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

| ***Rickettsia* spp.** | **Primer/Probe** | **Sequence (5' → 3')** |
|---|---|---|
| *R. africae* | Sca1_africae_fwd | CGT GGT ATG TAC GGC ACT AAT AA |
| | Sca1_africae_rev | TTT CAG CAT CGA ACC CGA TAG |
| | Sca1_africae | /56-FAM/ACC GGT CAT/ZEN/ATT CTC AAC GCG TCC/3IABkFQ/ |
| | | |
| *R. rickettsii* | Rr Sca1 F5271 | CAA GCT CGT TAT TAC CCC GAA T |
| | Sca1_RR_R5371 | CTA CCG CTC CTT GGA ATG TTA GAC C |
| | Sca1_RC_RR_Probe | /56-FAM/TCG GCT TAA/ZEN/GAT ACG GGA AGT/3IABkFQ/ |
| | | |
| *R. parkeri* | Rpp Sca-1 (316 bp) FWD | TGA TTC GTA ACA GAT TAG ATG C |
| | Rpp Sca-1 (316 bp) REV | CCG TAA ATA GAA ACC ACA TGA C |
| | Rpp Sca-1 PRB Set 2 | /56-FAM/ACC GGT CAT/ZEN/ATT CTC AAC GCG TCC/3IABkFQ/ |
| | | |
| *R. akari* | Sca1akari_444_fwd | ACT AAC AGA GCA AAC GCC TAA |
| | Sca1akari_568_rev | CGG TGA TGC CAG AGA AGT ATT |
| | Sca1_akari(494-518)probe | /56-FAM/CGC CTA CTG/ZEN/TTA GCC CAG CTT CAA/3IABkFQ/ |
| | | |
| *R. bellii* | Sca1bellii_13_fwd | GAC AGG GTA GCT GCA GAT ATA AA |
| | Sca1bellii_162_rev | CCC AAG GAG CTA TGT TCA TTA GT |
| | Sca1_bellii(57-83)probe | /56-FAM/<br>TGC AGC GAA/ZEN/AGG CTT AAA CGA TCA AC<br>/3IABkFQ/ |
| | | |
| **Host Cell Actin** | **Primer/Probe** | **Sequence (5' → 3')** |
| pEC3 | Actin-F420 | CCT GTA TGC CTC TGG TCG TA |
| | Actin-R681 | CCA TCT CCT GCT CGA AGT CT |
| | Actin_MS_Probe | /5MAXN/ ACT GTG CCC/ZEN/ATC TAC GAG/3IABkFQ/ |

All primers and probes for *Rickettsia* species were designed from the rickettsial antigen, Sca1.

## Supplemental Figure Legends

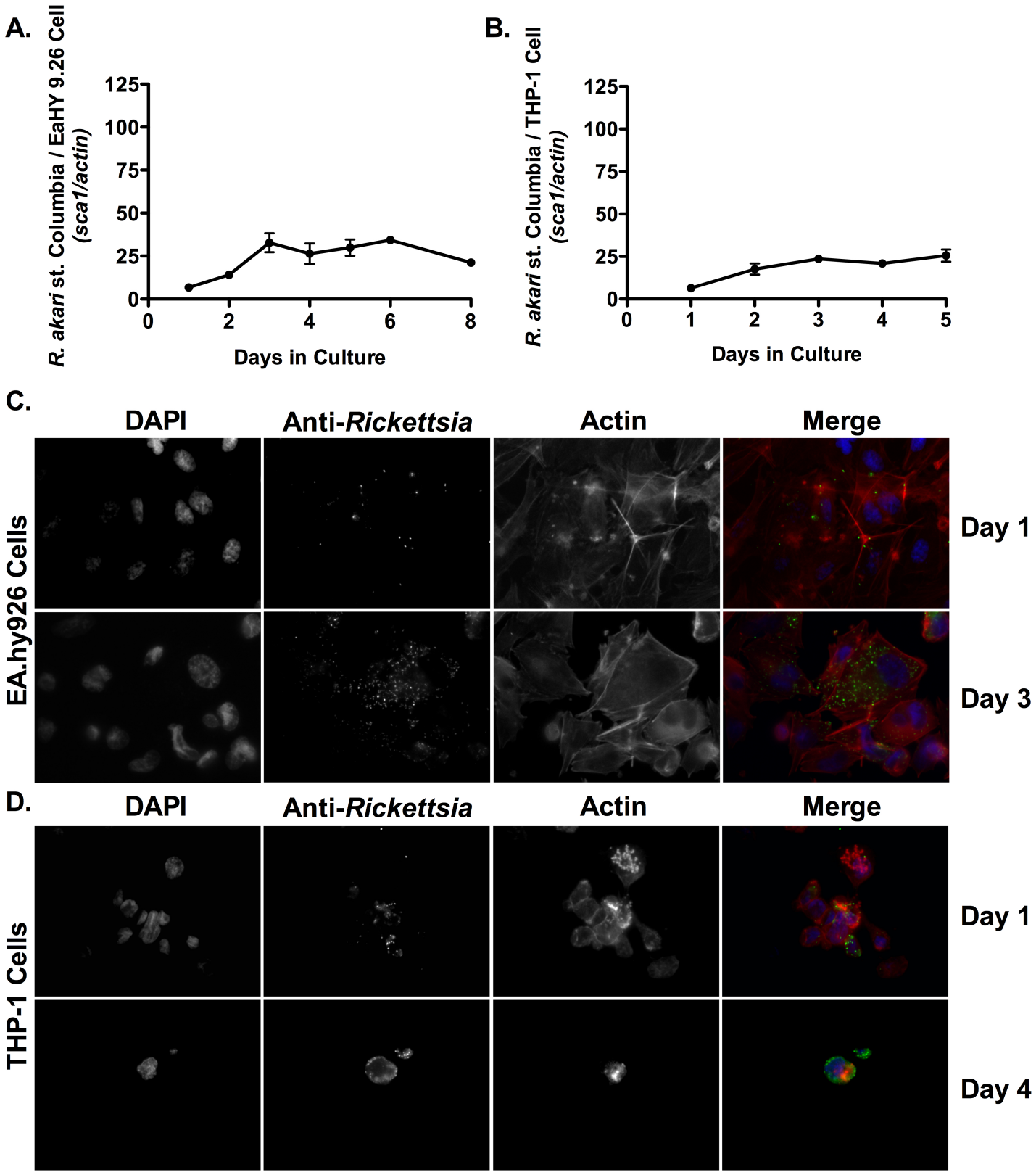
**Supplemental Figure 1: *R. akari* st. Columbia significantly grows within endothelial cells (EA.hy926) and human derived macrophage cells (THP-1). (A,B)** EA.hy926 cells and PMA-differentiated THP-1 cells were infected with *R. akari* st. Columbia (MOI=2.5), and genomic DNA was extracted at each time 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**.

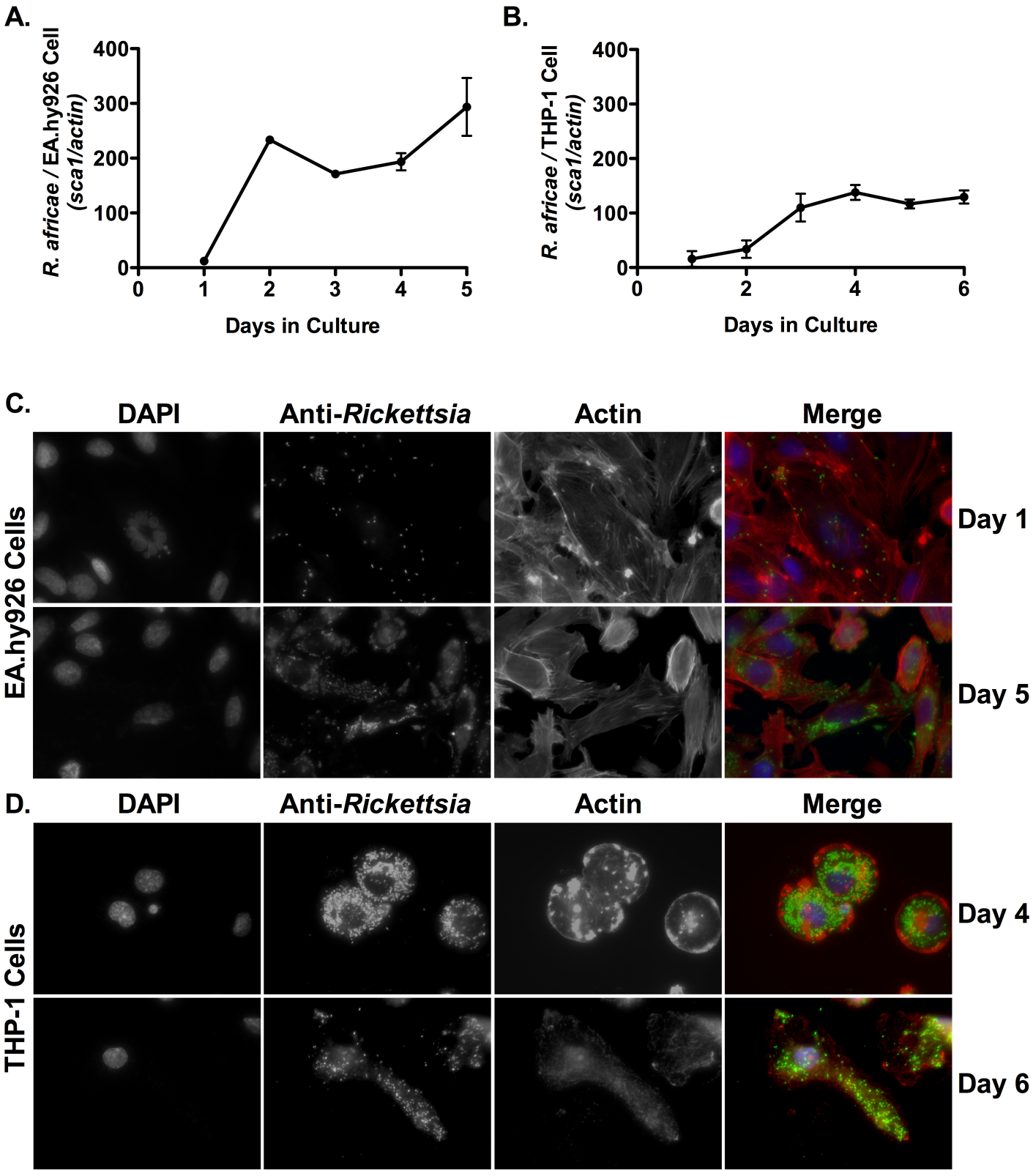
**Supplemental Figure 2: *R. africae* proliferates within endothelial cells (EA.hy926) and human derived macrophage cells (THP-1). (A,B)** EA.hy926 cells and PMA-differentiated THP-1 cells were infected with *R. africae* (MOI=2.5), and genomic DNA was extracted at each time point post-infection. Each 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

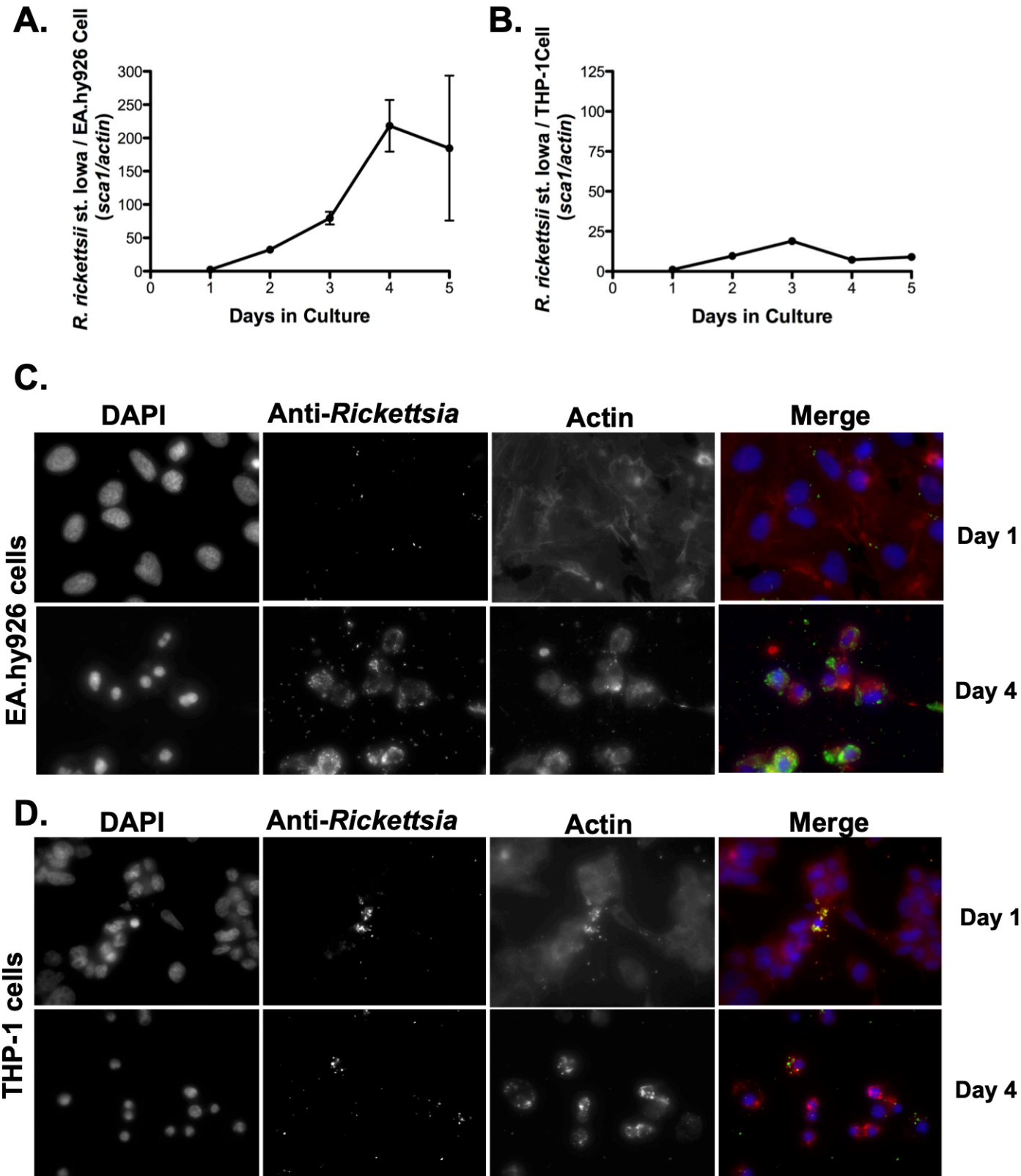
demonstrate significant intracellular proliferation. DAPI (blue) was used to visualize host cell nuclei, anti-*Rickettsia* antibody (RcPFA) followed by Alexa Fluor 488 (green) was utilized to reveal *R. africae*, and Alexa Fluor 546 Phalloidin (red) was used to indicate the host actin cytoskeleton in **C** and **D**. Scale bar= 10 μm.

**Supplemental Figure 3: *R. rickettsii* strain Iowa exhibits significant intracellular replication within endothelial cells (EA.hy926) but not in human derived macrophage cells (THP-1). (A,B)** EA.hy926 cells and PMA-differentiated THP-1 cells were infected with *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 **(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-*Rickettsia* antibody (RcPFA) followed by Alexa Fluor 488 (green) was utilized to reveal *R. rickettsii*, and Alexa Fluor 546 Phalloidin (red) was used to indicate the host actin cytoskeleton in **C** and **D**.

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


A.
R. akari st. Columbia / EaHY 9.26 Cell (sca1/actin)
125
100
75
50
25
0
0
2
4
6
8
Days in Culture
B.
R. akari st. Columbia / THP-1 Cell (sca1/actin)
125
100
75
50
25
0
0
1
2
3
4
5
Days in Culture
C.
DAPI
Anti-Rickettsia
Actin
Merge
EA.hy926 Cells
Day 1
Day 3
D.
DAPI
Anti-Rickettsia
Actin
Merge
THP-1 Cells
Day 1
Day 4


A.
R. africae / EA.hy926 Cell (sca1/actin)
400
300
200
100
0
0
1
2
3
4
5
Days in Culture
B.
R. africae / THP-1 Cell (sca1/actin)
400
300
200
100
0
0
2
4
6
Days in Culture
C.
DAPI
Anti-Rickettsia
Actin
Merge
EA.hy926 Cells
Day 1
Day 5
D.
DAPI
Anti-Rickettsia
Actin
Merge
THP-1 Cells
Day 4
Day 6


A.
R. rickettsii st. Iowa / EA.hy926 Cell (sca1/actin)
300
250
200
150
100
50
0
0 1 2 3 4 5
Days in Culture
B.
R. rickettsii st. Iowa / THP-1Cell (sca1/actin)
125
100
75
50
25
0
0 1 2 3 4 5
Days in Culture
C.
DAPI
Anti-Rickettsia
Actin
Merge
EA.hy926 cells
Day 1
Day 4
D.
DAPI
Anti-Rickettsia
Actin
Merge
THP-1 cells
Day 1
Day 4

**A**

| Species | RefSeq number | Amino acids | % identity |
|---|---|---|---|
| *R. rickettsii* "Sheila Smith" | WP_012151259.1 | 299 | |
| *R. rickettsii* "Iowa" | WP_0121511259.1 | 299 | 100 |
| *R. conorii* | WP_010977712.1 | 299 | 99.7 |
| *R. africae* | WP_012719992.1 | 299 | 99.7 |
| *R. parkeri* | WP_014411035.1 | 299 | 99.0 |
| *R. akari* | WP_012150023.1 | 301 | 96.6 |
| *R. bellii* | WP_011477962.1 | 301 | 82.4 |

**B**

| Species | RefSeq number | Amino acids | % identity |
|---|---|---|---|
| *R. rickettsii* "Sheila Smith" | WP_012151375.1 | 200 | |
| *R. rickettsii* "Iowa" | WP_012151375.1 | 200 | 100 |
| *R. conorii* | WP_010977832.1 | 200 | 98 |
| *R. parkeri* | WP_014411111.1 | 200 | 97.5 |
| *R. africae* | WP_012720066.1 | 200 | 96.5 |
| *R. akari* | WP_012150121.1 | 200 | 92 |
| *R. bellii* | WP_011476870.1 | 201 | 79.1 |