Supplementary Information

Cell-selective proteomics reveal novel effectors secreted by an obligate intracellular bacterial pathogen

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Supplementary Fig. 1 | Tryptic peptides mapping to autotransporter proteins Sca1 and OmpA. Positions of unique peptides (black boxes) and passenger and autotransporter (AT) domains are indicated.







Supplementary Fig. 2 | Genomic positions and gene neighborhoods of *srf* loci.

a *R. parkeri* genome map displaying loci encoding SrfA–G; autotransporter proteins Sca1 and OmpA; known secreted effectors RARP-2, Pat1, Risk1, and Sca4; and components of the type IV (T4SS: RvhBD) and putative type I (T1SS: ToIC, AprDE) secretion systems.

b Gene neighborhoods for *srfA*–*G* (black) across select members of the *Rickettsia* genus. Shared flanking genes are colored if found in at least three of the aligned genomes, and continuous gene neighborhoods for *srfG* and *srfA* and for *srfD* and *srfC* are highlighted for clarity. The putative *srfE* homolog of *R. bellii* (*RBE_1196*) is located distal to the flanking genes shared by other species and is therefore absent from the alignment. hp, hypothetical protein; *pa*, *R. parkeri*; *ri*, *R. rickettsii*; *lp*, *Rickettsia* endosymbiont of *Ixodes pacificus*; *fe*, *R. felis*; *ty*, *R. typhi*; *ca*, *R. canadensis*; *be*, *R. bellii*. Scale bar, 1 kbp.













Supplementary Fig. 3 | GSK-tagged SrfB and SrfE are not obviously expressed by *R. parkeri*. Western blots from Fig. 3a with enhanced contrast. SrfB and SrfE (expected 37 and 50 kDa, respectively) were not detected. Source data are provided as a Source Data file.



Supplementary Fig. 4 | SrfD does not impact secretion of *Gaussia* luciferase.

HEK293T cells stably expressing *Gaussia* luciferase were transiently transfected in triplicate (n = 3) with either empty vector or 3xFLAG-tagged SrfD and treated with DMSO or brefeldin A (BFA) prior to measuring luciferase activity of the culture supernatants. Relative light units (RLU) from a representative experiment were used to calculate the means \pm SD and p-values (one-way ANOVA with post hoc Dunnett's test, F = 74.15, q = 10.42 and 0.24, df = 6). Results are representative of two independent experiments. Source data are provided as a Source Data file.

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Supplementary Table 1. Strains and plasmids used in this study.

Supplementary References

- 1. Sanderlin, A. G., Hanna, R. E. & Lamason, R. L. The ankyrin repeat protein RARP-1 is a periplasmic factor that supports Rickettsia parkeri growth and host cell invasion. *J. Bacteriol.* **204**, e00182-22 (2022).
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