Supporting information
for

Host cell-catalyzed S-palmitoylation mediates Golgi targeting of the *Legionella* ubiquitin ligase GobX[*]

Yi-Han Lin, Eric Cheng, Alexandra G. Doms, Byoungkwan Kim, Timothy R. Evans, and Matthias P. Machner[#]

Unit on Microbial Pathogenesis, Cell Biology and Metabolism Program, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA, 20892

[*]Running title: *S-Palmitoylation of Legionella GobX*

Table S1. Plasmids used in this study.

Name	Properties	Source
E. coli		
pDEST17	Gateway^{TM} destination vector generating an N-terminal His$_6$ tag	Invitrogen
pDEST17-Lpg2455	*lpg2455* in pDEST17	This study
pGEX-6p-1	*E. coli* expression vector generating an N-terminal GST fusion	GE
pGEX-6p-1-Lpg2455	*lpg2455* in pGEX-6p-1	This study
pGEX-6p-1-Lpg2455^{I58A}	pGEX-6p-1-Lpg2455 with mutation I58A	This study
pGEX-6p-1-Lpg2455^{W87A}	pGEX-6p-1-Lpg2455 with mutation W87A	This study
pGEX-6p-1-Lpg2455$^{I58A/W87A}$	pGEX-6p-1-Lpg2455 with mutation I58A and W87A	This study
Legionella		
pJB908	*Legionella* expression vector; a derivative of pKB5 (Amp^r *td*Δ*i*)	(1)
pJB908D	pJB908 containing the nucleotides between *att*R1-*att*R2 from pDEST17; Gateway^{TM} compatible	This study
pJB908D-Lpg2455	*lpg2455* in pJB908D	This study
pSR47S	R6K suicide vector (Kan^r *sacB*)	(2)
pSR47S-Δlpg2455	pSR47S containing flanking regions of *lpg2455*	This study
pXDC61	*Legionella* expression vector derived from pMMB207C	(3)
pXDC61-Lpg2455	*lpg2455* in pXDC61	This study
pXDC61.1-HA	pXDC61 backbone with the β-lactamase region replaced by a hemagglutinin (HA) tag at the *Nde*I and *Kpn*I sites	This study
pXDC61.1-HA-Lpg2455	*lpg2455* in pXDC61.1-HA	This study
pXDC61.1-HA-Lpg2455^{C175A}	pXDC61.1-HA-Lpg2455 with mutation C175A	This study
S. cerevisiae		
pYES2/NTA	*S. cerevisiae* expression vector containing the *URA3* gene and 2μ origin	Invitrogen
pYES2-VipD	*vipD* in pYES2/NTA	(4)
pYES2-Lpg2455	*Lpg2455* in pYES2/NTA	This study
pYES2-Lpg2455^{I58A}	pYES2-Lpg2455 with mutation I58A	This study
pYES2-Lpg2455^{W87A}	pYES2-Lpg2455 with mutation W87A	This study
pYES2-Lpg2455$^{I58A/W87A}$	pYES2-Lpg2455 with mutation I58A and W87A	This study
Cell culture		
pcDNA6.2-EmGFP	Gateway^{TM} destination vector generating an N-terminal EmGFP fusion	Invitrogen
pcDNA6.2-EmGFP-Lpg2455	*lpg2455* in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{1-50}$	*lpg2455* residues 1-50 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{51-120}$	*lpg2455* residues 51-120 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{1-120}$	*lpg2455* residues 1-120 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{101-209}$	*lpg2455* residues 101-209 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{121-209}$	*lpg2455* residues 121-209 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{141-209}$	*lpg2455* residues 141-209 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{161-209}$	*lpg2455* residues 161-209 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{181-209}$	*lpg2455* residues 181-209 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{161-200}$	*lpg2455* residues 161-200 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{161-190}$	*lpg2455* residues 161-190 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{161-180}$	*lpg2455* residues 161-180 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455$_{171-190}$	*lpg2455* residues 171-190 in pcDNA6.2-EmGFP	This study
pcDNA6.2-EmGFP-Lpg2455^{C56S}	pcDNA6.2-EmGFP-Lpg2455 with mutation C56S	This study
pcDNA6.2-EmGFP-Lpg2455^{C175S}	pcDNA6.2-EmGFP-Lpg2455 with mutation C175S	This study
pcDNA6.2-EmGFP-Lpg2455^{C175A}	pcDNA6.2-EmGFP-Lpg2455 with mutation C175A	This study
pcDNA6.2-EmGFP-Lpg2455$_{161-209}$^{C175A}	pcDNA6.2-EmGFP-Lpg2455$_{161-209}$ with mutation C175A	This study
pcDNA6.2-EmGFP-Lpg2455^{Q171A}	pcDNA6.2-EmGFP-Lpg2455 with mutation Q171A	This study
pcDNA6.2-EmGFP-Lpg2455^{S172A}	pcDNA6.2-EmGFP-Lpg2455 with mutation S172A	This study
pcDNA6.2-EmGFP-Lpg2455^{R173A}	pcDNA6.2-EmGFP-Lpg2455 with mutation R173A	This study
pcDNA6.2-EmGFP-Lpg2455^{S174A}	pcDNA6.2-EmGFP-Lpg2455 with mutation S174A	This study
pcDNA6.2-EmGFP-Lpg2455^{W176A}	pcDNA6.2-EmGFP-Lpg2455 with mutation W176A	This study
pcDNA6.2-EmGFP-Lpg2455^{S177A}	pcDNA6.2-EmGFP-Lpg2455 with mutation S177A	This study
pcDNA6.2-EmGFP-Lpg2455^{L178A}	pcDNA6.2-EmGFP-Lpg2455 with mutation L178A	This study
pcDNA6.2-EmGFP-Lpg2455^{F179A}	pcDNA6.2-EmGFP-Lpg2455 with mutation F179A	This study

pcDNA6.2-EmGFP-Lpg2455^{Y181A}	pcDNA6.2-EmGFP-Lpg2455 with mutation Y181A	This study
pcDNA6.2-EmGFP-Lpg2455^{S182A}	pcDNA6.2-EmGFP-Lpg2455 with mutation S182A	This study
pcDNA6.2-EmGFP-Lpg2455^{I183A}	pcDNA6.2-EmGFP-Lpg2455 with mutation I183A	This study
pcDNA6.2-EmGFP-Lpg2455^{F184A}	pcDNA6.2-EmGFP-Lpg2455 with mutation F184A	This study
pcDNA6.2-EmGFP-Lpg2455^{K187A}	pcDNA6.2-EmGFP-Lpg2455 with mutation K187A	This study
pcDNA6.2-EmGFP-Lpg2455^{D188A}	pcDNA6.2-EmGFP-Lpg2455 with mutation D188A	This study
pcDNA6.2-EmGFP-Lpg2455^{N189A}	pcDNA6.2-EmGFP-Lpg2455 with mutation N189A	This study
pcDNA6.2-EmGFP-Lpg2455^{R190A}	pcDNA6.2-EmGFP-Lpg2455 with mutation R190A	This study
pcDNA6.2-EmGFP-Lpg2455^{L178S}	pcDNA6.2-EmGFP-Lpg2455 with mutation L178S	This study
pcDNA6.2-EmGFP-Lpg2455^{L180S}	pcDNA6.2-EmGFP-Lpg2455 with mutation L180S	This study
pEGFP-C1	Mammalian expression vector generating an N-terminal GFP fusion	Clontech
pEGFP-C1-Lpg2455$_{171-190}$	*lpg2455* residues 171-190 in pEGFP-C1	This study
pEGFP-C1-Lpg2455$_{172-186}$	*lpg2455* residues 172-186 in pEGFP-C1	This study
pEGFP-C1-Lpg2455$_{175-184}$	*lpg2455* residues 175-184 in pEGFP-C1	This study
pEGFP-C1-Lpl2374$_{171-190}$	*lpl2374* residues 171-190 in pEGFP-C1	This study
pEGFP-C1-Lpo2646$_{171-190}$	*lpo2646* residues 171-190 in pEGFP-C1	This study
pEF-BOS/HA-DHHS4	pEF-BOS/HA-DHHC4 with a Cys->Ser substitution in the DHHC motif	This study
pEF-BOS/HA-DHHS21	pEF-BOS/HA-DHHC21 with a Cys->Ser substitution in the DHHC motif	This study
pmCherry-C1	Mammalian expression vector generating an N-terminal mCherry fusion	Clontech
pmCherry-C1-Lpg2455	*lpg2455* in pmCherry-C1	This study

1. Sexton, J. A., Pinkner, J. S., Roth, R., Heuser, J. E., Hultgren, S. J., and Vogel, J. P. (2004) The Legionella pneumophila PilT Homologue DotB Exhibits ATPase Activity That Is Critical for Intracellular Growth. *Journal of Bacteriology* **186**, 1658-1666

2. Merriam, J. J., Mathur, R., Maxfield-Boumil, R., and Isberg, R. R. (1997) Analysis of the Legionella pneumophila fliI gene: intracellular growth of a defined mutant defective for flagellum biosynthesis. *Infection and Immunity* **65**, 2497-2501

3. de Felipe, K. S., Glover, R. T., Charpentier, X., Anderson, O. R., Reyes, M., Pericone, C. D., and Shuman, H. A. (2008) Legionella Eukaryotic-Like Type IV Substrates Interfere with Organelle Trafficking. *PLoS Pathog* **4**, e1000117

4. Gaspar, A. H., and Machner, M. P. (2014) VipD is a Rab5-activated phospholipase A1 that protects Legionella pneumophila from endosomal fusion. *Proceedings of the National Academy of Sciences* **111**, 4560-4565

Table S2. Oligonucleotides used in this study.

Name	Sequence (5'→3', **bold**: mutation; <u>underline</u>: RE site; *italic*: *att*B)	Cloning
BamHI_lpg2455_for	AAAA<u>GGATCC</u>ACGAAAATTGTTTATCTACACAATGACCC	pGEX-6p-1
EcoRI_lpg2455_rev	AAAA<u>GAATTC</u>TTAATGATGGGGCTGTATATCATACG	pGEX-6p-1
lpg2455(I58A)_for	TTTTATTGTGCA**GCT**TGTATGGAACTTCC	SD
lpg2455(I58A)_rev	GGAAGTTCCATACA**AGC**TGCACAATAAAA	SD
lpg2455(W87A)_for	GTGAATTAGTCAAA**GCG**TTTTCAAGAGACC	SD
lpg2455(W87A)_rev	GGTCTCTTGAAAA**CGC**TTTGACTAATTCAC	SD
pdest (SacI)	TCACCAT<u>GAGCTC</u>GAATCAACAAG	pJB908
pdest (KpnI)	GCTTCCTTTCGGGCTTT<u>GGTACC</u>AGCC	pJB908
lpg2455 upst_SalI	AAAA<u>GTCGAC</u>GAGTCATAACTTCAGCATAATGCATCG	pSR47S
lpg2455 downst_SacI	AAAA<u>GAGCTC</u>GGATGAGATTTGGCAAAATGACTGACCG	pSR47S
Δlpg2455_for	TTCTATGGAGAGGTTATGACGAAAATTGCCCATCATTAATGAAAATAAATCAAGT	Δ*lpg2455*
Δlpg2455_rev	ACTTGATTTATTTTCATTAATGATGGGCAATTTTCGTCATAACCTCTCCATAGAA	Δ*lpg2455*
KpnI_lpg2455_for	GATC<u>GGTACC</u>ATGACGAAAATTGTTTATCTACACAATG	pXDC61
XbaI_lpg2455_rev	GATC<u>TCTAGA</u>TTAATGATGGGGCTGTATATCATAC	pXDC61 pYES2
5'P_NdeI_HA_KpnI_sense	<u>TATG</u>GCTGGATCTTACCCATACGATGTTCCAGATTACGCT<u>GGTAC</u>	pXDC61.1-HA
5'P_NdeI_HA_KpnI_anti	<u>C</u>AGCGTAATCTGGAACATCGTATGGGTAAGATCCAGC<u>CA</u>	pXDC61.1-HA
BamHI_AG_lpg2455_for	AAAA<u>GGATCC</u>AGATGACGAAAATTGTTTATCTACACAATG	pYES2
attB1_lpg2455_1	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGACGAAAATTGTTTATCTACACAATGA	GW
attB1_lpg2455_51	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGTTAGATTGGTTTTATTGTGCAATTTG	GW
attB1_lpg2455_101	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGCTTGGTTTAAATGTTACTATATCTCA	GW
attB2_lpg2455_50	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTAATCAACTACTTCGAGTAGTTTTAATTT	GW
attB2_lpg2455_120	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTAATCAAGCAATTCTTTTGGAGTAGGAGT	GW
attB2_lpg2455_209	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTAATGATGGGGCTGTATATCATACGATGT	GW
attB1_lpg2455_121	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGTTTGTTATTAAATCAACAGGATTTGC	GW
attB1_lpg2455_141	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGCTAGCAAAAAAAATTTCCCCTGAGG	GW
attB1_lpg2455_161	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGCGCAATGAAATAGATAGCCCCACTC	GW
attB1_lpg2455_181	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGTATAGTATTTTTGGTGGTAAAGATAA	GW
attB2_lpg2455_200	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTATAGGGAATCAGTTTTTTCCTGCATTAC	GW
attB2_lpg2455_190	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTATCTGTTATCTTTACCACCAAAAATAC	GW
attB2_lpg2455_180	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CCTACAAAAAGAGACTCCAGCAGGATCTTG	GW
attB1_lpg2455_171	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCATGCAGTCAAGATCCTGCTGGAGT	GW
lpg2455(C56S)_for	GATTGGTTTTAT**TCT**GCAATTTGTATGGAAC	SD
lpg2455(C56S)_rev	GTTCCATACAAATTGC**AGA**ATAAAACCAATC	SD
lpg2455(C175S)_for	CACAGTCAAGATCC**TCC**TGGAGTCTCTTTTG	SD
lpg2455(C175S)_rev	CAAAAAGAGACTCCA**GGA**GGATCTTGACTGTG	SD
lpg2455(C175A)_for	CACAGTCAAGATCC**GCC**TGGAGTCTCTTTTTG	SD
lpg2455(C175A)_rev	CAAAAAGAGACTCCA**GGC**GGATCTTGACTGTG	SD
lpg2455(Q171A)_for	AGCCCCACTCATTCA**GCG**TCAAGATCCTGC	SD
lpg2455(Q171A)_rev	GCAGGATCTTGA**CGC**TGAATGAGTGGGGCT	SD
lpg2455(S172A)_for	ACTCATTCACAG**GCA**AGATCCTGCTGG	SD
lpg2455(S172A)_rev	CCAGCAGGATCT**TGC**CTGTGAATGAGT	SD

lpg2455(R173A)_for	CTCATTCACAGTCA**GCA**TCCTGCTGGAGTC	SD
lpg2455(R173A)_rev	GACTCCAGCAGGA**TGC**TGACTGTGAATGAG	SD
lpg2455(S174A)_for	CATTCACAGTCAAGA**GCC**TGCTGGAGTCTC	SD
lpg2455(S174A)_rev	GAGACTCCAGCA**GGC**TCTTGACTGTGAATG	SD
lpg2455(W176A)_for	CAGTCAAGATCCTGC**GCG**AGTCTCTTTTTG	SD
lpg2455(W176A)_rev	CAAAAAGAGACT**CGC**GCAGGATCTTGACTG	SD
lpg2455(S177A)_for	CAAGATCCTGCTGG**GCT**CTCTTTTTGTATAG	SD
lpg2455(S177A)_rev	CTATACAAAAAGAG**AGC**CCAGCAGGATCTTG	SD
lpg2455(L178A)_for	GATCCTGCTGGAGT**GCC**TTTTTGTATAGTATT	SD
lpg2455(L178A)_rev	AATACTATACAAAAA**GGC**ACTCCAGCAGGATC	SD
lpg2455(F179A)_for	CCTGCTGGAGTCTC**GCT**TTGTATAGTATTTTTG	SD
lpg2455(F179A)_rev	CAAAAATACTATACAA**AGC**GAGACTCCAGCAGG	SD
lpg2455(Y181A)_for	GGAGTCTCTTTTTG**GCT**AGTATTTTTGGTGG	SD
lpg2455(Y181A)_rev	CCACCAAAAATACT**AGC**CAAAAAGAGACTCC	SD
lpg2455(S182A)_for	GTCTCTTTTTGTAT**GCT**ATTTTTGGTGGTAAAG	SD
lpg2455(S182A)_rev	CTTTACCACCAAAAAT**AGC**ATACAAAAAGAGAC	SD
lpg2455(I183A)_for	CTCTTTTTGTATAGT**GCT**TTTGGTGGTAAAG	SD
lpg2455(I183A)_rev	CTTTACCACCAAA**AGC**ACTATACAAAAAGAG	SD
lpg2455(F184A)_for	CTTTTTGTATAGTATT**GCT**GGTGGTAAAGATAAC	SD
lpg2455(F184A)_rev	GTTATCTTTACCACC**AGC**AATACTATACAAAAAG	SD
lpg2455(K187A)_for	GTATTTTGGTGGT**GCA**GATAACAGAAAAG	SD
lpg2455(K187A)_rev	CTTTTCTGTTATC**TGC**ACCACCAAAAATAC	SD
lpg2455(D188A)_for	GTATTTTGGTGGTAAA**GCT**AACAGAAAAGTAATGC	SD
lpg2455(D188A)_rev	GCATTACTTTTCTGTT**AGC**TTTACCACCAAAAATAC	SD
lpg2455(N189A)_for	GGTGGTAAAGAT**GCC**AGAAAAGTAATGCAG	SD
lpg2455(N189A)_rev	CTGCATTACTTTTCT**GGC**ATCTTTACCACC	SD
lpg2455(R190A)_for	GGTGGTAAAGATAAC**GCA**AAAGTAATGCAG	SD
lpg2455(R190A)_rev	CTGCATTACTTT**TGC**GTTATCTTTACCACC	SD
lpg2455(L178S)_for	GATCCTGCTGGAGT**TCC**TTTTTGTATAGTATTTTTGG	SD
lpg2455(L178S)_rev	CCAAAAATACTATACAAAAA**GGA**ACTCCAGCAGGATC	SD
lpg2455(L180S)_for	GCTGGAGTCTCTTTT**TCG**TATAGTATTTTTGGTGG	SD
lpg2455(L180S)_rev	CCACCAAAAATACTATA**CGA**AAAGAGACTCCAGC	SD
5'P_lpg2455_171-190_sense	<u>AATTC</u>TCAGTCAAGATCCTGCTGGAGTCTCTTTTTGTATAGTATTTTT GGTGGTAAAGATAACAGATAA<u>G</u>	pEGFP-C1
5'P_lpg2455_171-190_anti	<u>GATCC</u>TTATCTGTTATCTTTACCACCAAAAATACTATACAAAAAGA GACTCCAGCAGGATCTTGACTGA<u>G</u>	pEGFP-C1
5'P_lpg2455_172-186_sense	<u>AATTC</u>TTCAAGATCCTGCTGGAGTCTCTTTTTGTATAGTATTTTTGGT GGTTAA<u>G</u>	pEGFP-C1
5'P_lpg2455_172-186_anti	<u>GATCC</u>TTAACCACCAAAAATACTATACAAAAAGAGACTCCAGCAGG ATCTTGAA<u>G</u>	pEGFP-C1
5'P_lpg2455_175-184_sense	<u>AATTC</u>TTGCTGGAGTCTCTTTTTGTATAGTATTTTTTAA<u>G</u>	pEGFP-C1
5'P_lpg2455_175-184_anti	<u>GATCC</u>TTAAAAAATACTATACAAAAAGAGACTCCAGCAA<u>G</u>	pEGFP-C1
5'P_lpl2374_171-190_sense	<u>AATTC</u>TCAGCAAAGATCCTGCTTGAGTCTCTTTTTGTACAGTATTTT TGGTTGTAAAGGAAAGAGATAA<u>G</u>	pEGFP-C1
5'P_lpl2374_171-190_anti	<u>GATCC</u>TTATCTCTTTCCTTTACAACCAAAAATACTGTACAAAAAGAG ACTCAAGCAGGATCTTTGCTGA<u>G</u>	pEGFP-C1
5'P_lpo2646_171-190_sense	<u>AATTC</u>TAAGCAAAGATCCTGTTTGGGTCTCTTGTTGTATAGTATTTT TGGTTGTAAAGACAACAGATAA<u>G</u>	pEGFP-C1
5'P_lpo2646_171-190_anti	<u>GATCC</u>TTATCTGTTGTCTTTACAACCAAAAATACTATACAACAAGA GACCCAAACAGGATCTTTGCTTA<u>G</u>	pEGFP-C1
Zdhhc4(C179S)_for	GTTCGACCATCAC**TCT**GTTTGGGTGAAC	SD
Zdhhc4(C179S)_rev	GTTCACCCAAAC**AGA**GTGATGGTCGAAC	SD
Zdhhc21(C120S)_for	GGATGGACCATCAC**TCT**CCTTGGATAAACAATTG	SD
Zdhhc21(C120S)_rev	CAATTGTTTATCCAAGG**AGA**GTGATGGTCCATCC	SD
lpg2455 SalI upst	AAAA<u>GTCGAC</u>ATGACGAAAATTGTTTATCTACACAATGAC	pmCherry-C1
lpg2455 BamHI down	AAAA<u>GGATCC</u>CTTGATTTATTTTCATTAATGATGGGGCTG	pmCherry-C1

SD, site-directed mutagenesis; GW, Gateway[TM] cloning