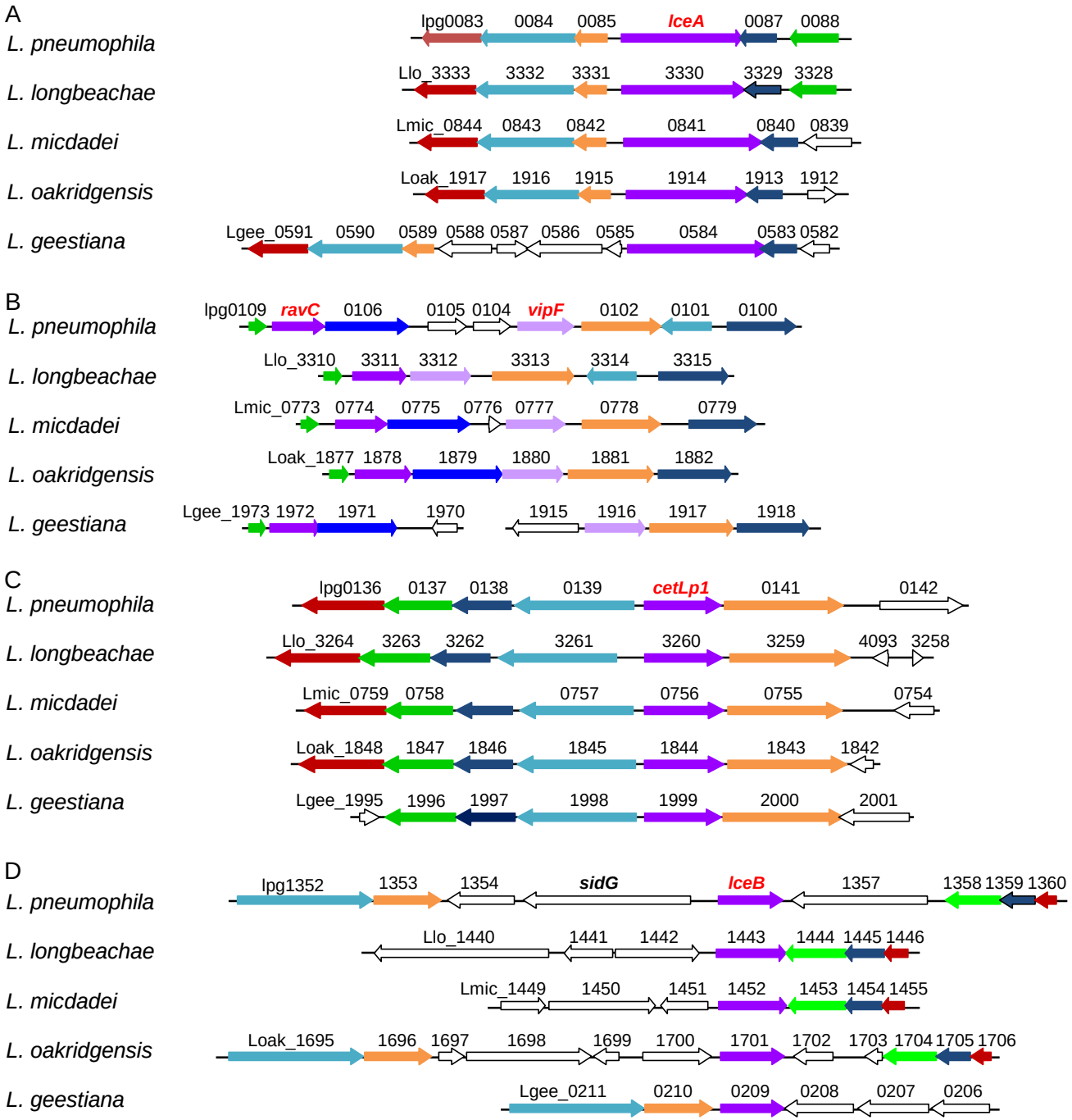

Fig. S1. legend is on the next page.

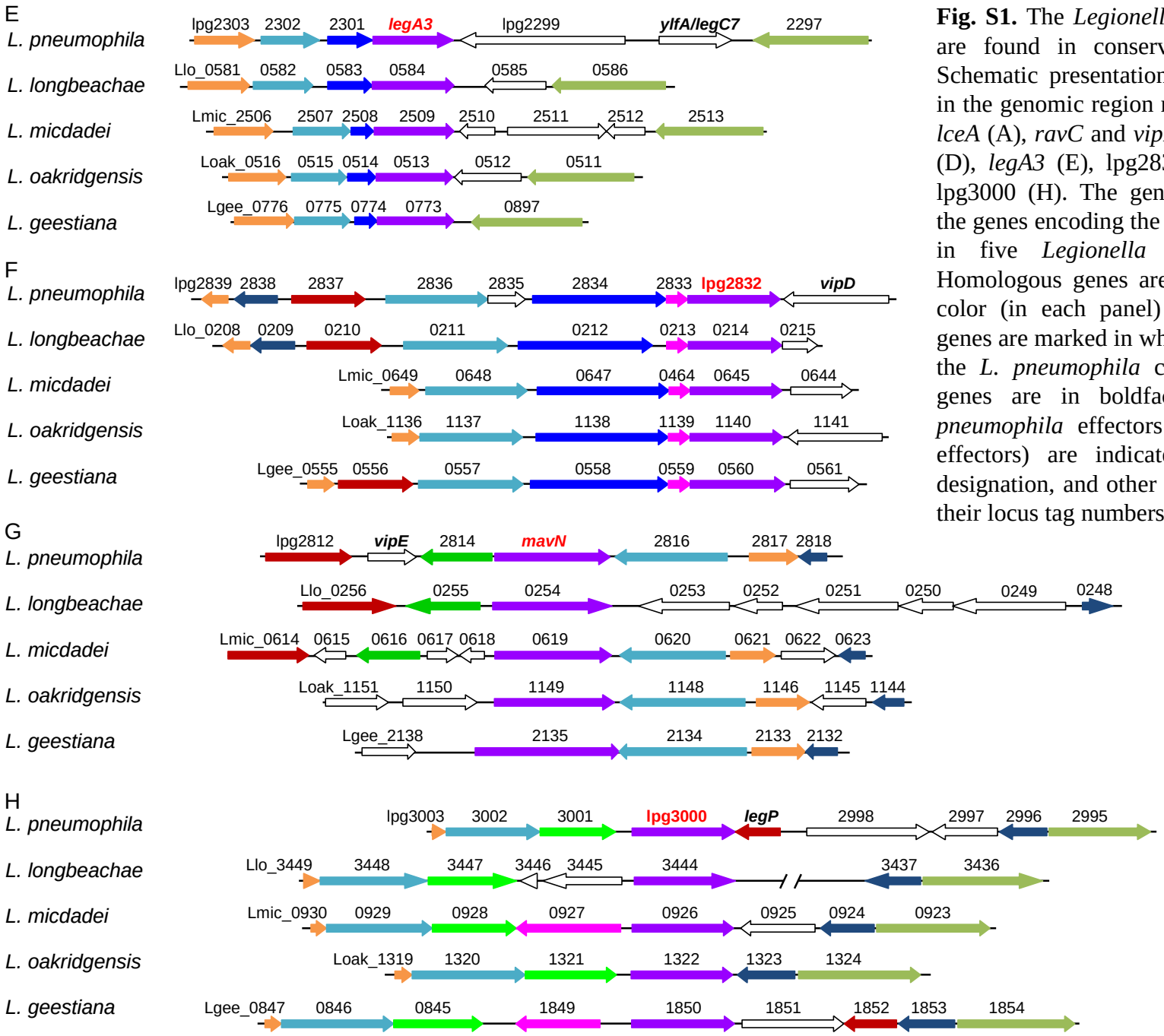

Fig. S1. The *Legionella* genus core effectors are found in conserved genomic regions. Schematic presentation of the genes located in the genomic region near the core effectors: *lceA* (A), *ravC* and *vipF* (B), *cetLp1* (C) *lceB* (D), *legA3* (E), lpg2832 (F) *mavN* (G) and lpg3000 (H). The genomic organizations of the genes encoding the core effector orthologs in five *Legionella* species are shown. Homologous genes are marked by the same color (in each panel) and non-homologous genes are marked in white. The designation of the *L. pneumophila* core effector encoding genes are in boldface red, validated *L. pneumophila* effectors (which are not core effectors) are indicated in bold by their designation, and other genes are indicated by their locus tag numbers.

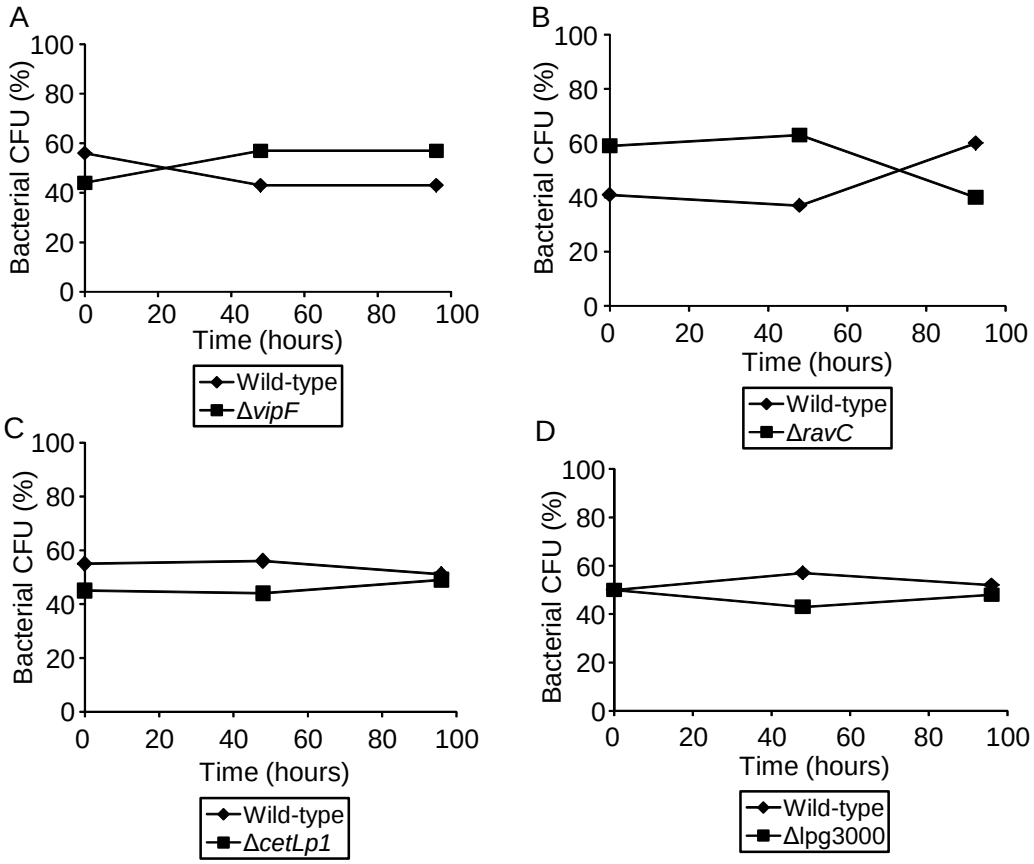

Fig. S2. Analysis of the intracellular growth phenotype of the *Legionella* genus core effectors. Intracellular competition assay in *A. castellanii* between *L. pneumophila* wild type strain JR32 and the *vipF* deletion mutant (A); the *ravC* deletion mutant (B); the *cetLp1* deletion mutant (C); and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

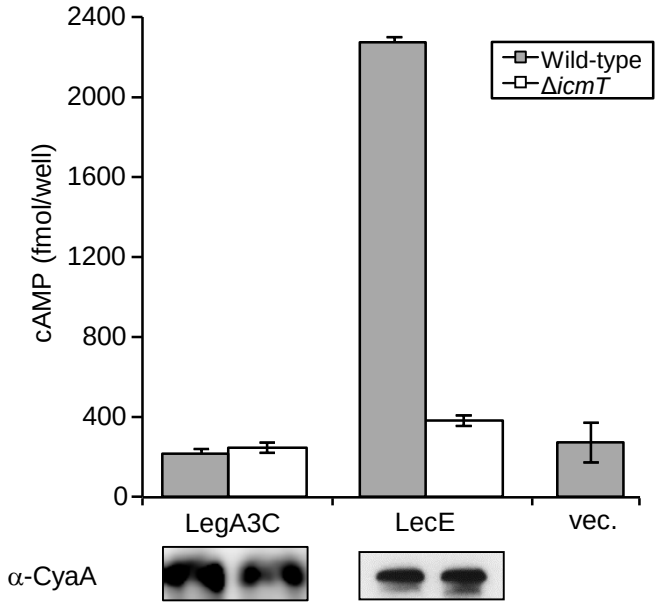

Fig. S3. LegA3C does not translocate into host cells during infection. The *L. pneumophila* wild-type strain JR32 (grey bars) and the *icmT* deletion mutant GS3011 (white bars) harboring the LegA3C-CyaA fusion protein or the LecE-CyaA fusion protein (positive control) were used to infect HL-60-derived 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.

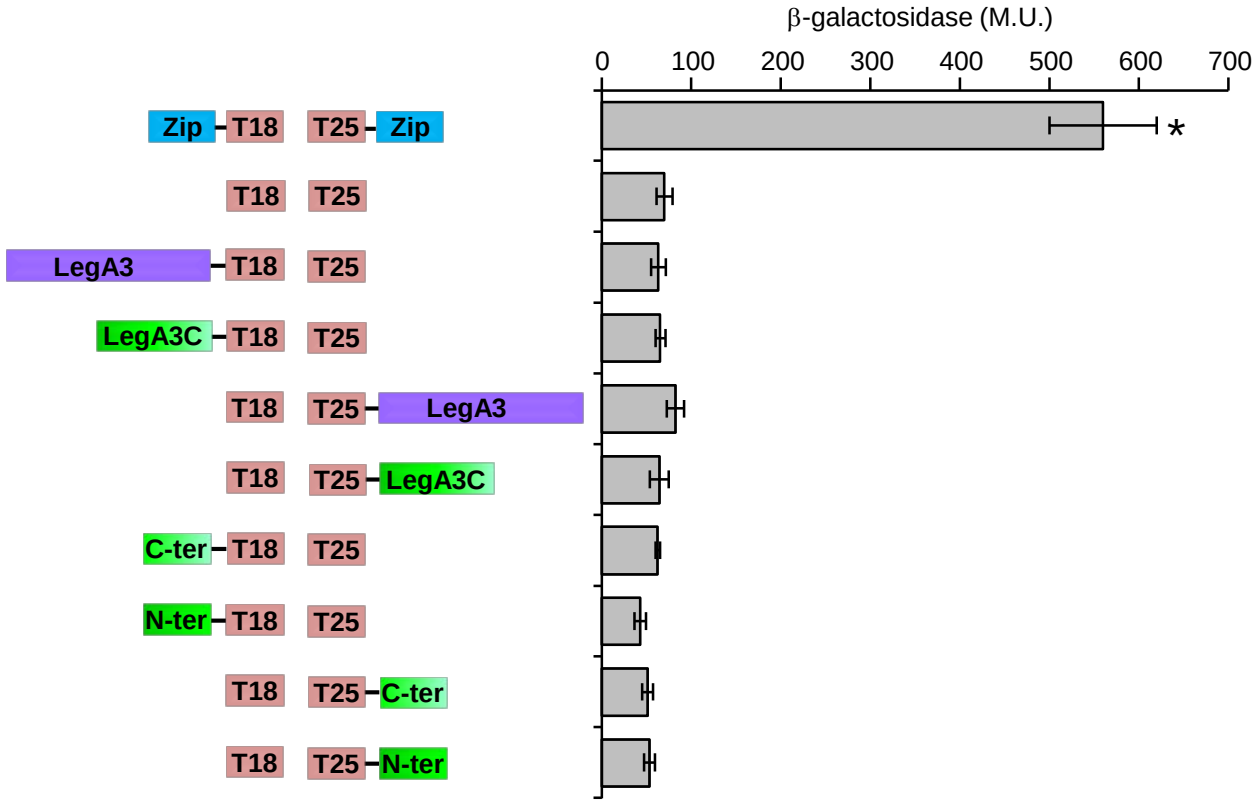

Fig. S4. Controls of the two-hybrid analysis of LegA3 and LegA3C. Full-length fusions of LegA3, LegA3C and LegA3C C-terminal and N-terminal fusions (C-ter and N-ter, respectively) to the CyaA domains (T18 and T25) were examined with the empty vectors (T18 and T25) controls. The T25 and T18 domains fused to the Zip domain were used as a positive control. Data (expressed in Miller units [M.U.]) are the average ± 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).

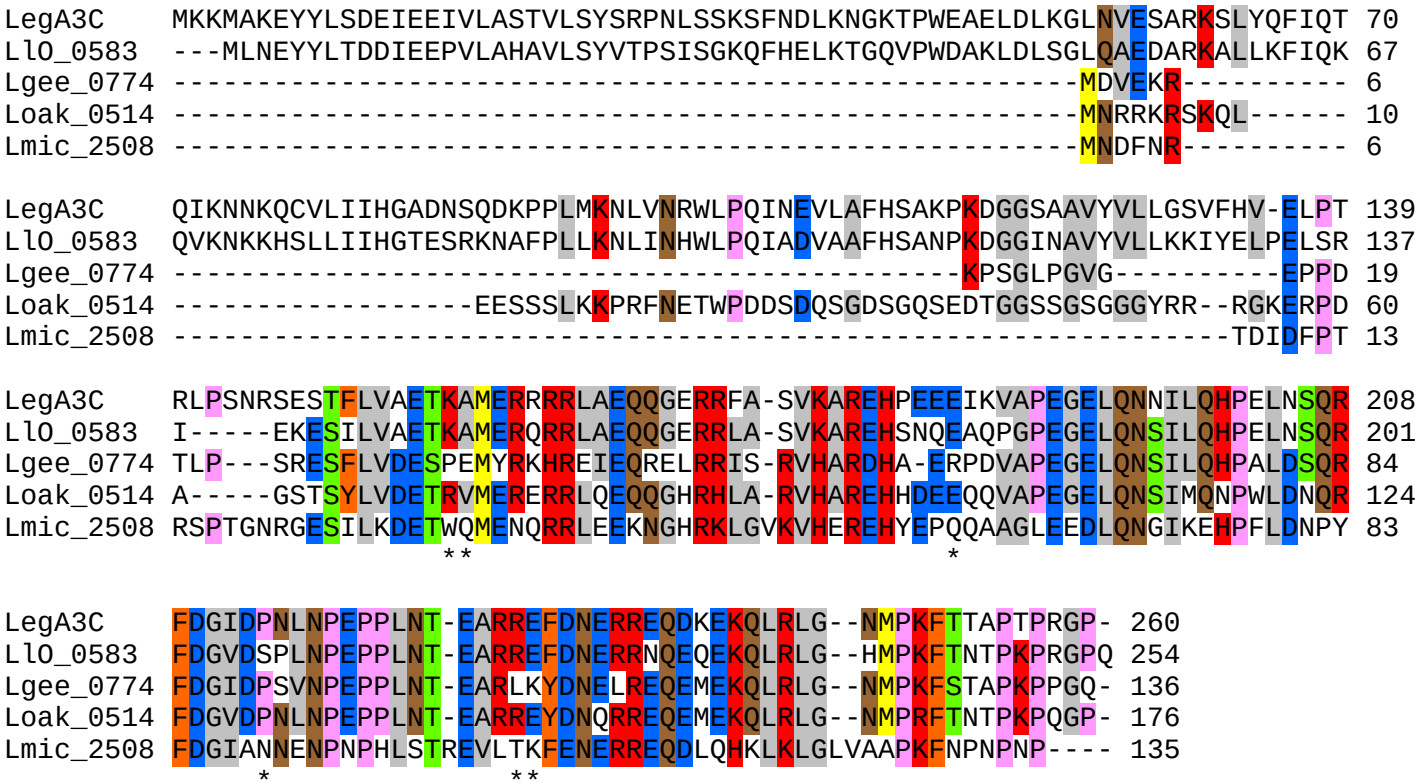

Fig. S5. Multiple sequence alignment of five orthologs of LegA3C. Multiple sequence alignment of five LegA3C orthologs that were examined for interaction with LegA3. Conserved amino acids are indicated in colors related to their properties. Amino acids which are identical in the *L. pneumophila*, *L. 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.

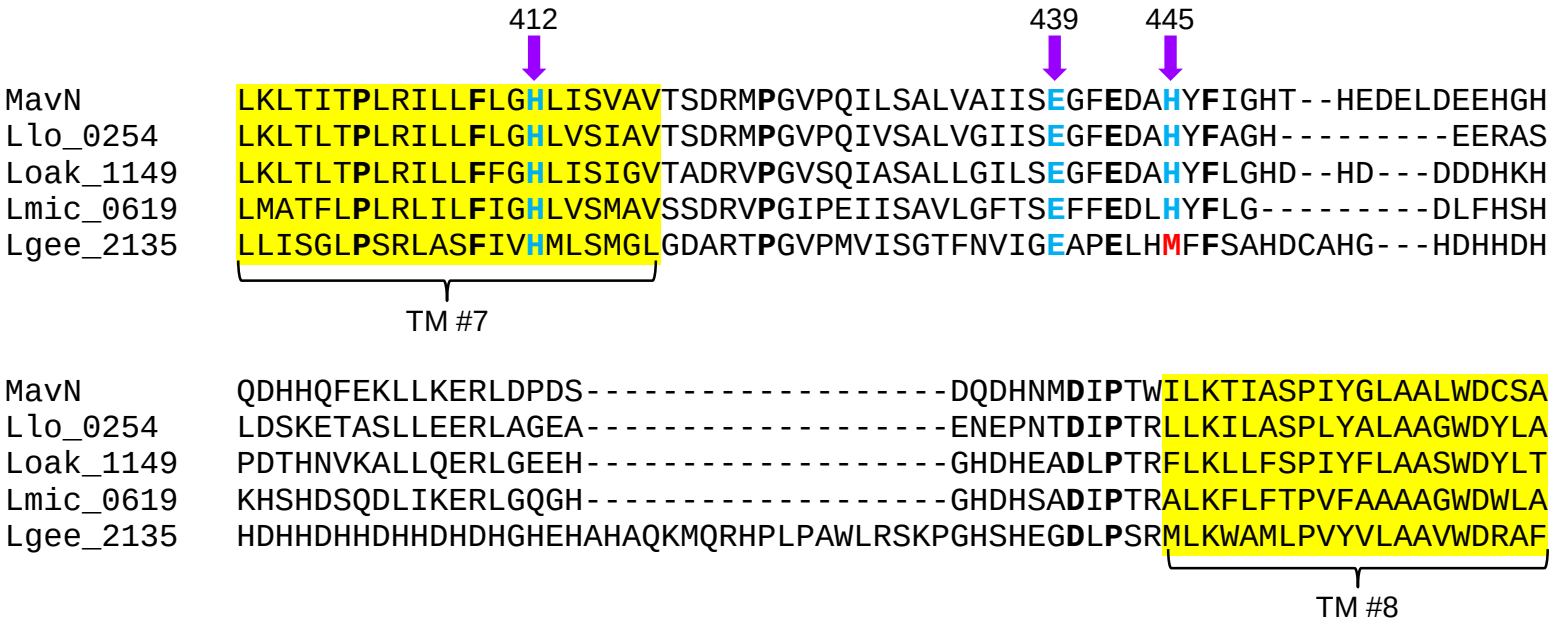

Fig. S6. Part of a multiple sequence alignment of five core effector orthologs of MavN. Multiple sequence alignment of loop number seven located between transmembrane domains number 7 and 8 (marked in yellow) of five MavN core effectors orthologs. Three amino acids (H412, E439 and H445) which were previously shown to be the required for MavN function (Isaac *et a*l., 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.

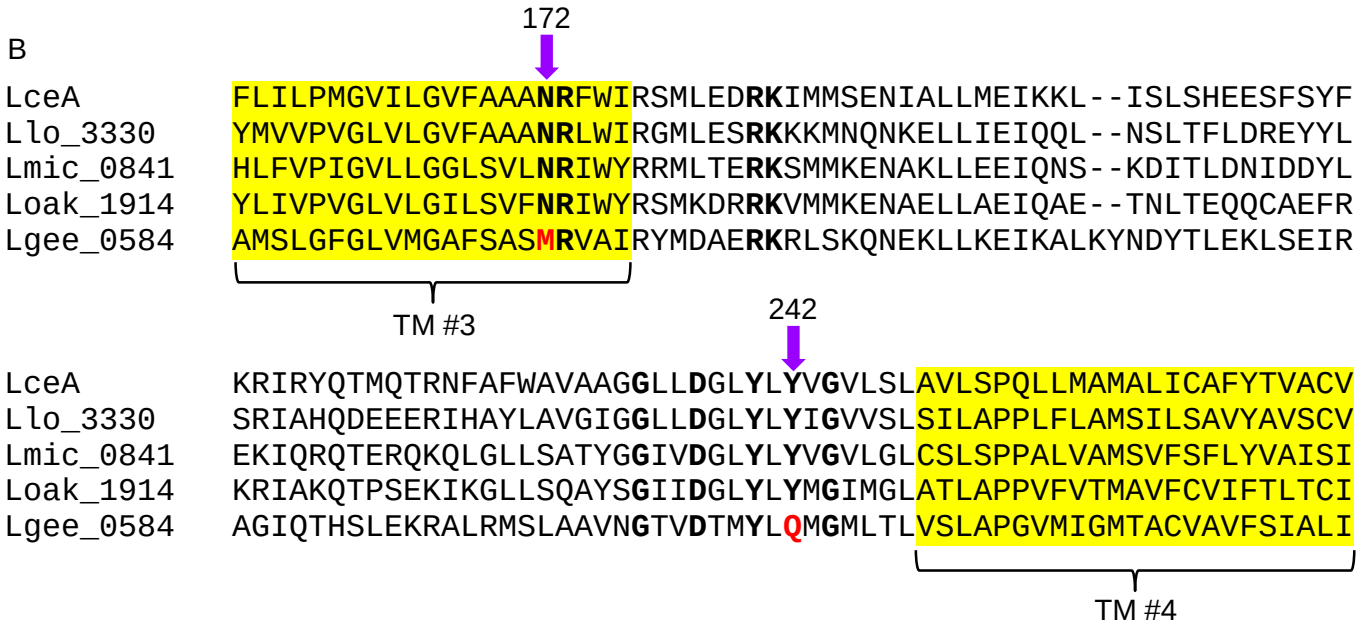

Fig. S7. Part of a multiple sequence alignment of five core effector orthologs of LceA. Multiple sequence alignment of the region located between transmembrane domains number 3 and 4 (marked in yellow) of five LceA core effectors orthologs. Two amino acids (N172 and Y242) are conserved in all the 58 *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.