

## Supplemental figure legends

### **The *Legionella pneumophila* effector LpdA is a palmitoylated phospholipase D virulence factor**

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**Figure S1. LpdA is conserved across the most commonly used sequenced prototype *L. pneumophila* isolates.** Alignment of LpdA-homologues from *L. pneumophila* 130b (Lpw\_19211), Philadelphia (Lpg1888), Corby (LPC\_1336), Alcoy (Lpa\_02723), Paris (Lpp1855) and Lens (Lpl1850). Not conserved residues are shaded in red.

**Figure S2. LpdA translocation assays.** TEM-1 translocation assays revealed that LpdA and LpdA KK165/376RR (KKRR) are translocated at low efficiency from wild type *L. pneumophila* 130b. No translocation was seen from the T4S-deficient  $\Delta dotA$  mutant. The T4SS effector LegC2 and the house keeping protein Fab1 were included as positive and negative controls respectively. Error bars represent mean standard deviation (SD). Representative of two independent experiments.

**Figure S3. LpdA lacking the C-terminal palmitoylation motif is active and induces redistribution of a PA reporter.** HeLa cells were co-transfected with pRK5 HA-LpdA, HA-LpdA $\Delta$ C5aa or pRK5 HA and a plasmid for the expression of GFP-tagged SPO PA reporter. Cells were immunostained and evaluated by IF microscopy. Representative of two independent experiments. Scale bar 10  $\mu$ m.

**Figure S4. LpdA lacking the C-terminal palmitoylation motif does not co-localize with Rab4 or Rab14.** HeLa cells were co-transfected with pRK5 HA-LpdA, HA-LpdA $\Delta$ C5aa or pRK5 HA and plasmids for the expression of GFP-Rab4 or GFP-Rab14 reporter. Cells were immunostained and evaluated by IF microscopy. Representative of three independent experiments. Scale bar 10  $\mu$ m.

**Figure S5. Co-localization of ectopically-expressed LpdA with different cellular markers.**

HeLa cells were co-transfected with pRK5 HA-LpdA and plasmids for the expression of (A) GFP-tagged Rab GTPases, (B) the autophagosome marker LC3 or (C) co-stained with MitoTracker®Red and processed for IF microscopy. HA-LpdA sporadically localized to Rab5 and Rab7 positive vesicles (Inset). Similar results were observed in at least two independent experiments. Scale bar 10  $\mu\text{m}$ .

**Figure S6. Ectopically-expressed LpdA does not disrupt the ER network.**

Immunofluorescence images of HeLa cells co-transfected with pRK5 HA or pRK5 HA-LpdA and pmCherryER expressing ER-targeted mCherry protein. Results are representative of at least two independent experiments. Scale bar 10  $\mu\text{m}$ .

**Figure S7. *L. pneumophila* infection induces dispersion of the Golgi apparatus.**

A549 cells were infected with *L. pneumophila* 130b wild type,  $\Delta dotA$  or  $\Delta lpdA$ , fixed 2h 30 min post infection and immunostained for the Golgi-protein Giantin and DNA. Selected bacteria are indicated with white arrows. Scale bar 10  $\mu\text{m}$ .

**Figure S8. *L. pneumophila* 130b  $\Delta lpdA$  is not attenuated for intracellular replication in THP-1 macrophages.**

THP-1 cells were infected with *L. pneumophila* 130b wild type or  $\Delta lpdA$  strains containing the pXDC31 GFP-expression plasmid (two clones (C1 and C2) each) for 1 hour at a MOI of 0.1. After 1 h of infection cells were incubated with 100  $\mu\text{g}/\text{mL}$  gentamicin for another 1h, then washed and GFP-fluorescence recorded over 92 h using a FLUOstar Omega plate reader. Error bars represent mean standard deviation of a sample triplicate. Representative of two experiments.