

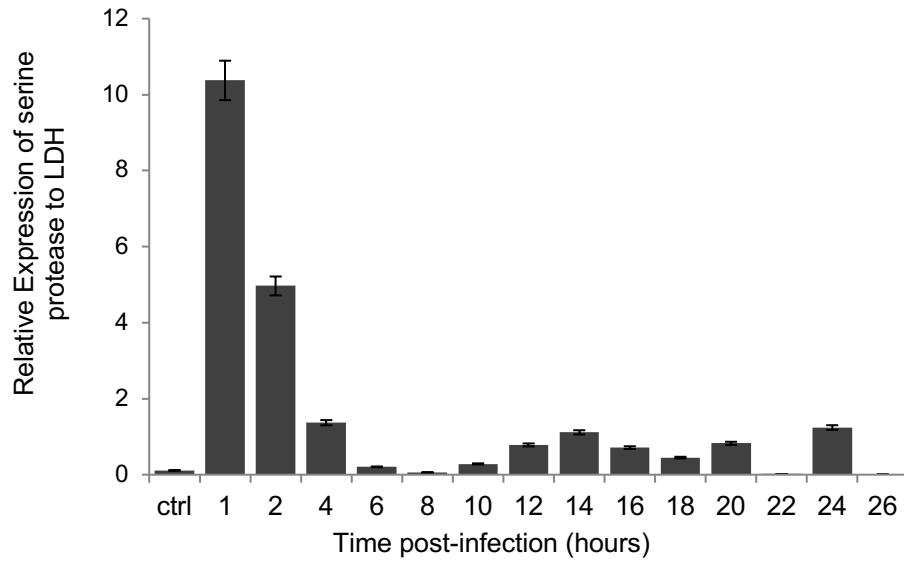
**Supplementary Table 1.** Primer sequences used for rtPCR analysis studies

<b>Primer</b>	<b>Sequence</b>
LDH Forward	GACGCAAGATTGCCGTTATT
LDH Reverse	TATGTGGAAGCCCAGAAACC
SUB1 Forward	TCAAGTTGTTGCCATATTTATTGGT
SBU1 Reverse	CCTAAATGTTTCCATGCCATCAA
H18s Forward	CCGATAACGAACGAGACTCTGG
H18s Reverse	TAGGGTAGGCACACGCTGAGCC

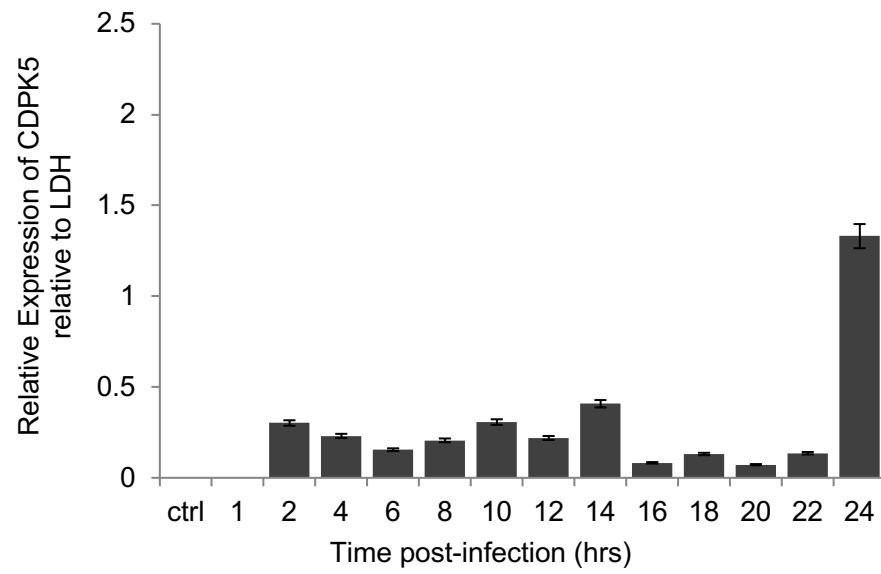
**Supplementary Table 2.** Single-stranded antisense sequences. Antisense sequences sorted by sequence, size and location from 5' end of SUB1 and CDPK5 mRNA, respectively.

<b>Target</b>	<b>ssRNA sequence</b>	<b>Size</b>	<b>Location</b>
SUB1 #1	GAU GAG CUU GUU GGA AUC AAA UCT G	25	544-569
SUB1 #2	GAA UGG AUU CUG AAG AAG UAA AAA	24	2837-2862
SUB1 #3	GTT GAA GGT CGG AGC TCT G	20	106-126
SUB1 #4	GAA CCA GAT CAG GAA GTT AGG CTT GCA	27	688-714
SUB1 #5	GAA GAA GAG GAT GAT GGA GA	20	3292-3372
CDPK5 #1	CAG GGG AGC UUC AGC AAA A	19	218-236
CDPK5 #2	GGG ACA CAT TTA TGC AGC TAT GTGT	25	183-207
CDPK5 #3	GAG AGG CTC TTA TGG ATC TGT TGTA	25	576-600
CDPK5 #4	CAT GCG CTT GTT AAC AGG AAT ATTA	25	517-541
CDPK5 #5	AGG GAA TAC AGG AAC AAG GC	20	128-153

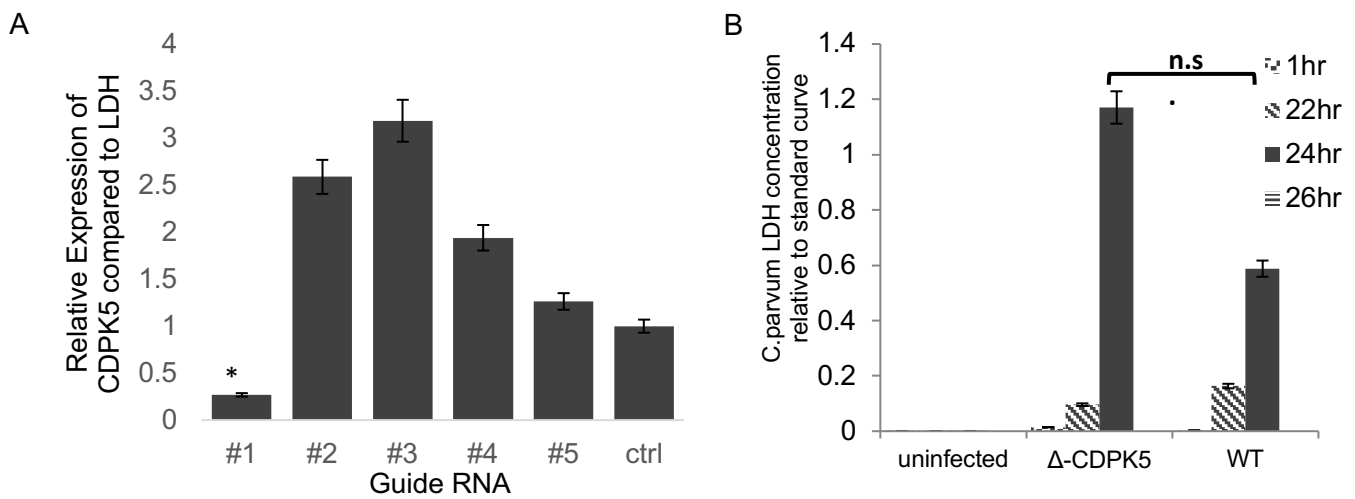
Supplementary Figure **S1**. The expression pattern of serine protease (*cgd2\_3660*) was observed using rtPCR. Expression peaks at the beginning of infection (1hour post-infection) and wanes to minimal levels for the rest of the asexual replication stage. This observation is contrary to what was observed for SUB1 expression levels.



**Supplementary Figure S2.** CDPK5 expression pattern in infection of HCT8-cells with *Cryptosporidium parvum*. We observed a peak of expression taking place at the time of egress (24 hours post-infection).



**Supplementary Figure S3.** A) Antisense sequences designed for CDPK5 silencing were tested for potency. Segment #1 was the only one that demonstrated significant silencing in oocysts and was used for additional experimentation. B.) CDPK5 silenced samples demonstrated similar egress compared to wild-type controls. We observed no changes in the levels of egressed parasites from collected supernatants compared to controls at 24 hours post-infection.



**Supplementary Figure S4.**  $\Delta$ SUB1 parasites exhibited decreased levels of egress in comparison to wild-type controls. At 1 and 20 hours post-infection, there is minimal or no parasites detected in supernatant in both treated and control samples. At 24 hours post-infection, there is a peak of egress observed in wild-type samples, where  $\Delta$ SUB1 parasites have a significant decrease. At 26 hours post-infection, there is no detection of parasites in both wild-type and treated samples.

