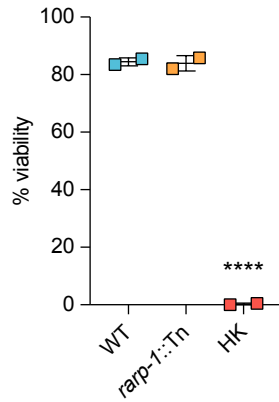
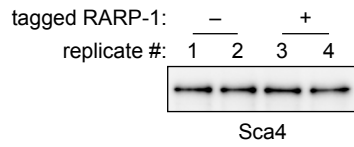
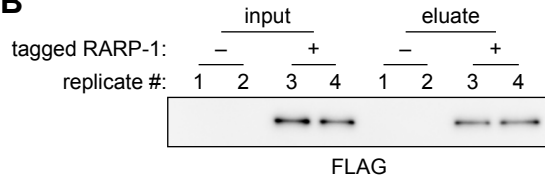


Supplementary Figure 1. Tagged RARP-1 constructs and endogenous RARP-1 are not secreted. (A) *R. parkeri* RARP-1 with insertion sites for 3xFLAG and Ty1 epitope tags indicated (arrowheads). Western blots for FLAG (top) and Ty1 (middle) after infection of A549 cells with *rarp-1::Tn* + 3xFLAG-RARP-1 (single-tagged) or *rarp-1::Tn* + 3xFLAG-Ty1-RARP-1 (dual-tagged) bacteria. (B) Infected cells per focus during infection of A549 cells. (C) Bacteria per focus during infection of A549 cells. In (B) and (C), the means from three independent experiments (squares) are superimposed over the raw data (circles) and were used to calculate the mean \pm SD and p-value (one-way ANOVA with post-hoc Dunnett's test, ** $p < 0.01$ relative to WT). (D) Western blots for RARP-1 (top) and Sca4 (middle) after infection of A549 cells with WT or *rarp-1::Tn* bacteria. Note the specific RARP-1 band in the pellet sample for WT bacteria only, in contrast to the identical non-specific bands in the supernatant samples for WT and *rarp-1::Tn* bacteria. In (A) and (D), infected host cells were selectively lysed after 48 h to separate supernatants (S) containing the infected host cytoplasm from pellets (P) containing intact bacteria. RpoA (bottom) served as a control for bacterial lysis or contamination of the infected cytoplasmic fraction.



Supplementary Figure 2. RARP-1 is dispensable for bacterial viability.

Bacteria were released from infected A549 cells after 48 h and viability was assessed by differential staining. Percentages were determined from two independent experiments (≥ 160 bacteria were counted for each infection) and were used to calculate the mean \pm SD and p-value (one-way ANOVA with post-hoc Dunnett's test, **** $p < 0.0001$ relative to WT). Heat-killed (HK) bacteria served as a positive control.

A**B**

Supplementary Figure 3. Inputs and eluates from co-immunoprecipitation of lysozyme-permeabilized bacteria. (A) Western blot for Sca4 (loading control) in input lysates. (B) Western blot for FLAG in input lysates and FLAG immunoprecipitation eluates. In (A) and (B), bacteria expressing tagged (+) or untagged (-) RARP-1 were purified and then permeabilized by lysozyme prior to immunoprecipitation. Two replicate samples were harvested from each strain.

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

Strain or plasmid	Genotype or feature	Reference or source
<i>R. parkeri</i> strains		
<i>R. parkeri</i> str. Portsmouth	Parental <i>R. parkeri</i> strain	Chris Paddock
WT	pRAM18dSGA+OmpApr-GFPuv	(1)
<i>rarp-1</i> ::Tn	<i>rarp-1</i> ::Tn	(2)
<i>sca2</i> ::Tn	<i>sca2</i> ::Tn + pRAM18dSGA+OmpApr-GFPuv	(2)
<i>ompB</i> ^{STOP} ::Tn	<i>ompB</i> ^{STOP} ::Tn	(3)
<i>rarp-1</i> ::Tn + 3xFLAG-RARP-1	<i>rarp-1</i> ::Tn + pRAM18dSGA- 3xFLAG-RARP-1	This study
<i>rarp-1</i> ::Tn + 3xFLAG-Ty1-RARP-1	<i>rarp-1</i> ::Tn + pRL0079	This study
GSK-BFP	pRL0284	This study
GSK-RARP-2	pRL0285	This study
GSK-RARP-1	pRL0286	This study
<i>E. coli</i> strains		
WT	Keio Knockout Collection parental K12 strain (BW25113)	(4); Horizon Discovery
$\Delta tolC$	$\Delta tolC$::Kan (JW5503-1)	(4); Horizon Discovery

WT + 3xFLAG-RARP-1 _{Rp}	WT + pRL0287	This study
WT + 3xFLAG-RARP-1 _{Rt}	WT + pRL0288	This study
$\Delta toIC$ + 3xFLAG-RARP-1 _{Rp}	$\Delta toIC$ + pRL0287	This study
$\Delta toIC$ + 3xFLAG-RARP-1 _{Rt}	$\Delta toIC$ + pRL0288	This study
WT + Myc-6xHis-RARP-1 _{Rt}	WT + pRL0290	This study
WT + 6xHis-YebF	WT + pRL0291	This study

Plasmids

pRAM18dSGA[MCS]	<i>Rickettsia</i> shuttle vector	Ulrike Munderloh
pRAM18dSGA+OmpApr-GFPuv	GFPuv	(1)
pRAM18dSGA-3xFLAG-RARP-1	3xFLAG-tagged <i>R. parkeri</i> RARP-1	This study
pRL0079	3xFLAG- and Ty1-tagged <i>R. parkeri</i> RARP-1	This study
pRL0284	GSK-tagged TagBFP	This study
pRL0285	GSK-tagged <i>R. parkeri</i> RARP-2	This study
pRL0286	GSK-tagged <i>R. parkeri</i> RARP-1	This study
pEXT20	IPTG-inducible <i>E. coli</i> expression vector	This study
pRL0287	3xFLAG-tagged <i>R. parkeri</i> RARP-1	This study
pRL0288	3xFLAG-tagged <i>R. typhi</i> RARP-1	This study
pRL0290	Myc-6xHis-tagged <i>R. typhi</i> RARP-1	This study
pRL0291	6xHis-tagged <i>E. coli</i> YebF	This study

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