

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

Jeremy W. Turck¹, Vikas Taank¹, Girish Neelakanta^{1,2,*}, and Hameeda Sultana^{1,2,*}

¹ Department of Biological Sciences, ² Center for Molecular Medicine, College of Sciences, Old Dominion University, Norfolk, VA, USA

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 quinquefasciatus*, *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 (BglIII-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

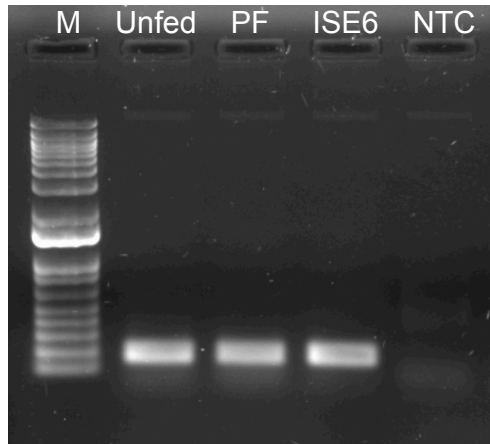
Supplementary Figure 5. Src expression in mock-buffer-treated, mock-dsRNA-

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 .

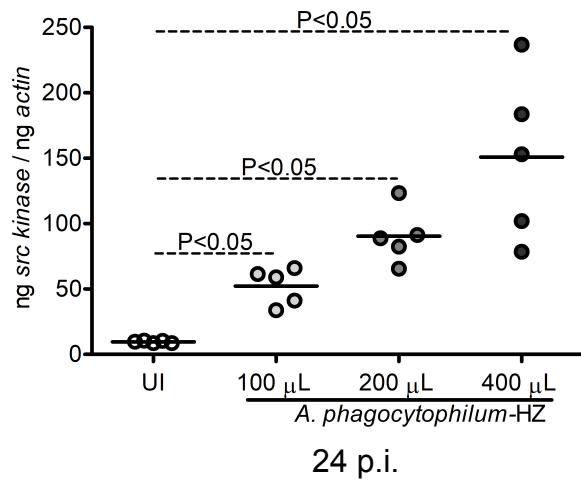
A Src amplification



B

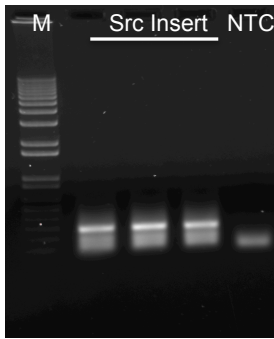
		Percent Identity								
		1	2	3	4	5	6	7		
Divergence	1	█	79.3	45.1	45.0	38.6	55.9	55.6	1	XP_002434467_Ixodes scapularis
	2	24.3	█	48.1	48.1	42.3	54.6	55.1	2	AAF57295_Drosophila melanogaster
	3	94.0	85.0	█	96.6	86.7	48.4	48.7	3	AAEL004592-PA_Aedes aegypti
	4	94.4	85.0	3.4	█	85.4	48.1	48.4	4	AGAP006510-PA_Anopheles gambiae
	5	116.8	103.1	14.6	16.3	█	41.8	42.1	5	CPIJ007458-PA_Culex quinquefasciatus
	6	65.3	68.3	84.2	85.1	104.9	█	99.1	6	AAX90616_Mus musculus
	7	66.1	67.2	83.4	84.2	103.8	0.9	█	7	NP_938033_Homo sapiens
		1	2	3	4	5	6	7		

Supplementary Figure 1



Supplementary Figure 2

A Src cloned product amplification



B Src clone sequence

```

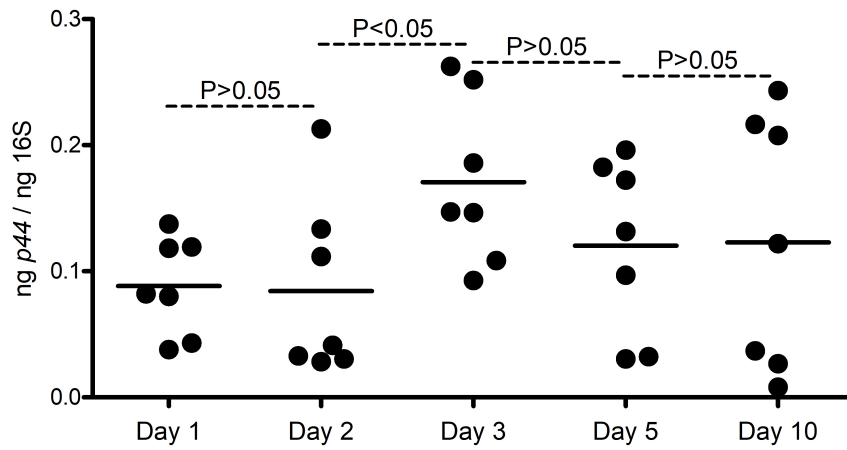
SN202_M13F43-3 AAANGNNAA GGATCNTTCT NAGATCCNTT TTTTNNNNN GTAATCTGCT NNTTGCAAAC NAAAAAACA CCGCTACCAG CCGTGGNTTG TTTGCCGGAT CAAGAGCTAC CAACTCNTTT
140 | 160 | 180 | 200 | 220 | 240 |
SN202_M13F43-3 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG CTCTGCTAAT
260 | 280 | 300 | 320 | 340 | 360 |
SN202_M13F43-3 CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGTT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGGTGAACG GGGGTTTCGT GCACACAGCC
380 | 400 | 420 | 440 | 460 | 480 |
SN202_M13F43-3 CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC TATGAGAAAG GCCCAGCTTT CCCGAAGGGA GAAAGCGGGA CAGGTATCCG GTAAGCGGCA GGTTCGGAAC
500 | 520 | 540 | 560 | 580 | 600 |
SN202_M13F43-3 AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA GTCTGTCTGG GTTTGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCCTCAGGGG GGCGGAGCCT
620 | 640 | 660 | 680 | 700 | 720 |
SN202_M13F43-3 ATGGAAAAAC GCCAGCAACG CCGCCCTTTT ACGGTTCCTG GCCTTTTGGT GGCTTTTGGC TCACATGTTT TTTCTCTGCT TATCCCTGA TCTGTGGAT AACCGTATTA CCGCCTTTGA
740 | 760 | 780 | 800 | 820 | 840 |
SN202_M13F43-3 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC CACCGAGCC AGCGAGTCAG TGAGCGAGGA AGCAACCTGG CTTATCGAAA TTAATACGAC TCACTATAGG GAGACCGGCA GATCTCGAGC
860 | 880 | 900 | 920 | 940 | 960 |
SN202_M13F43-3 TCCAAGAACA CCAAGAAGGA AGTGGTCCAG GCGCCCGTCG AGAGGGGCGA CGTCTCGCTG ATCAATATCG GCCAAGTGAC CACCCACGAG CCCGTGGGTA GTGGCGGGGG CGGCGGGCTT
980 | 1,000 | 1,020 | 1,040 | 1,060 |
SN202_M13F43-3 CGTTCGGTC ATCACATCGA ACTCGGGGT AGGAATGGAC CCACGATTC GAGCGCGTA TCAATTCCG CCTATAGTGA GTCGTATACG CGCGNTCNNN NNNNNNNN NNNNNNNN
1,080 | 1,100 | 1,120 | 1,140 | 1,160 |

```

Supplementary Figure 3

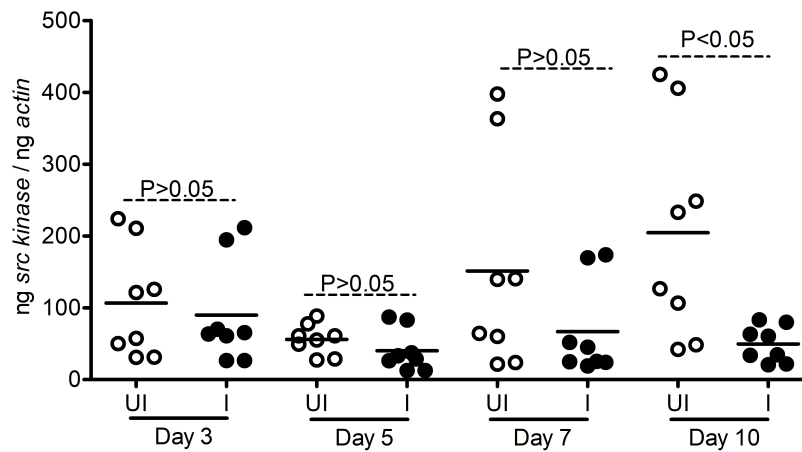
A

***Anaplasma phagocytophilum*-HZ loads in tick**

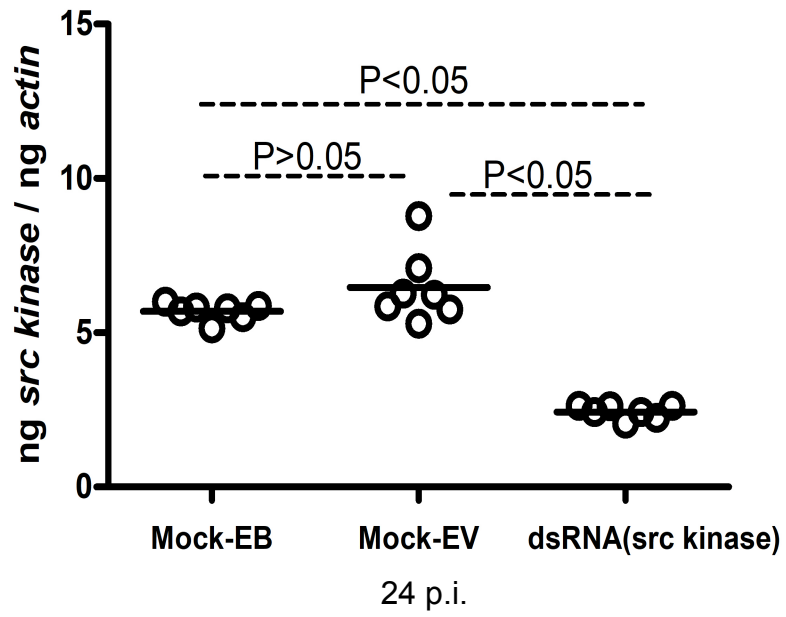


B

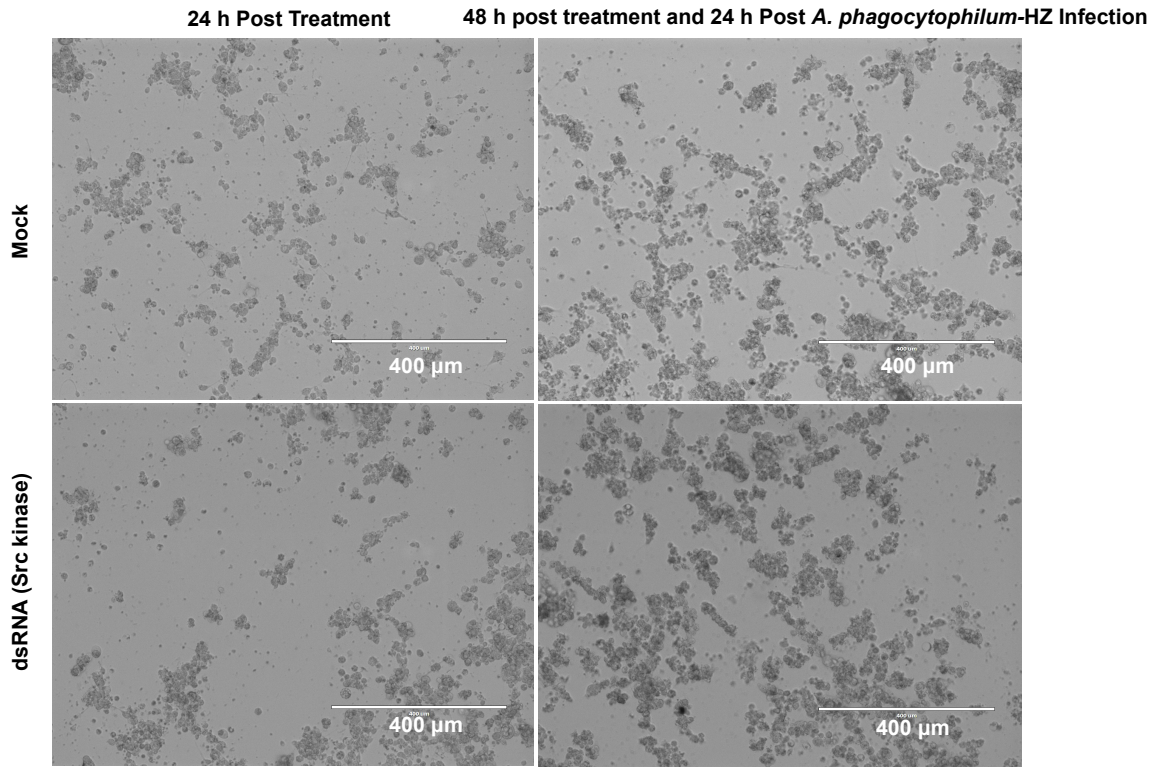
Src kinase expression in tick cells infected with *Ap*-HZ



Supplementary Figure 4



Supplementary Figure 5



Supplementary Fig. 6