### Supplementary Table 1. Primers and probes used for quantitative PCR (qPCR).

<table>
<thead>
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<th>Rickettsia spp.</th>
<th>Primer/Probe</th>
<th>Sequence (5’ → 3’).</th>
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<tr>
<td><strong>R. africae</strong></td>
<td>Sca1_africae_fwd</td>
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<td>Sca1_africae_rev</td>
<td>TTT CAG CAT CGA ACC CGA TAG</td>
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<td>Sca1_africae</td>
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<td>Rpp Sca-1 (316 bp) REV</td>
<td>CCG TAA ATA GAA ACC ACA TGA C</td>
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<td><strong>Host Cell Actin</strong></td>
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All primers and probes for *Rickettsia* species were designed from the rickettsial antigen, Sca1.

### Supplemental Figure Legends
Supplemental Figure 1: *R. akari* st. Columbia significantly grows within endothelial cells (EA.hy926) and human derived macrophage cells (THP-1). (A,B)

EA.hy926 cells and PMA-differentiated THP-1 cells were infected with *R. akari* st. Columbia (MOI=2.5), and genomic DNA was extracted at each time point post-infection. Each time point represents the ratio of *R. akari* sca1 to host cell actin genes amplified from genomic DNA and determined by quantitative PCR (qPCR). Immunofluorescence microscopy growth analyses in EA.hy926 cells at days 1 and 3 post-infection (C) and in PMA-differentiated THP-1 cells at days 1 and 4 post-infection demonstrate significant intracellular proliferation. DAPI (blue) was used to visualize host cell nuclei, anti-*Rickettsia* antibody (RcPFA) followed by Alexa Fluor 488 (green) was utilized to reveal *R. rickettsii* st. Sheila Smith, and Alexa Fluor 546 Phalloidin (red) was used to indicate the host actin cytoskeleton in C and D. Scale bar= 10 µm. A logistic regression test was used to measure significance (p<0.05) in growth over time in both mammalian cell lines in A and B.

Supplemental Figure 2: *R. africae* proliferates within endothelial cells (EA.hy926) and human derived macrophage cells (THP-1). (A,B) EA.hy926 cells and PMA-differentiated THP-1 cells were infected with *R. africae* (MOI=2.5), and genomic DNA was extracted at each time point post-infection. Each time point represents the ratio of *R. africae* sca1 to host cell actin genes amplified from genomic DNA and determined by quantitative PCR (qPCR). A logistic regression test was used to measure significance (p<0.05) in growth over time in both mammalian cell lines in A and B. Immunofluorescence microscopy growth analyses in EA.hy926 cells at days 1 and 5 post-infection (C) and in PMA-differentiated THP-1 cells at days 4 and 6 post-infection
demonstrate significant intracellular proliferation. DAPI (blue) was used to visualize host cell nuclei, anti-\textit{Rickettsia} antibody (RcPFA) followed by Alexa Fluor 488 (green) was utilized to reveal \textit{R. africae}, and Alexa Fluor 546 Phalloidin (red) was used to indicate the host actin cytoskeleton in \textbf{C} and \textbf{D}. Scale bar= 10 \textmu m.

\textbf{Supplemental Figure 3:} \textit{R. rickettsii} strain \textit{Iowa} exhibits significant intracellular replication within endothelial cells (EA.hy926) but not in human derived macrophage cells (THP-1). (\textbf{A,B}) EA.hy926 cells and PMA-differentiated THP-1 cells were infected with \textit{R. rickettsii} st. Iowa (MOI=2.5), genomic DNA was extracted at each indicated time point post-infection and then growth was determined by qPCR. A logistic regression test was used to measure significance (p<0.05) in growth over time and indicated growth in EA.hy926 cells (A), but not in THP-1 cells (B). Immunofluorescence microscopy growth analyses in EA.hy926 cells at days 1 and 4 post-infection (\textbf{C}) and in PMA-differentiated THP-1 cells at days 1 and 4 post-infection confirms results from the qPCR analyses. DAPI (blue) was used to visualize host cell nuclei, anti-\textit{Rickettsia} antibody (RcPFA) followed by Alexa Fluor 488 (green) was utilized to reveal \textit{R. rickettsii}, and Alexa Fluor 546 Phalloidin (red) was used to indicate the host actin cytoskeleton in \textbf{C} and \textbf{D}.

\textbf{Supplemental Figure 4.} TlyC and Pld protein sequence conservation in pathogenic and non-pathogenic \textit{Rickettsia} species. Percent identities of TlyC (\textbf{A}) and Pld (\textbf{B}) protein homologues were generated from protein sequences (RefSeq) for each indicated \textit{Rickettsia} species when compared to \textit{R. rickettsii} “Sheila Smith” proteins using the NCBI Blastp algorithm.
### A

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### B

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