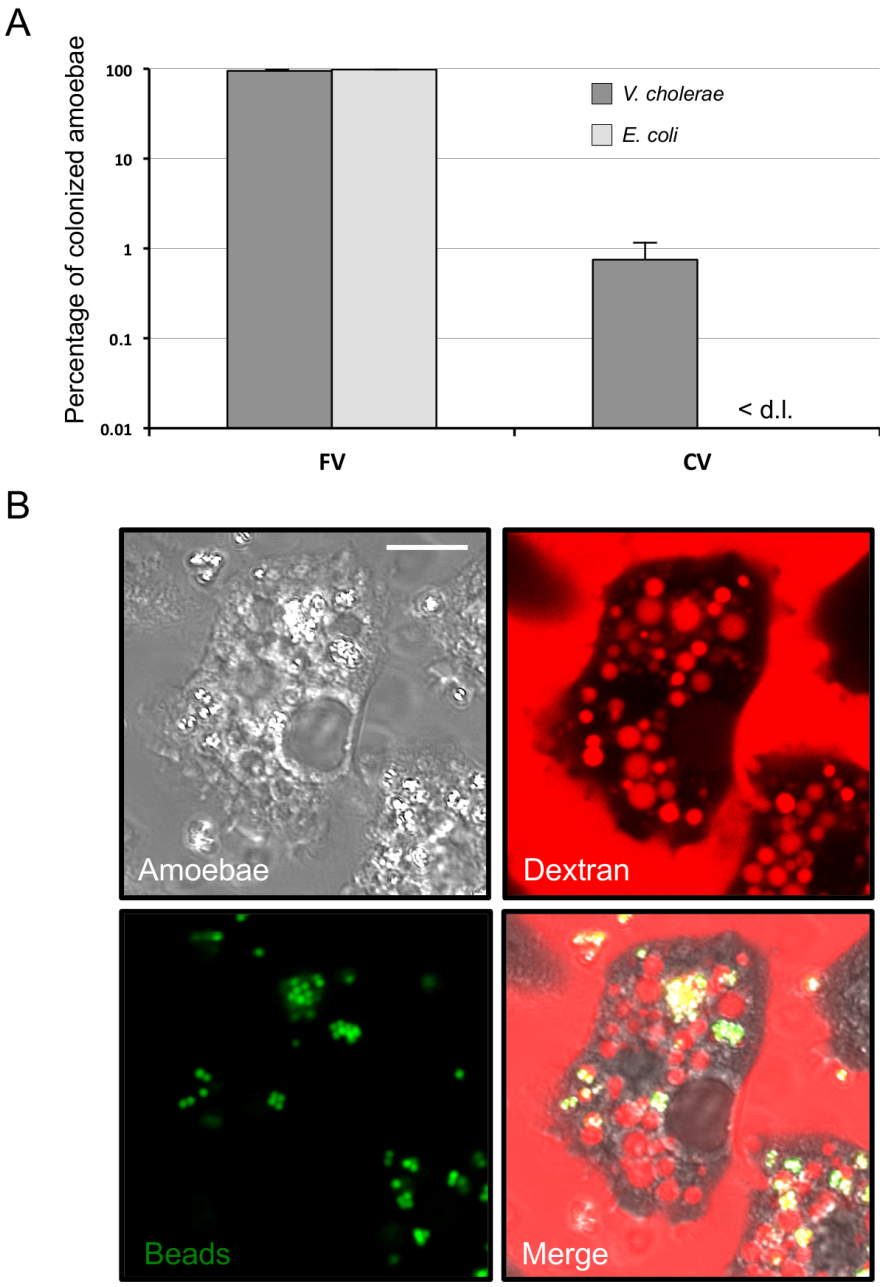
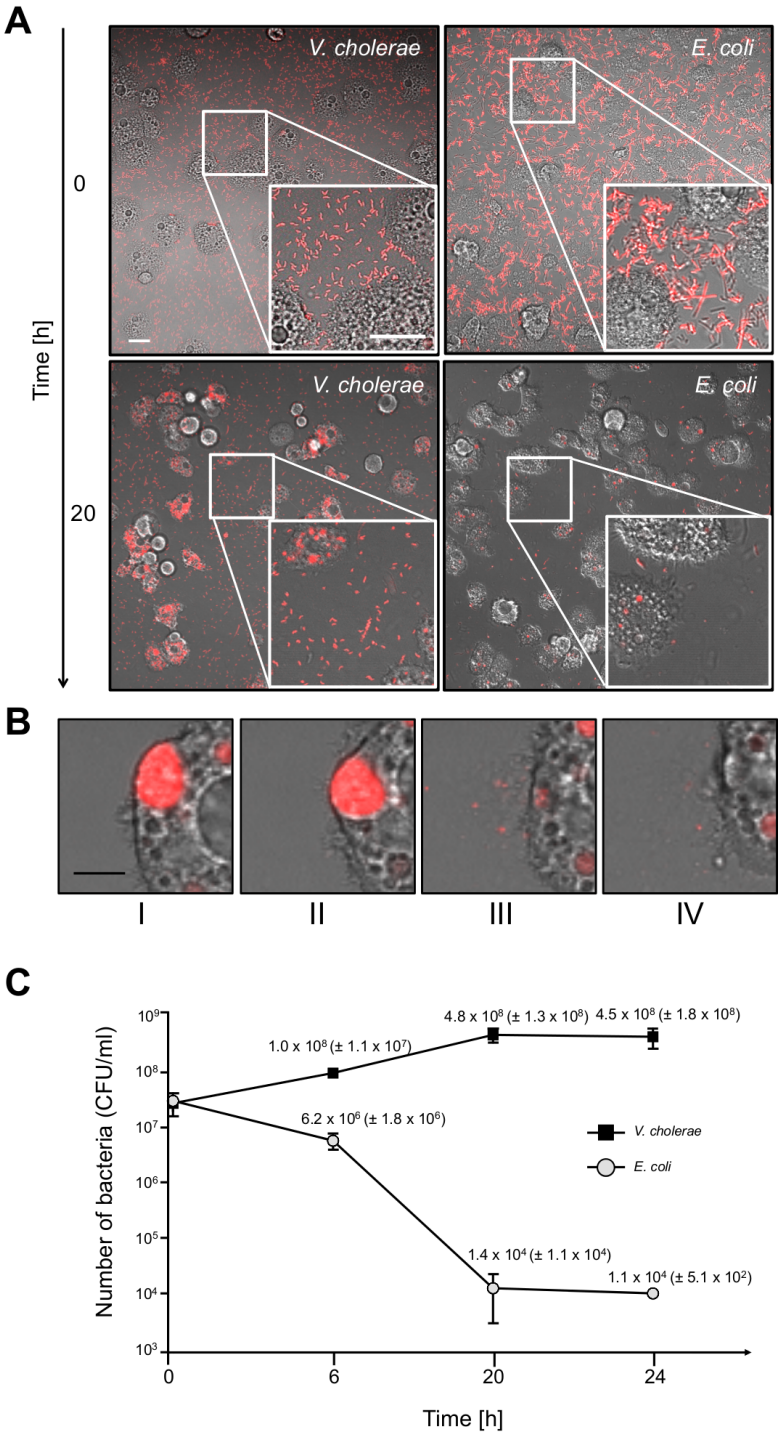
Supplementary Material

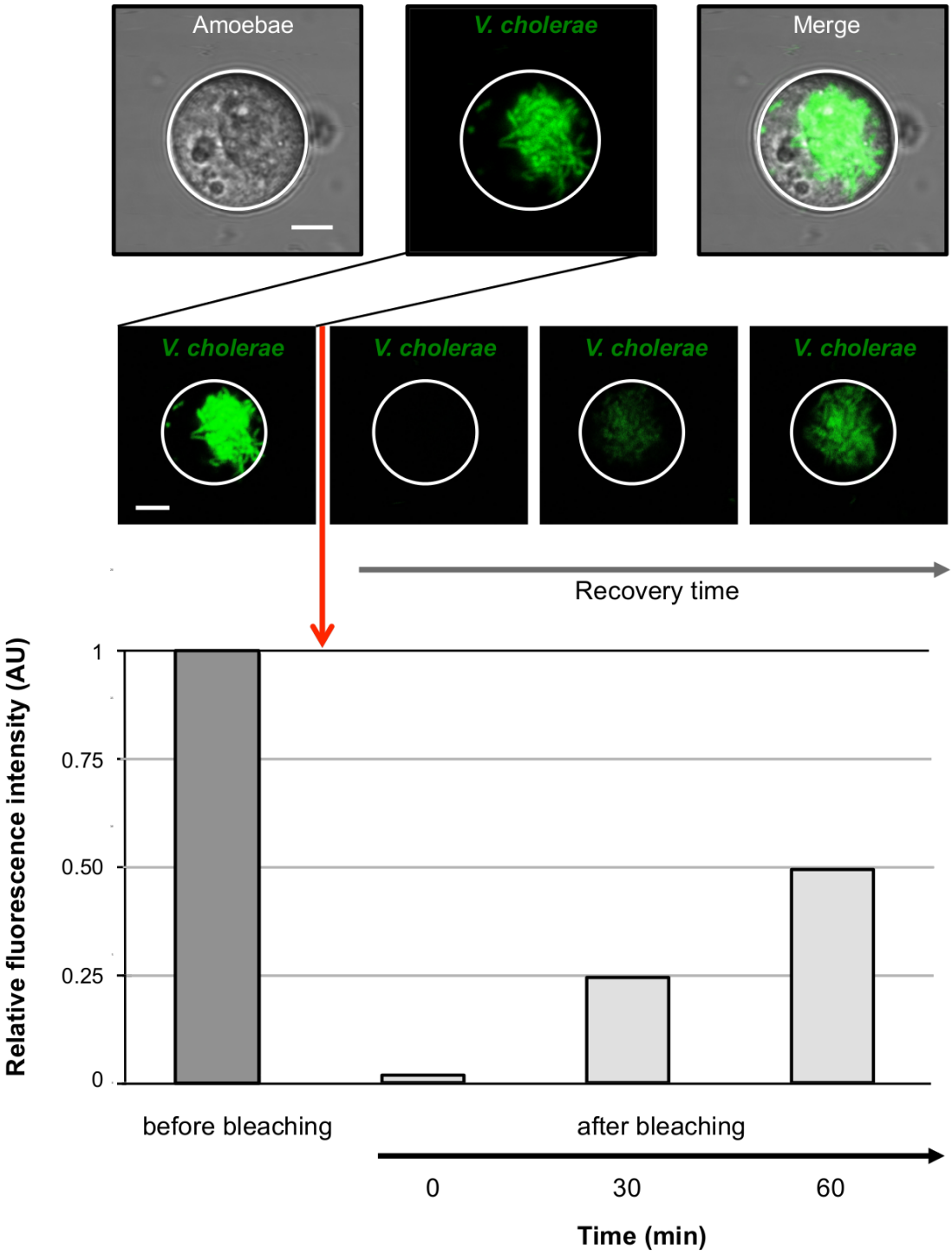
Supplementary Figures



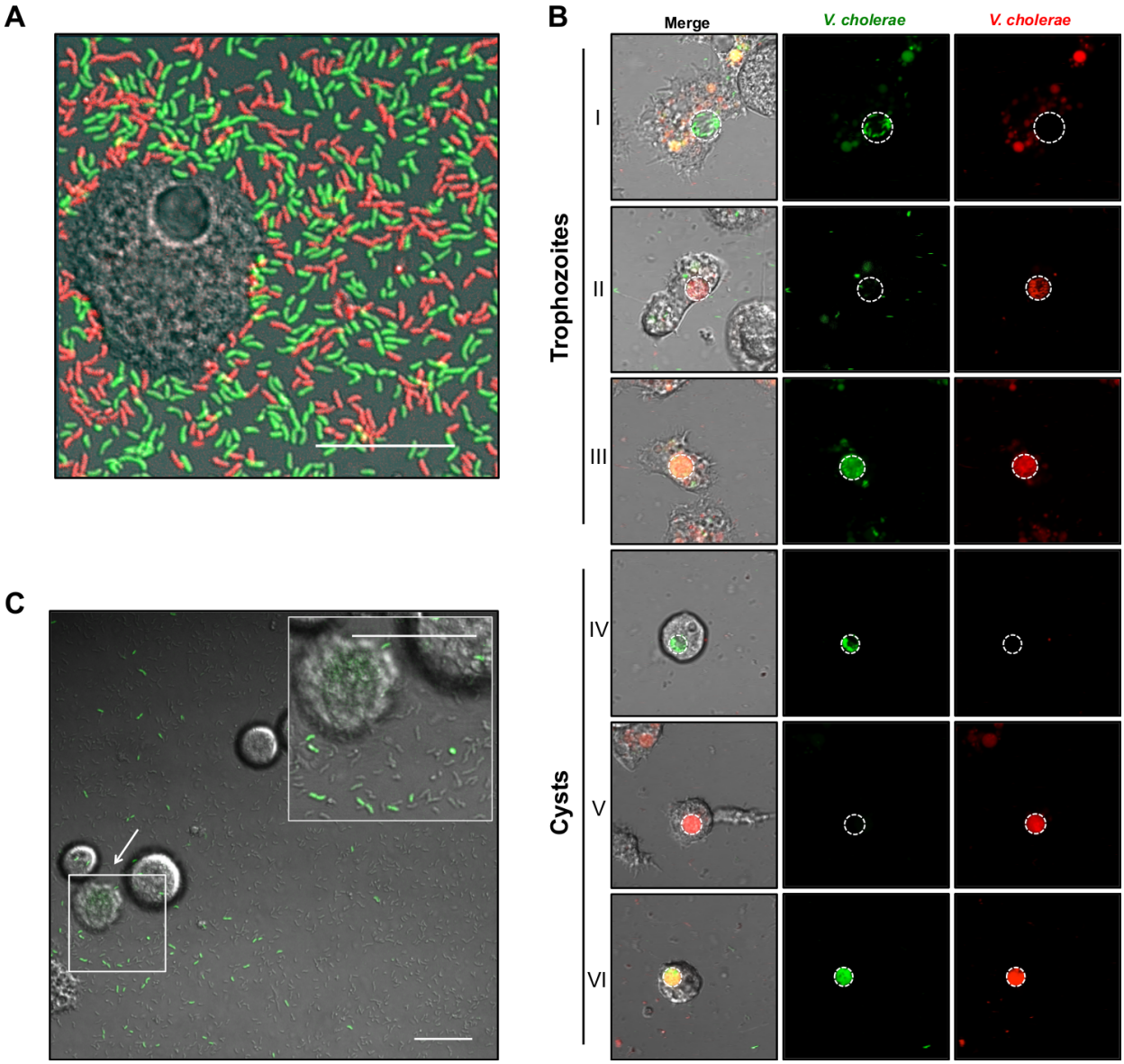
Supplementary Figure S1. Intracellular localization of *V. cholerae* and *E. coli* after phagocytosis by *A. castellanii*. (A) Quantification of colonized amoebae containing bacteria (*V. cholerae* and *E. coli*) inside food vacuoles (FV) and the contractile vacuole (CV). < d.l., below detection limit of 0.027%; additional visual inspection of 10^5 amoebae for *E. coli*-colonized CVs confirmed these data (d.l. < 0.001% in three independent experiments). (B) Fluorescent beads follow the phago-/endosomal pathway. Confocal images showing the co-localization of Fluoresbrite Yellow Green (YG) microspheres and Alexa 647-conjugated dextran (MW=10,000 Da). Image arrangement: transmission light image (labeled Amoebae), far-red channel (labeled Dextran), green channel (labeled Beads) and a merged image of all channels (labeled Merge). Scale bar: 10µm.



Supplementary Figure S2. *A. castellanii* digests phagocytosed *E. coli*. (A) Confocal images of *dsRed*-expressing *V. cholerae* and *E. coli* at the time of initial contact with the amoebae and 20h later. The time is indicated on the left. *V. cholerae* bacteria are detected around and inside amoebae at 20h p.p.c., whereas undigested extracellular *E. coli* cells are rarely detectable. Scale bars: 20µm. (B) Time-lapse confocal microscopy image series showing the exocytosis of a food vacuole without the release of intact *E. coli*. The relative time points (starting at 14h p.p.c.) are: I, t=0; II, t=90s; III, t= 180s and IV, t=300s. Scale bar: 10µm. (C) Kinetics of bacterial killing by *A. castellanii*. Survival was assessed by CFU enumeration of extra- and intracellular bacteria after 6h, 20h and 24h p.p.c., as indicated on the X-axis.



Supplementary Figure S3. **Bacterial viability within the amoebal CV.** Fluorescence recovery after photobleaching indicates intracellular viability of *V. cholerae*. A GFP-producing WT strain was incubated for 17h with *A. castellanii* before a colonized CV was bleached as described in the materials and methods section. The recovery of the GFP signal (shown on the Y-axis) within the bacteria was monitored over 60 min as indicated on the X-axis. Scale bar: 5µm.



Supplementary Figure S4. Intracellular growth of *V. cholerae*. (A+B) Visualization of intracellular proliferation based on a mixed-color inoculation procedure. *V. cholerae* WT strains (GFP- and dsRed-producing) were mixed at a ratio of 1:1 and co-cultured with *A. castellanii*. (A) Enlarged image of *gfp*- and *dsRed*-expressing *V. cholerae* surrounding *A. castellanii* at t=0h. (B) Confocal images showing single-colored CVs within trophozoites (I, II) or cysts (IV, V) indicating the proliferation of a single or very few bacteria at 20h p.p.c. Mixed-color CVs (III, VI) were also observed (see Table 2 for quantification). Images from left to right: merged image of the two fluorescent channels and the transmitted light channel (amoebae), green channel (green-written *V. cholerae*), and red channel (red-written *V. cholerae*). (C) Release of growing bacteria from lysed cysts. The amoebae were co-incubated with the *V. cholerae* growth reporter strain for 24h. The lysis of the CV followed by the disintegration of the cyst (white arrow) results in the release of actively growing *gfp*(ASV)-expressing *V. cholerae*. Note the extracellular and non-fluorescent bacteria in the surrounding area. Scale bar for all panels: 20µm.

Supplementary Movie legends

Supplementary Movie S1. Internalization of *V. cholerae* by *A. castellanii*. Time-lapse imaging of a GFP-producing WT *V. cholerae* strain co-cultured with *A. castellanii*. The movie starts at the first point of contact (t=0h) and lasts for 5h as indicated in the upper left corner. Shown are the merged signals between the transmission light channel (amoebae) and the GFP confocal channel (fluorescent *V. cholerae* cells).

Supplementary Movie S2. Release of undigested *V. cholerae* cells from *A. castellanii*. Time-lapse confocal microscopy imaging starting at 4.5h p.p.c. (t=0h, as indicated in the upper left corner). Shown are merged images of the transmission light (amoebae) and GFP signals (GFP-producing *V. cholerae*). The white boxed-area and the arrow highlight the release of undigested *V. cholerae*. See also Figure 2.

Supplementary Movie S3. Colonization of the contractile vacuole (CV). Time-lapse imaging as described for Supplementary Movie S2 starting at 5h p.p.c. The movie shows the vacuolar fusion and the bacterial transfer from digestive food vacuoles into a CV. See also Figure 3B.

Supplementary Movie S4. CV lysis releases *V. cholerae* into the cyst's cytosol. Time-lapse imaging starting at 16h p.p.c. (relative time t=0h as indicated in the left corner). Image arrangement as for Figure 4.

Supplementary Movie S5. Viability of *V. cholerae* within the cyst's cytosol. The time-lapse is a continuation of Supplementary Movie S4 (~ 18h p.p.c.) showing the motility of the bacteria inside the cytosol of the cyst. An increase of the fluorescent signal occurs over time. Image arrangement as for Figure 4.

Supplementary Movie S6. Lysis occurs after disruption of the *V. cholerae*-containing cyst. The time-lapse is a continuation of Supplementary Movie S5 (~21h p.p.c.) showing the release of *V. cholerae* cells into the surrounding, followed by the influx of dextran into the lysed cyst. Image arrangement as for Figure 4.

Supplementary Table S1: Quantification of colonized CVs.

***A. castellanii* +**	**Percentage of colonized CVs[#]**
E. coli	0% (± 0)
V. cholerae	1.0% (± 0.14)
beads	0% (± 0)
beads (in the presence of *E. coli*)	0% (± 0)
beads (in the presence of *V. cholerae*)	0.4 % (± 0.10)

[#] Percentage values are averages derived from three independent biological experiments (± SD). 4400 amoebae were counted in total for each condition.