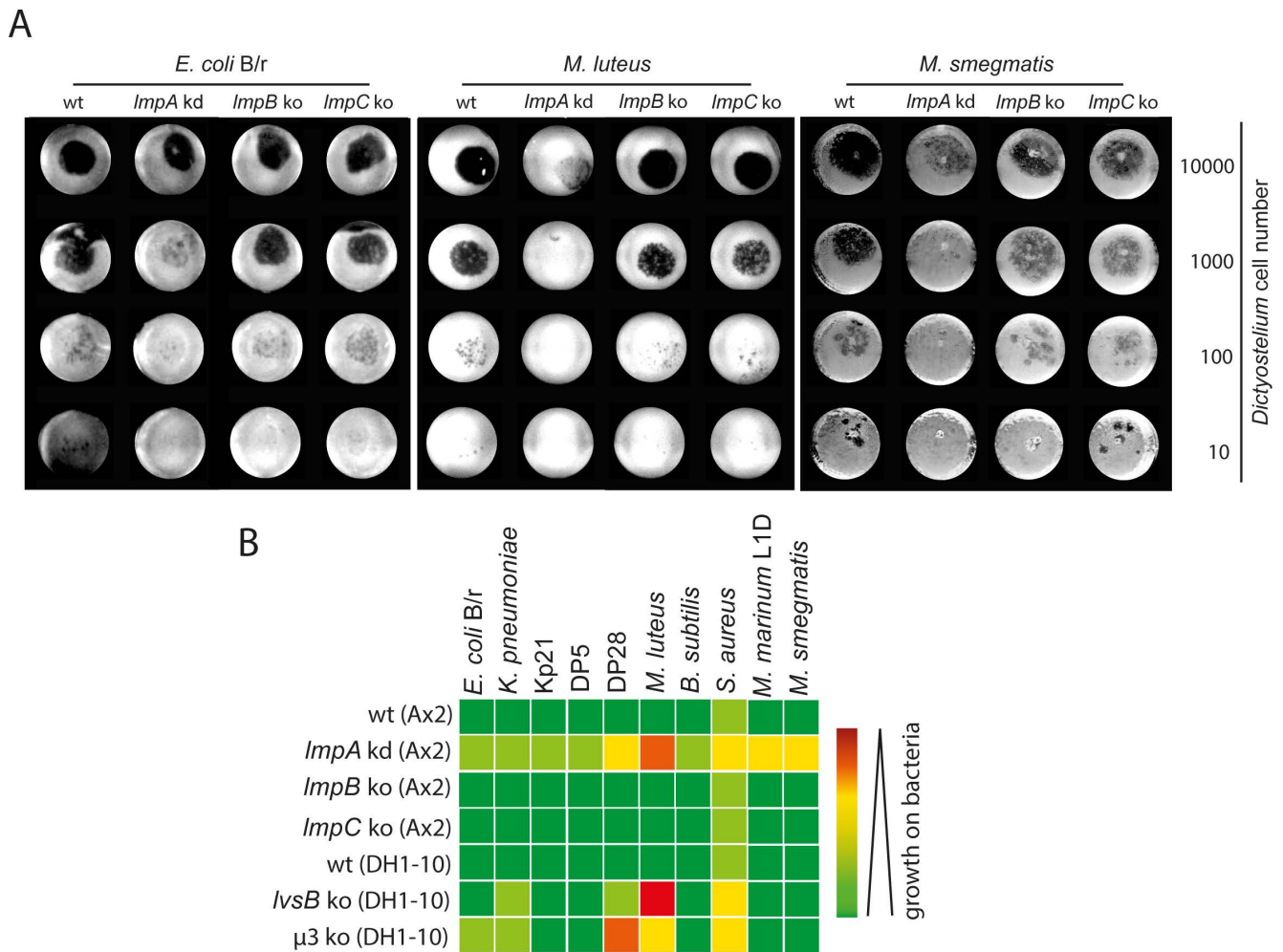
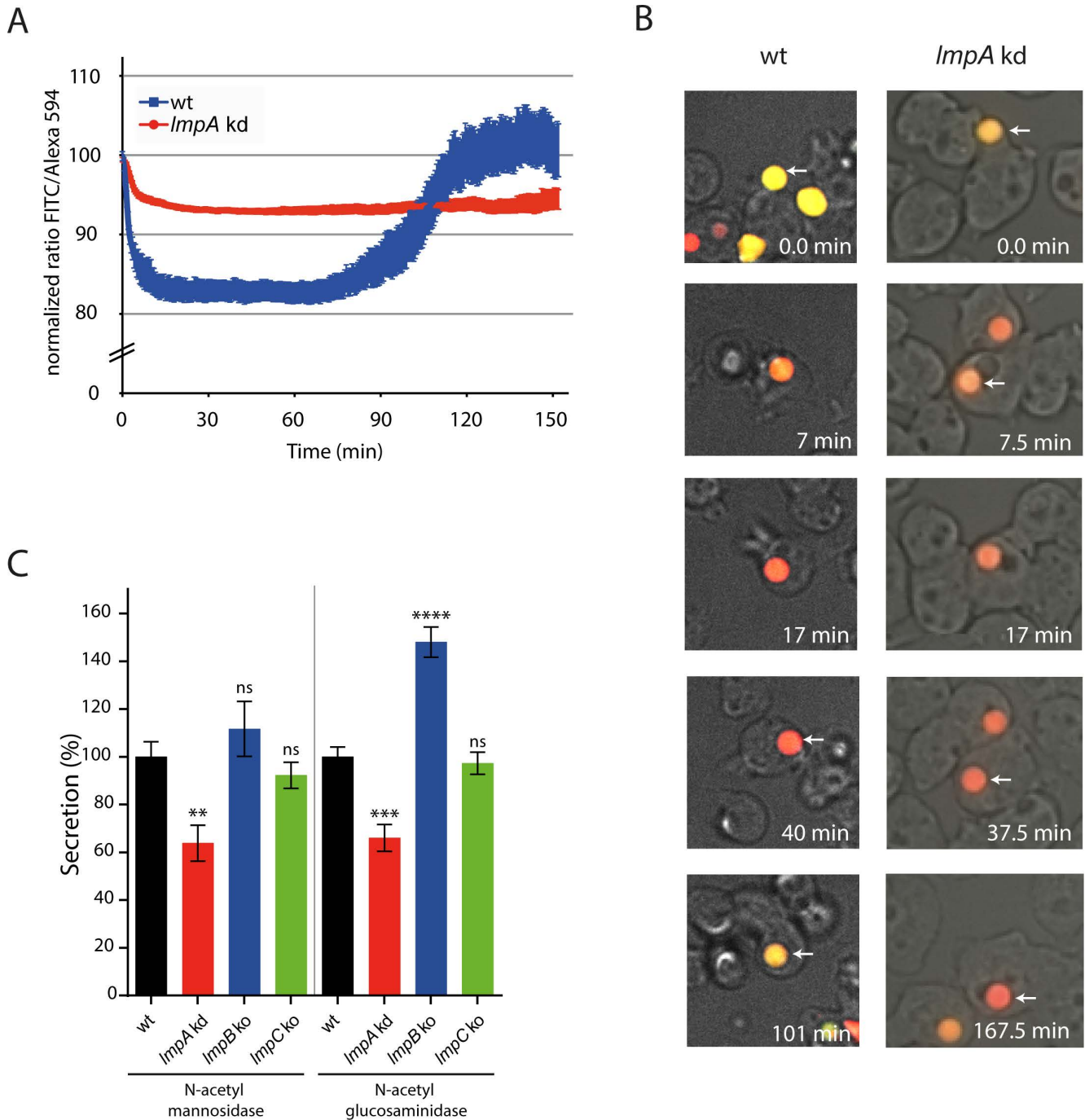


**Sup Fig. 1. Fluid phase uptake and random motility of wt, *ImpA* kd, *ImpB* ko, *ImpC* ko cells.** **A.** To measure the fluid phase uptake, cells were incubated 20 minutes with Alexa 647-coupled dextran, and the internalized fluorescent signal was measured by flow cytometry and expressed as the percentage of the levels in wt cells. Graphs depict the mean and SEM of four independent experiments. **B-E.** Cells were plated in medium onto a glass slide and their random cell motility was monitored every 30 seconds for 60 minutes. Metamorph Office software was used for cell tracking. Shown are representative experiments with tracks of 20 cells each, aligned at origin. The distribution of all cell positions around the origin corresponds to a Gaussian distribution, shown in the upper right corner of each graph.

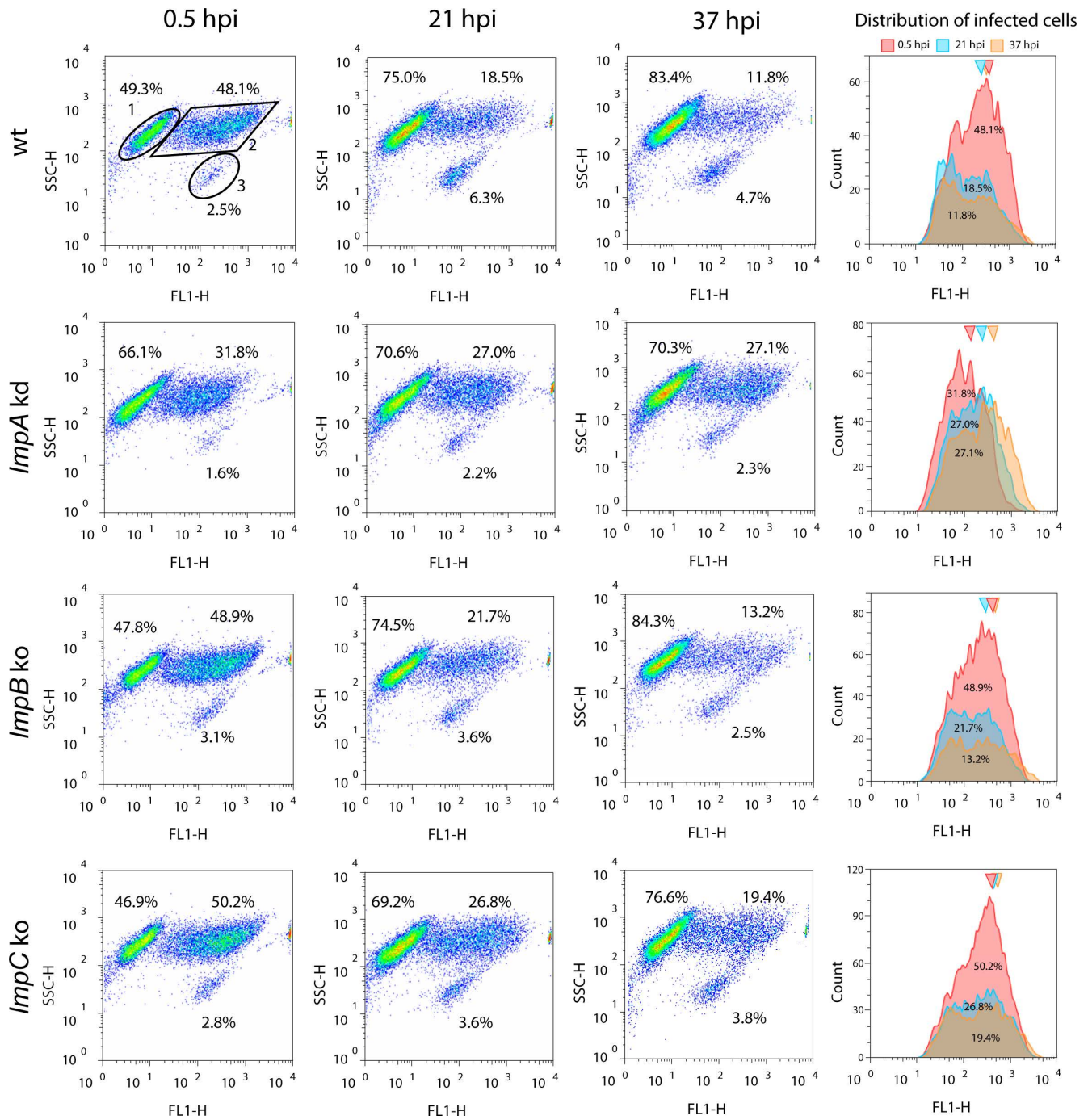


**Sup Fig. 2. Growth of *ImpA* kd, *ImpB* ko and *ImpC* ko cells on diverse bacteria. A.** 10, 10<sup>2</sup>, 10<sup>3</sup> or 10<sup>4</sup> cells of either *D. discoideum* wt, *ImpA* kd, *ImpB* ko or *ImpC* ko were deposited on a lawn of various bacterial strains. Representative images are shown for *E. coli*, *M. luteus* and *M. smegmatis*. **B.** Colour-coded summary of 3 to 22 plaque formation experiments. Plaque formation up to a dilution of 10, 10<sup>2</sup>, 10<sup>3</sup> or 10<sup>4</sup> cells is indicated in dark green, light green, yellow and red, respectively.



**Sup. Fig. 3. Acidification and secretion of lysosomal enzymes are strongly perturbed in *ImpA* kd cells.**

**A** and **B**. Acidification of single phagosomes measured by live microscopy. wt and *ImpA* kd cells were incubated with silica beads coupled to FITC and Alexa 594, and fluorescence emission was measured live for three hours. **A**. Graphs show the mean and SEM of 13 to 23 beads from 2 to 3 independent experiments per cell line normalized to time zero. **B**. Snapshots from two movies showing the fluorescence transitions of FITC/Alexa 594-labeled beads; yellow colour, neutral pH; red colour, acidic pH **C**. Secretion of lysosomal N-acetyl-glucosaminidase and N-acetyl-mannosidase enzymes measured in wt, *ImpA* kd, *ImpB* ko and *ImpC* ko. Cells were incubated for 6 hours in HI5 medium and their supernatant was incubated in an ELISA microplate reader with their respective chromogenic substrates. Graphs indicate the mean normalised to wt and SEM of 3 independent experiments performed in up to technical sextuplicates (\*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$ ; Student's *t*-test).



**Sup. Fig. 4. *ImpA* kd cells are heavily infected by *M. marinum*.** wt, *ImpA* kd, *ImpB* ko and *ImpC* ko cells infected with GFP-producing *M. marinum* were analyzed by flow cytometry. Plotting the side scatter (SSC) as a function of fluorescence (FL1) revealed three populations: (1) non-infected cells, (2) infected cells and (3) fluorescent extracellular bacteria, with the percentage of events in each gate shown. The fluorescence of the population of infected cells over time is also plotted, with the percentage of infected cells on each histogram as well as arrowheads depicting the mean fluorescence at each time point. At 0.5 hours post infection (hpi) all strains had a similar percentage of infected cells. While the proportion of infected wt, *ImpB* and *ImpC* cells decreased until 37 hpi, *ImpA* kd cells showed a higher degree of infection. In addition, *ImpA* kd infected cells, in contrast to the other cell lines, show an increase in fluorescence over time, suggesting that cells have a higher bacterial load.