Figure S7-S21. Related to Figures 3 and 4. All ejection trajectories. In each trajectory, the decrease of fluorescence in the phage is accompanied by a rise in fluorescence inside the cell, indicating that DNA is being transferred from the phage into the cell. These trajectories are the first direct *in vivo* measurements of DNA translocation, a single-molecule analog of the experiments by Hershey and Chase in 1952. The distribution of time to complete ejection is highly variable. In Figures S7—S21, we see that for the most part there is fluorescence conservation between ejecting phages (red lines) and increase in fluorescence in the cells (blue lines). The masks for ejecting phages are displayed in green, the masks for non-ejecting phages are displayed in red, and the masks for the cell are displayed in white. Often, trajectories contain steps and sometimes the fluorescence is not conserved. The lack of conservation could be due to dye being stripped off the phage as it translocates its DNA or photobleaching. It could also be due to nonlinearities introduced by the EM gain in the Andor iXon camera, as lack of conservation was not observed to the same degree in non electron-multiplying cameras. Note that the images were acquired with the phages optimally focused which may have rendered some of the phase images of cells less than ideal.

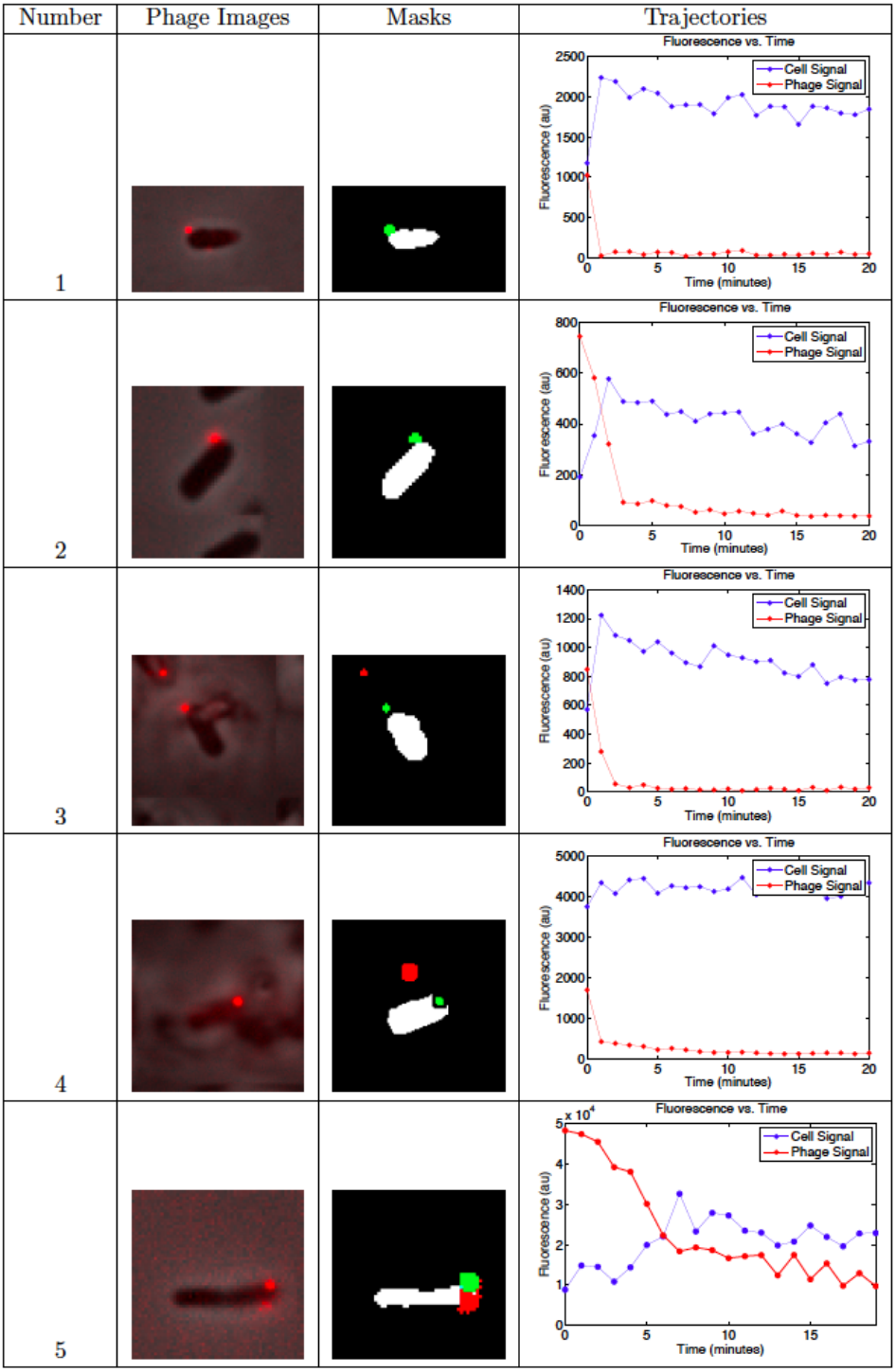

Figure S7. Single-molecule trajectories for λcI60: Group 1. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

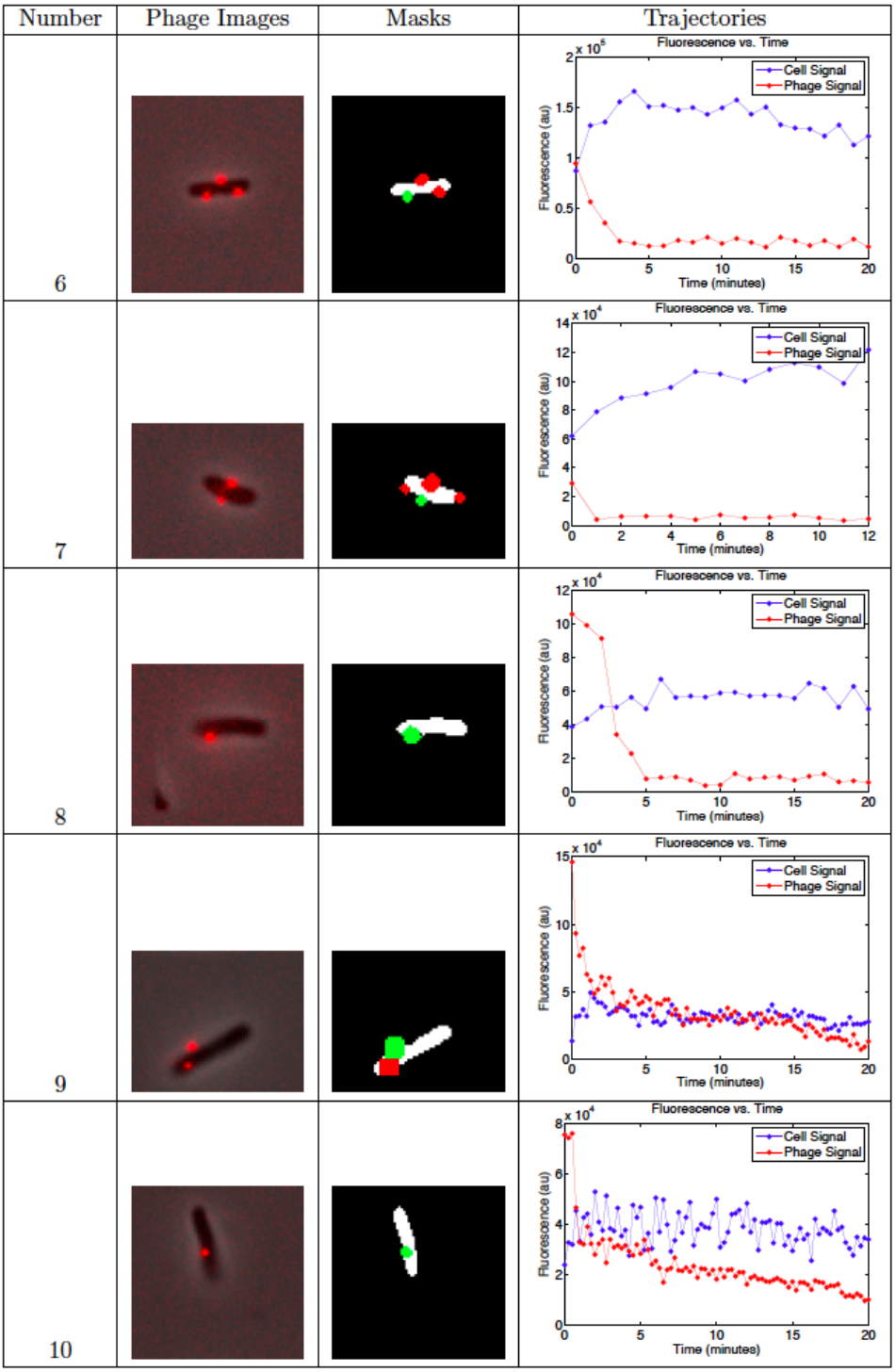

Figure S8. Single-molecule trajectories for λcI60: Group 2. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

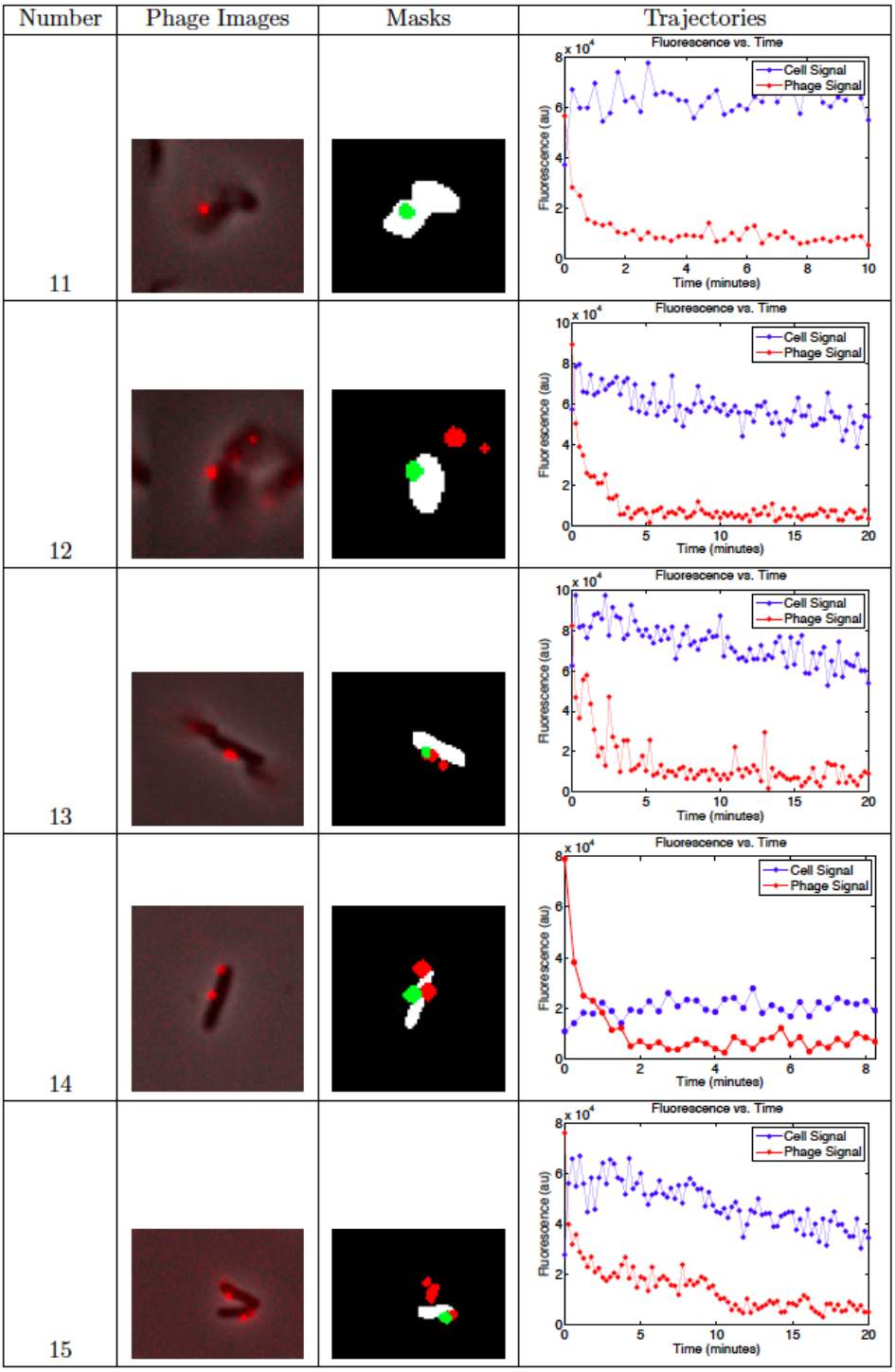

Figure S9. Single-molecule trajectories for λcI60: Group 3. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

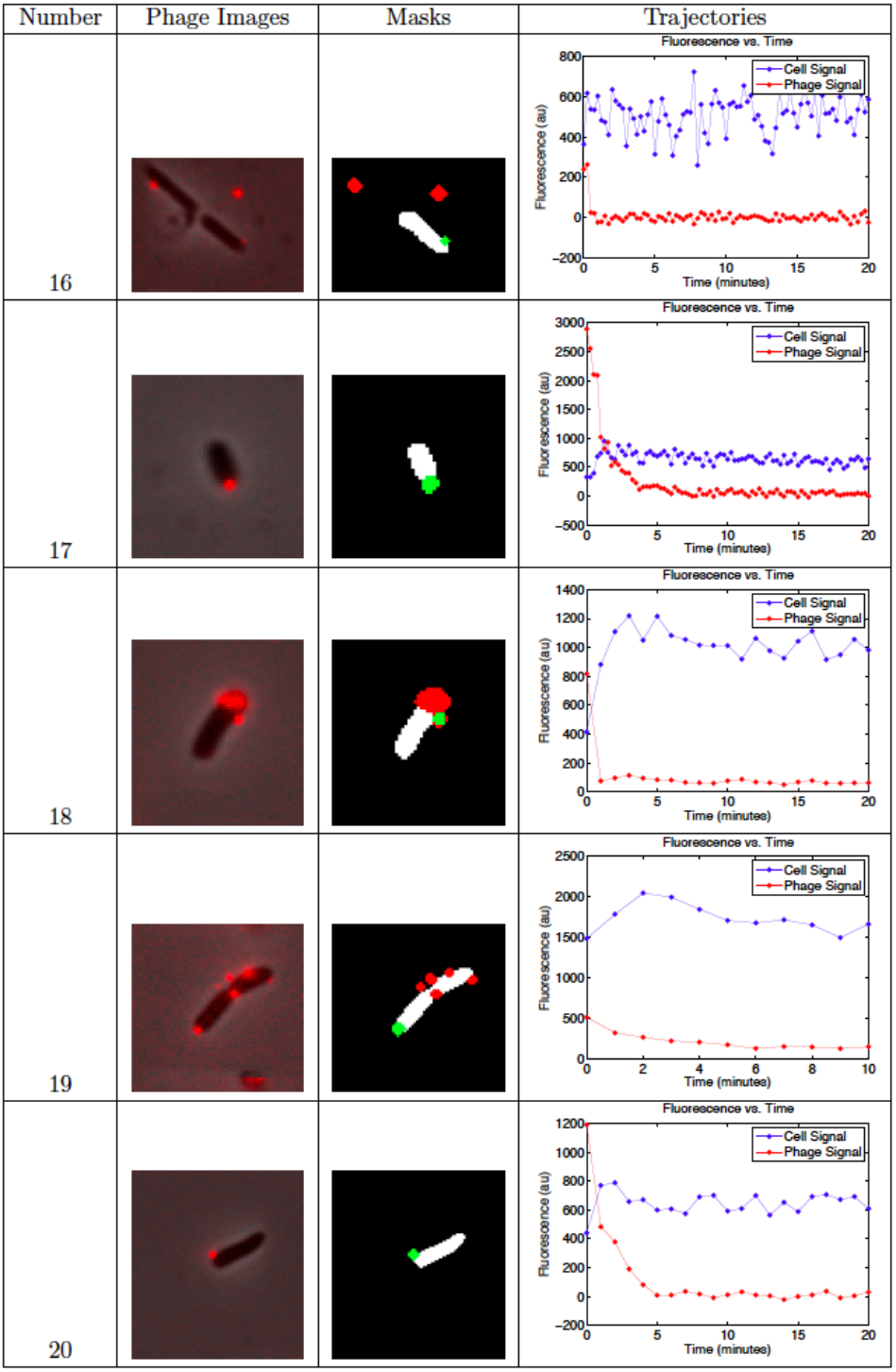

Figure S10. Single-molecule trajectories for λcI60: Group 4. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

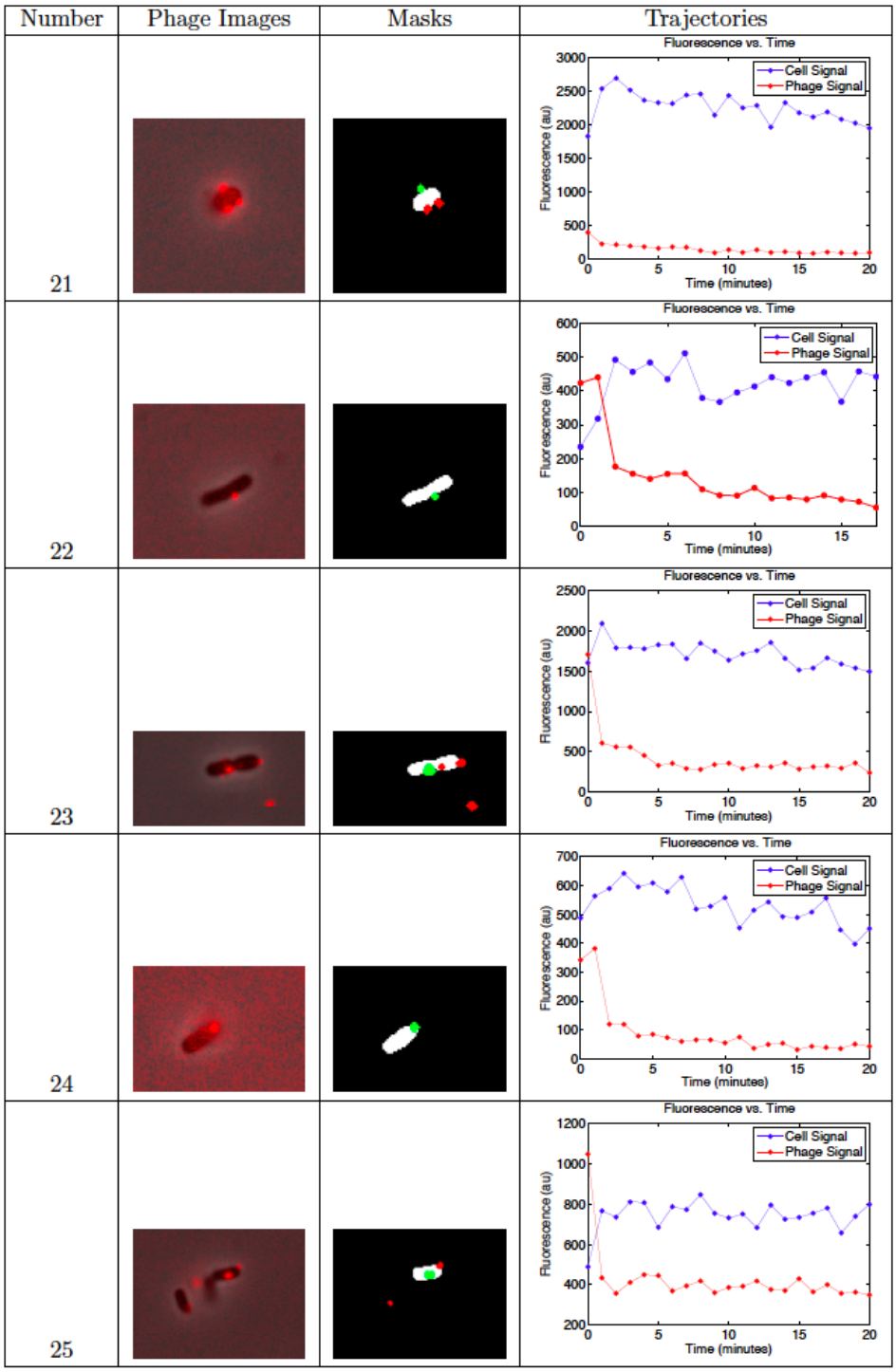

Figure S11. Single-molecule trajectories for λcI60: Group 5. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

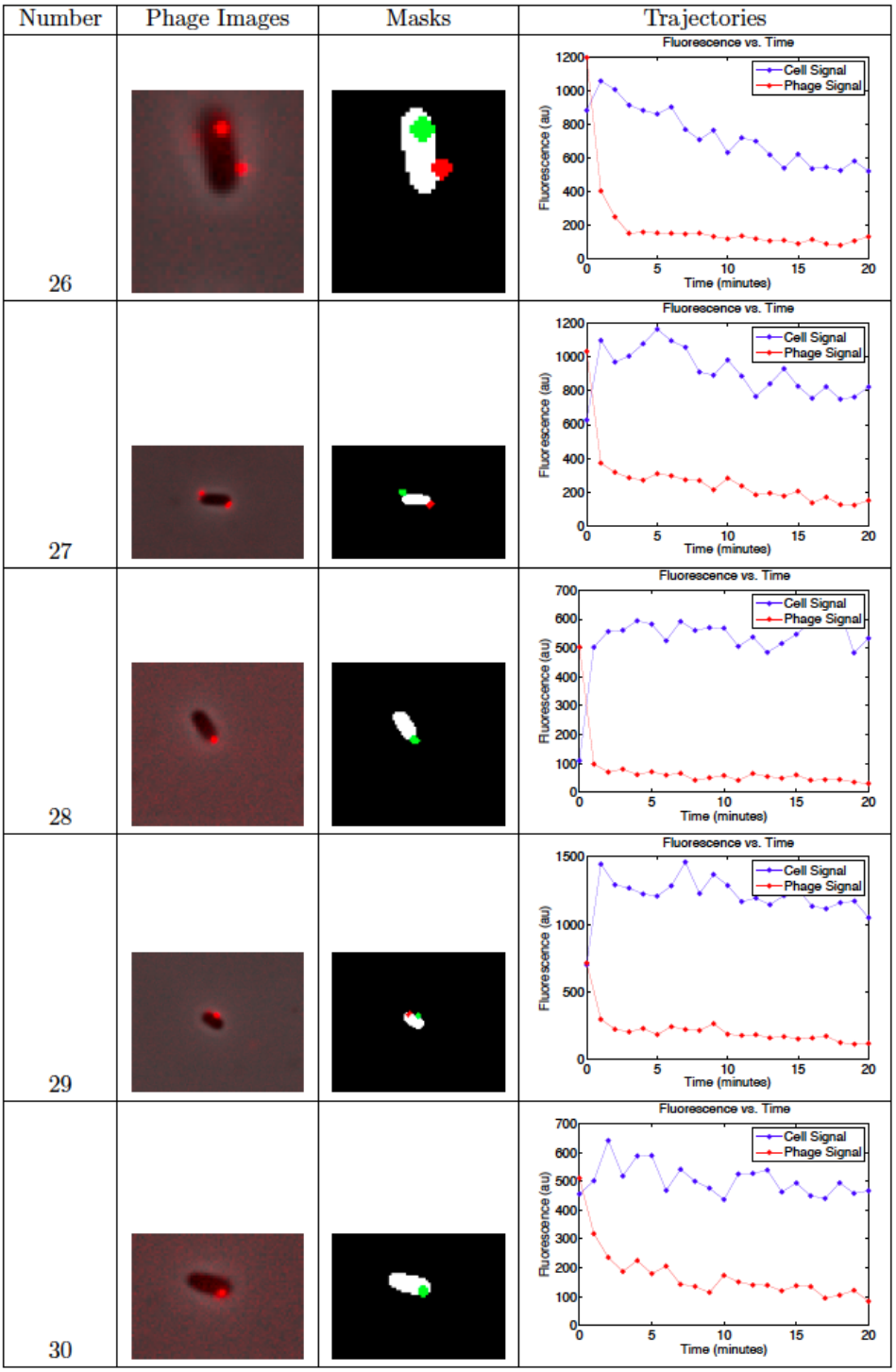

Figure S12. Single-molecule trajectories for λcI60: Group 6. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

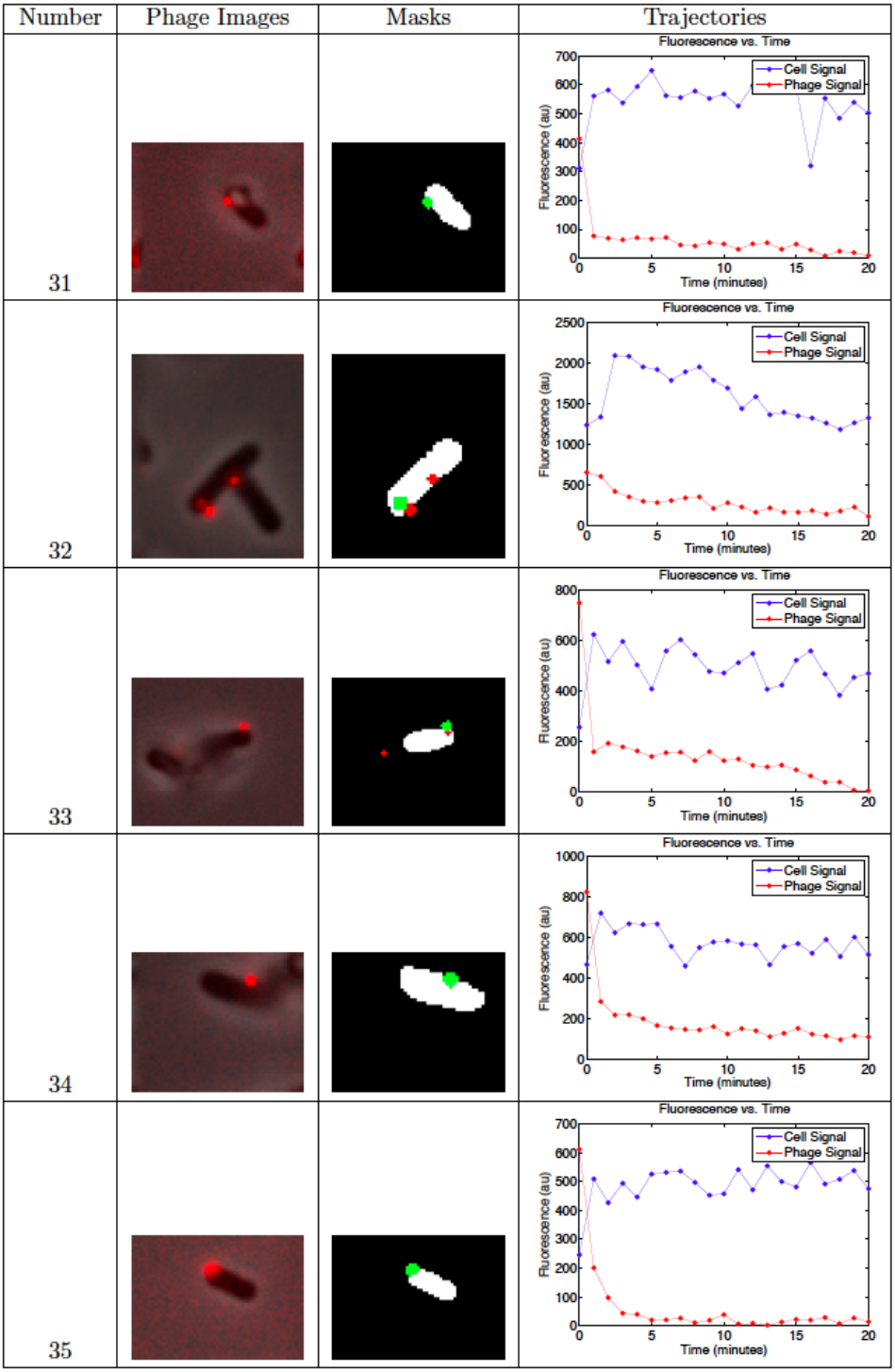

Figure S13. Single-molecule trajectories for λcI60: Group 7. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

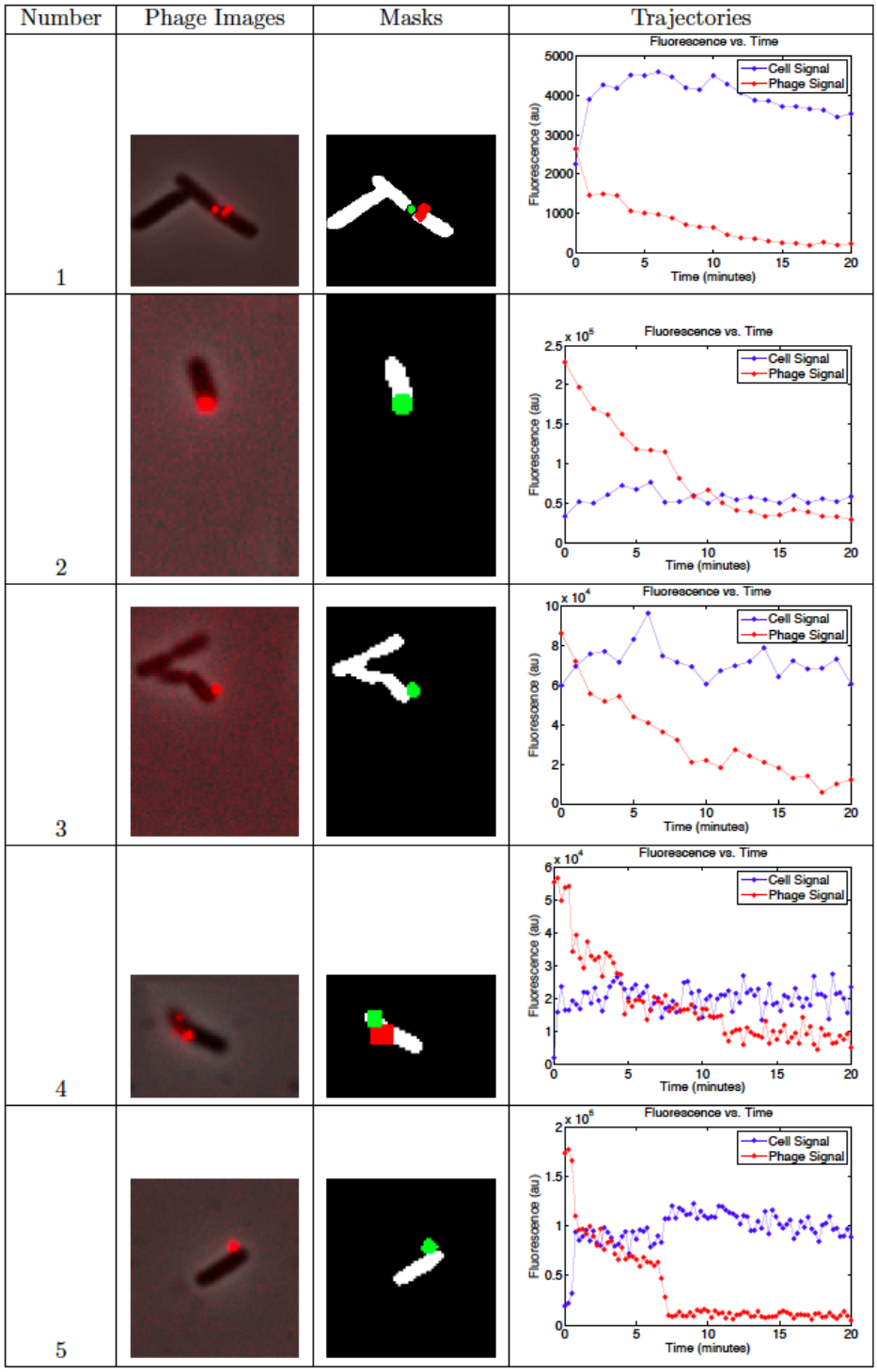

Figure S14. Single-molecule trajectories for λcI60 (paused): Group 1. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

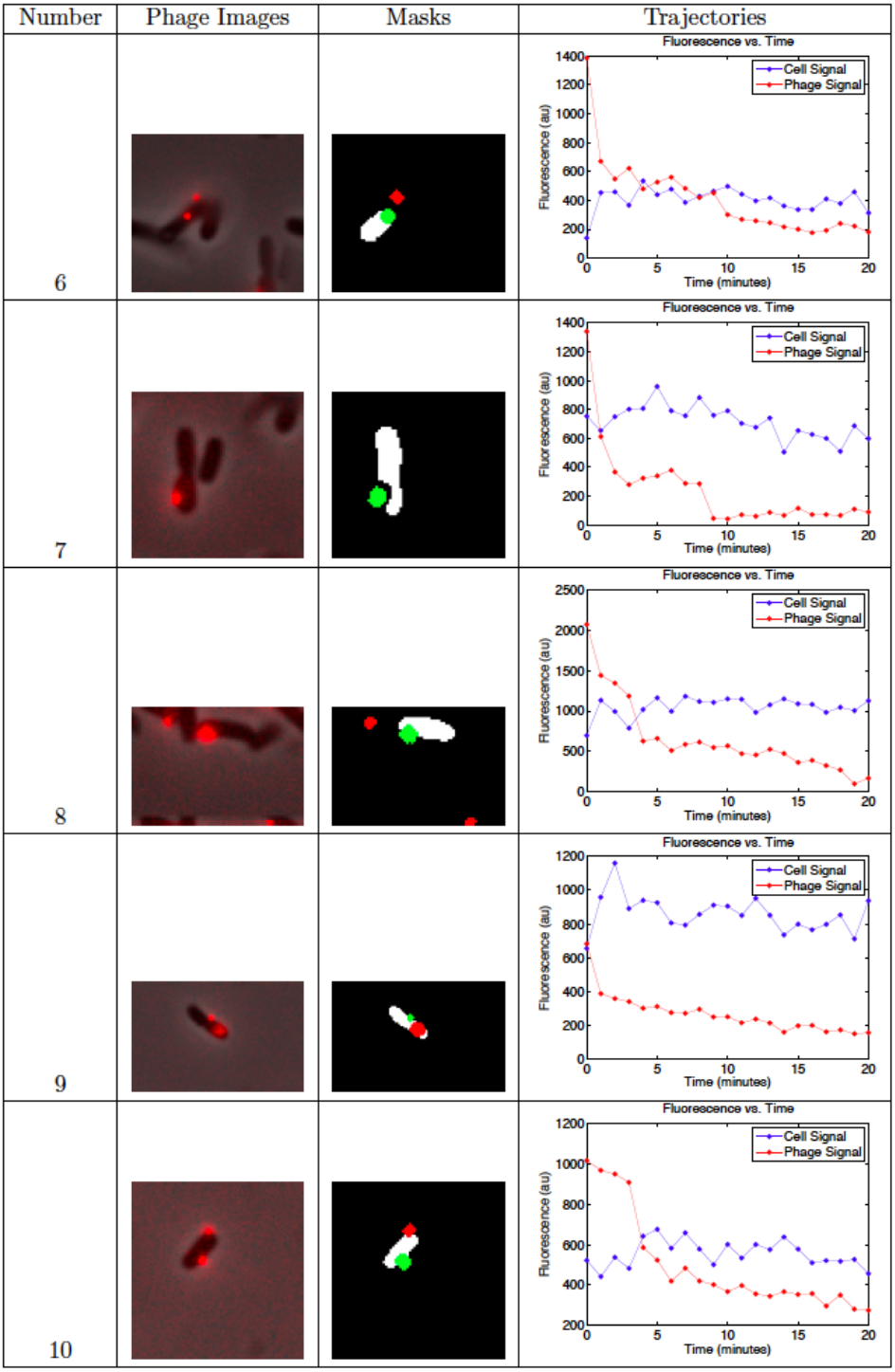

Figure S15. Single-molecule trajectories for λcI60 (paused): Group 2. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

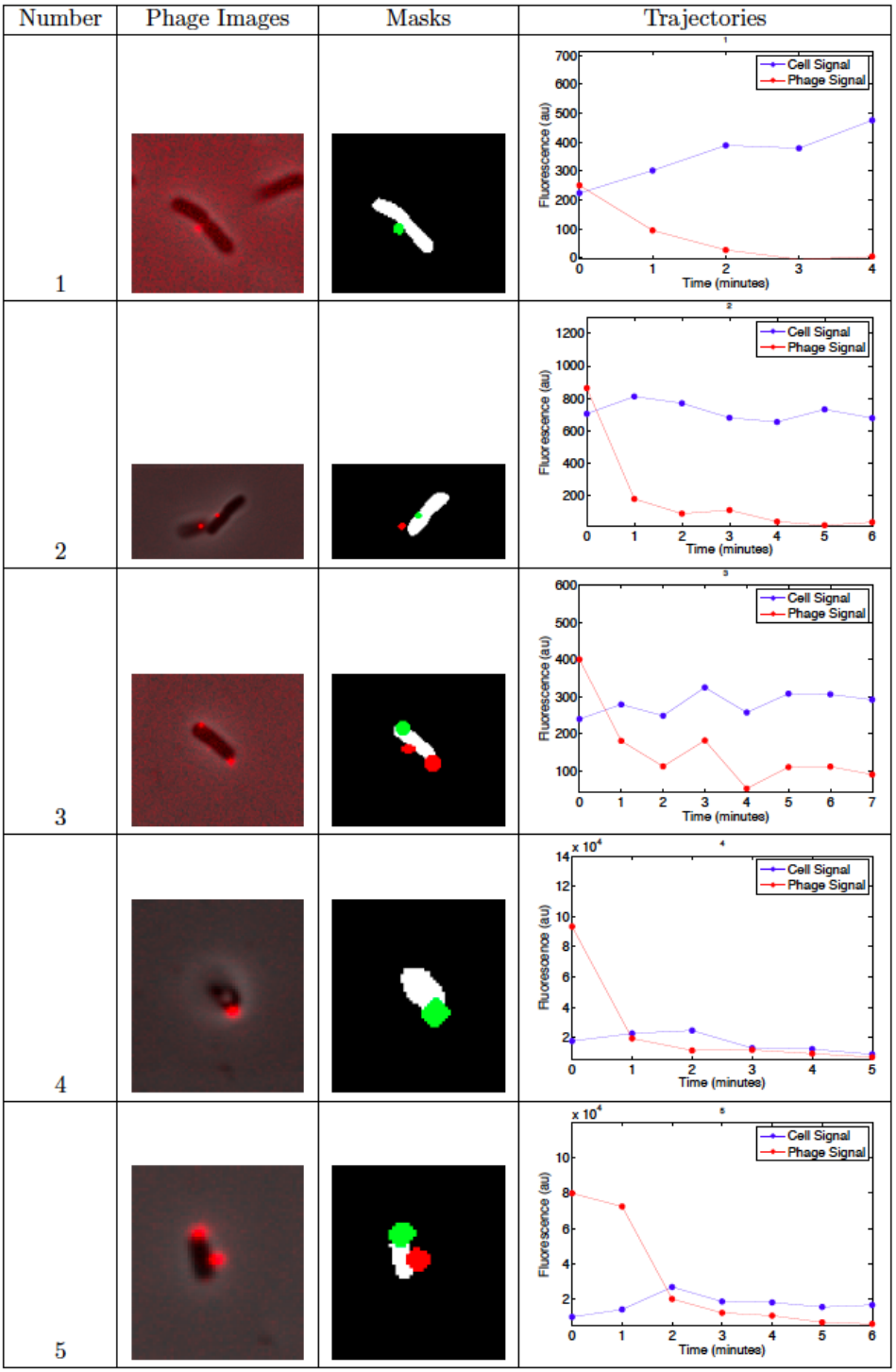

Figure S16. Single-molecule trajectories for λb221: Group 1. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

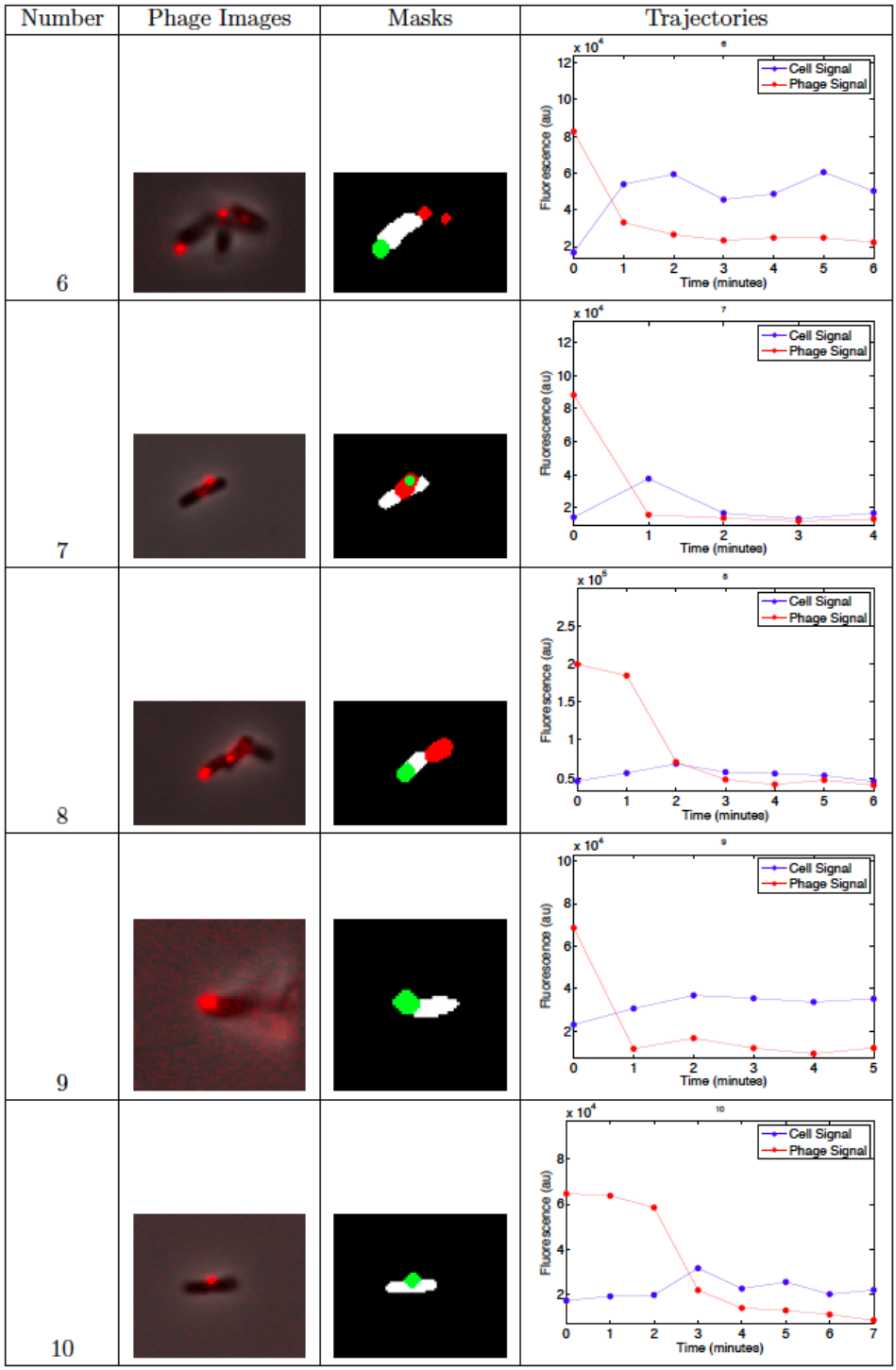

Figure S17. Single-molecule trajectories for λb221: Group 2. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

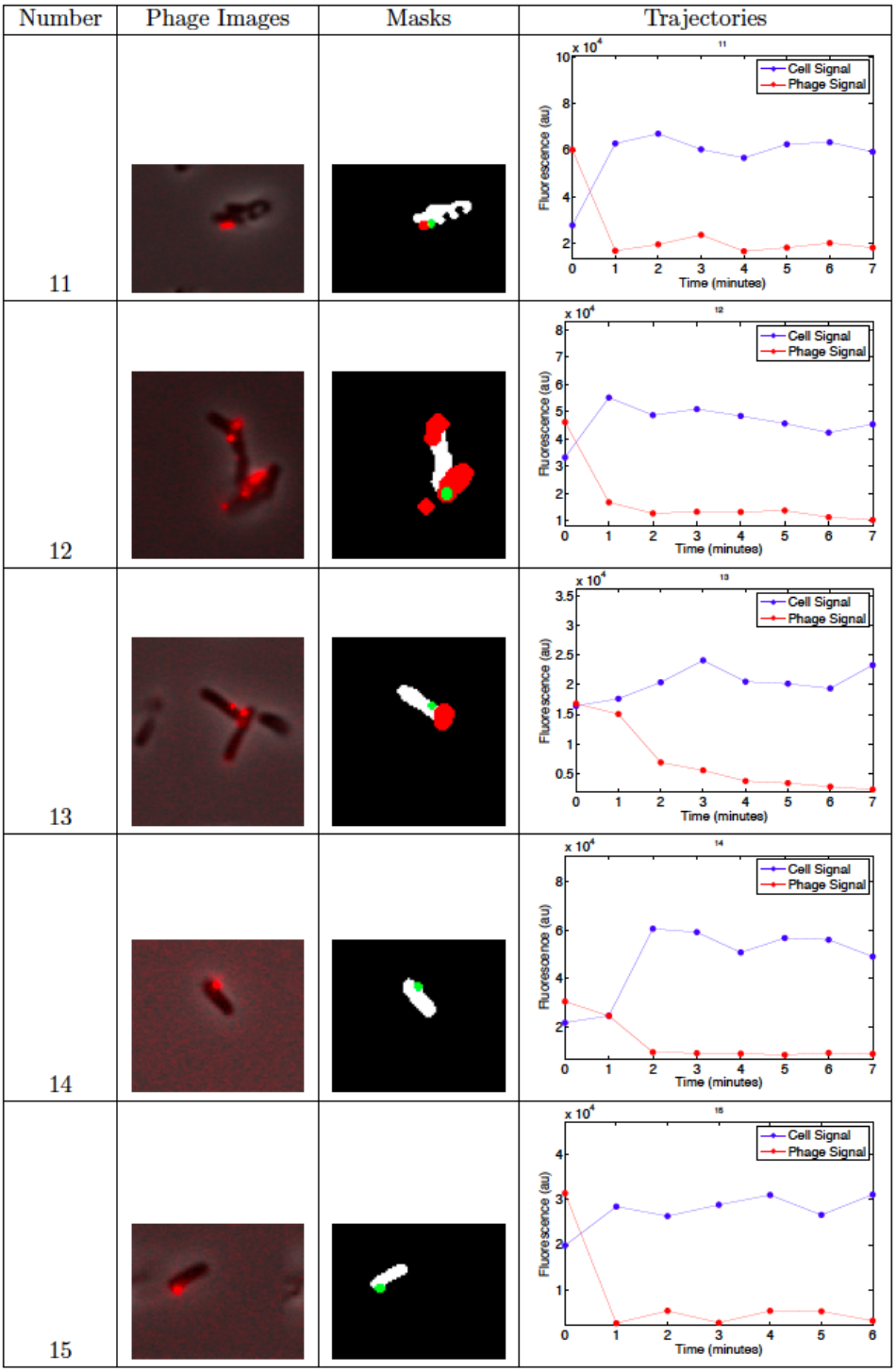

Figure S18. Single-molecule trajectories for λb221: Group 3. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

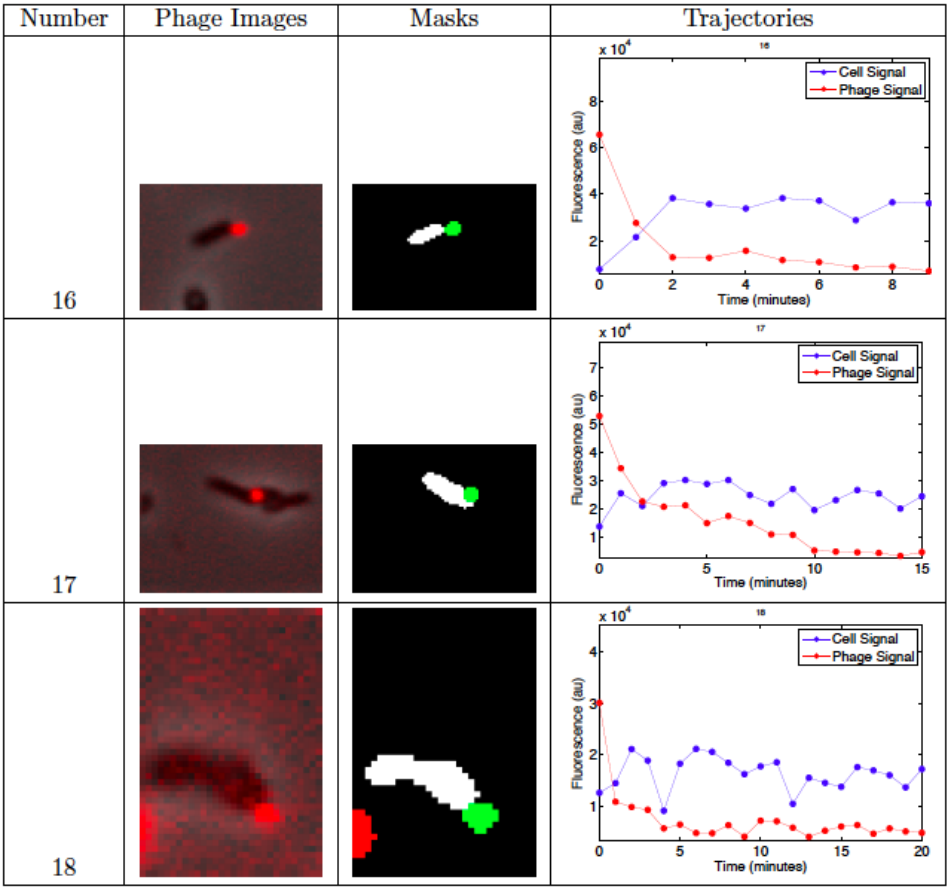

Figure S19. Single-molecule trajectories for λb221: Group 4. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

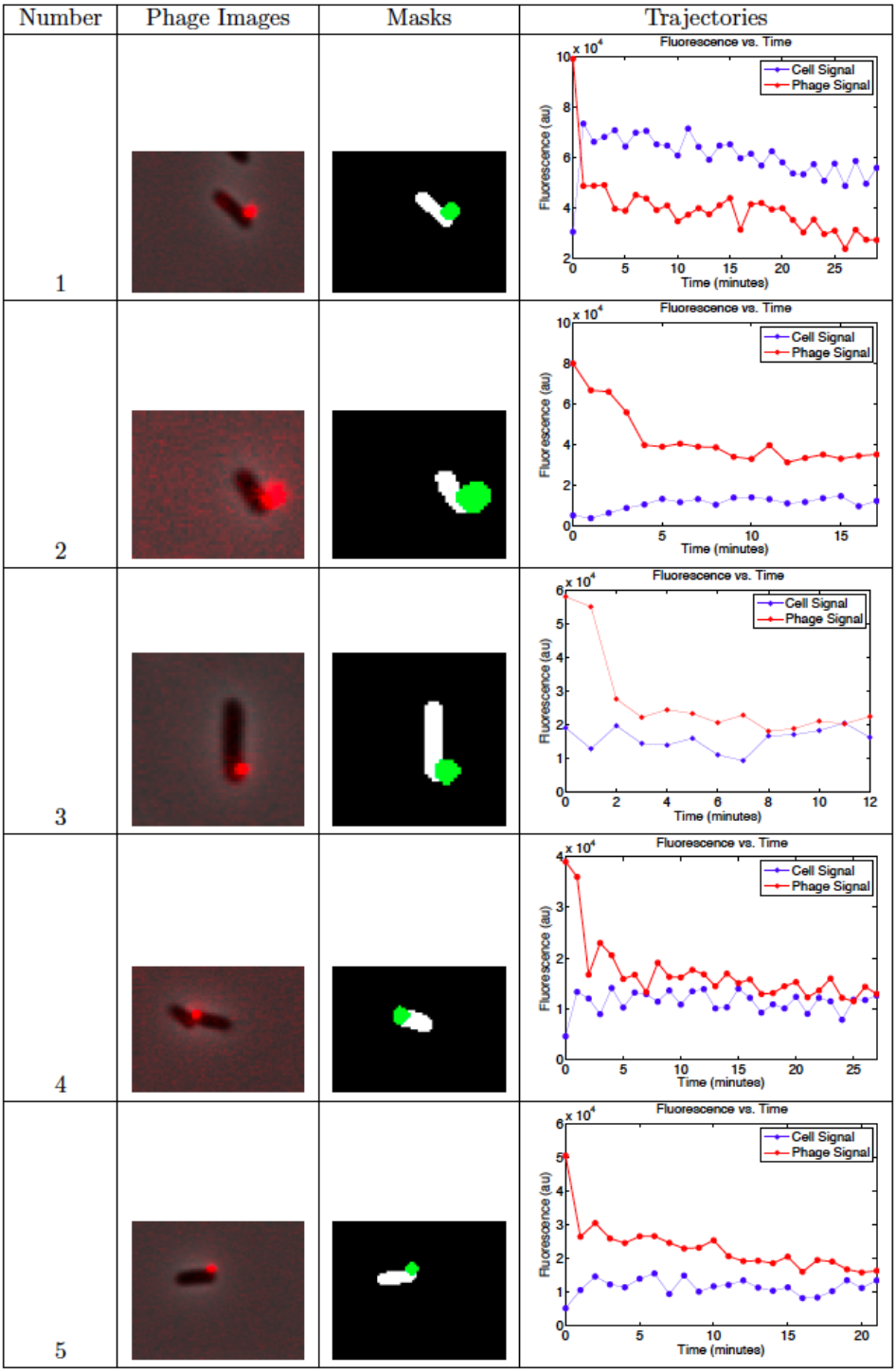

Figure S20. Single-molecule trajectories for λb221 (stalled): Group 1. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.

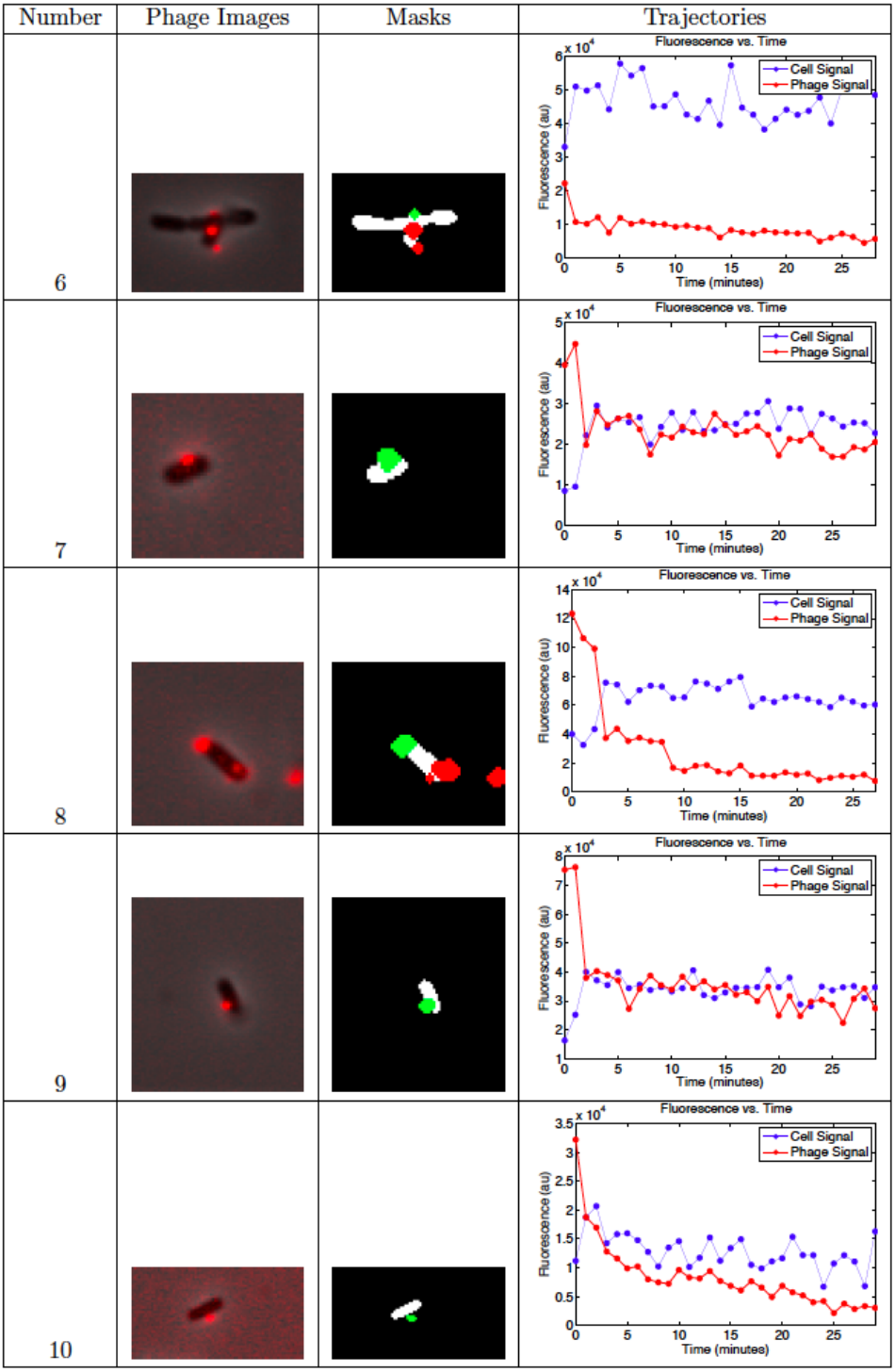

Figure S21. Single-molecule trajectories for λb221 (stalled): Group 2. Each one of the rows in this series of images shows the enumerated (1st column) phase and fluorescence image of a particular cell (2nd column) that has been segmented, the corresponding masks and the resulting fluorescence both from the virus-segmented region (non-ejecting phages have a red mask, ejecting phages have a green mask) and from the whole cell (white mask) (3rd column). The ejection curves (4th column) show the decrease in fluorescence in the ejecting phage (red) and the concomitant fluorescence change in the cell (blue). All images were scaled to a fixed width of 1 inch.}