Figure S1

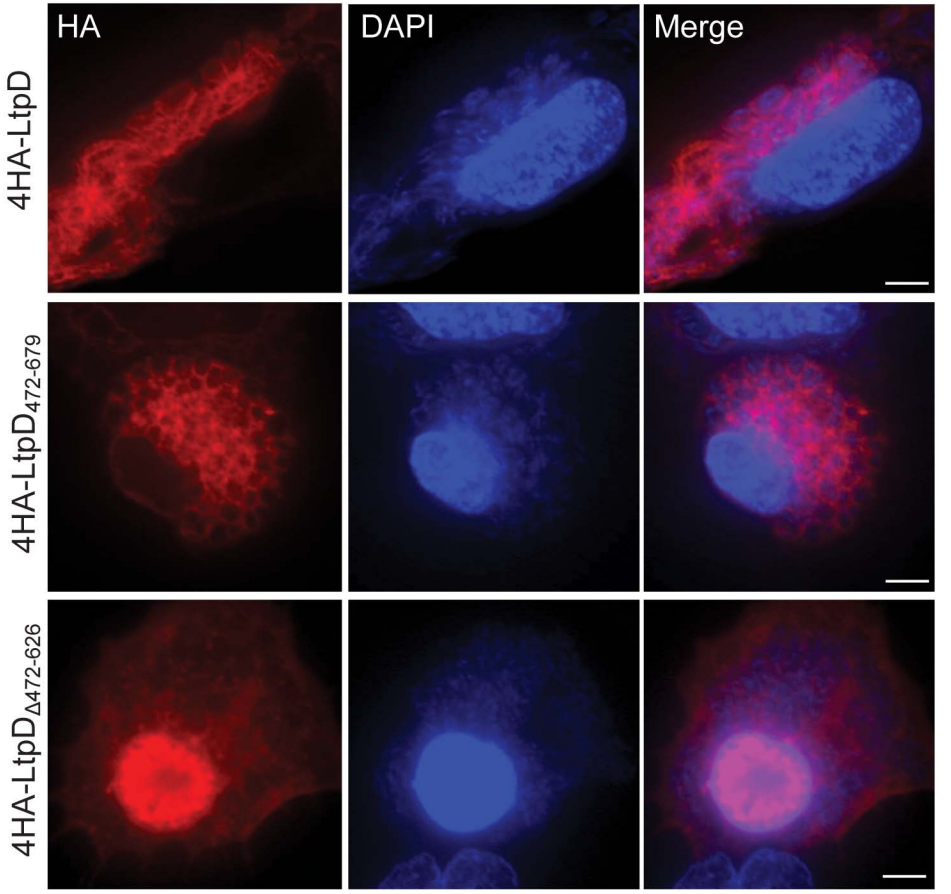

Figure S1. The internal region of LtpD, LtpD$_{472-626}$, is required for localization of LtpD to the LCV. A549 cells were infected for 24 hours with WT *L. pneumophila* 130b expressing 4HA-LtpD, 4HA-LtpD$_{472-679}$ or 4HA-LtpD$_{\Delta 472-626}$ and stained using anti-HA antibody (red) and DAPI DNA stain (blue). 4HA-LtpD and 4HA-LtpD$_{472-679}$ localized to the LCV, while 4HA-LtpD$_{\Delta 472-626}$ displayed diffuse, cytosolic and strong nuclear localisation. Results are representative of two separate experiments. Scale bar 10 μm.

Figure S2

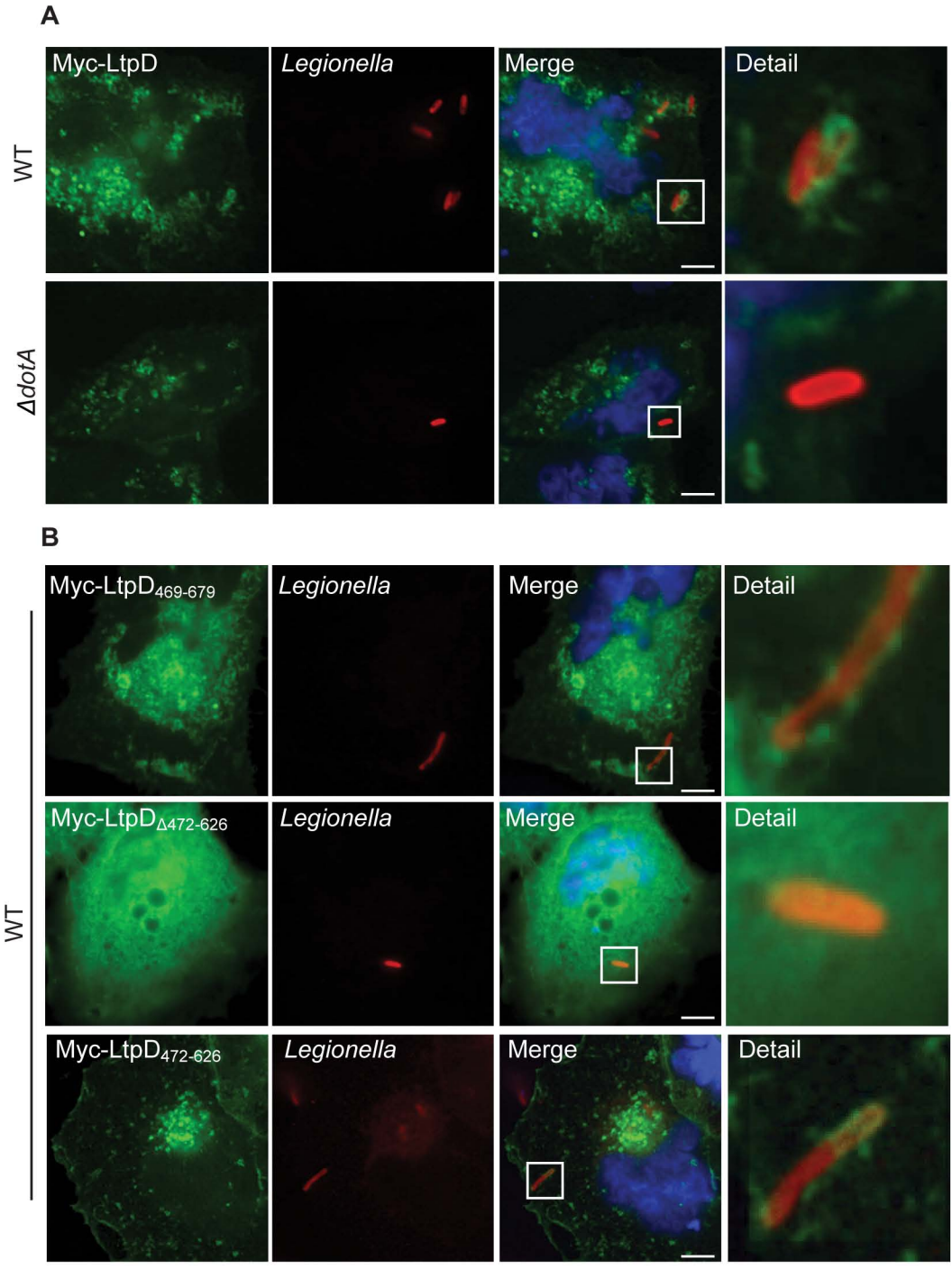

Figure S2. LtpD$_{472-626}$ is necessary and sufficient for the recruitment of ectopically-expressed Myc-LtpD to the LCV. Immunofluorescence images of A549 cells transfected with Myc-LtpD, Myc-LtpD$_{\Delta 472-626}$ or Myc-LtpD$_{472-626}$ and then infected with WT or Δ*dotA L. pneumophila*. (A) Myc-LtpD is recruited to the LCV of WT but not Δ*dotA* bacteria during infection. (B) Transfection of Myc-LtpD$_{472-679}$, Myc-LtpD$_{472-626}$ or Myc-LtpD$_{\Delta 472-626}$ demonstrates that LtpD$_{472-626}$ is sufficient for the recruitment of Myc-LtpD to the LCV during infection. Results are representative of at least three experiments. Scale bar 10 μm.

Figure S3

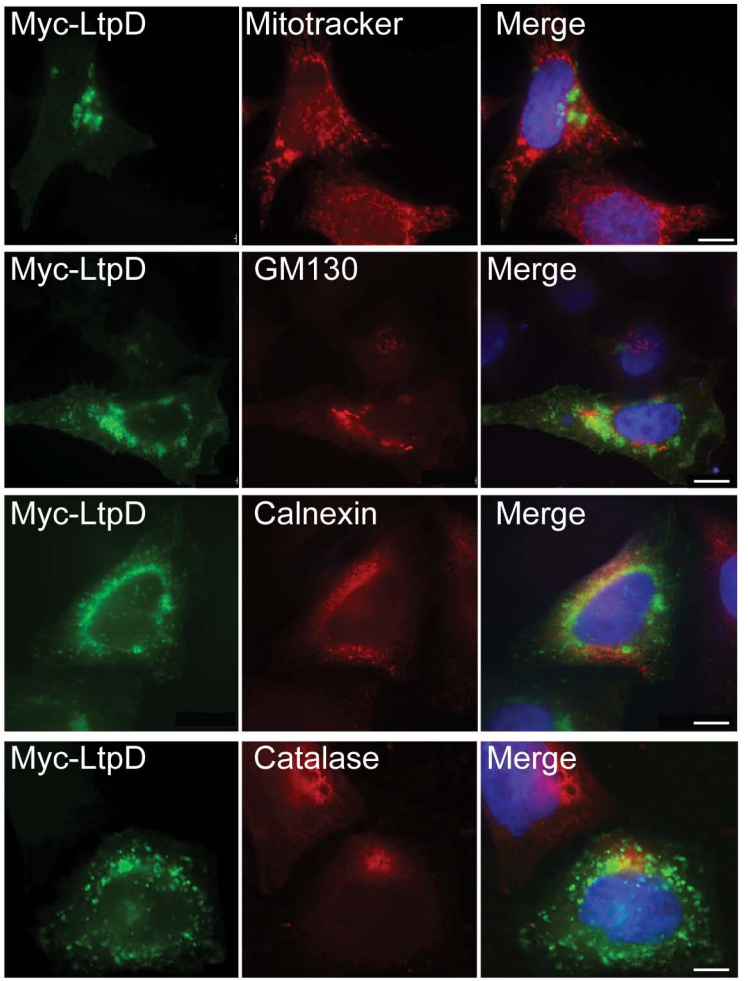

Figure S3. **Myc-LtpD does not co-localize with selected cellular organelles.** HeLa cells were transfected with Myc-LtpD and stained using antibodies for selected cellular markers. To visualize mitochondria, live cells were loaded with Mitotracker, cells were then fixed and stained with anti-Myc antibody. No significant co-localization between Myc-LtpD and Mitotracker, calnexin, GM130 or catalase was observed. Results are representative of at least three independent experiments. Scale bar 10 μm.

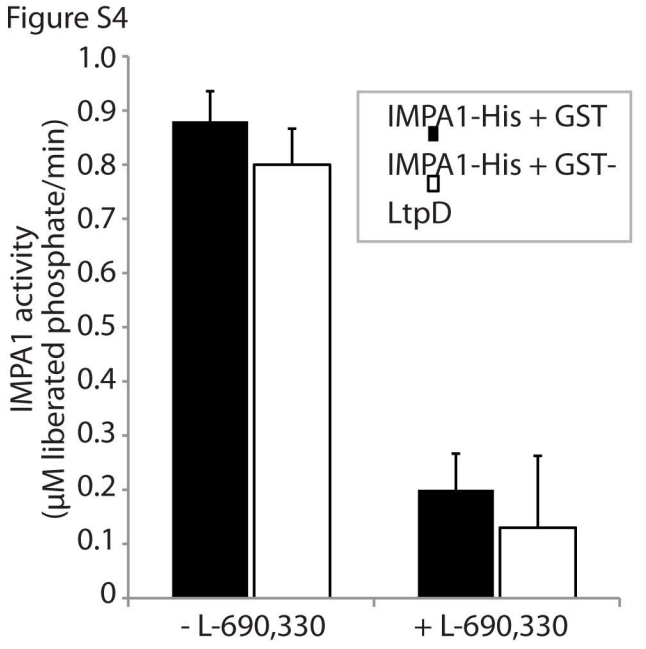

Figure S4. **LtpD does not affect IMPA1 activity *in vitro*.** Free phosphate released by His-IMPA1 from the substrate inositol monophosphate was quantified using the Malachite green assay. Incubation of IMPA1 with the substrate resulted in the release of phosphate however the addition of GST alone or GST-LtPD did not affect the quantity of phosphate released. The specific inhibitor L-690,330 was able to prevent IMPA1 activity. Results mean of two separate experiments performed in triplicate ± standard deviation.

Table S1. Plasmids and primers used in this study

Plasmid	Description			Reference
pRK5	**Vector for the expression of proteins with N-terminal HA or Myc tag in mammalian cells**			**Clontech**
pRK5-derived	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1247	Myc-LtpD	CACTGACCCGGGGAACCAAGAAACATTTAAATCCAGAC GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	HindIII BamHI	This study
pICC1248	Myc-LtpD$_{1-469}$	CACTGACCCGGGGAACCAAGAAACATTTAAATCCAGAC CAGTCAAGCTTCTACTGAGTTTCAGGTAGGAGGGGC	HindIII BamHI	This study
pICC1249	Myc-LtpD$_{472-679}$	GTATGGAATTCATGGACAGAGACAGAGACAGAGACA GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	EcoRI BamHI	This study
pICC1250	Myc-LtpD$_{556-679}$	CACTAACCCGGGAAGCAACAAATGGGCAAGAAC GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	XmaI BamHI	This study
pICC1251	Myc-LtpD$_{626-679}$	CACTAACCCGGGAATCAAATTCATCAGACGAAACG GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	XmaI BamHI	This study
pICC1252	Myc-LtpD$_{472-556}$	CACTAACCCGGGAAGAGACAGAGACAGAGACAG GTCGCAAAGCTTCTAGTTTTCAAACGCTGATTTG	XmaI HindIII	This study
pICC1253	Myc-LtpD$_{472-626}$	CACTAACCCGGGAAGAGACAGAGACAGAGACAG GTCGCAAAGCTTGCGCTACCACTTTGCGTCCCTTTTC	XmaI HindIII	This study
pICC1254	Myc-LtpD$_{\Delta 472-626}$	See text for details		This study
pICC1255	Myc-LtpD$_{\Delta 472-556}$	See text for details		This study
pICC1341	**pRK5-HA, Vector for the expression of proteins with N-terminal HA in mammalian cells**; generated by replacing the Myc-tag of pRK5-Myc by an phosphorylated, annealed oligo cassette encoding the HA tag			This study
	CCACCATGGCGTACCCATACGATGTTCCAGATTACGCTGGCGCGG		NA	
	GATCCCGCGCCAGCGTAATCTGGAACATCGTATGGGTACGCCATGGTGG		NA	
pICC1342	HA-SetA$_{PIP3(401-631)}$	CACTGAGGATCCGTAAATCCAACGGCTCAAGAAT TCGCAGAATTCTTATATTCTTAAACCATGATTGTTATCGT	BamHI EcoRI	This study
pICC1343	HA-SidC$_{PIP4}$	GTCATAGGATCCAAATATTCCTCCAAGCCATTATTGG GATACCCTGCAGCTATTTCTTTATAACTCCCGTGTAC	BamHI PstI	This study
pGBT9	**Vector for the expression of GAL4 DNA-binding domain (DNA-BD) fusion proteins in yeast**			**Clontech**
pGBT9-derived	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1268	DNA-BD-LtpD	CACTGAGAATTCACCAAGAAACATTTAAATCCAGACTC AGCTGCTGCAGCTAAATCTTTAAACCAGTGTCGC	EcoRI PstI	This study
pICC1270	DNA-BD-LtpD$_{1-469}$	CACTGAGAATTCACCAAGAAACATTTAAATCCAGACTC CAGTCAAGCTTCTACTGAGTTTCAGGTAGGAGGGGC	EcoRI BamHI	This study
pICC1269	DNA-BD-LtpD$_{472-679}$	CACTAACCCGGGAGAGACAGAGACAGAGACAG AGCTGCTGCAGCTAAATCTTTAAACCAGTGTCGC	XmaI PstI	This study
pGADT7	**Vector for the expression of activation domain (AD) fusion proteins in yeast**			**Clontech**
pGADT7-derived	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1261	AD-IMPA1	CTAATCCCGGGAATGGCTGATCCTTGGCAGGAATGC GCGATCATCGATTTAATCTTCGTCGTCTCGTTGCAAAGG	XmaI ClaI	This study
pICC562	**pMMB207c-4HA; Vector for the expression of proteins with four N-terminal HA-tags in *L. pneumophila***			(1)
pICC562 *-derived*	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1265	4HA-LtpD	CACTGACCCGGGGAACCAAGAAACATTTAAATCCAGAC GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	Xmal HindIII	This study
pICC1266	4HA-LtpD$_{1-469}$	CACTGACCCGGGGAACCAAGAAACATTTAAATCCAGAC CAGTCAAGCTTCTACTGAGTTTCAGGTAGGAGGGGC	XmaI HindIII	This study
pICC1267	4HA-LtpD$_{472-679}$	GTATGGTACCATGGGGACAGAGACAGAGACAGAGACA GTCGCAAAGCTTCTAAATCTTTAAACCAGTGTCGC	KpnI HindIII	This study
pICC1344	4HA-SetA$_{PIP3(401-631)}$	CACTGAGGTACCGTAAATCCAACGGCTCAAGAAT TCGCATCTAGATTATATTCTTAAACCATGATTGTTATCGT	KpnI XbaI	This study

pGEX-6P-2	**Vector for the expression of a GST fusion protein in *E. coli***			**GE Healthcare**
pGEX-6P2 *-derived*	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1345	GST-LtpD	GTATGGAATTCAAACCAAGAAACATTTAAATCCAGACTC GACGTCGCGGCCGCCTAAATCTTTAAACCAGTGTCGC	EcoRI NotI	This study
pICC1346	GST-LtpD$_{472-626}$	GTATGGAATTCATGAGACAGAGACAGAGACAGAGACA GACGTCGCGGCCGCCTAAATCTTTAAACCAGTGTCGC	EcoRI NotI	This study
pET28a(+)	**Vector for the expression of a 6 x His tag fusion protein in *E.coli***			Novagen
pET28a(+)-derived	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1259	His-IMPA1	GGCTAGAGCTCATGGCTGATCCTTGGCAGGAATGC GGACTGCGGCCGCTTAATCTTCGTCGTCTCGTTGCAAAGG	SacI NotI	This study
pcDNA	**Vector for the expression FLAG tag fusion proteins in mammalian cells**			**Invitrogen**
pcDNA-derived	*Expressed protein*	*Primers 5' -> 3'*	*RS*	
pICC1263	FLAG-IMPA1	CTAATCCCGGGAAATGGCTGATCCTTGGCAGGAATGC GGACTCTGCAGTTAATCTTCGTCGTCTCGTTGCAAAGG	XmaI PstI	This study
Primers for construction of the Δ*ltpD* strain				
	Amplification of the 5' flanking region	GATGTCTCTGATATCCATAAGATTTTC GGTACGAATTCCATGATATTCTCCTGAATAATTCGC	NA	This study
	Amplification of the 3' flanking region	GGTACGAATTCGGACGCAAAGTGGTAGCGCAATCAAATTC GGTGCAAATCATTATGGCTTCGCGACTTATTTATGG	NA	This study
	Confirmation primer upstream 5' flanking region	CGCTAATTATCAAACGTACATTTAACTC	NA	This study
	Confirmation primer upstream 5' flanking region	CCATTTTATATGTTGCAGTCGCC	NA	This study

RS = restriction site

References

1. **Dolezal P, Aili M, Tong J, Jiang JH, Marobbio CM, Lee SF, Schuelein R, Belluzzo S, Binova E, Mousnier A, Frankel G, Giannuzzi G, Palmieri F, Gabriel K, Naderer T, Hartland EL, Lithgow T.** 2012. *Legionella pneumophila* Secretes a Mitochondrial Carrier Protein during Infection. PLoS Pathog **8:**e1002459.

Table S2. Hits of the LtpD yeast-2-hybrid screen

Gene Name	**No. of hits**
Structural maintenance of chromosomes 4 (SMC4)	1
Myotrophin (MTPN) Leucine repeat protein	1
(myo)-Inositol-1(or 4)-monophosphatase 1 (IMPA1)	1
E3 ubiquitin-protein ligase (RNF8)	2
Leucine rich repeat containing 59 (LRRC59)	1
Splicing factor, arginine/serine-rich 11 (SFRS11)	1
COX11 homolog	1
Activating transcription factor 7 interacting protein (ATF71P)	1