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online data

SUPPLEMENTARY ONLINE DATA

Molecular mechanism of elongation factor 1A inhibition by a *Legionella pneumophila* glycosyltransferase

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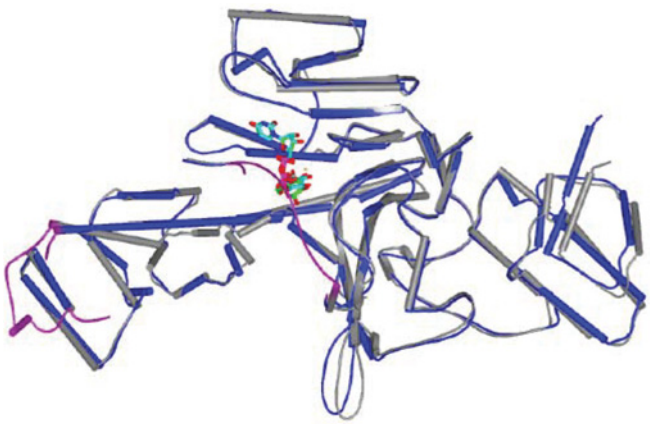

Figure S1 Superposition of *Lpp*GT and *LplGT* crystal structures

*Lpp*GT is shown in grey and *LplGT* in blue. UDP and glucose are shown in green sticks in *Lpp*GT and UDP-glucose is shown in cyan sticks in *Lpl*GT. Disordered regions in the *Lpl*GT structure are indicated in purple.

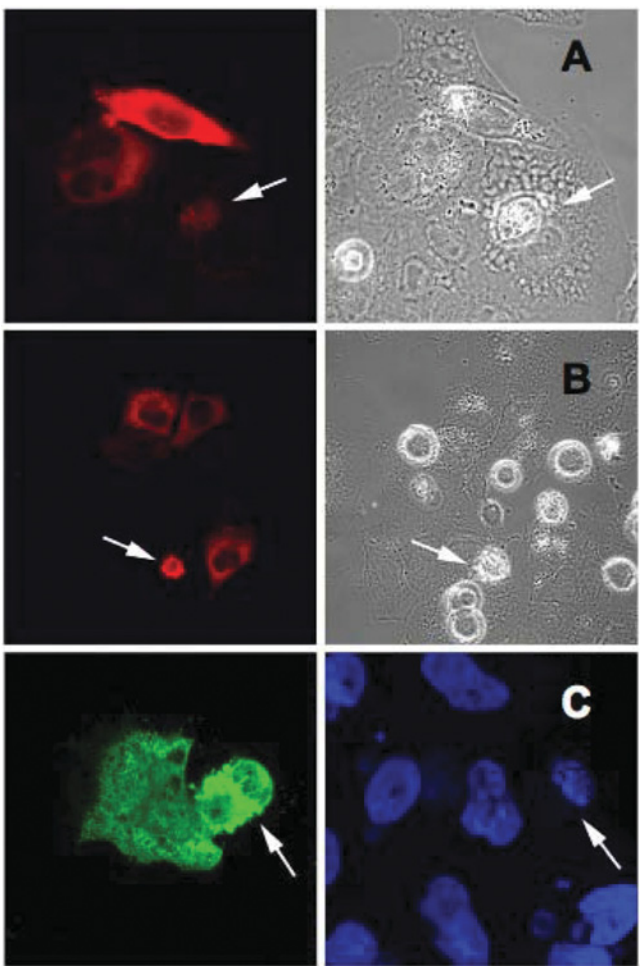

Figure S2 Cells microinjected with *Lpp*GT and incubated for 24 h post-injection exhibited characteristics of programmed cell death

(**A**) A dead injected cell, as indicated by the Texas Red–dextran fluorescence (arrow in the left-hand image) has been phagocytosed by a neighbouring uninjected cell (arrow in right-hand phase contrast microscopy image). Other injected cells, as indicated by red fluorescence, are still intact. (**B**) An injected cell (arrow) has many visible cell surface blebs. Left-hand image, Texas Red–dextran fluorescence; right-hand image, phase contrast microscopy image. (**C**) A cell (arrow) injected with FLAG-tagged *Lpp*GT (counterstained with Alexa Fluor® 488; green in the left-hand image) at an earlier phase of cell death than the cell in (**B**). This cell also shows cell surface blebbing and clumped DAPI-stained DNA in the nucleus (right-hand image).

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The structural co-ordinates reported will appear in the Protein Data Bank under accession codes 2WZF and 2WZG.

Table S1 Primers used in the present study

Restriction enzymes sites used during the cloning are underlined.

Gene	Primer name	Sequence (5′-3′)
Primers used to clone the glucosyltransferase genes in pGEX6P1		
Lpg1368	1368-for-EcoRI	GCGAATTCATGAAAGCAAGAAGGGATCAACAACTCTC
	1368-rev-NotI	CTGCGGCCGCCTACCCTACTGAAGGCAACCAACTC
Lpg1319	1319-for-EcoRI	GCGAATTCATGAAAGCAAGAAGGAGTAACGAACTTTCCAAATTG
	1319-rev-NotI	CTGCGGCCGCCTACCCTACTGAAGGCAACCAACTC
Lpg1488/Lgt3	1488-for-ApaI	AAAAAAGGGCCCATGAAAGAGCAACAAAAGGCAATTTTTATTGAGAATATC
	1488-rev-SmaI	CTGCGGCCGCCCCGGGCTAACAGTTTTTTTGACTCAATTCGCTATTGATTGC
Lpg2862/Lgt2	2862-for-BamHI	AAAAGGATCCATGAGCGAACAATATTGGCGTTTCAAATTGATGAC
	2862-rev-XhoI	AAAACTCGAGTTATTTTGATAATCGTGCTTGCTCACTAGGCATC
Primer used for construction of CyaA fusions		
Lpg1368	1368-cyaA-EcoRI	GACGGAATTCGATGAAAGCAAGAAGGGATCAAC
	1368-cyaA-PstI	GAGACTGCAGCTACCCTACTGAAGGCAACC
	1368-cyaA-T-EcoRI	GACGGAATTCGTTAAAGGAAGCTCAAAGCAAATAC
	1368-cyaA-N-SalI	GAGCGTCGACACTCCTACTGAAGGCAACCAACTC
Lpg1488/Lgt3	LegC5-XbaI	GAGGTCTAGAGATGAAAGAGCAACAAAAGGCA
	LegC5-PstI	GACGCTGCAGCATTCTATGTCTAGCCTAATTCC
Lpg2862/Lgt2	LegC8-EcoRI	GAGGGAATTCGATGAGCGAACAATATTGGCG
	LegC8-BamHI	GACCGGATCCTTATTTTGATAATCGTGCTTGC

Table S2 Plasmids used with *L. pneumophila* srtain JR32

Plasmid name	Feature(s)	Reference or source
CyaA fusions		
pZT-lpg1368-cyaA	C-terminal fusion of lpg1368 to CyaA	Present study
pZT-lpg1368-cyaA-T	C-terminal fusion of the 144 C-terminal amino acids of lpg1368 to CyaA	Present study
pZT-lpg1368-cyaA-N	N-terminal fusion of lpg1368 to CyaA	Present study
pZT-Lgt3-cyaA	C-terminal fusion of *leg*C5-lpg1488 to CyaA	Present study
pZT-Lgt2-cyaA	C-terminal fusion of *leg*C8-lpg2862 to CyaA	Present study
Additional plasmids		
pMMB-cyaA-C	*ori*V(RSF1010) C-terminal CyaA fusion vector Cm^r	[1]
pMMB-cyaA-N	*ori*V(RSF1010) N-terminal CyaA fusion vector Cm^r	[1]

REFERENCE

1 Fritz, T. A., Raman, J. and Tabak, L. A. (2006) Dynamic association between the catalytic and lactin domains of human UDP-GalNAc:polypeptide α-N-acetylgalactosaminyltransferase-2. J. Biol. chem. **281**, 8613–8619

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