

Figure S5. T4SS structure and activity influence cell-pole tethering at late infection stages.

(A) Position of subunits of the T4SS and sub-tomogram averages of T4SS particles from L. pneumophila wild-type,  $\Delta icmE$ , and  $\Delta icmN$  mutant bacteria. Images taken from Ghosal et~al. (2019) Nat~Microbiol~4: 1173-1182. Scale bars, 10 nm. (B) Representative fluorescence micrographs of D.~discoideum~Ax3 producing P4C-mCherry (pWS032) and AmtA-GFP (pHK121), infected (MOI 5; 8 h) with mCerulean-producing L.~pneumophila wild-type and  $\Delta icmT$  mutant bacteria harboring plasmid pNP99. Examples are shown for contact between bacteria and the LCV membrane (top, white arrowheads) or no contact (bottom, red arrowheads). Scale bars: 2  $\mu$ m (overviews), 1  $\mu$ m (inserts). (C) Quantification of data shown in (B) ( $n^{events}$ =60). Data are represented as mean  $\pm$  SD from three independent experiments (\*\*\*P<0.001).