A L pneumonhila	lpg0083 0084 0085 /ceA 0087 0088
L. Jongbeachae	Llo_3333 3332 3331 3330 3329 3328
L. micdadei	Lmic_0844 0843 0842 0841 0840 0839
L. ookridaansis	Loak_1917 1916 1915 1914 1913 1912
L. Oaknugensis	Lgee 0591 0590 0589 0588 0587 0586 0585 0584 0583 0582
L. geesiiana	
B L. pneumophila	lpg0109 ravC 0106 0105 0104 vipF 0102 0101 0100
L. longbeachae	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
L. micdadei	Lmic_0773 0774 0775 0776 0777 0778 0779
L. oakridgensis	Loak_1877 1878 1879 1880 1881 1882
L. geestiana	Lgee_1973 1972 1971 1970 1915 1916 1917 1918
C L pneumophila	lpg0136 0137 0138 0139 cetLp1 0141 0142
E. prioarnoprina	
L. longbeachae	Llo_3264 3263 3262 3261 3260 3259 4093 3258
L. longbeachae I micdadei	Llo_3264 3263 3262 3261 3260 3259 4093 3258 Lmic_0759_0758 0757 0756 0755 0754
L. longbeachae L. micdadei L. oakridgensis	Llo_3264 3263 3262 3261 3260 3259 4093 3258 Lmic_0759 0758 0757 0756 0755 0754 Loak_1848 1847 1846 1845 1844 1843 1842
L. longbeachae L. micdadei L. oakridgensis	Llo_3264 3263 3262 3261 3260 3259 4093 3258 Lmic_0759 0758 0757 0756 0755 0754 Loak_1848 1847 1846 1845 1844 1843 1842 Lage 1995 1996 1997 1998 1999 2000 2001
L. longbeachae L. micdadei L. oakridgensis L. geestiana	Llo_3264 3263 3262 3261 3260 3259 4093 3258 Lmic_0759 0758 0757 0756 0755 0754 Loak_1848 1847 1846 1845 1844 1843 1842 Lgee_1995 1996 1997 1998 1999 2000 2001
L. longbeachae L. micdadei L. oakridgensis L. geestiana D	Llo_3264 3263 3262 3261 3260 3259 4093 3258 Lmic_0759 0758 0757 0756 0755 0754 Loak_1848 1847 1846 1845 1844 1843 1842 Lgee_1995 1996 1997 1998 1999 2000 2001 Ipg1352 1353 1354 sidG IceB 1357 1358 1359 1360
L. longbeachae L. micdadei L. oakridgensis L. geestiana D L. pneumophila	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
L. longbeachae L. micdadei L. oakridgensis L. geestiana D L. pneumophila L. longbeachae	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
L. longbeachae L. micdadei L. oakridgensis L. geestiana D L. pneumophila L. longbeachae L. micdadei	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
L. longbeachae L. micdadei L. oakridgensis L. geestiana D L. pneumophila L. longbeachae L. micdadei L. oakridgensis	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Fig. S1. legend is on the next page.





Fig. S2. Analysis of the intracellular growth phenotype of the *Legionella* genus core effectors. Intracellular competition assay in *A. castellanii* between *L. pneumophila* wild type strain JR32 and the *vipF* deletion mutant (A); the *ravC* deletion mutant (B); the *cetLp1* deletion mutant (C); and the lpg3000 deletion mutant (D). CFU – colony forming units. The data shown are representative of three independent experiments.



Fig. S3. LegA3C does not translocate into host cells during infection. The *L. pneumophila* wild-type strain JR32 (grey bars) and the *icmT* deletion mutant GS3011 (white bars) harboring the LegA3C-CyaA fusion protein or the LecE-CyaA fusion protein (positive control) were used to infect HL-60-derived human macrophages, and the cAMP levels of the infected cells were determined as described in Experimental Procedures. Vector control is indicated as "vec." The bar heights represent the mean amounts of cAMP per well obtained in at least three independent experiments; error bars indicate standard deviations. The cAMP levels of the LecE fusion was found to be significantly different (*, P < 0.01, Student's t test) between the wild-type strain and the *icmT* deletion mutant. The effectors were examined by Western blot analysis for their expression in the wild-type strain (left) and the *icmT* deletion mutant (right) using an anti-CyaA antibody.



Fig. S4. Controls of the two-hybrid analysis of LegA3 and LegA3C. Full-length fusions of LegA3, LegA3C and LegA3C C-terminal and N-terminal fusions (C-ter and N-ter, respectively) to the CyaA domains (T18 and T25) were examined with the empty vectors (T18 and T25) controls. The T25 and T18 domains fused to the Zip domain were used as a positive control. Data (expressed in Miller units [M.U.]) are the average \pm standard deviations (error bars) of the results of at least three different experiments. The levels of interaction obtained between the different constructs were found to be significantly higher (*, $P < 10^{-4}$, Student's *t* test), when comparing to the background interaction of the two vectors control (T18 and T25).

LegA3C	MKKMAKEYYLSDEIEEIVLASTVLSYSRPNLSSKSFNDLKNGKTPWEAELDLKGL <mark>NVE</mark> SAR <mark>K</mark> SLYQFIQT	70
LlO_0583	MLNEYYLTDDIEEPVLAHAVLSYVTPSISGKQFHELKTGQVPWDAKLDLSGLQAEDARKALLKFIQK	67
Lgee_0774	MDVEKRMDVE	б
Loak_0514	<mark>mn</mark> rrk <mark>r</mark> s <mark>k</mark> QL	10
Lmic_2508		б
LegA3C	QIKNNKQCVLIIHGADNSQDKPPLM <mark>K</mark> NLV <mark>N</mark> RWLPQINEVLAFHSAKPKDGGSAAVYVLLGSVFHV-ELPT	139
LlO_0583	QVKNKKHSLLIIHGTESRKNAFPLL <mark>K</mark> NLINHWLPQIADVAAFHSANP <mark>K</mark> DGGINAVYVLLKKIYELPELSR	137
Lgee_0774	KPSGLPGVGEPPD	19
Loak_0514	EESSSLK <mark>K</mark> PRF <mark>N</mark> ETWPDDS <mark>D</mark> QSGDSGQSEDTGGSSGSGGGYRRRGK <mark>E</mark> RPD	60
Lmic_2508	TDIDFPT	13
LegA3C	RLPSNRSES <mark>TF</mark> LVA <mark>ETK</mark> AME <mark>R</mark> RRRLAEQQGERRFA-SVKAREHPEEEIKVAPEGELQNNILQHPELN <mark>SQR</mark>	208
LlO_0583	IEK <mark>ES</mark> ILVA <mark>ETK</mark> AMERQRRLAEQQGERRLA-SVKAREHSNQEAQPGPEGELQNSILQHPELN <mark>SQR</mark>	201
Lgee_0774	TLPSR <mark>ESF</mark> LV <mark>DES</mark> PE <mark>MYR</mark> KHREIEQREL <mark>RR</mark> IS-RVHARDHA-ERPDVAPEGELQNSILQHPALDSQR	84
Loak_0514	AGST <mark>SYLVDETRVMERERRLQE</mark> QQGH <mark>RH</mark> LA-RVHAREHHDEEQQVAPEGELQNSIMQNPWLDNQR	124
Lmic_2508	RSPTGNRG <mark>ES</mark> ILK <mark>DET</mark> WQ <mark>ME</mark> NQ <mark>RR</mark> LE <mark>E</mark> KNGH <mark>RK</mark> LGV <mark>KVHEREHYE</mark> PQQAAGL <mark>EED</mark> LQNGIKEHPFL <mark>D</mark> NPY	83
	** *	
LegA3C	FDGIDPNLNPEPPLNT-EARREFDNERREQDKEKQLRLGNMPKFTTAPTPRGP- 260	
LlO_0583	<mark>FD</mark> GV <mark>D</mark> SPL <mark>NPE</mark> PPLNT-EA <mark>RREF</mark> DNERRNQEQEKQLRLGHMPKFTNTPKPRGPQ 254	
Lgee_0774	<mark>FD</mark> GI <mark>D</mark> PSVNPEPPLNT-EARLK <mark>YDNELREQEMEK</mark> QL <mark>R</mark> LGNMPKFSTAPKPPGQ- 136	
Loak_0514	FDGVDPNLNPEPPLNT-EARREYDNQRREQEMEKQLRLGNMPRFTNTPKPQGP- 176	
Lmic_2508	FDGIANNENPNPHLS <mark>TRE</mark> VLTK <mark>FENERREQD</mark> LQ <mark>H</mark> KL <mark>K</mark> LGLVAAPKFNPNPNP 135	
	* **	

Fig. S5. Multiple sequence alignment of five orthologs of LegA3C. Multiple sequence alignment of five LegA3C orthologs that were examined for interaction with LegA3. Conserved amino acids are indicated in colors related to their properties. Amino acids which are identical in the *L. pneumophila*, *L. longbeachae* and *L. oakridgensis* orthologs (which interact with the *L. pneumophila* LegA3 orthologs) and different in the *L. micdadei* and *L. geestiana* LegA3 orthologs (which do not interact with the *L. pneumophila* LegA3 orthologs) are indicated by asterisks.



Fig. S6. Part of a multiple sequence alignment of five core effector orthologs of MavN. Multiple sequence alignment of loop number seven located between transmembrane domains number 7 and 8 (marked in yellow) of five MavN core effectors orthologs. Three amino acids (H412, E439 and H445) which were previously shown to be the required for MavN function (Isaac *et al.*, 2015, Christenson *et al.*, 2019) are marked blue (in *L. geestiana* the unique M445 is marked in red). Amino acids conserved in all available MavN sequences are marked in bold.



Fig. S7. Part of a multiple sequence alignment of five core effector orthologs of LceA. Multiple sequence alignment of the region located between transmembrane domains number 3 and 4 (marked in yellow) of five LceA core effectors orthologs. Two amino acids (N172 and Y242) are conserved in all the 58 *Legionella* species examined except for *L. geestiana*. These residues are marked in bold and in red in the *L. geestiana* sequence. Other amino acids which were found to be conserved in all available LceA sequences are marked in bold.