Ixodes scapularis Src tyrosine kinase facilitates *Anaplasma phagocytophilum* survival in its arthropod vector

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Percent identity and divergence of *I. scapularis* Src kinase with other orthologs. A) Agarose gel image showing PCR amplification of *src* gene used for QRT-PCR analysis. Band of approximately 159 bp was evident in both unfed, post-fed *I. scapularis* ticks (PF) and ISE6 tick cells. M indicates marker and NTC indicates no template control. **B**) Percent identity (horizontally above black boxed diagonal) and divergence (vertically below black boxed diagonal) of *I. scapularis* Src kinase with *Drosophila melanogaster*, *Aedes aegypti*, *Anopheles gambiae, Culex quinquefaciatus, Mus musculus* and *Homo sapiens* sequences is shown.

Supplementary Figure 2. *A. phagocytophilum*-HZ strain upregulates Src kinase levels in tick cells. A) QRT-PCR analysis showing *src* transcript levels upon treatment with different doses of *A. phagocytophilum*-HZ strain in tick cells at 24 p.i. Each circle represents data from one independent well of the culture plate. The mRNA levels of *src*

and *P44* (*A. phagocytophilum* gene) are normalized to tick beta-actin levels. P value from non-paired Student's t-test is shown.

Supplementary Figure 3. PCR amplification and nucleotide sequence of *I. scapularis* Src kinase dsRNA fragment. A) Agarose gel image showing PCR amplification of *src* gene fragment (334 bp) used for synthesis of dsRNA is shown. M indicates marker and NTC indicates no template control. **B**) Nucleotide sequence of *I. scapularis src*-dsRNA fragment (BglII-KpnI) clone is shown.

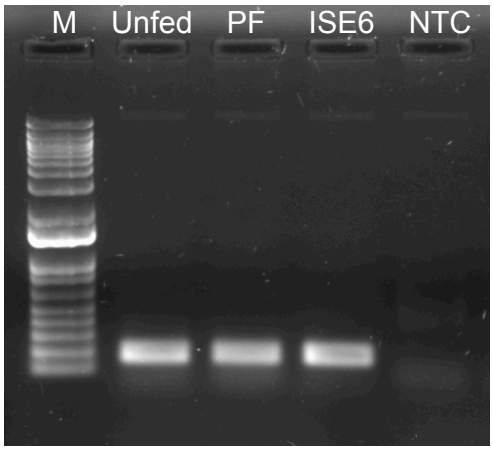
Supplementary Figure 4. Src kinase gene expression is not affected upon *A. phagocytophilum* at later time points of infection in tick cells. QRT-PCR showing levels of *A. phagocytophilum*-HZ strain (A) and *src* transcript levels (B) in uninfected (UI) or *A. phagocytophilum*-infected (I) tick cells at different time points of days 1, 3, 5, 7 and 10 post infection. Open circle represents uninfected (UI) and closed circles represent infected (I) tick cells group. Each circle represents data from one independent well of the culture plate. The *A. phagocytophilum* burden (p44 gene amplification) was normalized to tick 16S DNA levels and mRNA levels of *src* are normalized to tick beta-actin mRNA levels. P value from non-paired Student's t-test is shown

Supplementary Figure 5. Src expression in mock-buffer-treated, mock-dsRNA-treated and *src*-dsRNA-treated tick cells. A) QRT-PCR analysis showing levels of src mRNA in mock buffer (mock-EB), mock-dsRNA (mock-EV) and *src*-dsRNA treated tick cells at 24 p.i. is shown. Levels of *src* mRNA was normalized to tick beta-actin levels. P value from non-paired Student's t-test is shown.

Supplementary Figure 6. Treatment of *src*-dsRNA has no effect on tick cells.

Microscopic images of mock or *src*-dsRNA treated at 24 h post treatment and 48 h post-treatment-24 post-infected tick cells are shown. Scale 400 μm.

A **Src amplification**



B

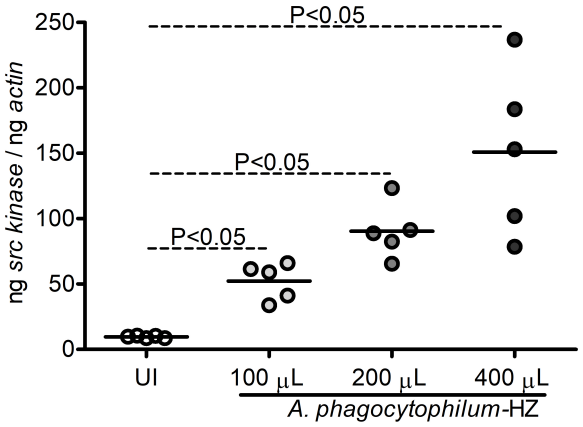
Percent Identity

	1	2	3	4	5	6	7	
1		79.3	45.1	45.0	38.6	55.9	55.6	**1**
2	24.3		48.1	48.1	42.3	54.6	55.1	**2**
3	94.0	85.0		96.6	86.7	48.4	48.7	**3**
4	94.4	85.0	3.4		85.4	48.1	48.4	**4**
5	116.8	103.1	14.6	16.3		41.8	42.1	**5**
6	65.3	68.3	84.2	85.1	104.9		99.1	**6**
7	66.1	67.2	83.4	84.2	103.8	0.9		**7**
	1	**2**	**3**	**4**	**5**	**6**	**7**	

Divergence

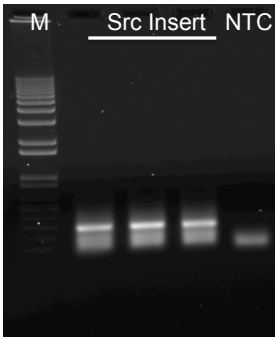
1 XP_002434467_Ixodes scapularis
2 AAF57295_Drosophila melanogaster
3 AAEL004592-PA_Aedes aegypti
4 AGAP006510-PA_Anopheles gambiae
5 CPIJ007458-PA_Culex quinquefaciatus
6 AAX90616_Mus musculus
7 NP_938033_Homo sapiens

Supplementary Figure 1

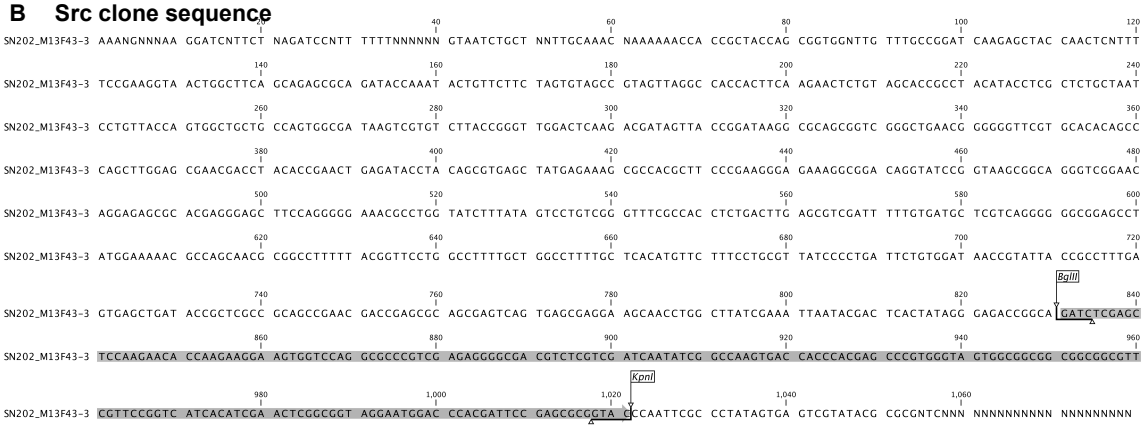


Supplementary Figure 2

A Src cloned product amplification

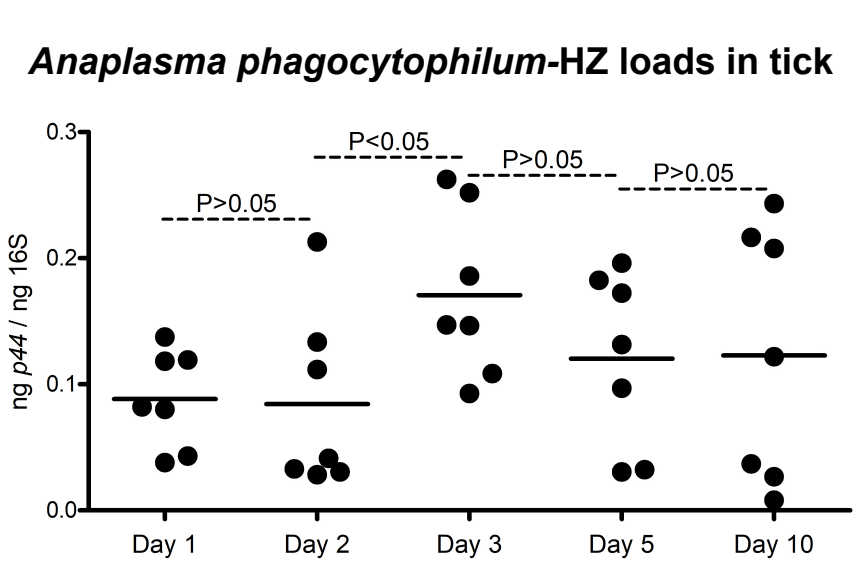


B Src clone sequence

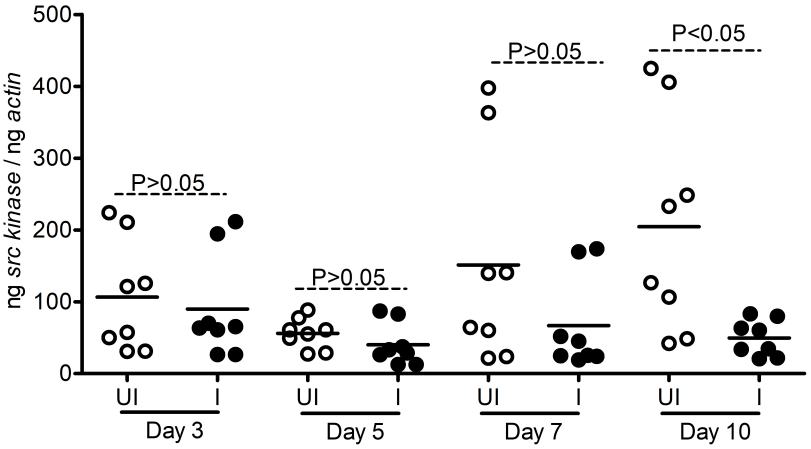


Supplementary Figure 3

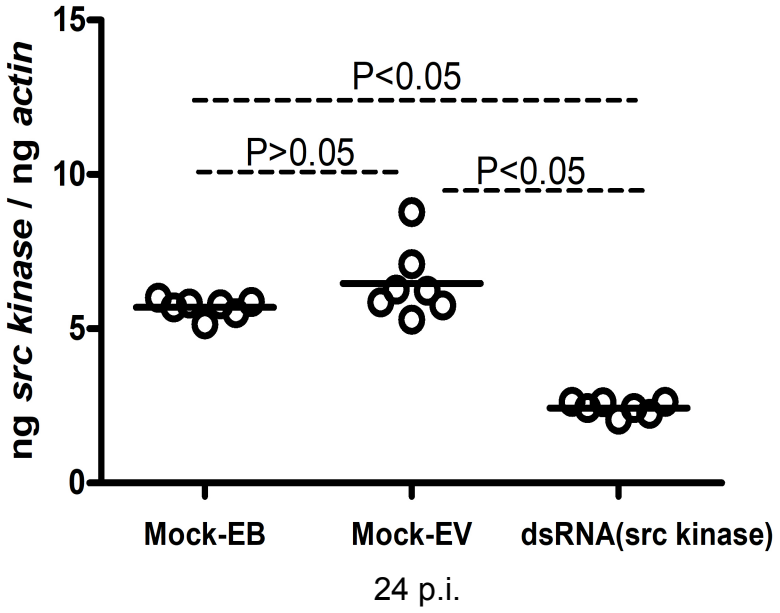
A



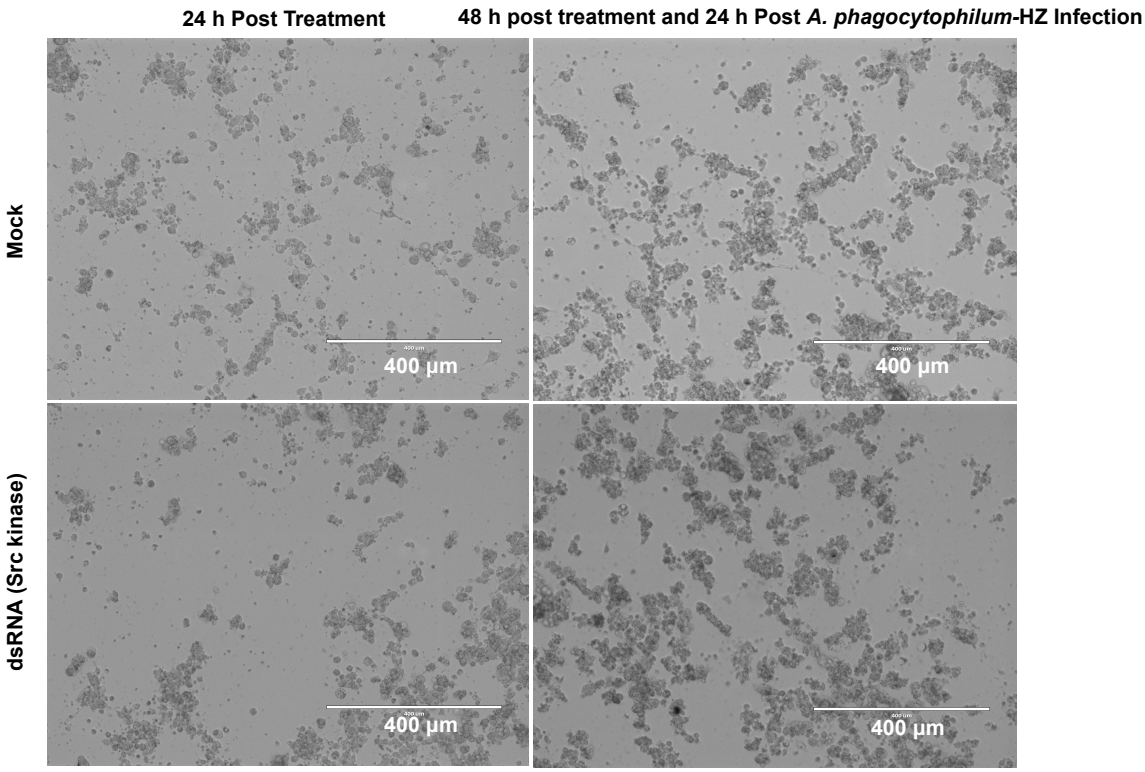
B



Supplementary Figure 4



Supplementary Figure 5



Supplementary Fig. 6