Supplementary Table 1: Dot/Icm translocated substrates

A: Substrates based on similarity to eukaryotic proteins

Protein	Gene	Domain/Function	Evidence for Translocation[a]
LepA[1]	lpg2793	coiled-coil domain/ bacterial egress	CA, BLA
LepB[1,2]	lpg2490	coiled-coil domain, Rab1 GAP, bacterial egress	CA
LegA2[3]	lpg2215	ankyrin repeat	BLA
LegA3/AnkH /AnkW [3-5]	lpg2300	ankyrin repeat	BLA
LegAS4/AnkI[3,4]	lpg1718	ankyrin repeat	BLA
LegA5/AnkK[3,4]	lpg2322	ankyrin repeat	BLA
LegA7/AnkG/ AnkZ[3-5]	lpg0401	ankyrin repeat	CA
LegA8/AnkN /AnkX[3-5]	lpg0695	ankyrin repeat	BLA
LegA9/AnkY[3,5]	lpg0402	ankyrin repeat	CA
LegA10/ AnkQ [3,4,6]	lpg0038	ankyrin repeat	CA, BLA
LegA11/ AnkJ [3,4,6]	lpg0436	ankyrin repeat	CA, BLA
LegA12/ AnkC[3,4]	lpg0483	ankyrin repeat	BLA
LegA14/Ceg31 /AnkF[3,4,7]	lpg2452	ankyrin repeat	BLA
LegA15/ AnkD[3,4]	lpg2456	ankyrin repeat	BLA
LegAU13/ Ceg27/AnkB[3,4,8]	lpg2144	F-box, ankyrin repeat	BLA

LegC3[3]	lpg1701	coiled-coil domain	BLA
LegC4[3]	lpg1953	coiled-coil domain	BLA
LegC5/Lgt3[3,9]	lpg1488	coiled-coil domain; glucosyltransferase of EF1A	CA, BLA
LegC6 [3,10]	lpg1588	coiled-coil domain	CA, BLA
LegC8/Lgt2[3,9]	lpg2862	coiled-coil domain; glucosyltransferase of EF1A	BLA
LegC7/YlfA[3,7]	lpg2298	coiled-coil domain	CA, BLA
LegC2/YlfB[3,7]	lpg1884	coiled-coil domain	CA, BLA
LegG1[3]	lpg1976	UVB-resistance protein UVR8	CA, BLA
LegG2[3]	lpg0276	Ras GEF	CA, BLA
LegK2[3]	lpg2137	serine/threonine protein kinase	BLA
LegK3[3]	lpg2556	serine/threonine protein kinase	BLA
LegL1[3]	lpg0945	leucine-rich repeat	BLA
LegL2[3]	lpg1602	leucine-rich repeat	BLA
LegL3[3]	lpg1660	leucine-rich repeat	CA, BLA
LegLC4[3]	lpg1948	leucine-rich repeat, coiled-coil domain	CA, BLA
LegL5 [3,10]	lpg1958	leucine-rich repeat	CA, BLA
LegL7[3]	lpg2400	leucine-rich repeat	CA, BLA
LegLC8 [3,10]	lpg1890	leucine-rich repeat, coiled-coil domain	CA, BLA
LegP[3,11]	lpg2999	astacin protease	BLA
LegS2[3]	lpg2176	sphingosine 1-phosphate lyase	BLA
LegU1[3]	lpg0171	F-box	BLA
LegU2/ LubX [3,10]	lpg2830	U-box; Ubiquitination of Clk	CYA, CA, BLA

B: Substrates identified by directly assaying for Dot/Icm-dependent translocation

Protein	Gene	Domain/Function	Evidence for Translocation[a]
SidA [12,13]	lpg0621	hypothetical protein	IT, CA
SidB [12,13]	lpg1642	Rtx toxin/lipase	IT, CA
SidC [12,13]	lpg2511	PI(4)P binding domain/vesicle trafficking	IF, PNS, ST, CA
SidD [12,13]	lpg2465	hypothetical protein	IT, CA
SidE [12,13]	lpg0234	hypothetical protein	IT, CA
SdeA[13]	lpg0257	hypothetical protein	IT
SdeC[13]	lpg2157	hypothetical protein	IT
SidF [12-14]	lpg2584	Bcl2-rambo and BNIP3 binding/anti-apoptosis	IT, IF, CA
SidG [12,13,15]	lpg1355	coiled-coil domain	CA, IT
SidH [12,13]	lpg2829	hypothetical protein	CA, IT
SdhA[16]	lpg0376	GRIP domain, coiled-coil domain/anti-apoptosis	SE

C: Substrates identified in yeast ectopic overexpression studies

Protein	Gene	Domain/Function	Evidence for Translocation[a]
VipA[17]	lpg0390	hypothetical protein /vesicle trafficking	CA
VipD[17,18]	lpg2831	patatin-like phospholipase/vesicle trafficking	CA, ST, SE
VipF[17]	lpg0103	acetyltransferase/vesicle trafficking	CA
YlfA/LegC7[3,7]	lpg2298	coiled-coil domain/vesicle trafficking	CA
YlfB/LegC2[3,7]	lpg1884	coiled-coil domain	CA

D: Substrates identified based on regulatory networks

Protein	Gene	Domain/Function	Evidence for Translocation[a]
Ceg7[6]	lpg0227	hypothetical protein	CA
Ceg18[6]	lpg0898	hypothetical protein	CA
Ceg33[6]	lpg2591	hypothetical protein	CA
Ceg10[8]	lpg0284	hypothetical protein	CA
Ceg23[8]	lpg1621	hypothetical protein	CA
Ceg29[8]	lpg2409	hypothetical protein	CA
CegC1[6]	lpg0012	hypothetical protein	CA
CegC2[6]	lpg0126	ninein, eukaryotic NIN gene	CA
CegC3[6]	lpg1144	hypothetical protein	CA
CegC4[6]	lpg2200	hypothetical protein	CA
LegA10/AnkQ[3,4,6]	lpg0038	ankyrin repeat	CA, BLA
LegA11/AnkJ[3,4,6]	lpg0436	ankyrin repeat	CA, BLA

E: Substrate identified by a putative Dot/Icm translocation signal

Protein	Gene	Domain/Function	Evidence for Translocation[a]
Lpg0045[10]	lpg0045	hypothetical protein	CA
Lpg0081[10]	lpg0081	hypothetical protein	CA
Lpg0294[10]	lpg0294	hypothetical protein	CA
Lpg0365[10]	lpg0365	hypothetical protein	CA
Lpg0518[10]	lpg0518	hypothetical protein	CA
Lpg0634[10]	lpg0634	hypothetical protein	CA

Lpg0963[10]	lpg0963	hypothetical protein	CA
Lpg1148[10]	lpg1148	hypothetical protein	CA
Lpg1158[10]	lpg1158	hypothetical protein	CA
Lpg1273[10]	lpg1273	hypothetical protein	CA
Lpg1689[10]	lpg1689	hypothetical protein	CA
Lpg1717[10]	lpg1717	hypothetical protein	CA
Lpg1751[10]	lpg1751	hypothetical protein	CA
Lpg2327[10]	lpg2327	hypothetical protein	CA
Lpg2407[10]	lpg2407	hypothetical protein	CA
Lpg2527[10]	lpg2527	coiled-coil domain	CA
Lpg2744[10]	lpg2744	hypothetical protein	CA

F: Substrates identified by alternative mechanisms

Protein	**Gene**	**Domain/Function**	**Evidence for Translocation**[a]
RalF[19]	lpg1950	Arf1 GEF, ARF1 recruitment	CA, IF
SidM/DrrA[20,21]	lpg2464	Rab1 GEF, Rab1 GDI/Rab1 recruitment	CA, IF, PNS
LidA[20,22]	lpg0940	coiled-coil domain/Rab1 sequestering	IF, PNS
SidJ[9,23]	lpg2155	hypothetical protein/vesicle recruitment	SE, ST, CA
SdjA[9,23]	lpg2508	hypothetical protein	ST, CA
VpdA[18]	lpg2410	patatin-like phospholipase	SE, ST
VpdB[18]	lpg1227	patatin-like phospholipase	SE, ST
WipA[15]	lpg2718	hypothetical protein	CA
WipB[15]	lpg0642	hypothetical protein	CA

[a] CA: cya-fusion assay; IF: immunofluorescence microscopy; IT: inter-bacterial transfer; PNS: protein present on phagosomes isolated from postnuclear supernatants of infected cells; SE: saponin extraction; ST: SidC-based translocation assay; BLA: fusions to β-lactamase[24].

SUPPLEMENTARY TABLE REFERENCES

1. Chen, J. et al. *Legionella* effectors that promote nonlytic release from protozoa. *Science* **303**, 1358-1361 (2004).
2. Ingmundson, A., Delprato, A., Lambright, D.G. & Roy, C.R. *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* **450**, 365-9 (2007).
3. de Felipe, K.S. et al. Evidence for acquisition of *Legionella* type IV secretion substrates via interdomain horizontal gene transfer. *J Bacteriol* **187**, 7716-26 (2005).
4. Habyarimana, F. et al. Role for the Ankyrin eukaryotic-like genes of *Legionella pneumophila* in parasitism of protozoan hosts and human macrophages. *Environ Microbiol* **10**, 1460-74 (2008).
5. Pan, X., Luhrmann, A., Satoh, A., Laskowski-Arce, M.A. & Roy, C.R. Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. *Science* **320**, 1651-4 (2008).
6. Altman, E. & Segal, G. The response regulator CpxR directly regulates the expression of several *Legionella pneumophila icm/dot* components as well as new translocated substrates. *J Bacteriol* **190**, 1986-96 (2008).
7. Campodonico, E.M., Chesnel, L. & Roy, C.R. A yeast genetic system for the identification and characterization of substrate proteins transferred into host