Supporting Information

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SI Materials and Methods

Bromouridine (BrU) Transfection and Immunogold Electron Microscopy. To visualize early viral mRNAs, *Acanthamoeba* cells were infected and transfected with BrU at 2 h postinfection (PI). Cells were grown in six-well plates in PYG medium (2% Proteose Peptone, 0.1% yeast extract, 100 mM glucose) and infected with Mimivirus at 100 multiplicity of infection; 50 mM 5′-bromouridine 5′-trisphosphate (Sigma) was preincubated for 30 min in 110 μL of transfection medium containing 20 μL of Transfect reagent (Qiagen). Samples were diluted to 1 mL with transfection medium and added to the cells. After 30 min of incubation, cells were washed two times, incubated in PYG for 1.5 h, and fixed with a freshly prepared solution of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer containing 5 mM $CaCl_2$ for 1 h at room temperature. Fixed cells pellets were incubated in 10% gelatin at 37 °C for 30 min and then centrifuged; excess of gelatin was removed at 37 °C followed by postfixation in the same fixative at 4 °C for 24 h. Fixed cells pellets were cryoprotected by overnight infiltration with 2.3 M sucrose in cacodylate buffer and frozen by injection into liquid nitrogen. Then, ultrathin (~75 nm) frozen sections were cut with a diamond knife at 115 °C. Sections were transferred to formvar-coated 200 mesh nickel grids and treated with conditional medium (0.5% BSA, 3% normal goat serum, 0.1% glycine, and 1%Tween-20 in PBS) for 5 min followed by 2 h of incubation with mouse anti-bromouridine (BrU) antibodies (Sigma). After extensive washing in 0.1% glycine in PBS, the primary antibody was detected with goat anti-mouse 5 nm colloidal gold conjugate. Grids were then stained with 2% uranyl acetate in H_2O for 10 min and embedded in 2% methyl cellulose/uranyl acetate. Samples were viewed using a FEI Tecnai G^2 Bio-Twin operating at 120 kV. Images were recorded on an FEI Eagle CCD.

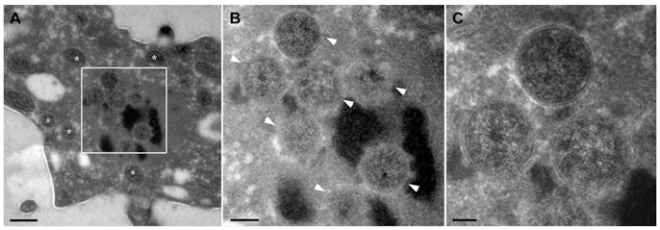


Fig. S1. Mimivirus cores in the host cytoplasm at early infection stages. *Acanthamoeba* cells were fixed and processed for transmission electron microscope (TEM) cryosectioning at 2 h PI. (*A*) TEM image of infected cell. Mitochondria are marked with white asterisks. (Scale bar, 500 nm.) (*B*) Magnification of the delineated area in *A* showing multiple cores (white arrowheads), several of which are undergoing DNA release and one (lower right corner) is surrounded by initial replication centers. (Scale bar, 200 nm.) (*C*) Magnification of the upper left region in *B* showing three viral cores. The structural similarity to Vaccinia Virus crescents (24) should be noted. (Scale bar, 100 nm.)

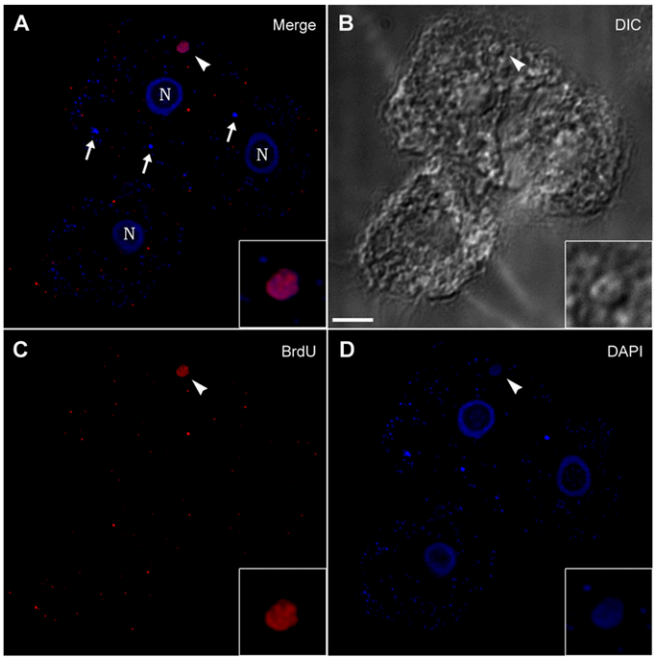


Fig. S2. Mimivirus replication occurs in the host cytoplasm. Amoeba cells infected with Mimivirus in the presence of bromodeoxyuridine (BrdU) were labeled with anti-BrdU antibody (red) and counterstained with DAPI (blue) at 2 h PI. (*A*) A merge view of 0.15-μm optical section depicting three infected cells. A cytoplasmic viral factory (white arrowhead; magnified in *Inset*) as well as three free viral cores (white arrows) are discernable. N, host nuclei. (*B*) Differential interference contrast (DIC) image of the cells in *A*. (*C* and *D*) Single-channel representations of the cells in *A*. The viral factory region is magnified in *C Inset* and *D Inset*. (Scale bar, 5 μm.)

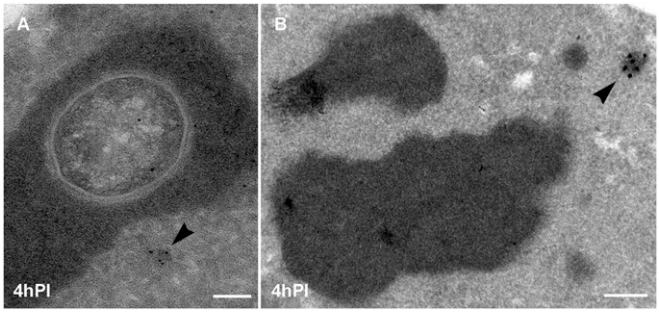
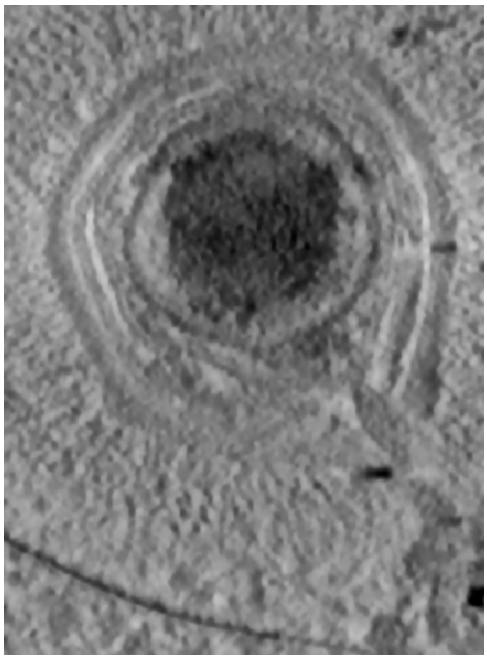
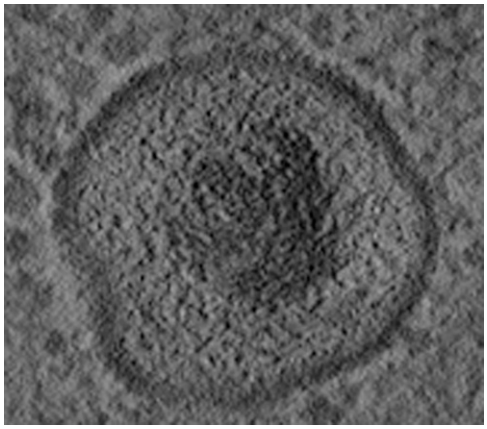


Fig. S3. Foci of newly transcribed RNA adjacent to viral cores. *Acanthamoeba* cells were infected and transfected with BrU for 30 min (from 2 h to 2.5 h PI), and they were processed for immuno-TEM at 4 h PI. (*A*) TEM image of a viral core surrounded with viral DNA. A BrU-positive focus (black arrowhead) is labeled with 5 nm colloidal gold-conjugated antibody. (Scale bar, 100 nm.) (*B*) TEM image of an early replication factory. A BrU positive focus (arrowhead) is labeled with 15 nm gold-conjugated antibody. (Scale bar, 200 nm.)



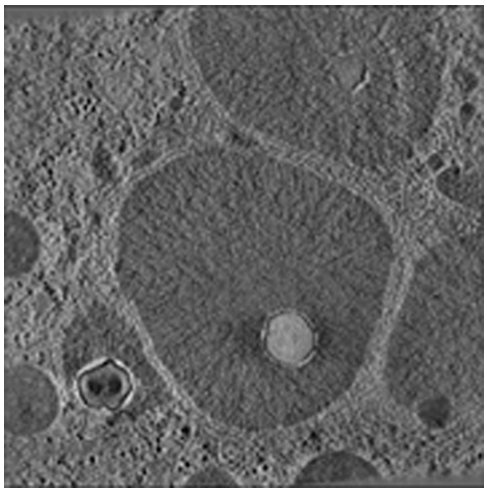
Movie S1. Electron tomogram of Mimivirus particle enclosed within a phagosome and undergoing initial uncoating accompanied by inner membrane protrusion (Fig. 2*A*).

Movie S1.



Movie S2. Electron tomogram of a free Mimivirus core in the host cytoplasm (Fig. 2*D*).

Movie S2.



Movie S3. Electron tomogram of three early cytoplasmic viral factories surrounding three cores at various stages of genome release, showing partially and completely empty cores (Fig. 2*G*).

Movie S3.