SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. A-C) RAW264.7 macrophages were labeled with calcein blue then exposed for 20 min to live *B. cenocepacia* expressing RFP (red in A and B), at a multiplicity of infection of 60 bacteria/cell. The dye FM1-43 (green in B and C) was added to the cells in cold solution immediately before imaging by spinning-disc microscopy. FM1-43 is a membraneimpermeant solvochromic dye that labels the extracellular bacteria (arrowheads), as well as the plasma membrane and its invaginations (asterisks in C). Note that intracellular bacteria, i.e. those generating voids in the calcein blue pattern, were not stained by FM1-43. Size bar = 10 μm.

Supplementary Figure 2. *Upper panels:* Representative transmission electron micrographs of RAW macrophages infected with live *B. cenocepacia.* Insets are enlarged sections of the areas indicated by dotted lines. Size bars = 5 μm. The top left panel is the same micrograph shown in Fig. 1D, but highlighting different BcCV. *Lower panel:* The fraction of live or dead *B. cenocepacia* inside vacuoles (i.e. membrane-lined and surrounded by an electron-lucent area distinct from the cytosol) was determined from electron micrographs like those shown above. The number of BcCV analyzed in each case is indicated in parenthesis.

Supplementary Figure 3. RAW macrophages transfected with PX-GFP (green) were infected with *B. cenocepacia* (red) and fluorescence images were acquired using spinning-disc microscopy at the indicated times after infection. Note the transient acquisition of PX-GFP, a probe for PI(3)P. Size bar = 10 μm.

Supplementary Figure 4. *Effect of MOI on active Rab7 acquisition and lysosome fusion.* Macrophages transfected with cDNA encoding GFP-tagged RILP-C33 were pre-loaded with Alexa 647-conjugated dextran (blue), then infected with different MOIs of either live *B. cenocepacia* or live *E. coli* expressing RFP for 20 min, followed by 100 min of maturation. *Top panels:* Representative confocal images. When cells are infected at a MOI=10 a sizable fraction of BcCV containing live *B. cenocepacia* fail to acquire RILP-C33, an indicator of active Rab7 (left) and do not fuse with lysosomes (right). *Lower panels:* RAW cells were infected with live *E. coli* or *B. cenocepacia* at the indicated MOI (abscissa). The fraction of RILP-C33-GFPpositive BcCV (left) or dextran-positive vacuoles (right) containing *E. coli* (open bars) or *B. cenocepacia* (black bars) was quantified in 3 similar experiments, each counting at least 10 infected cells. Size bar = 10 μm.

Supplementary Figure 5. *Assessment of active Rab7 acquisition by vacuoles formed by B. cenocepacia opsonized with heat-inactivated serum.* Macrophages transfected with cDNA encoding GFP-tagged RILP-C33 were infected with live *B. cenocepacia* (red), opsonized with heat-inactivated human serum (56ºC for 45 min) for 20 min and allowed to mature for a further 100 min. Arrows point to BcCV devoid of RILP-C33; arrowheads point to RILP-C33-positive BcCV. Size bar = 10 μm.

Supplementary Figure 6. A-B) Effect of GFP-Rab7Q67L on the fusion of BcCV with lysosomes. Macrophages were transfected with cDNA encoding GFP-Rab7Q67L (green), then pulsed with 50 μg/mL of Alexa 647-conjugated dextran (blue) for 16 h. Opsonized live bacteria expressing RFP (red) were exposed to the macrophages at an MOI of 60 for 20 min, followed by 100 min of maturation. Size bar = 10 μm, (C) The fraction of BcCVs containing live bacteria that merged with lysosomes containing Alexa 647-dextran were quantified in cells transfected

with either wild-type GFP-Rab7 or GFP-Rab7Q67L. For comparison, the fraction of BcCV with dead bacteria that fuse with lysosomes is also shown. Data are means of 3 experiments ± SE. The difference between BcCV containing live and dead bacteria in C is highly significant ($p<0.01$).