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Supplemental information

**SdhA blocks disruption
of the *Legionella*-containing vacuole
by hijacking the OCRL phosphatase**

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Supplementary Table S1. Conserved Motifs. Linked to Fig. 1.

Motifs	Pattern	No.	Positions in SdhA
Clathrin box	L[IVLMF]X[IVLMF][DE]	2	355-359 (LLSIE) 1177-1181 (LLYIE)
AP2 β binding	[DE]x(1,2)Fxx [FL] xxxR	1	360-370 (DSRFLALRLER)
OCRL-binding F&H motif	xFxx[KRIL]Hxx[YLMFH] xxxx	1	1194-1206 (AFCNRHIGLQYTD)

Supplementary Table S2. From Dataset 1: Icm/Dot Translocated Effectors Having Two or More of the Conserved Motifs. Linked to Fig. 1.

Orf	Gene	Clathrin Box	AP2 beta	F&H	Unique motifs
LPG0376	<i>sdhA</i>	2	1	1	3
LPG0090	<i>lem1</i>	1	1	0	2
LPG2433	<i>ceg30</i>	1	1	0	2
LPG2745	---	1	1	0	2
LPG3000	---	1	0	1	2

Supplemental Table S3: Identification of proteins associated with the SdhA CBM2A region. Linked to Figs. 1 and 2.

Gene Symbol	Annotation	Process	Relative abundance	CBM2A/GST
RANGAP1	GAP domain	Nuclear Egress	4.4E+07	1.1E+04
EEF1A1	Elongation factor 1 GTPase	Translation	8.6E+06	3.6E+02
EIF3A	Initiation factor 3A subunit	Translation	2.0E+06	5.4E+01
DDX3X	RNA Helicase	Translation	1.1E+06	3.3E+02
EIF3C	Initiation factor 3C subunit	Translation	1.1E+06	7.9E+01
YARS	Tyr tRNA synthetase	Translation	7.4E+05	4.1E+02
DHX9	RNA helicase A	RNA metabolism	5.9E+05	3.7E+01
AP3D1	AP-3 delta subunit	Vesicle adaptor	5.7E+05	6.9E+01
EIF5B	Initiation factor 5B GTP binding	Translation	5.7E+05	5.2E+01
EIF4G2	Initiation factor 4G2 subunit	Translation	4.0E+05	5.7E+00
EIF3D	Initiation factor 3D subunit	Translation	2.6E+05	2.2E+01
EEF1G	Elongation factor 1 subunit	Translation	2.3E+05	3.3E+01
OCRL	Inositol 5-phosphatase (GAP)	Vesicle traffic	1.9E+05	1.2E+01
IQGAP1	GAP domain	Membrane/cytoskeleton	1.7E+05	3.3E+01
EIF3L	Initiation factor 3L	Translation	1.2E+05	5.2E+01
G3BP2	GAP-binding protein 2	Stress Granules	1.2E+05	5.5E+00
G3BP1	GAP-binding protein 1	Stress Granules	9.8E+04	4.5E+01
SERBP1	RNA-binding protein	Translation	7.6E+04	2.6E+01
EIF3E	Initiation factor 3E	Translation	7.2E+04	1.6E+01
YTHDF3	YTH domain-protein 3 OS	m6A reader	7.1E+04	5.1E+00
CLASP2	CLASP 2 (IQGAP Binding)	Clathrin Associated Sorting	3.1E+04	7.9E+00

Extracts were incubated with either GST or CBM2A-GST immobilized on glutathione beads, and eluted proteins were analysis by LC MS/MS analysis (STAR Methods). Relative quantities of peptides identified from each protein were determined from both the GST and CBM2A-GST eluates and expressed as a ratio (CBM2A/GST). Proteins in which (CBM2A/GST) ≥ 5 are displayed.

Supplementary Table S4. Oligonucleotides used in this study. Linked to STAR Methods, Key resources.

Name	Sequences	Purpose
Owy77	gtgaagaattcga aaa aggagaagagttactcagtatag	SdhA-CBM1
Owy63	gtgaactc gag ttagggggtccaatattttgt	
Owy81	gtgaaGAATTCggaatgatatgtataaaacaccaatagatc	SdhA-CBM2
Owy82	gtgaaCTCGAGttaaagatgataatatttctcaaaattatctct	
Owy84	tgctcagtgtattgtaaacctatagcacgggtgcaggcagcatctaattgtttattaaatg	CBM2 (F1195A H1199A) mutation
Owy83	catttaataaacaattagatgctGCCtgaaccgtGCTataggtttacaatacactga ca	
Owy72	cgactcaggaacaagaagatgccgcttatatagaagaatacactacagaatc	CBM2 (L1177A L1178A) mutation
Owy73	gattctgtagtgtattcttctatataagcggcatcttctgttcctgagtcg	
Owy89	aaggaatatgcacaaaacttagagaata	CBM2 Δ FH deletion
Owy90	atctaattgtttattaatgattctgtagtgtattc	
Owy81	gtgaaGAATTCggaatgatatgtataaaacaccaatagatc	CBM2A
Owy113	gtgaaCTCGAGttaagtggtttgcaccttttg	
Owy114	gtgaaGAATTCaaaggtggcaaaaccactgat	CBM2B
Owy115	gtgaaCTCGAGttaaggatcaggatctgtattctgg	
Owy79	gtgaaGAATTCagctaccatcattatgaaggctt	CBM2C
Owy117	gtgaaCTCGAGttatgtagtgtattcttctatataaaggag	
Owy116	gtgaaGAATTCattcaacgctcaaaatccgcag	CBM2D
Owy82	gtgaaCTCGAGttaaagatgataatatttctcaaaattatctct	
Owy100	gtgaaGGATCCaaggttgggatgaacgaagg	OCRL- ASH/RhoGAP
Owy101	gtgaaCTGCAGttagtcttctcgtcccaag	
Owy100	gtgaaGGATCCaaggttgggatgaacgaagg	OCRL-ASH

Owy133	gtgaa <u>CTGCAG</u> Gttaactgggaggttaattccactg	
Owy134	gtgaa <u>GGATCC</u> agttgtttggcacatccttagag	OCRL-RhoGAP
Owy101	gtgaa <u>CTGCAG</u> ttagtcttcttcgctccaag	
Owy14	gtgaag <u>tcgac</u> atttcagaaaagatcaagctttagaatcc	pJB908-3xflag- <i>sdhA</i>
Owy15	gtgaact <u>gcag</u> ttatgcggatggcgctaata	
Owy137	5'phos/gatgcagtaagagtcgctttagc	<i>sdhA</i> Δ1029-1260aa
Owy138	5'phos/aatatgtgctttggaatgcagcgc	(ΔCBM2)deletion
Owy139	5'phos/gatgatgaattaatcgaaactataaaaaag	<i>sdhA</i> Δ1029-1080aa
Owy138	5'phos/aatatgtgctttggaatgcagcgc	(ΔCBM2A)deletion

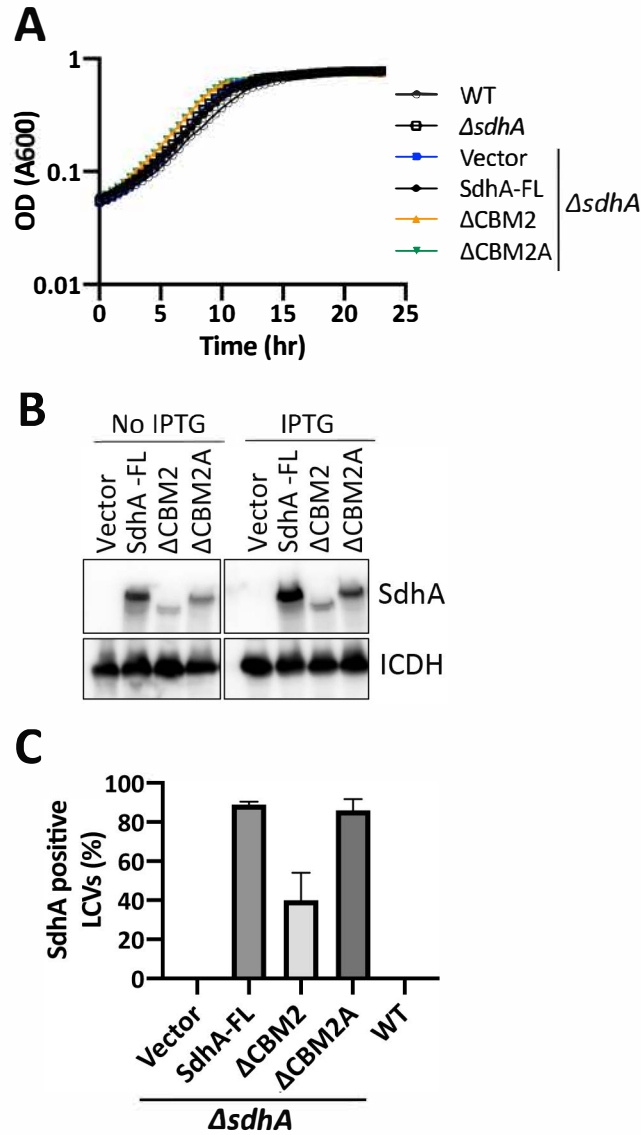


Figure S1. Characterization of SdhA mutants. Linked to Figs. 2 and 3.

(A) Growth of noted strains in AYE broth. Strains with $\Delta sdhA$ allele have noted genes inserted into pJB908 (Vector). Shown are singlicate cultures (B) Western blot analysis of expression levels of SdhA variants. Isocitrate dehydrogenase (ICDH) was used for loading control. (C) The presence of SdhA variants on LCV surface was assessed by fluorescence microscopy as in Fig. 3A. SdhA is undetectable in WT strain and must be overproduced on pJB908 to identify by immunofluorescence microscopy. Each of the $\Delta sdhA$ strains harbor pJB908 with noted chromosomal fragments. Data represents means and SDs of biological triplicates. More than 100 LCVs were counted per replicate.

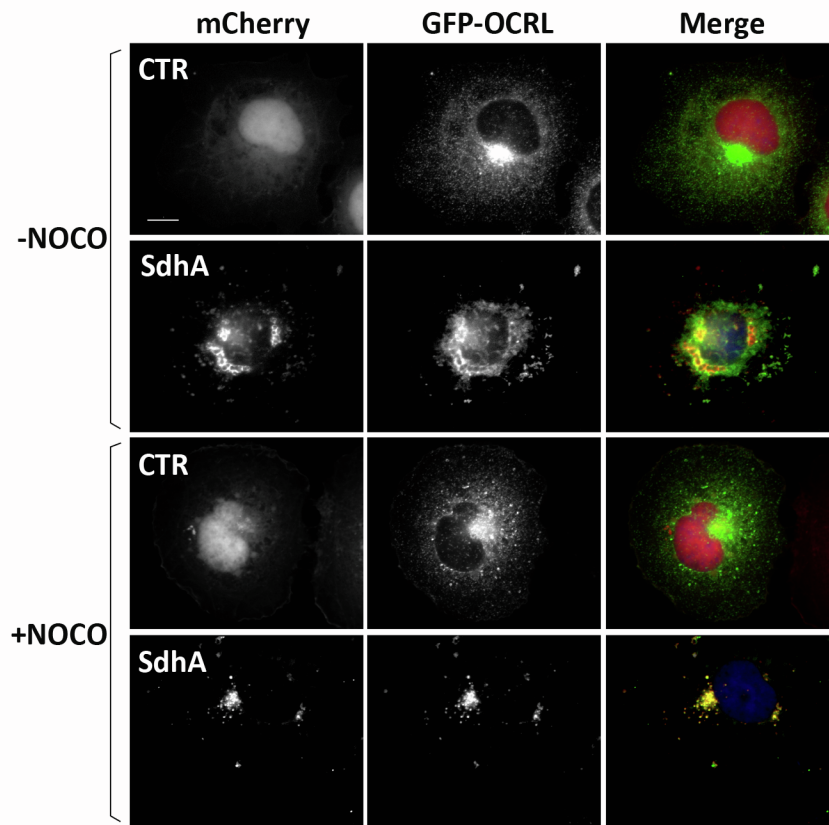


Figure S2. Ectopically expressed SdhA associates with OCRL positive compartments. Linked to Fig. 4.

Representative micrographs of fixed COS-7 cells coexpressing mCherry-SdhA (red) and GFP-tagged OCRL. DNA was labeled by Hoechst stains (blue). Cells were treated with nocodazole (NOCO) to release aggregation of the compartments. (Scale bar, 10 μ m)

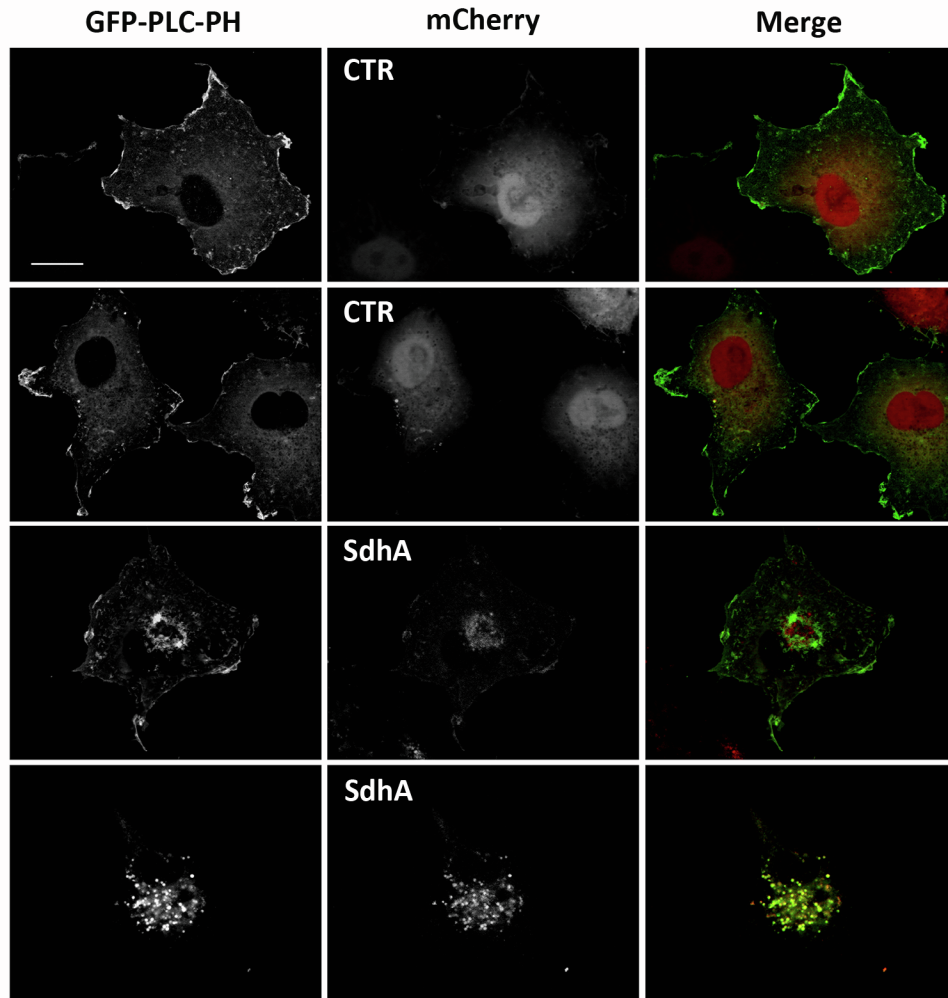


Figure S3. COS-7 cells coexpressing mCherry-SdhA and GFP-PLC δ -PH demonstrate rearrangement and internalization of PI(4,5)P₂. Linked to Fig. 4.

Scale bar represents 20 μ m.

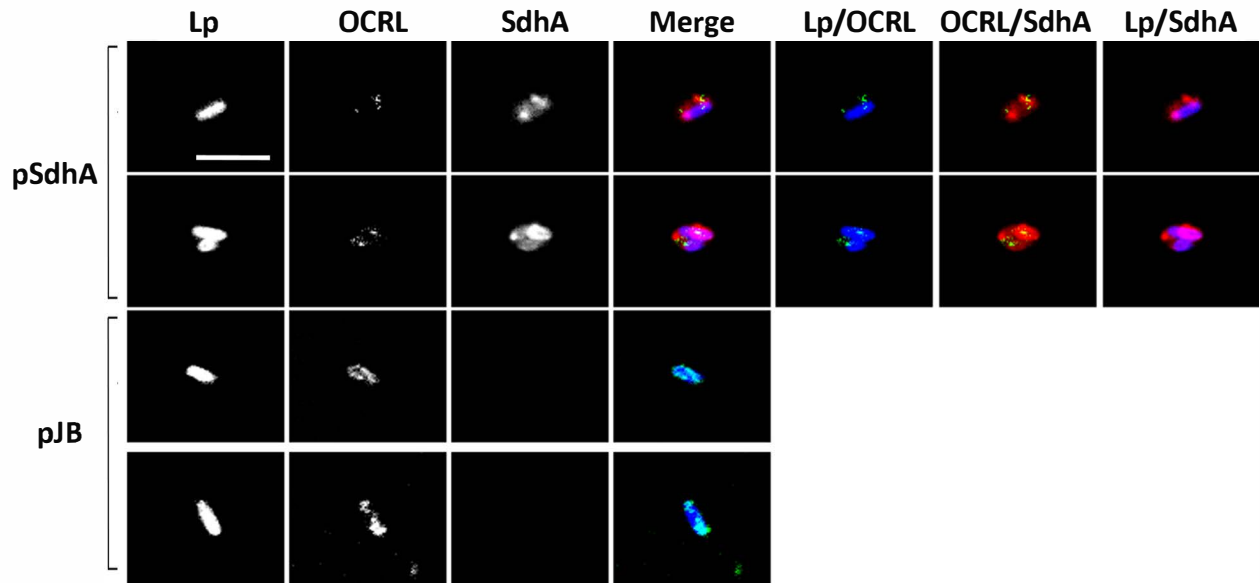


Figure S4. OCRL localization about the LCV surface. Linked to Fig. 6D.

Confocal images of vacuoles isolated from infected U937 cells (MOI=10, 3h) with Δ sdhA mutant harboring pSdhA or pJB vector. The presence of OCRL and SdhA on LCVs was assessed by immunofluorescence using antibodies against OCRL, SdhA and *L. pneumophila* (Scale bar, 4 μ m).