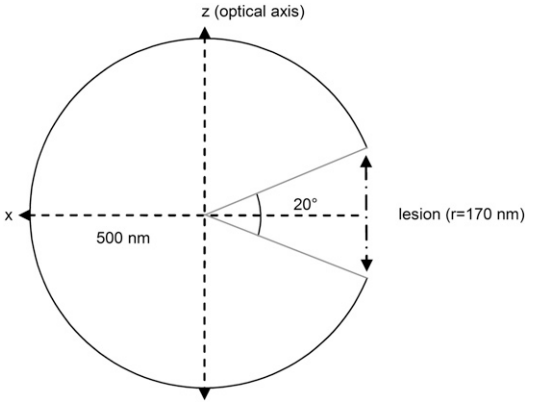

Fig. S1. Diagram depicting the calculations for the probability of observation of a 340-nm lesion at a tilt of 0°. The circle represents the circumference of a rod-shaped cell along its width (cells observed had an average width of ~1,000 nm). A 340-nm lesion (which spans a range of ~40°) can be observed when part of it is located at the x–y plane. r, radius.

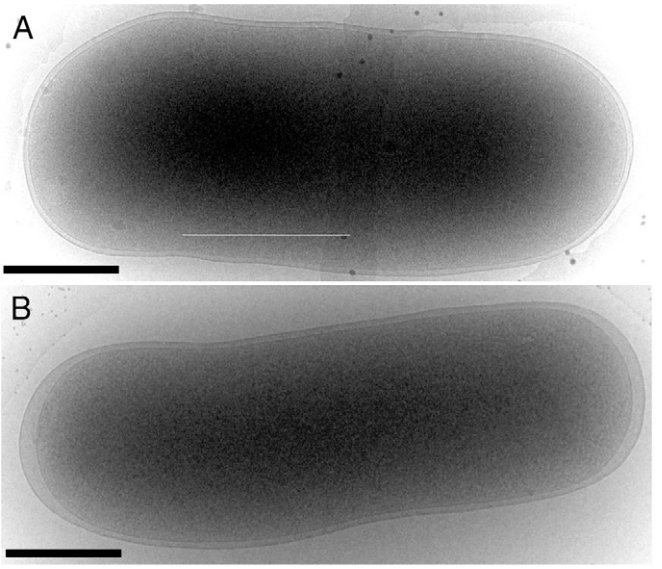

Fig. S2. Cells expressing *S105* (pS) (*A*) or *Sam7* (p0) (*B*). A continuous inner membrane was characteristic of all cells expressing *Sam7*. (Scale bars: 500 nm.)

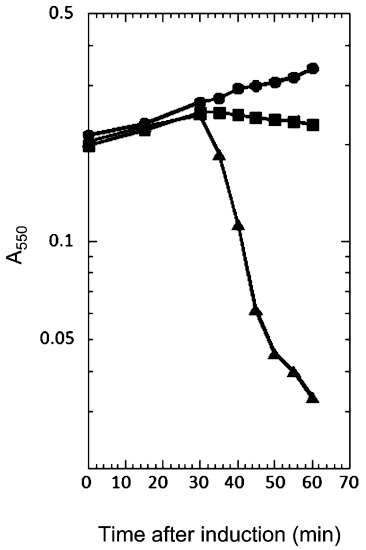

Fig. S3. Growth curves of cultures carrying no S105 plasmid (●), pS (■), or pSRRzRz1(▲), grown in succinate-minimal media and induced with isopropyl-β-D-thiogalactopyranoside at $t = 0$.

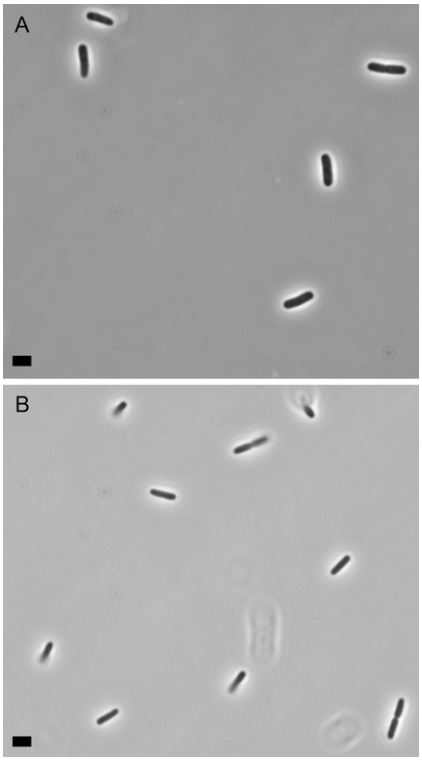

Fig. S4. Light micrographs of cells grown in LB (*A*) and succinate media (*B*). The average length of cells grown in LB and succinate is 3.8 μm and 2.9 μm, respectively, and the average width measures 0.97 μm in LB and 0.77 μm in succinate (as measured by cryo-EM). Measurements using light microscopy closely matched these results. (Scale bars: 2 μm.)

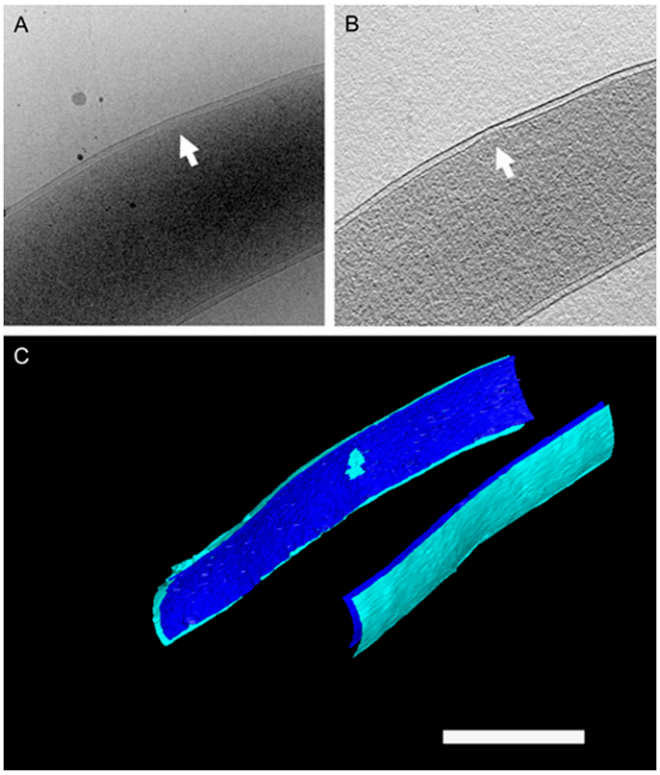

Fig. S5. Cryoelectron tomography of a cell expressing S105. A zero-tilt projection image (*A*) and a ~25-nm-thick slice through the tomogram (*B*) are shown. (*C*) Observed lesion is highlighted by arrows in *A* and *B* and displayed in 3D after segmentation of the inner (dark blue) and outer (light blue) membrane densities. (Scale bar: 500 nm.)

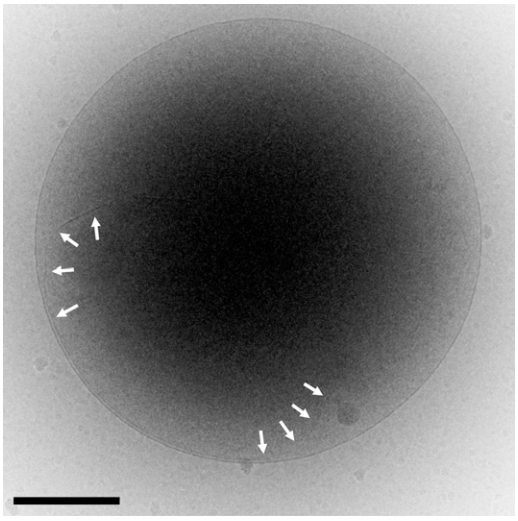

Fig. S6. Cell expressing *S105* and *R*, without divalent cations present. For these conditions, most cells had undergone complete lysis, as evident from the observed cell debris. Arrows trace the discernible inner membrane at the point of separation from the cell envelope. (Scale bar: 500 nm.)

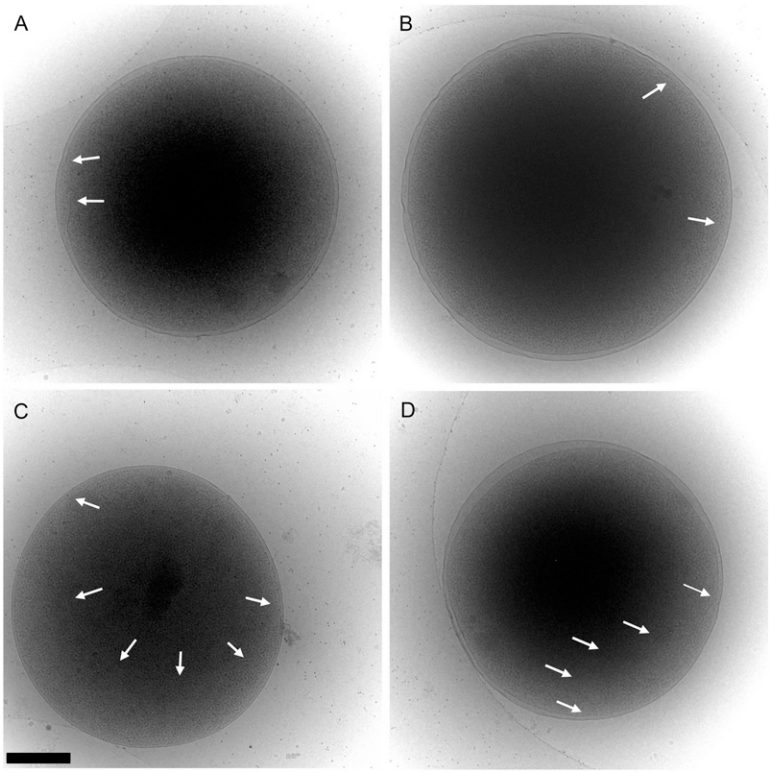

Fig. S7. Cryo-EM of cells coexpressing holin (*S105*) and endolysin (*R*) in the presence of Mg^{2+} ions. Cells were captured at different stages of inner membrane disruption, including gap formation (*A* and *B*) and a more pronounced collapse of the inner membrane (*C* and *D*). White arrows indicate the border of membrane discontinuity (*A* and *B*) or the trace of the collapsed membrane (*C* and *D*). (Scale bar: 500 nm.)

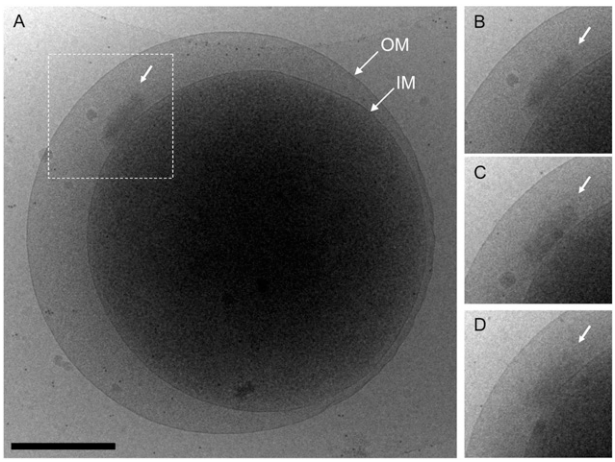

Fig. S8. Early stages of cytoplasmic leakage from a cell coexpressing *S105* and *R*. (*A* and *B*) At a tilt of 0°, the image indicates a cloud of density escaping from the cytoplasm. White arrows point to the protein density escaping from the cytoplasm. The white dashed box indicates the area depicted in *B*. IM, inner membrane; OM, outer membrane. Tilting of the stage by 20° (*C*) and 30° (*D*) revealed the membrane lesion from which the leakage originated. (Scale bar: 500 nm.)