Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

An *in vivo* microfluidic study of bacterial transit in *C. elegans* nematodes

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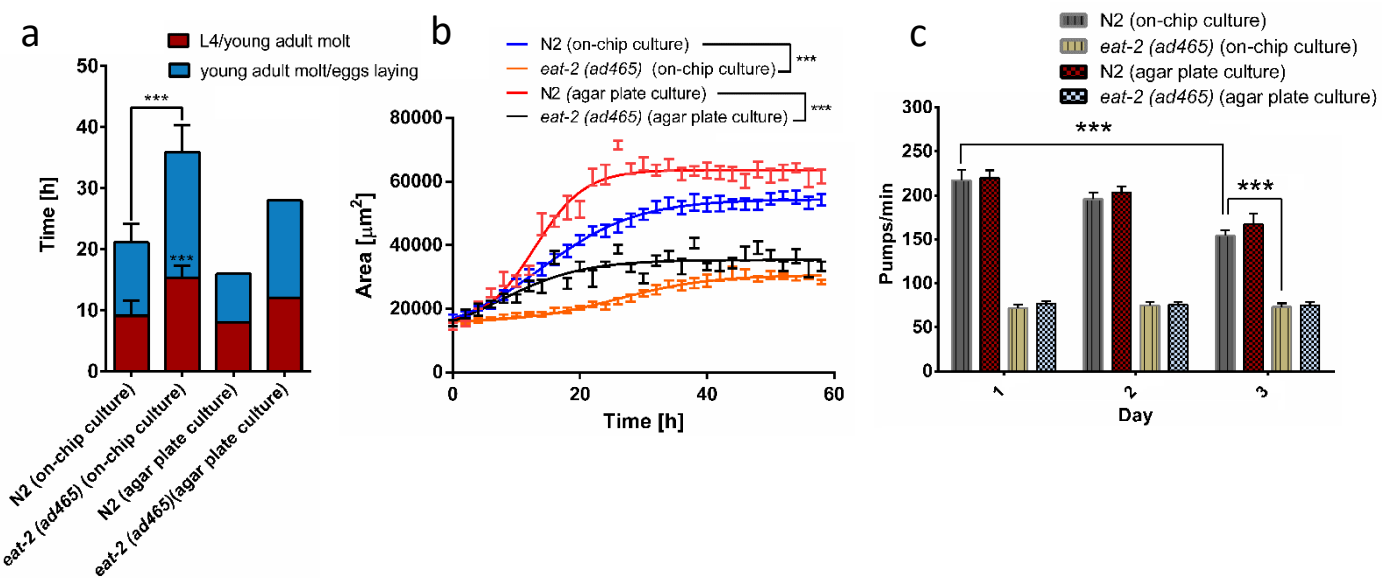

Fig. S1 (Supplementary). Monitoring of development parameters of N2 worms and *eat-2 (ad465)* mutants for on-chip and agar plate culture. a) Development time of N2 worms and *eat-2 (ad465)* mutants for two different stages from L4 to adulthood. Free-swimming worms have been maintained in the culture part of the microfluidic chamber and were continuously perfused with *E. coli* HT115 (n = 17). b) Body area evolution over time (starting from L4 stage) for N2 worms and *eat-2 (ad465)* mutants, measured by optical microscopy (data bars in the graphs express mean ± SEM ,*** $p \leq 0.001$, n = 17). c) Pharyngeal pumping frequency recorded at Day 1, Day 2 and Day 3 after L4 stage (bar graphs are expressed as mean ± SD, *** $p \leq 0.001$, n = 10).

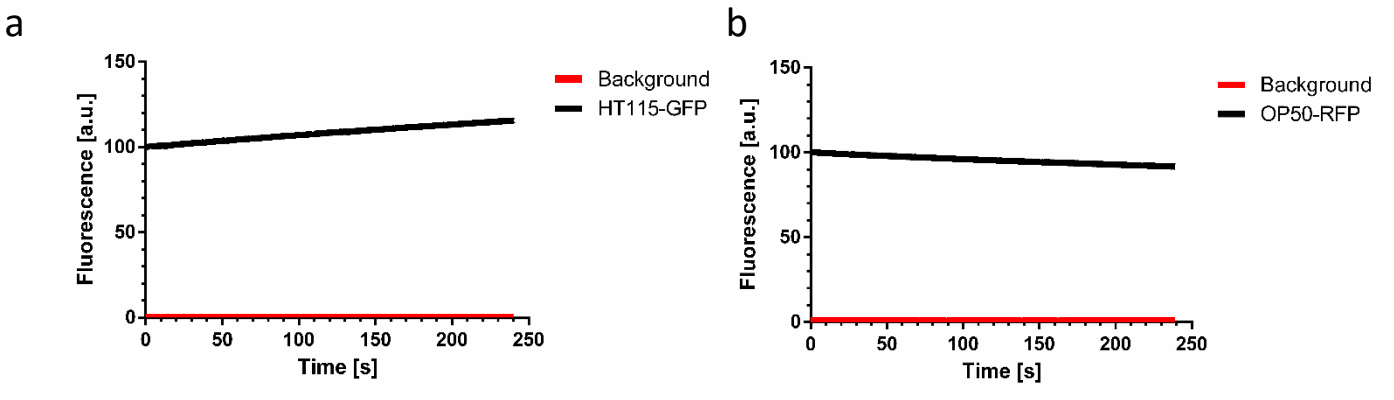

Fig. S2 (Supplementary). Monitoring of the fluorescence signal expressed by HT155 GFP (a) and OP50 RFP (b) *E. coli* bacteria in order to assess photo-bleaching of the fluorophores. Frames were acquired for a total duration of 240 s with a sampling interval of 200 ms. Excitation wavelengths selected for HT115 GFP and OP50 RFP *E. coli* bacteria are 488 nm and 545 nm, respectively. Photo-bleaching did not significantly affect the fluorescence signal expressed by the plasmid in both cases. Accordingly, the same parameters were selected to perform bacterial load measurements on worms.

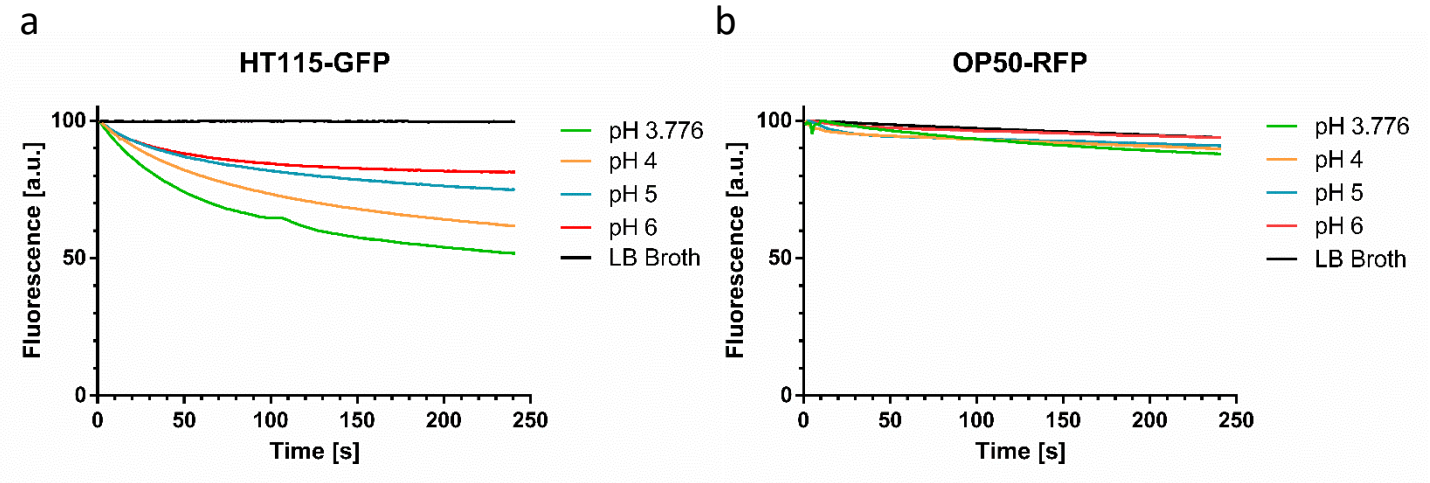

Fig. S3 (Supplementary). Monitoring of fluorescence expressed by HT155 GFP (a) and OP50 RFP (b) *E. coli* bacteria suspended in buffer solutions ranging from pH 3.776 to 6 in order to assess the fluorophores sensitivity to pH. 50 µl of bacterial suspension was diluted and mixed in 1 ml of buffer solution with known pH value and fluorescence was measured over 240 s. pH values in the intestine of viable *C. elegans* worms ranges from 5.96 in the pharynx to 3.59 in the posterior intestine, buffer solutions pH values were selected accordingly.

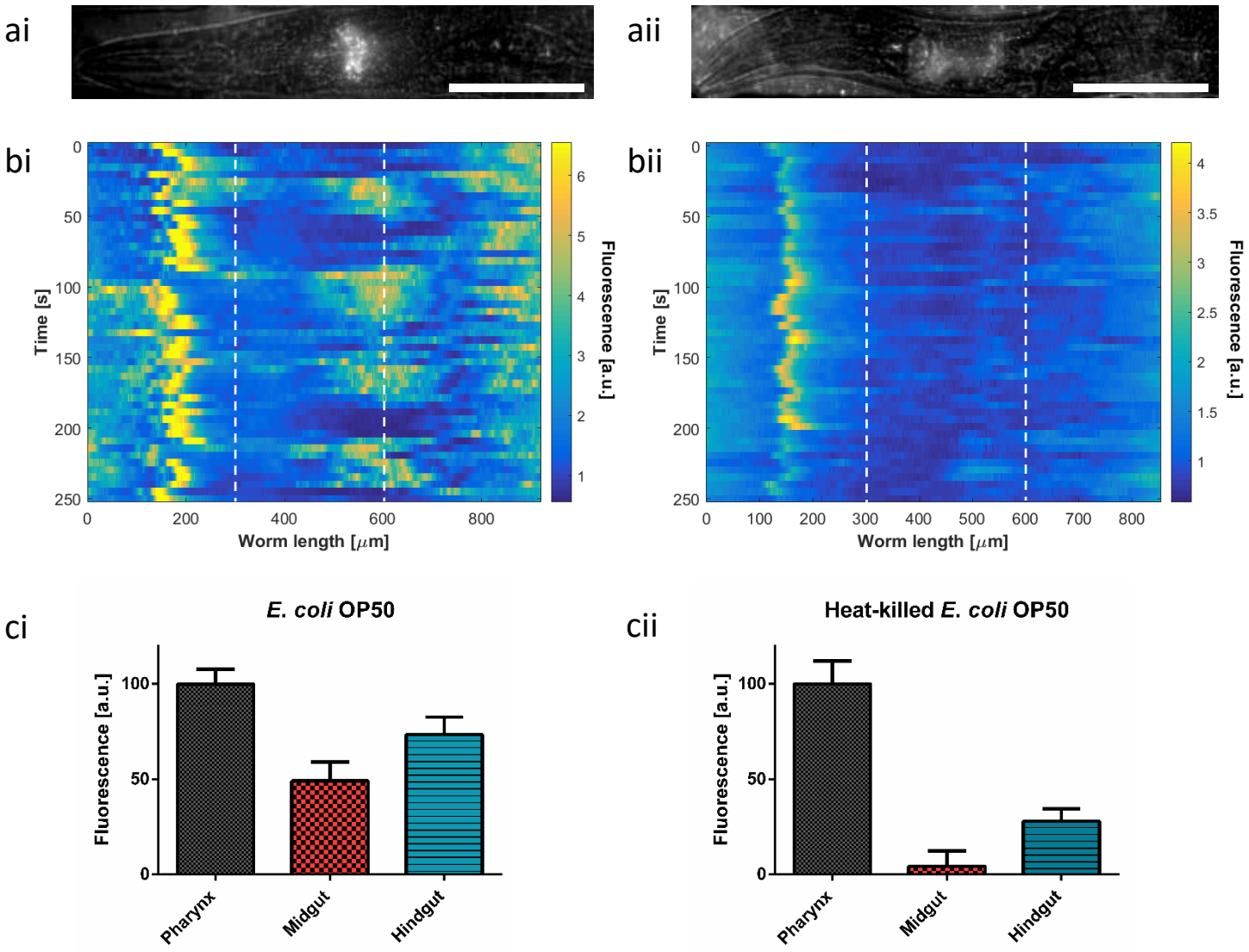

Fig. S4 (Supplementary). Analysis of the bacterial load in adult wild-type *C. elegans* worms fed with live and heat-killed *E. coli* OP50 RFP. a) High-resolution brightfield/fluorescence (40x) images of a N2 worm (pharynx) fed with live (ai) and heat-killed (aii) *E. coli* OP50 RFP. Scale bar = 50 μm. b) Time-lapse recordings of the fluorescence intensity in a representative single N2 worm over the whole gut. The worm was fed with live (bi) and heat-killed (bii) *E. coli* OP50 RFP, respectively. Pharynx, midgut and hindgut regions of the worms are delimited by vertical dashed lines in the plots (l_{worm} = 900 μm). c) Bacterial load as measured by fluorescence of the worm gut in adult N2 fed with live (ci) and heat-killed (cii) *E. coli* OP50 RFP. Bars correspond to average fluorescence values measured in the indicated intestine section, normalized with respect the maximum fluorescence value measured in the pharynx. Heat-killed *E. coli* OP50 RFP show a lower persistency in the posterior section of the worms' gut (graphs are expressed as mean ± SD, n = 10).

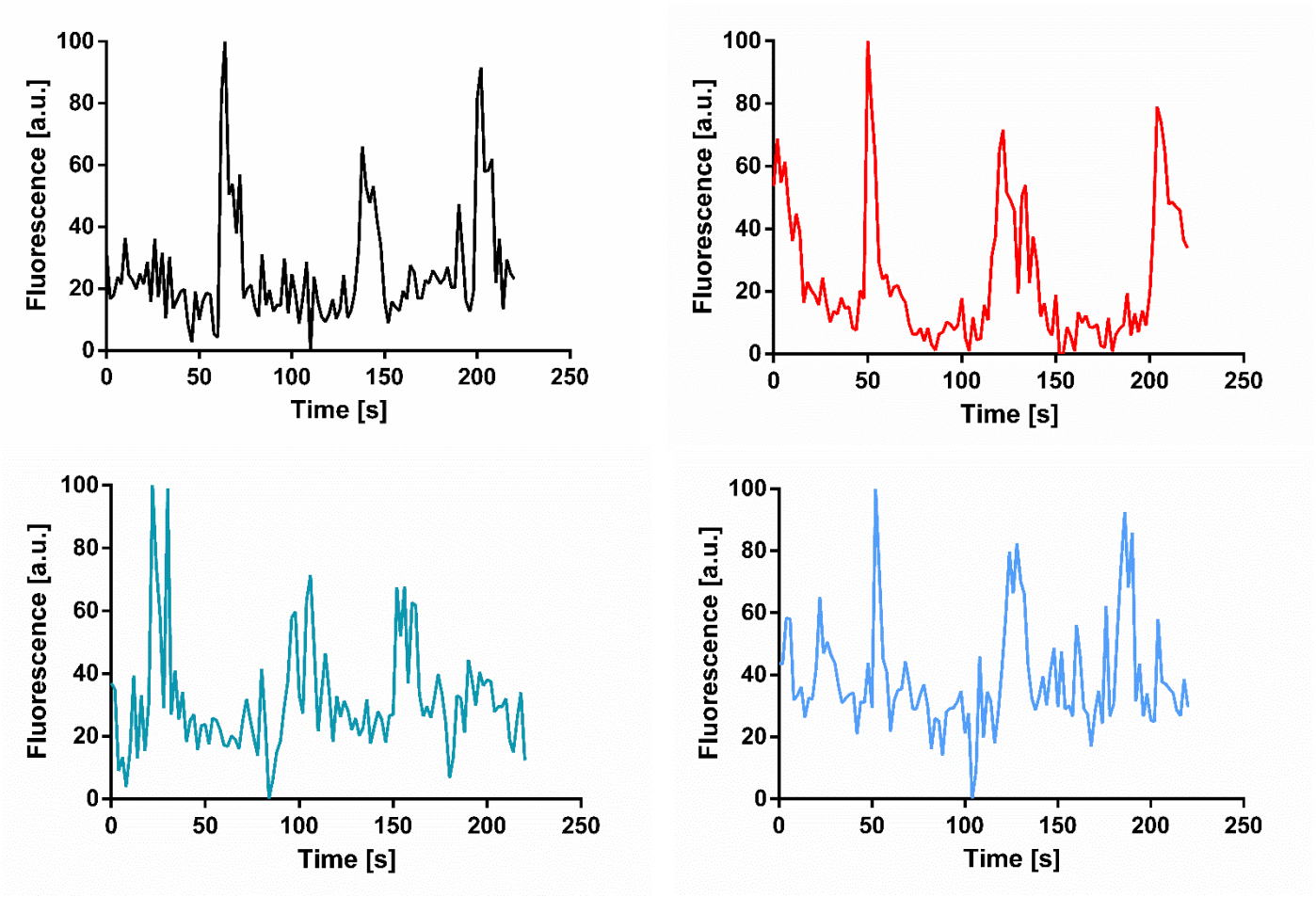

Fig. S5 (Supplementary). Average fluorescence signal corresponding to the hindgut region for 4 representative worms fed with *E. coli* HT115 GFP bacteria. The signals have been normalized for analysing the periodicity by Fast Fourier Transform (as shown in Fig. 3).

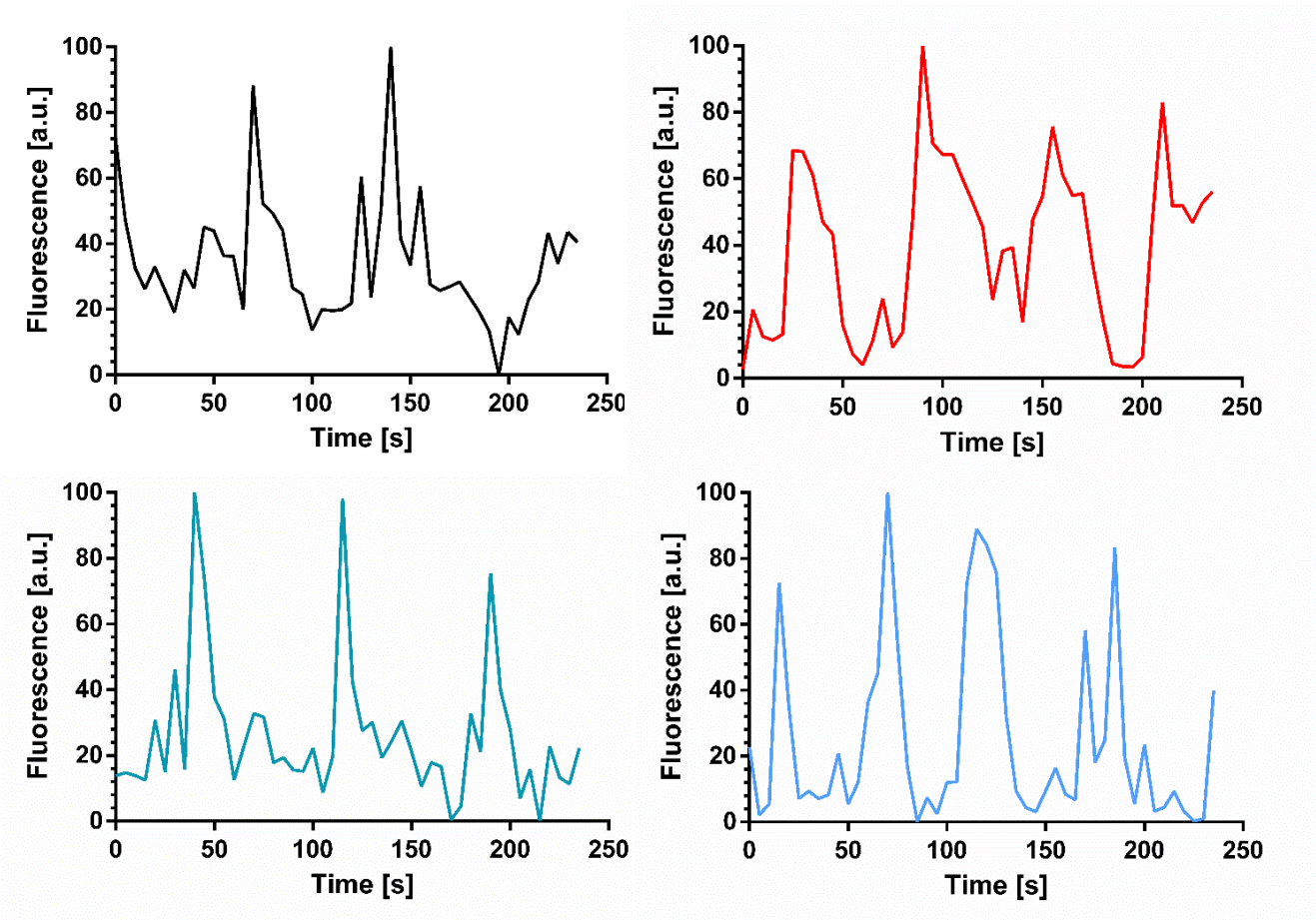

Fig. S6 (Supplementary). Average fluorescence signal corresponding to the hindgut region for 4 representative worms fed with *E. coli* OP50 RFP bacteria. The signals have been normalized for analysing the periodicity by Fast Fourier Transform (as shown in Fig. 3).

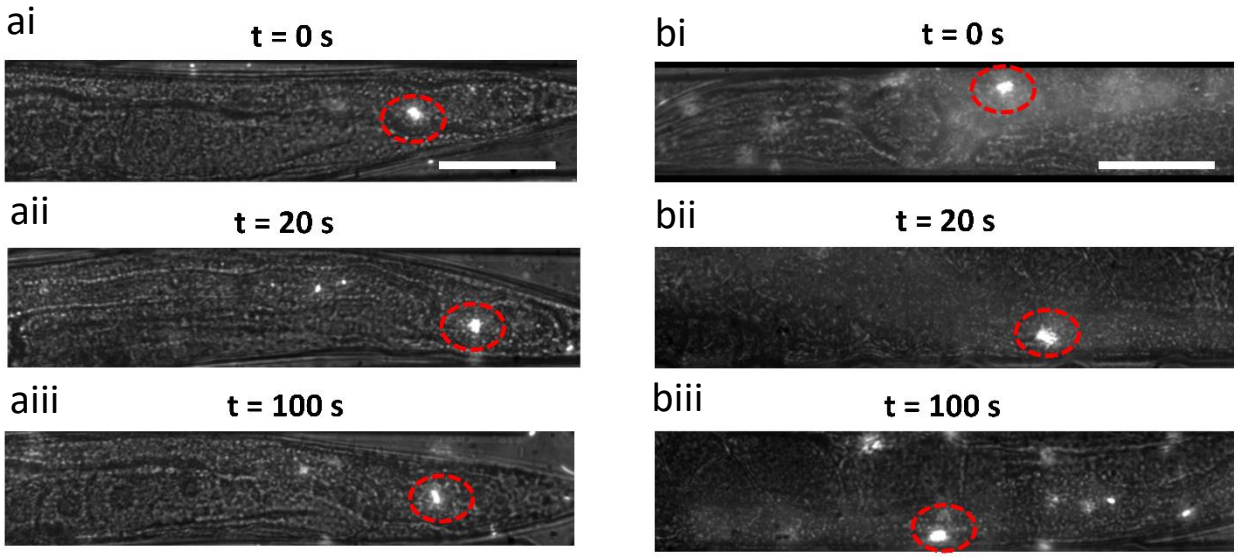

Fig. S7 (Supplementary). High-resolution brightfield/fluorescence (40x) time-lapse images of an immobilized N2 *C. elegans* worm fed with *E. coli* HT115 GFP. The image sequences show: (a) a few bacteria persisting in the region of the hindgut and (b) a few bacteria passing through the whole intestinal lumen, in particular the pharynx (bi), the midgut (bii) and the hindgut (biii). Single bacteria are highlighted by red dashed circles. Scale bar = 50 μm.