

FIG S1. Reactivity of the candidate proteins with the suicide probes. Recombinant proteins purified from *E. coli* were reacted with Ub-VME (A) or Ub-PA (B) at 37 °C for 2 h. Proteins separated by SDS-PAGE were subjected to silver staining. Similar results are observed in three independent experiments.



FIG S2. Hydrolysis of diubiquitins by the DUB modules of the *L*. *Iongbeachae* SidE family proteins. The DUB domains of SidE orthologue proteins in *L. longbeachae* were purified and incubated with a panel of diubiquitins at 37 °C for 2 h. Cleavage of diubiquitins were visualized after SDS-PAGE followed by Coomassie brilliant blue staining (CBB). Data shown are one representative from three independent experiments.



FIG S3. Cleavage of diubiquitins requires the catalytic cysteine in DUBs.

The indicated diubiquitins were incubated with WT or their active cysteine mutant DUBs at 37 °C for 1 h. Proteins in the reactions were separated by SDS-PAGE and detected by CBB. Data shown are one representative from three independent experiments.

Α			в		
LLO_2238 Lem27		48 60	LLO_1014 Ceg23	—MYDKKDENTKVRVKNAVDVSGSPDNCFPHNYALYLLTNHQALPEDLFNFKSTLE-NS MOTKKDKRVTSLQERVENAVDVSGAFDNCFFHNFALYLLTNNLPLPDDLFHFKSTINENS ***: **:********:******************	56 60
LLO_2238 Lem27	VRLRVLLSKSPEQEFSSSEIKNIIEPILGKATRNLAAEVIKNEFKLSPQDAPLFSSANYG ARLEKLLSKDPDRAFTRDEIKTIEPILGRATRDLAAEHKKVEFKSSPHDTFLSSLHYA . *** ****.*:::::::::::::::::::::::::::	108 120	LLO_1014 Ceg23	KATQLIHYFPNQKSINQISLIDISKDNASNYLFEKTLILGFLFREWFFTQIMEHPELG KAEQLFEFFHNPESINLFSILDKENDVSEFSGYLFEKSLILGFLIREWFFTQIVRNSAVK ** **:* * :*** :*:**:* : *.*****:******:******	114 120
LLO_2238 Lem27	LEFCFNCSMRLNGSELSHLIEHEFDNPDYIGAEIYKVKGMUMAMQEYSLARLPYVIEKFN VEFGFKSLQINESELTLLINNFSNPDYTEAEIYKVGGLDALQEYLIEKTFSVIEEFN :** ** :::: ***: **:: **:: **:::*	168 180	LLO_1014 Ceg23	NEMLEGENGVFSGFENYKEYRKMIKELYTSEFSVLYEANQEFLEYYYNRSLG-KIDKN AEMLEGENGVFSAFKNYKEYRSFMIKEELKISTEFGALYEANEAFLEYFYNRSESTLINKD ******::****.*:*******************::********:******:**	173 180
LLO_2238 Lem27	REWSIREQEFIEQKKELTERFIQSKKSTLLDKILRNETIEFFLGEEDKOLDQV/OOLQQE RQVENKSQELKEQ-SLTEKEIQV/KQATLDNILKKETIDFLLAENEIGUDEVREHLRR *:*. ::**: *****:*** :::*:**********	228 238	LLO_1014 Ceg23	SAFEEYFAHASSNEEAIRNYWNIEGYASYCKYMAQPQVRLSHIEIMAMORLYNQPLIIYD SPFEKYFWGSSSDEEAIRNYWDAEGYTLYCQHLARPQVRLSYIEIMIMORYINQPLIIYD * **:**. :**:****::***: ***:: **:::*:********	233 240
LLO_2238 Lem27	GVNGTEETLFVLHRAIQGEHVVRN-EGRANIVVDREIILHLYRNDGISVDQSGSPEIJDN FVNGSEETLMVLHRAIQGEHVVRNEGRIEFVVDNEITLHAVRBGASFSVQAGSFBMILN ***:****:**********::********::***	287 298	LLO_1014 Ceg23	RSTAAVVDEYRNINATSPKFEVALMASEGHYFLIKTDETKEDLEEYVQSQRQYKKDRSEL RSTSSIVAEYVNPKVNIPDFEVAIDALQGHYFLIKTEETEKELEEYERSYAQYKRDRSEI ***:::* ** *: *, ****::* :********::*::::****::* ***::	293 300
LLO_2238 Lem27	NKGNVHWTSIIPDSIFVLKYTPQEQKLYKLLDUMQMEFESIPQDSEKAVL/SSDWILALR NEGRVHWTSIIPDAIFTSKLTDKERKLLDMLERMQSEFGSKELGVEKK-SSISDWISDLM *:***********************************	347 357	LLO_1014 Ceg23	LSKSERPVSSLFVRATCPKGHLDDEPFDALIERVSELENLRKVEDKKPVGQKSTTASQNT LAHSDKPVSSLLVRATCPKGHLDEDFFIALIESLSEINSLSQIDTNLK——NENTDIP *:*:::*****:*************************	353 355
LLO_2238 Lem27	AQLDIHKDNFSREAKEKALGVSMQIIGKMMTTLGNVFYWRALLTHFFSALLECIFTFMAD NGIELIKTSFTAVTNGKEIELFFQILAKATFKLASEFALMNSLGTLFSNFLZCIFALMVE *::::*.*::::::::::::::::::::::::::::::	407 417	LLO_1014 Ceg23	NHNFLINLGSSIVGTSAALVTLVGIAILTGQVDFGPSLVEBAVATTVISSVAGLGVSLVN NCNFLLKVGASVVGTSSALITINGIAILSGQVDFGNSLAEBALATTVVSSIVGLGVGLVS * ****::::::::::::::::::::::::::::::::	413 415
LLO_2238 Lem27	KKISSGLIQCESLITTKQRPASVIPERKDISLAPENKEVSLATVTYNEESSSQSQKGLVS NKLSTGLAKFGLNSPVISQKKEDLSLTKSSEEKVNSDQPQKGVLS :*:*:**:	467 462	LLO_1014 Ceg23	FFTSNAQSNSSKEPEQVSNLSQKQKLN 440 FPSSSPKVSER DISTNGLSQKLKIN 439 **:*.: : : **:*	
LLO_2238 Lem27	KFYQHTLFKHPSATLIGKTYPEETARYITENNQTGPSVAKALRHNTQBGLNGHEYSPEHC QFSHTTLLKVSEDVSFHYFTELNFYVRHNNQSGFRVARALTQHTQKSLEGDENTTFHC :* :***:* * ** *: *: *:*:*:*:*:*:*	527 522			
LLO_2238 Lem27	REIAQRYISYVKYQGRNFVLADVVSFIKEMDDLIKQKEEQIDEFIEHIGGMKFK 581 KDIAQRYIEYSKSHCRNFILDDVIFVQEMUKITKQQESISENSSK-AAAM 572 ::******* * : ***:* ***: *:**:**:**:**: ::: * 572				

FIG S4. Sequence alignments of *L. longbeachae* OTU proteins and their orthologs in *L. pneumophila*. The alignments are generated via Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) using LLO_2238/Lem27 (A)

or LLO_1014/Ceg23 (B) as queries.



FIG S5. Translocation of *L. longbeachae* OTUs by the *L. pneumophila* **Dot/Icm system.** Plasmids expressing the indicated TEM fusion proteins were electroporated into both wild-type *L. penumophila* and *dotA*⁻ mutant. These strains were used to infect RAW264.7 cells for 2 h at an MOI of 50. After loading the samples with CCF4/AM, translocation of the TEM-fused OTUs were visualized by the Olympus IX83 fluorescence microscope equipped with a β -lactamase FL-Cube. Scale bar: 100 µm. Expression of the TEM fusion proteins in *L. pneumophila* strains were confirmed by probing the bacterial cells with a TEM antibody. Three randomly selected images were used to count the cells emitting blue fluorescence signals (n = 500). Data are one representative of three independent experiments.



FIG S6. *L. longbeachae* DUBs are not required for bacterial intracellular replication. U937 cells were challenged with wild-type, $\Delta dotB$, ΔLLO_1014 , ΔLLO_2238 , ΔLLO_2491 , and ΔLLO_3391 at an MOI of 10. At indicated time after infection, infected cells were lysed and plated on CYE plates. The CFUs were counted after culturing at 37 °C for 4 days. Results are presented as mean \pm SD and are one representative from three independent assays done in triplicate.

Bacterial Strains	Relevant properties	Reference
E. coli		
DH5α(λpir)	supE44 d <i>lacU169</i> (φ80 <i>lacZ</i> Δ <i>M15) hsdR17</i> recA1 endA1 gyrA96 thi-1 relA1 pir tet::Mu recA	Our collection
BL21(DE3)	F ⁻ ompT hsdSB (rB ⁻ mB ⁻) gal dcm (DE3)	Our collection
L. longbeachae		
L. longbeachae serogroup 1	ATCC ^a 33462; type strain	ATCC 33462
YS0001	ATCC 33462 <i>L. longbeachae</i> serogroup 1 strain with <i>dotB</i> deletion mutation	This study
YS0002	LLO+pXDC61JQ-Flag	This study
YS0003	LLO dotB+pXDC61JQ-Flag	This study
YS0004	LLO <i>ΔLLO_1014</i>	This study
YS0005	LLO ∆ <i>LLO_1014</i> +pXDC61JQ-Flag	This study
YS0006	LLO ∆ <i>LLO_1014</i> +pXDC61JQ-Flag- LLO_1014	This study
YS0007	LLO Δ <i>LLO_1014</i> +pXDC61JQ-Flag- LLO_1014 _{C26A}	This study
YS0008	LLO <i>ΔLLO_2238</i>	This study
YS0009	LLO ∆ <i>LLO_</i> 2238+pXDC61JQ-Flag	This study
YS0010	LLO Δ <i>LLO_2238</i> +pXDC61JQ-Flag- LLO_2238	This study
YS0011	LLO Δ <i>LLO_2238</i> +pXDC61JQ-Flag- LLO_2238 _{C12A}	This study
YS0012	LLO <u>ALLO_2491</u>	This study
YS0013	LLO <i>ALLO</i> _3391	This study
YS0014	LLO+pXDC61JQ-Flag-LLO_1014	This study
YS0015	LLO dotB+pXDC61JQ-Flag-LLO_1014	This study
YS0016	LLO+pXDC61JQ-Flag-LLO_2238	This study
YS0017	LLO dotB+pXDC61JQ-Flag-LLO_2238	This study
L. pneumophila		
Lp02	Philadelphia-1 rpsL hsdR thyA	(1)
Lp03	Lp02 dotA	(1)
Lp02 (pZL507)	Lp02+pZL507	(2)
Lp03 (pZL507)	Lp03+pZL507	(2)
YS0018	LP02 ∆ceg23	(3)
YS0019	LP02 ∆ <i>ceg</i> 23+pZL507	(3)
YS0020	LP02 ∆ <i>ceg</i> 23+pZL507-Ceg23	(3)
YS0021	LP02 ∆ <i>ceg</i> 23+pZL507-LLO_1014	This study
YS0022	LP02 ∆ <i>ceg</i> 23+pZL507-LLO_1014 _{C26A}	This study
YS0023	LP02 ∆ <i>lem</i> 27	(4)

Table S1. Bacterial strains used in the study.

YS0024	LP02 ∆ <i>lem</i> 27+pZL507	(4)	
YS0025	LP02 ∆ <i>lem</i> 27+pZL507-Lem27	(4)	
YS0026	LP02 ∆ <i>lem</i> 27+pZL507-LLO_2238	This study	
YS0027	LP02 ∆ <i>lem</i> 27+pZL507-LLO_2238c12A	This study	
YS0028	LP02 TEM- <i>LLO_1014</i>	This study	
YS0029	Lp03 dotA ⁻ TEM-LLO_1014	This study	
YS0030	LP02 TEM- <i>LLO_2238</i>	This study	
YS0031	Lp03 dotA ⁻ TEM-LLO_2238	This study	
	American Type Culture Collection		

^a ATCC, American Type Culture Collection

Plasmids	Properties	Reference	
	Kan ^R F Coli expression vectors for	Novagen	
pET28a	His-tagged proteins	(CAT#60861)	
	$\frac{110}{100} \frac{100}{100} \frac{110}{100} 11$		
pET28a- <i>LLO_0424</i> 1-200	1-200) in pET282	This study	
	1-200 III perzoa		
pET28a- <i>LLO_04</i> 25 ₁₋₂₀₀	1 200) in pET285	This study	
pET28a- <i>LLO_0426</i> 1-200	1 200) in pET282	This study	
pET28a- <i>LLO_3092₁₋₂₀₀</i>	1 200) in pET222	This study	
pET28a- <i>LLO_3095₁₋₂₀₀</i>		This study	
pET28a- <i>LLO_ 1014∆TM</i>	LLO_1014 Δ TMI (LLO_1014 residues	This study	
	1-358) IN PE 1288	-	
p∈128a-LLU_	$p = 128a - LLO_1014\Delta I M$ with mutation	This study	
1014Д I Mc26A		This study	
p∈128a- <i>LLO_2238</i>	Full length <i>LLO_2238</i> in pE128a	I his study	
pET28a- <i>LLO_2238</i> C12A	pE128a-LLO_2238 with mutation	This study	
	C12A		
pET28a-	LLO_2491AC102 (LLO_2491	This study	
LLO_2491AC102	residues 1-270) in pET28a	· - ,	
pET28a- <i>LLO_3391₁₀₀₉₋</i>	LLO_3391 ₁₀₀₉₋₁₂₀₀ (LLO_3391	This studv	
1200	residues 1009-1200) in pET28a	,	
pET28a- <i>LLO_</i> 3391 ₁₀₀₉₋	pET28a-LLO_33911009-1200 with	This study	
1200 C1124A	mutation C1124A		
pET28a- <i>LLO_0794</i>	Full length <i>LLO_0794</i> in pET28a	This study	
pET28a- <i>LLO_1369</i>	Full length <i>LLO_1369</i> in pET28a	This study	
pET28a- <i>LLO_1631</i>	Full length <i>LLO_1631</i> in pET28a	This study	
pET28a- <i>LLO_3118</i>	Full length <i>LLO_3118</i> in pET28a	This study	
pET28a- <i>LLO_2210</i>	Full length <i>LLO_2210</i> in pET28a	This study	
pET28a- <i>LLO_2179</i>	Full length <i>LLO_2179</i> in pET28a	This study	
pET28a- <i>LLO_2985</i>	Full length <i>LLO_2985</i> in pET28a	This study	
pET28a- <i>LLO_20</i> 66	Full length <i>LLO_2066</i> in pET28a	This study	
pSR47s	R6K suicide vector (Kan ^R , sacB)	(5)	
	pSR47s containing the flanking region	This study	
por4/s-allo_1014	of LLO_1014	THIS SLUDY	
	pSR47s containing the flanking region	This study	
p5K4/S-∆LLU_2238	of LLO_2238	i nis study	
	pSR47s containing the flanking region	This start	
pSR47s-∆ <i>LLO_2491</i>	of <i>LLO_2491</i>	This study	
	pSR47s containing the flanking region	T 1 1 1 1	
p5R4/s-∆LLU_3391	of <i>LLO_3391</i>	This study	

Table S2. Plasmids used in the study.

pSR47s-∆ <i>LLO_2066</i>	pSR47s containing the flanking region of <i>LLO_2066</i>	This study
peGFPC1	For expressing N-terminal GFP fusion proteins in mammalian cells	Clontech
peGFP-sdeA _{Dub}	sdeA _{Dub} (residues 1-200) in peGFPC1	(6)
peGFP- <i>LLO_1014</i>	<i>LLO_1014</i> in peGFPC1	This study
peGFP- <i>LLO_1014</i> _{C26A}	LLO_1014 _{C26A} in peGFPC1	This study
peGFP- <i>LLO_2238</i>	LLO_2238 in peGFPC1	This study
peGFP- <i>LLO_2491</i>	<i>LLO_2491</i> in peGFPC1	This study
peGFP-LLO_33911009-1200	LLO_33911009-1200 in peGFPC1	This study
peGFP- <i>LLO_2066</i>	LLO_2066 in peGFPC1	This study
pZL507	For expression His ₆ -tagged protein <i>L.</i> pneumophila	(7)
pZL507-4×Flag- <i>LLO_1014</i>	4×Flag- <i>LLO_1014</i> in pZL507	This study
pZL507-4×Flag- <i>LLO_1014</i> _{C264}	4×Flag- LLO_1014 _{C26A} in pZL507	This study
pZL507-4×Flag- <i>LLO_223</i> 8	4×Flag- <i>LLO_2238</i> in pZL507	This study
pZL507-4×Flag- <i>LLO_2238</i> _{C12A}	4×Flag- <i>LLO_2238c12A</i> in pZL507	This study
p3×HACDNA3.1- <i>ub</i>	Ubiquitin in p3×HACDNA3.1	(6)
p3xHACDNA3.1- <i>ub</i> -63K	Ub63K in p3×HACDNA3.1	(4)
pXDC61m	Encodes IPTG-inducible with N- terminal BlaM fusion; Cm ^R	(8)
pXDC61m- <i>LLO_1014</i>	<i>LLO_1014</i> in pXDC61m	This study
pXDC61m- <i>LLO_2238</i>	<i>LLO_2238</i> in pXDC61m	This study
	Encodes IPTG-inducible expressing	
pXDC61JQ	N-terminal Flag fusion proteins in <i>L.</i> <i>Longbeachae</i> ; Cm ^R	This study
pXDC61JQ- <i>LLO_1014</i>	LLO_1014 in pXDC61JQ	This study
pXDC61JQ- <i>LLO_2238</i>	<i>LLO_2238</i> in pXDC61JQ	This study

Table S3.	Primers	used in	the study	

Table	SS. Frimer's used in the study.	
Primers	Sequence ^a	Note
pYS1001	CTG <u>GGATCC</u> ATGTACGATAAAAAAGATG	<i>LLO_1014</i> 5F BamHI
pYS1002	CTG <u>GTCGAC</u> TTAATTCAACTTTTGTTT	<i>LLO_1014</i> 3R Sall
pYS1003	CTG <u>GTCGAC</u> TCAAAGGAAGTTATGATTTG	<i>LLO_1014∆TM</i> 3R Sall
pYS1004	CTG <u>GGATCC</u> ATGTTTCAAGTGGATAATAG	<i>LLO_2238</i> 5F BamHI
pYS1005	CTG <u>GTCGAC</u> TTACTTGAACTTCATTCC	<i>LLO_2238</i> 3R Sall
pYS1006	CTG <u>GGATCC</u> ATGCCTTTTAGAATAGAT	<i>LLO_2491</i> 5F BamHI
pYS1007	CTG <u>GTCGAC</u> TTAGTAGCTTAAAATTCG	<i>LLO_2491</i> 3R Sall
pYS1008	CTG <u>GTCGAC</u> TCAAGAAATTTCCCGGGGT	<i>LLO_2491∆C10</i> 2 3R Sall
pYS1009	CTG <u>GGATCC</u> ATGAAAAGCATTACAGAG	<i>LLO_2066</i> 5F BamHI
pYS1010	CTG <u>GTCGAC</u> TTACCCGAGTGTTGAAGA	<i>LLO_2066</i> 3R Sall
pYS1011	CTG <u>GGATCC</u> ATGCCTGAATACATAAAAG	<i>LLO_0424₁₋₂₀₀</i> 5F BamHI
pYS1012	CTG <u>GTCGAC</u> TTATATTTCTTTTAGAACAGT	<i>LLO_0424₁₋₂₀₀</i> 3R Sall
pYS1013	CTG <u>AGATCT</u> ATGCCTAAGTATGTAAAAG	<i>LLO_04</i> 25 ₁₋₂₀₀ 5F BgIII
pYS1014	CTG <u>GTCGAC</u> TTACGGAAGTACTCTGAGATG	LLO_04251-200 3R Sall
pYS1015	CTG <u>GGATCC</u> ATGCCTAAATACGTAAAAG	<i>LLO_0426₁₋₂₀₀</i> 5F BamHI
pYS1016	CTG <u>GTCGAC</u> TTAAGGGATTTCTATATGTAA	<i>LLO_0426₁₋₂₀₀</i> 3R Sall
pYS1017	CTG <u>GGATCC</u> ATGCCAAAATACATAAAAG	<i>LLO_3092₁₋₂₀₀</i> 5F BamHI
pYS1018	CTG <u>GTCGAC</u> TTAAGTTGGAGTTTCTATATG	<i>LLO_3092₁₋₂₀₀</i> 3R Sall
pYS1019	CTG <u>AGATCT</u> ATGCCCGAGTTTGTGCAAG	<i>LLO_3095₁₋₂₀₀</i> 5F BgIII
pYS1020	CTG <u>GTCGAC</u> TTATGCGGGTATGTGTATGTT	<i>LLO_3095₁₋₂₀₀</i> 3R Sall
pYS1021	CTG <u>GGATCC</u> ATGTTATACGTAGAAGAAATG	LLO_33911009-1200 5F BamHI
pYS1022	CTG <u>GTCGAC</u> TCATTCACTTTCTTGCCTTA	<i>LLO_3391₁₀₀₉₋₁₂₀₀</i> 3R Sall
pYS1023	CTG <u>GGATCC</u> ATGATTCCAGTAGAAATAG	<i>LLO</i> _1631 5F BamHI
pYS1024	CTG <u>GTCGAC</u> TTAAGAAACGGAGCCCAT	<i>LLO</i> _1631 3R Sall
pYS1025	CTG <u>GGATCC</u> ATGCGACTAGAGATATCAA	<i>LLO</i> _3118 5F BamHI
pYS1026	CTG <u>GTCGAC</u> TTATCGATTATGCTTTAT	<i>LLO</i> _3118 3R Sall
pYS1027	CTG <u>GAGCTC</u> ATGAAAGATGATAAGTCAG	<i>LLO</i> _2179 5F Sacl
pYS1028	CTG <u>GCGGCCGC</u> TTAAACTTTAAATTGGCT	LLO_2179 3R Notl
pYS1029	CTG <u>GGATCC</u> ATGCAAAACTCATGGAAAG	<i>LLO</i> _0794 5F BamHI
pYS1030	CTG <u>GTCGAC</u> TCATTTCTCTGCACTAAT	<i>LLO</i> _0794 3R Sall
pYS1031	CTG <u>GCGGCCGC</u> ATGAGAGATGATAAGTTAG	LLO_2985 5F NotI
pYS1032	CTG <u>CTCGAG</u> CTAAAAGTTAAATTGTCT	LLO_2985 3R Xhol
pYS1033	CTG <u>GGATCC</u> ATGTTTACCGAAGAATTCA	<i>LLO</i> _1369 5F BamHI
pYS1034	CTG <u>GTCGAC</u> TTAGACACTTGTAACGTT	<i>LLO</i> _1369 3R Sall
pYS1035	CTG <u>GCGGCCGC</u> ATGACTCTACCAATATTT	LLO_2210 5F NotI
pYS1036		<i>LLO</i> _2210 3R Sall
pYS1107	CTG <u>GTCGAC</u> TACCAAGCTGGTAAGA	 LLO dotB ⁻ knockout up Sall-F
pYS1108	ATTAATTTATATACTCGAGTAAAACGAGTAGGCTC	LLO dotB knockout up-R
	ATCAGG	
pYS1109	CCTGATGAGCCTACTCGTTTTACTCGAGTATATAA ATTAAT	LLO dotB ⁻ knockout down-F
pYS1110	CTGGGATCCGGTAAGGGGGATGAAT	<i>LLO dotB</i> knockout down
F		

		BamHI-R
pYS1037	ACGC <u>GTCGAC</u> AACCCGCTCATTATGTTACC	LLO_1014knockout up Sall-F
pYS1038	AAATTGGATACTTGTTCTGGATTTTTTACTCGAAC TTTAA	LLO_1014 knockout up-R
pYS1039	TTAAAGTTCGAGTAAAAAATCCAGAACAAGTATC CAATTT	LLO_1014 knockout down-F
pYS1040	CGC <u>GGATCC</u> TAATCGATTTTTTTAGCAGG	<i>LLO_1014</i> knockout down BamHI-R
pYS1041	ACGC <u>GTCGAC</u> ACTCACAATGTTTTCCTATT	LLO_2238knockout up Sall-F
pYS1042	TGCTCAATAAACTCATCAATATAATACATACAATTG CCTT	LLO_2238 knockout up-R
pYS1043	AAGGCAATTGTATGTATTATATTGATGAGTTTATTG AGCA	LLO_2238 knockout down-F
pYS1044	CGC <u>GGATCC</u> AAATTTATCATTTGTAGGAA	<i>LLO_2238</i> knockout down BamHI-R
pYS1045	ACGC <u>GTCGAC</u> TTTAGAAGATGATACTGGTG	LLO_2491 knockout up Sall-F
pYS1046	TCTGCTTCTTGTAAGCGGGTAAATCCACAATCAC CACCAC	LLO_2491 knockout up-R
pYS1047	GTGGTGGTGATTGTGGATTTACCCGCTTACAAGA AGCAGA	LLO_2491 knockout down-F
pYS1048	CGC <u>GGATCC</u> AAAAGTTCCTTAAGTCAATG	<i>LLO_2491</i> knockout down BamHI-R
pYS1049	ACGC <u>GTCGAC</u> GCTTTAATAAATCCCTCAAG	LLO_3391 knockout up Sall-F
pYS1050	TTCTGTTCAGCATTAGTGTCTTTTTCCTTAGAACC TTCGT	LLO_3391 knockout up-R
pYS1051	ACGAAGGTTCTAAGGAAAAAGACACTAATGCTG AACAGAA	LLO_3391 knockout down-F
pYS1052	CGC <u>GGATCC</u> TCTGTCTCTGTTTTCATCCA	<i>LLO_3391</i> knockout down BamHI-R
pYS1053	AAGAAATACTATACGCATAATACATAGCATTGCCT TTACCACTATTATCCACTTG	LLO_2238 _{C12A} -1
pYS1054	CAAGTGGATAATAGTGGTAAAGGCAATGCTATGT ATTATGCGTATAGTATTTCTT	LLO_2238c12A-2
pYS1055	AGTAAATAAAGTGCATAGTTATGAAAAAAAGCATT ATCAAAACTTCCAGAAACATCAACTGC	LLO_1014 _{C26A} -1
pYS1056	GCAGTTGATGTTTCTGGAAGTTTTGATAATGCTT TTTTTCATAACTATGCACTTTATTTACT	LLO_1014 _{C26A} -2
pYS1057	GCAACCCAATACCCAGCTAATCCTCTATTTAATCC TTTGGCATTCG	LLO_3391 _{C1124A} -1
pYS1058	CGAATGCCAAAGGATTAAATAGAGGATTAGCTGG GTATTGGGTTGC	LLO_3391 _{C1124A} -2

^aRestriction enzyme sites are underlined.

REFERENCES

1. Berger KH, Isberg RR. 1993. Two distinct defects in intracellular growth complemented by a single genetic locus in *Legionella pneumophila*. *Mol Microbiol* 7:7-19 https://doi.org/10.1111/j.1365-2958.1993.tb01092.x.

2. Liu Y, Luo ZQ. 2007. The *Legionella pneumophila* effector SidJ is required for efficient recruitment of endoplasmic reticulum proteins to the bacterial phagosome. *Infect Immun* 75:592-603 https://doi.org/10.1128/iai.01278-06.

3. Ma K, Zhen X, Zhou B, Gan N, Cao Y, Fan C, Ouyang S, Luo ZQ, Qiu J. 2020. The bacterial deubiquitinase Ceg23 regulates the association of Lys-63-linked polyubiquitin molecules on the *Legionella* phagosome. *J Biol Chem* 295:1646-1657 https://doi.org/10.1074/jbc.RA119.011758.

 Liu S, Luo J, Zhen X, Qiu J, Ouyang S, Luo ZQ. 2020. Interplay between bacterial deubiquitinase and ubiquitin E3 ligase regulates ubiquitin dynamics on *Legionella* phagosomes. *Elife* 9:e58114 https://doi.org/10.7554/eLife.58114.
Duménil G, Isberg RR. 2001. The *Legionella pneumophila* IcmR protein exhibits chaperone activity for IcmQ by preventing its participation in highmolecular-weight complexes. *Mol Microbiol* 40:1113-27 https://doi.org/10.1046/j.1365-2958.2001.02454.x.

Qiu J, Sheedlo MJ, Yu K, Tan Y, Nakayasu ES, Das C, Liu X, Luo ZQ. 2016.
Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. *Nature* 533:120-4 https://doi.org/10.1038/nature17657.

7. Xu L, Shen X, Bryan A, Banga S, Swanson MS, Luo ZQ. 2010. Inhibition of host vacuolar H+-ATPase activity by a *Legionella pneumophila* effector. *PLoS*

Pathog 6:e1000822 https://doi.org/10.1371/journal.ppat.1000822.

8. Zhu W, Banga S, Tan Y, Zheng C, Stephenson R, Gately J, Luo ZQ. 2011. Comprehensive identification of protein substrates of the Dot/Icm type IV transporter of *Legionella pneumophila*. *PLoS One* 6:e17638 https://doi.org/10.1371/journal.pone.0017638.