

SUPPLEMENTARY MATERIAL

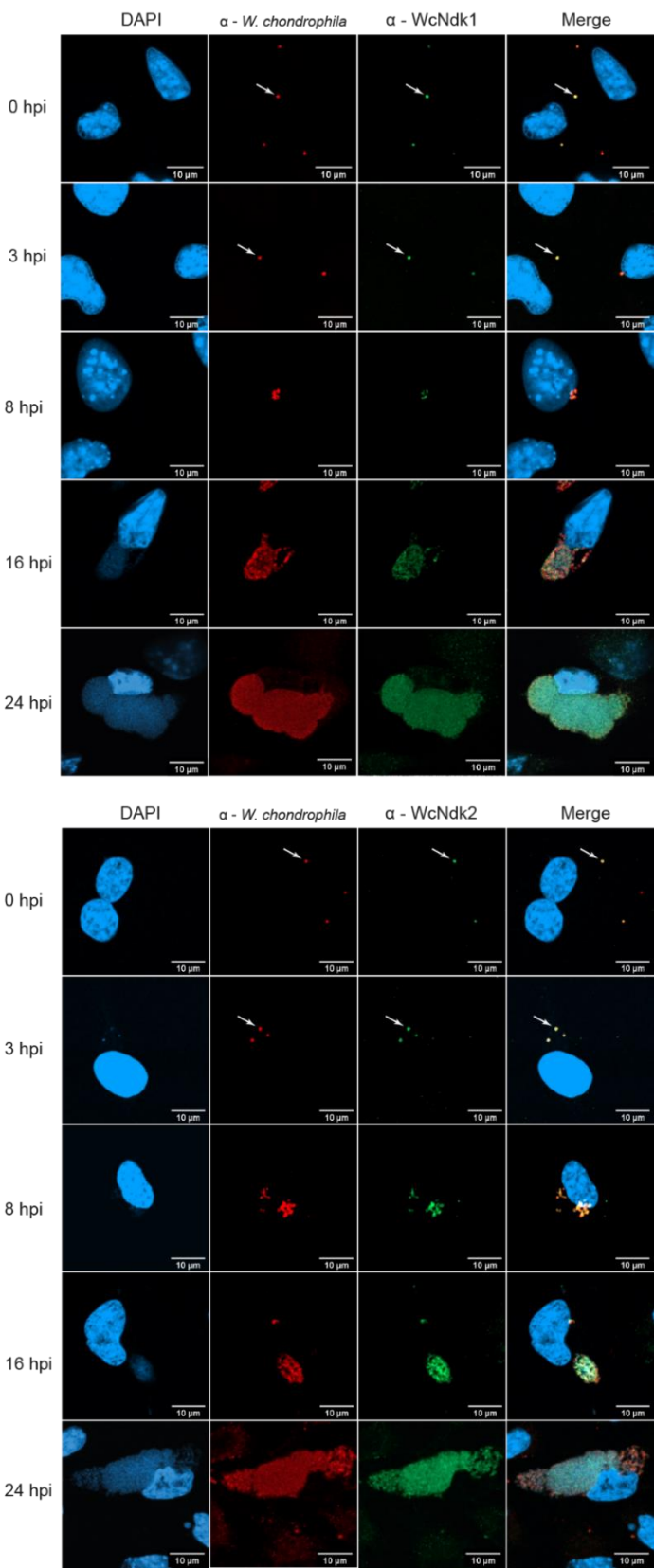


Figure S1: Immunofluorescence detection of WcNdk1 and WcNdk2 during *W. chondrophila* infection. DAPI staining (blue) marks host nuclei, anti-*W. chondrophila* (red) labels bacteria, and anti-WcNdk1 or anti-WcNdk2 (green) detect the corresponding proteins. Merged images show colocalization in yellow. Arrows indicate the localization of bacteria, WcNdks, and their colocalization.

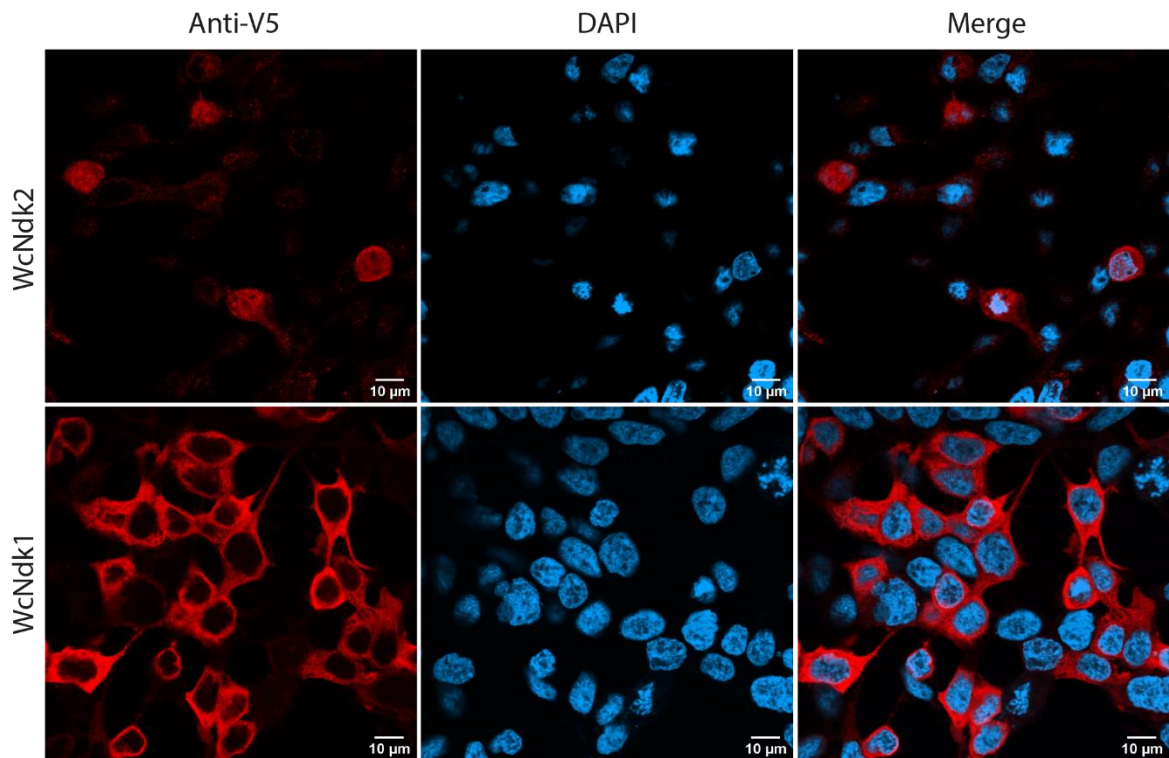


Figure S2: HeLa cells expressing WcNdk1-V5 or WcNdk2-V5. Cells were transiently transfected with WcNdk1 and WcNdk2 constructs carrying a C-terminal V5 tag.

Target gene	Oligo type	Sequence (5' -> 3')	Label	Amplicon size (bp)
wcw_1543 (<i>wcndk1</i>)	Forward primer	tgg-tat-tgg-aag-gag-acg-atg-c	-	160
wcw_1543 (<i>wcndk1</i>)	Reverse primer	tcc-gtt-ttt-gcc-gtt-tct-gg	-	160
wcw_1545 (<i>wcndk2</i>)	Forward primer	agt-tgt-tgc-gat-ggt-act-gga	-	150
wcw_1545 (<i>wcndk2</i>)	Reverse primer	gaa-tct-gat-cca-tgc-acg-gc	-	150
<i>Waddlia</i> 16S rRNA	Forward primer	ggc-cct-tgg-gtc-gta-aag-ttc-t	-	101
<i>Waddlia</i> 16S rRNA	Reverse primer	cgg-agt-tag-ccg-gtg-ctt-ct	-	101
<i>Waddlia</i> 16S rRNA	TaqMan prob	FAM-cAt-ggg-aaC-aag-aga-agG-ATg-BHQ	FAM/BHQ-1	-

Table S1: Primer sequences used for RT-qPCR in this study. Sequence letters in capital are Locked Nucleic acids.

	C. sequanensis_Ndk2	E. lausannensis_Ndk2	W. chondrophila_Ndk2	Neochlamydia_a sp_Ndk2	P. cantlamoebae_Ndk2	Neochlamydia_sp_Ndk1	C. sequanensis_Ndk1	E. lausannensis_Ndk1	P. acanthamoebae_Ndk1	W. chondrophila_Ndk1	Rabdochlamydia_Ndk	S. negevensis_Ndk	C. trachomatis_Ndk	Paeruginosa_Ndk	Thioalbus_dentrifivans_Ndk	Legionella_pneumophila_Ndk	Halofilum_ochreaceum_Ndk	M. tuberculosis_Ndk	E. coli_Ndk	A.flau_Ndk	A.niger_Ndk
C. sequanensis_Ndk2	100%																				
E. lausannensis_Ndk2	62%	100%																			
W. chondrophila_Ndk2	49%	56%	100%																		
Neochlamydia_sp_Ndk2	48%	52%	57%	100%																	
P. cantlamoebae_Ndk2	55%	60%	62%	64%	100%																
Neochlamydia_sp_Ndk1	51%	56%	55%	57%	57%	100%															
C. sequanensis_Ndk1	50%	56%	62%	56%	60%	77%	100%														
E. lausannensis_Ndk1	48%	57%	60%	56%	57%	82%	81%	100%													
P. acanthamoebae_Ndk1	51%	57%	56%	56%	56%	81%	78%	83%	100%												
W. chondrophila_Ndk1	49%	52%	54%	59%	53%	76%	73%	79%	79%	100%											
Rabdochlamydia_Ndk	46%	50%	57%	53%	52%	65%	64%	66%	66%	66%	100%										
S. negevensis_Ndk	51%	55%	57%	54%	57%	77%	73%	77%	78%	76%	69%	100%									
C. trachomatis_Ndk	47%	53%	59%	53%	55%	71%	70%	72%	70%	66%	67%	69%	100%								
Paeruginosa_Ndk	48%	52%	56%	54%	54%	72%	70%	78%	76%	80%	68%	77%	66%	100%							
Thioalbus_dentrifivans_Ndk	48%	52%	55%	56%	55%	75%	73%	80%	79%	82%	70%	81%	72%	88%	100%						
Legionella_pneumophila_Ndk	52%	54%	59%	55%	58%	75%	72%	76%	74%	72%	69%	79%	72%	77%	80%	100%					
Halofilum_ochreaceum_Ndk	46%	51%	55%	54%	53%	71%	70%	77%	76%	79%	67%	77%	66%	99%	88%	77%	100%				
M. tuberculosis_Ndk	38%	45%	48%	45%	47%	57%	55%	58%	57%	57%	56%	59%	54%	88%	80%	58%	57%	100%			
E. coli_Ndk	43%	48%	49%	48%	49%	66%	66%	72%	68%	67%	65%	68%	63%	70%	71%	68%	69%	56%	100%		
A.flau_Ndk	36%	37%	44%	40%	41%	53%	52%	54%	53%	53%	51%	51%	49%	49%	51%	49%	51%	54%	89%	100%	
A.niger_Ndk	36%	37%	43%	39%	41%	51%	51%	52%	52%	53%	49%	52%	51%	49%	51%	52%	49%	54%	51%	89%	100%
Homo sapiens_Ndk	34%	36%	44%	39%	39%	51%	49%	55%	53%	53%	50%	51%	51%	51%	51%	52%	51%	52%	73%	73%	73%

Table S2: Sequence similarity matrix of Ndk proteins across selected species. Heatmap showing pairwise amino acid identity (%) between Ndk proteins from diverse organisms. A: *Aspergillus*, C. *trachomatis*: *Chlamydia trachomatis*, C. *sequanensis*: *Criblamydia sequanensis*, E. coli: *Escherichia coli*, E. *lausannensis*: *Estrella lausannensis*, M: *Mycobacterium*, P. *acanthamoebae*: *Parachlamydia acanthamoebae*, P. *aeruginosa*: *Pseudomonas aeruginosa*, S: *Simkania*, W: *Waddlia*.

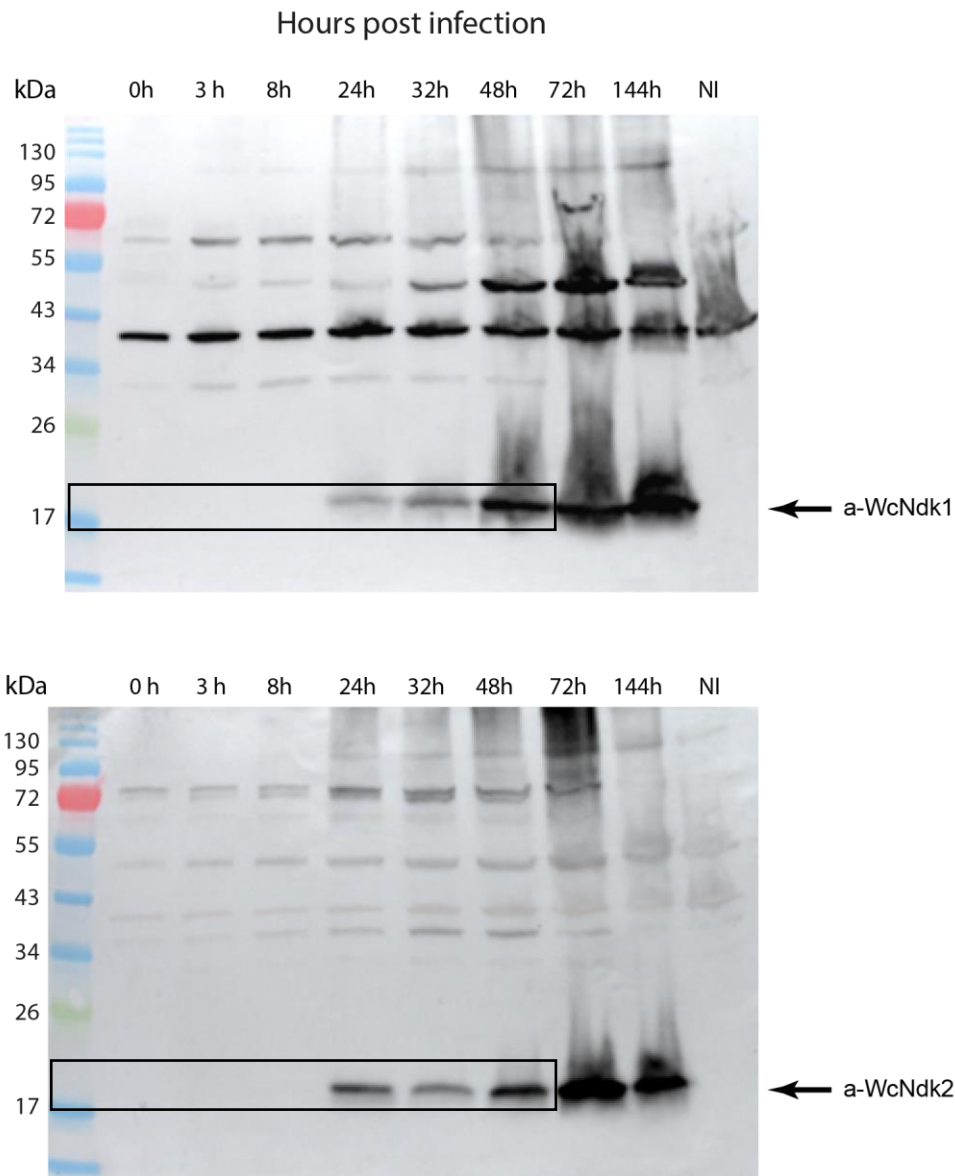


Figure S3: Full, uncropped immunoblot corresponding to Figure 3C. Arrows indicate the WcNdk1 and WcNdk2 proteins. Bands inside the black boxes were used for density quantification. Bands with similar molecular weight is not present in non-infected (NI) lysates, consistent with the antibody not detecting the host NDPKs at this molecular weight. Time points 72 hpi and 144 hpi are shown here for completeness but were not included in the main figure and not used for quantification because they fall outside the developmental period analyzed in this study.