Supplemental Material

Quantitative imaging flow cytometry of *Legionella*-infected *Dictyostelium* reveals the impact of retrograde trafficking on pathogen vacuole composition

Amanda Welin, Stephen Weber and Hubert Hilbi[#]

Institute of Medical Microbiology, University of Zürich, Gloriastrasse 30, 8006 Zürich, Switzerland.

[#]**Correspondence:** E-mail hilbi@imm.uzh.ch,

Tel.: +41 (0)44 634 2650, Fax: +41 (0)44 634 4906

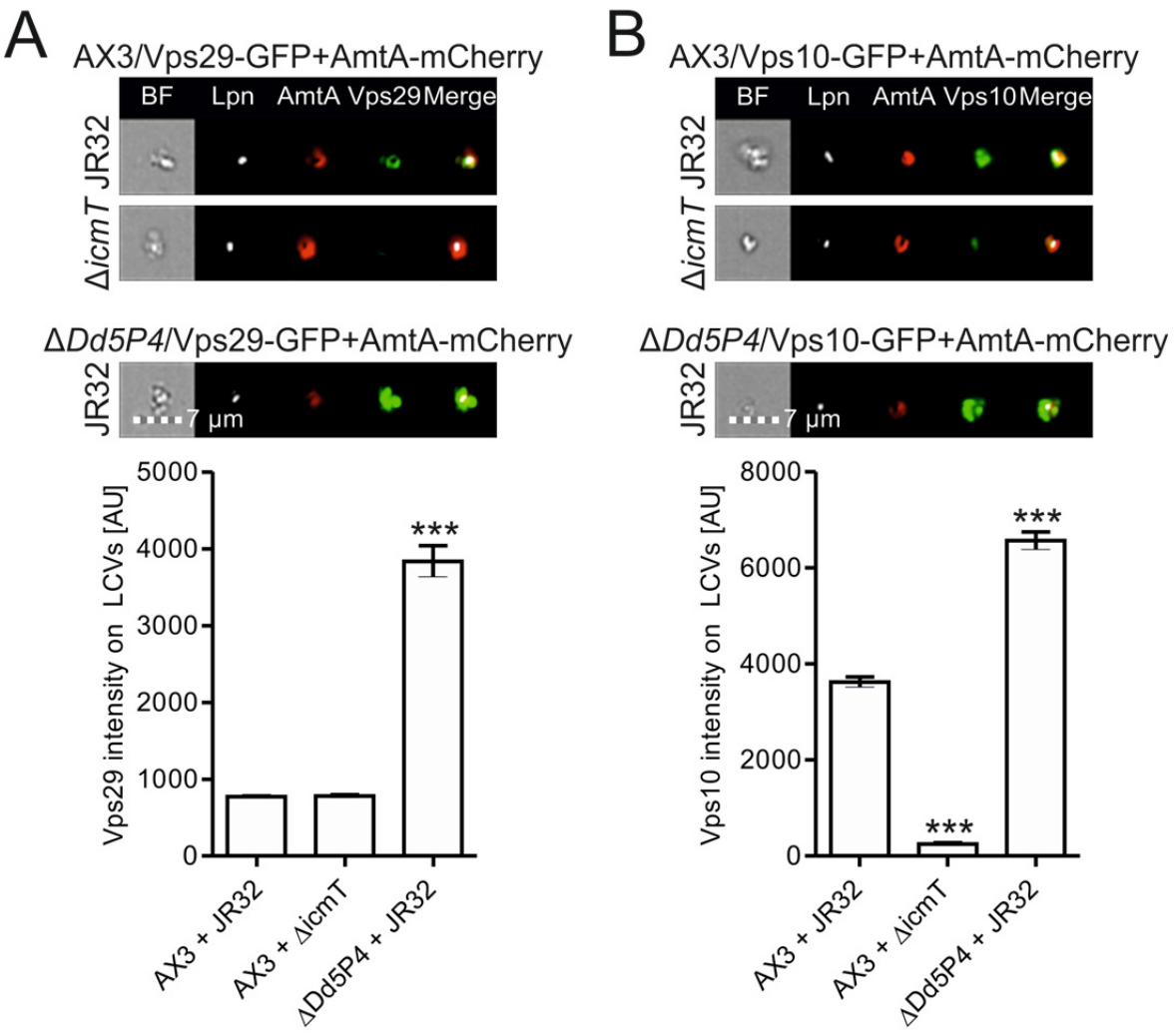

FIG S1. Effect of Dd5P4 deletion on Vps29 and Vp10 on the LCV. LCVs from homogenized *D. discoideum* Ax3 or Δ*Dd5P4* dually producing AmtA-mCherry and (A) GFP-Vps29 (pAW2), or (B) Vps10-GFP (pAW5), infected (MOI 50) for 2 h with mPlum-producing virulent *L. pneumophila* JR32 or Δ*icmT* (pAW14). Shown are representative IFC images and IFC quantifications of GFP intensities on AmtA-positive LCVs in >300 LCVs per sample. Data show means and SEM of one representative experiment out of three independent experiments (**, $P < 0.01$; ***, $P < 0.001$).

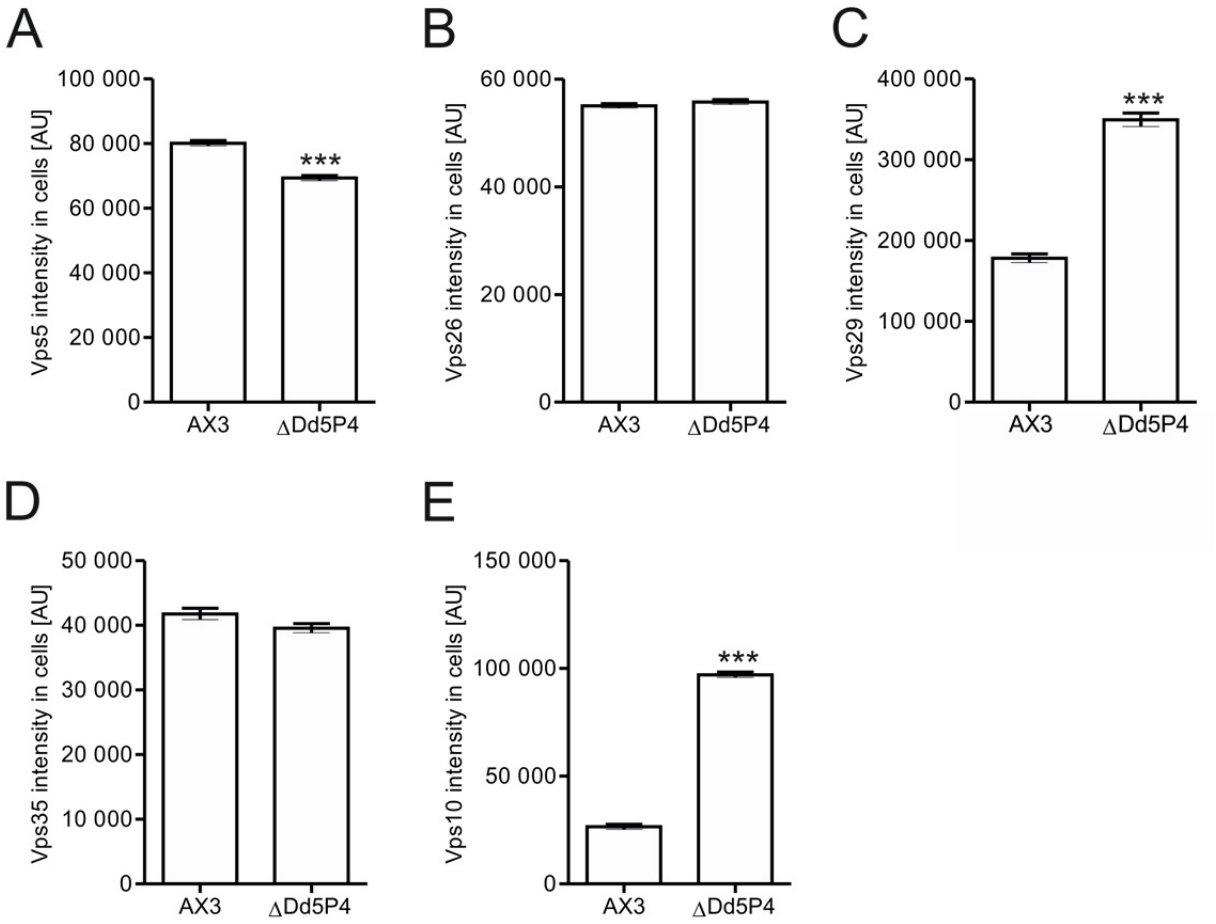

FIG S2. Whole-cell GFP fluorescence intensity in *D. discoideum* strains producing retrograde components. *D. discoideum* Ax3 or Δ*Dd5P4* dually producing AmtA-mCherry and (A) GFP-Vps5 (pAW18), (B) Vps26-GFP (pAW1), (C) Vps29-GFP (pAW2), (D) Vps35-GFP (pAW3), or (E) Vps10-GFP (pAW5) were fixed, and the total intensity of GFP was analyzed in >600 cells by IFC (***, $P < 0.001$).

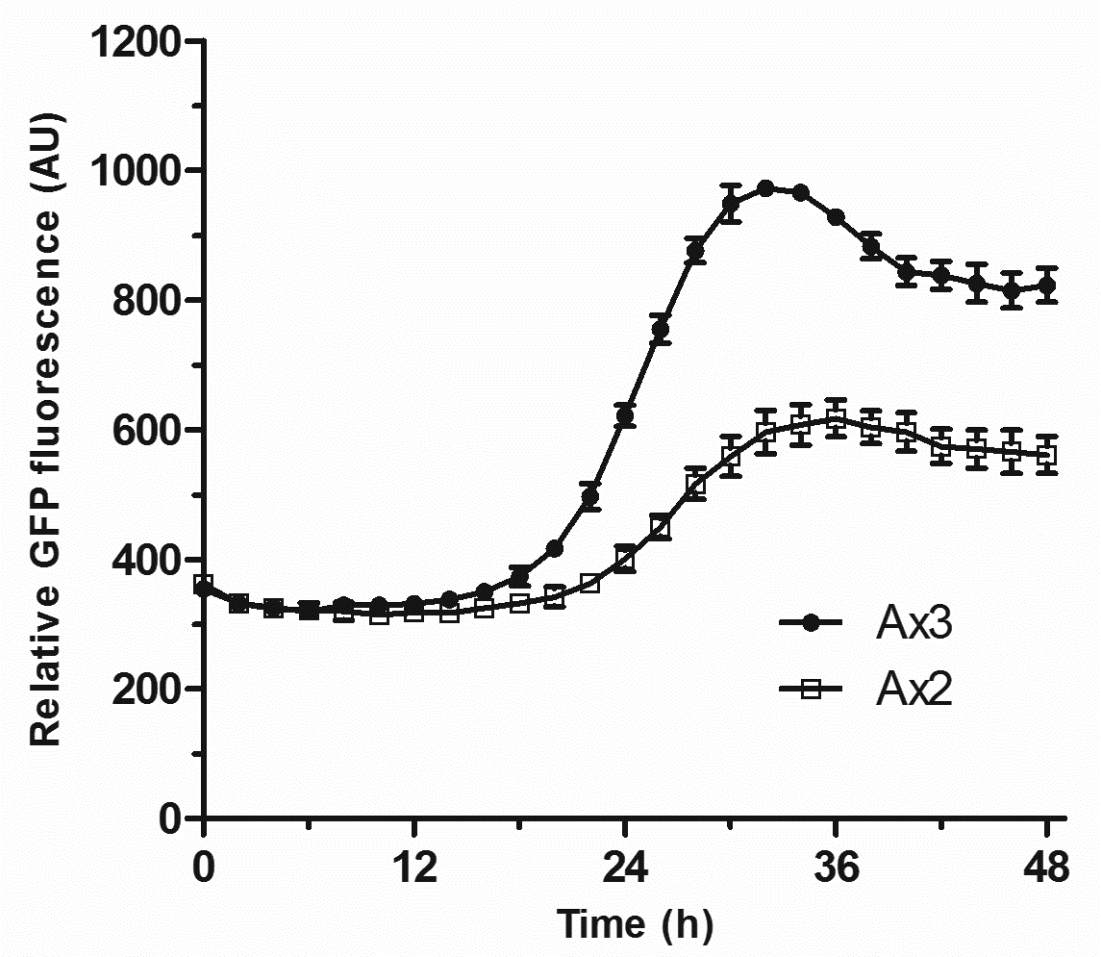

FIG S3. Comparison of intracellular replication of *L. pneumophila* in *D. discoideum* Ax3 and Ax2. *D. discoideum* Ax3 or Ax2 was infected (MOI 1) with GFP-producing *L. pneumophila* JR32 (pNT-28), and intracellular replication was assessed by fluorescence. Data show means and standard deviations of three biological replicates (means of technical replicates each).