**Supplementary Information (SI)**

**SI Figures**



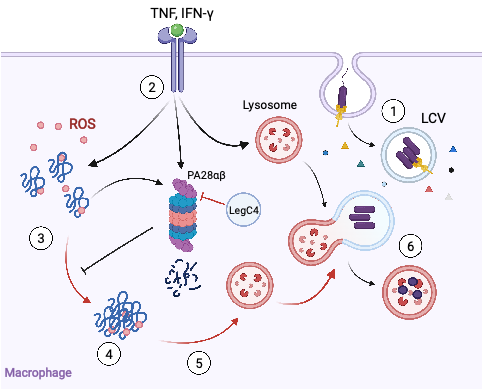
**Figure S1.** Neither LegC4 nor PA28ab increase lysis or TNF secretion from *L. pneumophila*-infected BMDMs. TNF was quantified by ELISA from supernatants of WT or *Psme1/2*-/- BMDMs infected for **(A)** 8 h or **(B)** 24 h with *L. pneumophila* ∆*flaA*, ∆*flaA*∆*legC4* (pEV), ∆*flaA*∆*legC4* (p*legC4*), or the avirulent ∆*dotA* control at a multiplicity of infection of 10. **(C)** WT BMDMs were infected in triplicates with *L. pneumophila* strains for 10 h at an MOI of 10 and LDH in cell supernatants was quantified by and percent cytotoxicity was calculated by normalizing to absorbance values to a lysis control (100% cytotoxicity). UI, uninfected cells. Plasmid expression of *legC4* was induced with 1 mM IPTG. Data are shown as mean ± s.d. of samples in triplicates and asterisks denote statistical significance by two-way ANOVA (\**P*<0.05; \*\**P*<0.01). Ns, not significant. Data shown are representative of three independent experiments.



**Figure S2.** LAMP1 localizes to LCVs harboring avirulent *L. pneumophila*. Representative epifluorescence images WT BMDMs infected with virulent (∆*flaA*) or avirulent (*dotA*::Tn) *L. pneumophila* strains for 9 h. LAMP1 and L. pneumophila were imaged with a-LAMP1 (red) and a-*L. pneumophila* (green) antibodies.



**Figure S3.** LegC4-mediated restriction does not require inflammasome activation. **(A)** Growth of *L. pneumophila* strains within WT BMDMs treated with 1 µM MCC950 or vehicle control. **(B)** Growth of *L. pneumophila* strains within WT or *Casp1*-/- BMDMs. Data are shown as mean ± s.d. on samples in triplicates and representative of two independent experiments and asterisks denote statistical significance by Students’ *t*-test (\*\**P*<0.01). Plasmid expression of *legC4* was induced with 1 mM IPTG. **(C)** WT BMDMs were primed with 1 µM PAM3CSK4 for 24 h and infected with the indicated *L. pneumophila* strains at an MOI of 10 for 6 h and cytotoxicity was quantified by LDH release assay. Data are shown as mean ± s.d. on samples in triplicates and representative of two independent experiments. Plasmid expression of *legC4* was induced with 1 mM IPTG.

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**Figure S4.** Schematic model of LegC4 and PA28ab in cell-autonomous restriction of L. pneumophila within macrophages. (1) *L. pneumophila* establishes a replicative LCV by translocating effector proteins (triangles and circles) into macrophages by a Dot/Icm T4SS (yellow). (2) Pro-inflammatory cytokines (green) trigger upregulation of antimicrobial lysosomal degradation pathways, reactive oxygen species, and PA28ab-CP proteasomes, and phagolysosomal fusion pathways. (3) ROS indiscriminately oxidize and denature cellular proteins, which are degraded by PA28ab-CP proteasomes. (4) Impaired PA28ab activity increases abundance of damaged proteins which can aggregate and cause proteotoxic stress. (5) Loss of proteasome activity results in compensatory upregulation of lysosomal degradation pathways, which (6) results in increased phagolysosomal fusion with LCVs, which augments existing lysosomal host defense mechanisms. Red arrows indicate consequences of LegC4 activity. Figure created with BioRender.com.

**SI Tables**

**Table S1**. Clones of proteasome regulators identified as LegC4 interacting partners by yeast two-hybrid.

See file: Table S1.xlsx

**Table S2.** Bacterial strains used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| **Strain** | **Description** | **Resistance** | **Ref(s)** |
| *Legionella pneumophila* SRS43 | | | |
| ∆*flaA* | FlaA-deficient parental strain | SmR | (1) |
| ∆*flaA*∆*legC4* | LegC4-deficient ∆*flaA* strain | SmR | (1) |
| ∆*flaA*∆*legC4* (pEV) | Harboring empty pSN85 vector | SmR, CmR | (1) |
| ∆*flaA*∆*legC4* (p*legC4*)a | Harboring pSN85::*legC4* | SmR, CmR | (1) |
| ∆*flaA*∆*legC4* (pJB) | Harboring empty pJB1806 vector | SmR, CmR | (1) |
| ∆*flaA*∆*legC4* (pJB*legC4*)b | Harboring pJB1806::p*legC4* | SmR, CmR | (1) |
| *dotA*::Tn | Avirulent control | SmR, CmR | (2) |

a – *legC4* expression induced with 1 mM IPTG

b – *legC4* expression from endogenous promoter

**Table S2.** Plasmids used in this study

|  |  |  |  |
| --- | --- | --- | --- |
| **Plasmid** | **Description** | **Resistance** | **Ref(s)** |
| *L. pneumophila* expression | | | |
| pSN85 (pEV) | *L. pneumophila* expression vector | CmR | (3) |
| pSN85::*legC4* (p*legC4*) | For expression of *3xflag-legC4* | CmR, IPTG | (1) |
| pJB1806 (pJB) | *L. pneumophila* expression vector | CmR | (4) |
| pJB1806::*legC4* (pJB*legC4*)b | *legC4* expression from endogenous promoter | CmR | (1) |
| Mammalian Expression |  |  |  |
| pcDNA::*3xflag* | Expression of FLAG-fusion proteins | AmpR | (5) |
| pcDNA::*3xflag-legC4* | Ectopic production of FLAG-LegC4 | AmpR | This study |
| pEGFPC1 | Expression of GFP-fusion proteins | KanR | Clontech |
| pEGFPC1::*psme1* | Ectopic production of GFP-PA28a | KanR | This study |
| pCMV-3Tag-4a::*psme1*c | Ectopic production of PA28a-Myc | KanR | This study |

a – *legC4* expression induced with 1 mM IPTG

b – *legC4* expression from endogenous promoter

c – purchased from Genscript (Piscataway, New Jersey)

**Table S3**. Oligonucleotide primers used in this study

|  |  |
| --- | --- |
| **Name** | **Sequence (5'🡪3')a** |
| LegC4BamHI-F | ATTGGATCCTTGATTCATTATGTATCCTTG |
| LegC4NotI-R | ATTGCGGCCGCTTATAGCTTAATATCAAAAG |
| Psme1Sal1-F | ATTGTCGACATGGCCACACTGAGGGTCCATCCC |
| Psme1BamHI-R | ATTGGATCCTCAATAGATCATTCCCTTGGTTTC |

a – restriction endonuclease cleavage sites are underlined.

**SI References**

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2. S. R. Shames, *et al.*, Multiple *Legionella pneumophila* effector virulence phenotypes revealed through high-throughput analysis of targeted mutant libraries. *Proc National Acad Sci* 63, 201708553 (2017).

3. M. Folly-Klan, *et al.*, A novel membrane sensor controls the localization and ArfGEF activity of bacterial RalF. *Plos Pathog* 9, e1003747 (2013).

4. J. P. Bardill, J. L. Miller, J. P. Vogel, IcmS-dependent translocation of SdeA into macrophages by the *Legionella pneumophila* type IV secretion system. *Mol Microbiol* 56, 90 103 (2005).

5. A. Ingmundson, A. Delprato, D. G. Lambright, C. R. Roy, *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* 450, 365–369 (2007).