

Supplementary Information

Supplementary Figure 1. Generation and characterization of PKG-HA and PKG T₆₁₉Q-HA lines in PbANKA 507c1.

Supplementary Figure 2. Maximum intensity projections of sporozoite motility patterns.

Supplementary Table 1. Effect of chemical and genetic inhibition of PKG on sporozoite infectivity.

Supplementary Table 2. Effect of CDPK4 inhibition on sporozoite infectivity.

Supplementary Figure 1

A.

Wild type *pkg* locus
in ANKA 507c1

Targeting vector

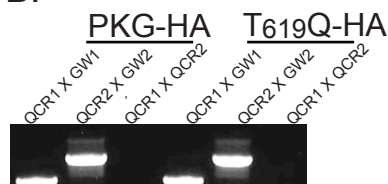


Mutated *pkg* locus
in ANKA 507c1

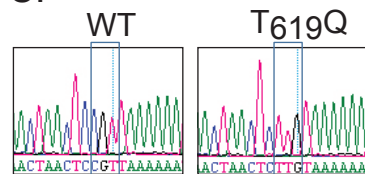


pkg QCR1 GCTGGACGTTTGCTTGAACCTCC
pkg QCR2 GGAACCAATGTGTTTTCCATGGGCA
 GW1 CATACTAGCCATTTTATGTG
 GW2 CTTTGGTGACAGATACTAC
pkg gkF TTGAAGGACCCATGTTAGCAC
pkg gkR TCAACTGTGCACCCATATCC

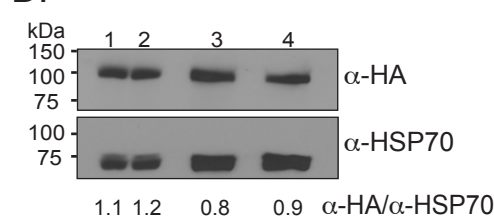
B.



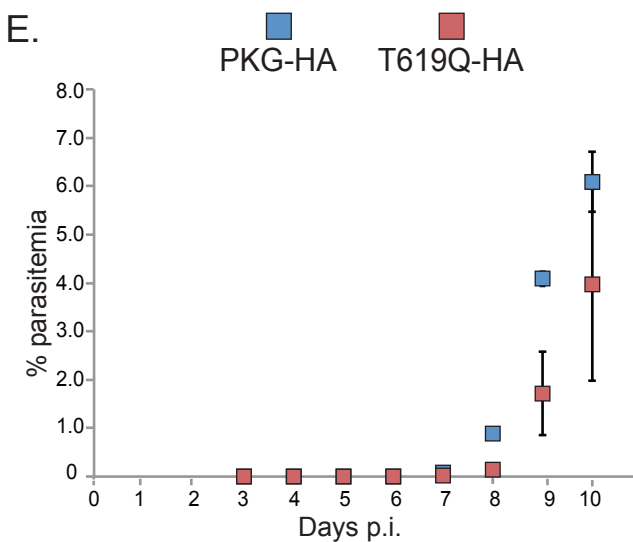
C.



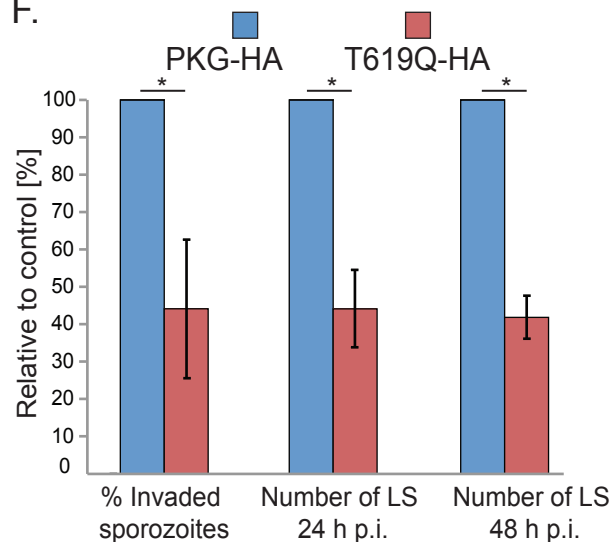
D.



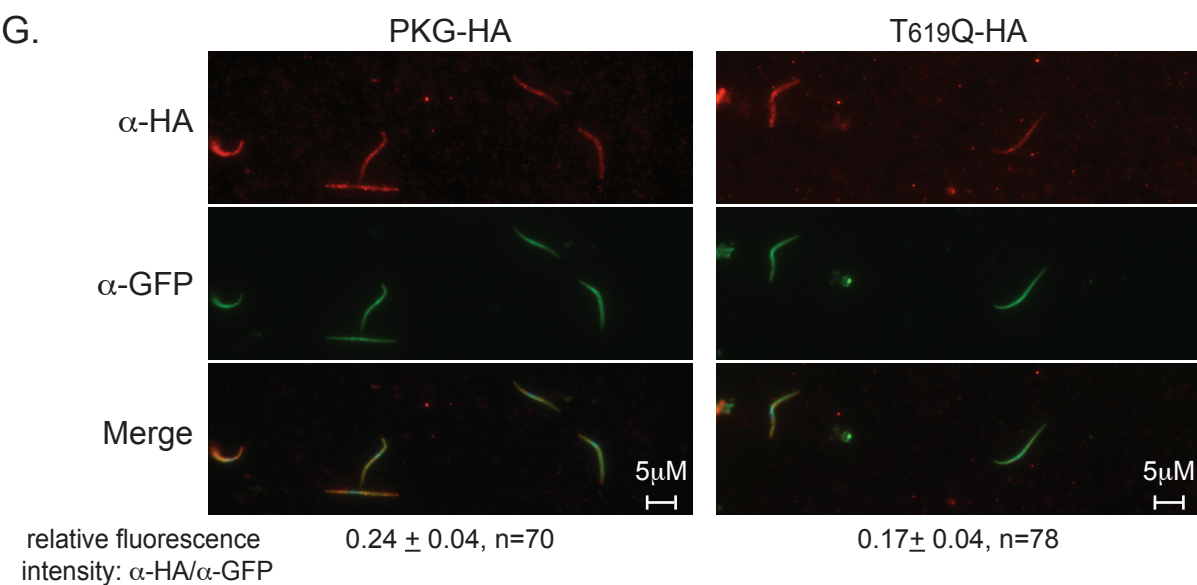
E.



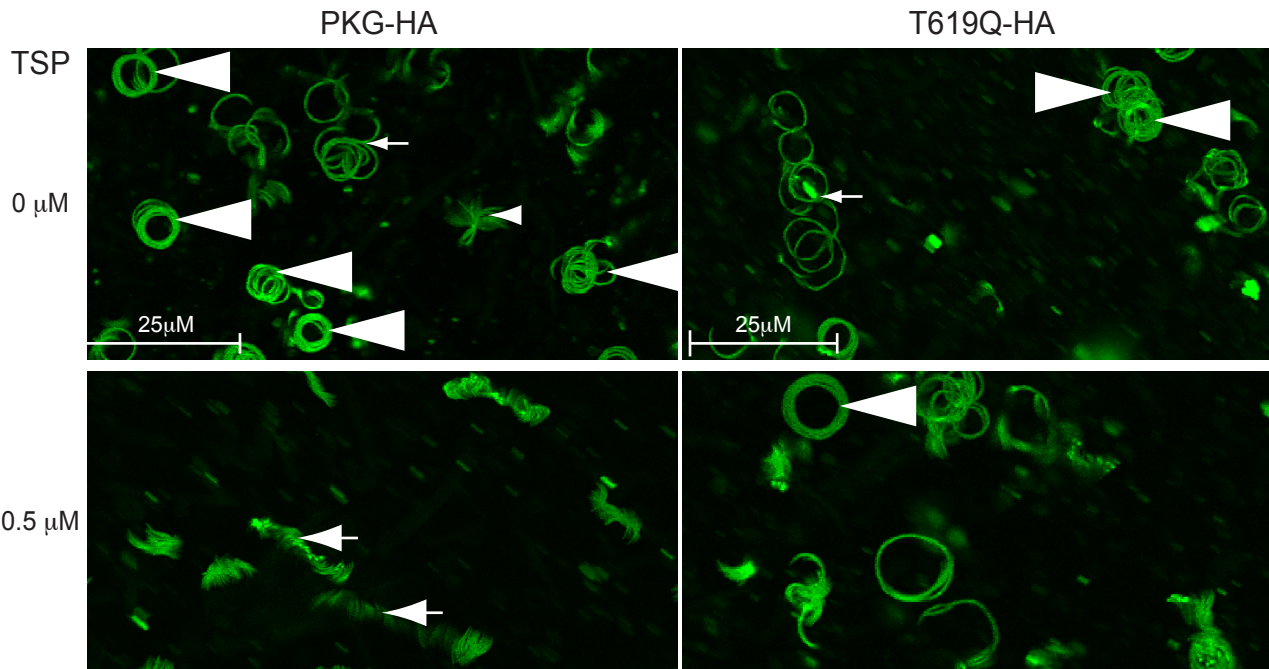
F.



G.



Supplementary Figure 2



Supplementary Table 1

A) Sensitivity to TSP: 0-14h treatment			Sensitivity to TSP: pre-treatment of sporozoites			Sensitivity to TSP: Invasion			Sensitivity to TSP: 24-65h treatment		
[TSP]	PKG-HA	T619Q-HA	[TSP]	PKG-HA	T619Q-HA	[TSP]	PKG-HA	T619Q-HA	[TSP]	PKG-HA	T619Q-HA
Experiment 1 (n=4)	# of LS, 40h p.i (mean ± SD)		Experiment 1 (n=4)	# of LS, 40h p.i (mean ± SD)		Experiment 1 (n=4)	% invaded sporozoites (mean ± SD)		Experiment 1 (n=3)	# of merosomes (mean ± SD)	
0 μM	328 ± 21	157 ± 19	0 μM	355 ± 15	117 ± 3	0 μM	53 ± 4	22 ± 3	0 μM	10000 ± 589	3750 ± 884
0.1 μM	292 ± 10	177 ± 9	0.5 μM	114 ± 6	110 ± 11	0.5 μM	10 ± 2	27 ± 2	0.5 μM	3333 ± 0	3750 ± 884
0.5 μM	0 ± 0	199 ± 6	2 μM	18 ± 6	101 ± 6	2 μM	3 ± 0	32 ± 1			
2 μM	0 ± 0	149 ± 16									
10 μM	0 ± 0	24 ± 3									
Experiment 2 (n=3)			Experiment 2 (n=4)			Experiment 2 (n=3)			Experiment 2 (n=3)		
0 μM	224 ± 12	150 ± 6	0 μM	615 ± 47	24.5 ± 1	0 μM	40 ± 3	35 ± 2	0 μM	2121 ± 1060	2500 ± 353
0.5 μM	0 ± 0	143 ± 16	2 μM	25 ± 1	77 ± 0.5	0.5 μM	12 ± 2	23 ± 1	2 μM	0 ± 0	1000 ± 0.0
2 μM	0 ± 0	89 ± 7				2 μM	5 ± 1	34 ± 2			
10 μM	0 ± 0	4 ± 0.6	Experiment 3 (n=4)						Experiment 3 (n=3)		
Experiment 3 (n=4)			0 μM	152 ± 5	75 ± 6				0 μM	9794 ± 676	7783 ± 2291
0 μM	328 ± 41	157 ± 38	0.5 μM	68 ± 3	59 ± 2				0.5 μM	4167 ± 413	8333 ± 1096
0.5 μM	0 ± 0	199 ± 13	2 μM	3 ± 1	79 ± 6				2 μM	567 ± 491	2783 ± 1274
2 μM	0 ± 0	149 ± 32									
10 μM	0 ± 0	24 ± 6									

B) Effect of TSP on motility						
Experiment 1	Gliding	Drifting	Waving	Adherent	Complex	Total observed
PKG-HA						
[TSP]	# of sporozoites	# of sporozoites	# of sporozoites	# of sporozoites	# of sporozoites	
0 μM	54	12	29	205	15	315
0.5 μM	4	4	53	43	93	197
T619Q-HA						
0 μM	13	7	69	257	2	348
0.5 μM	15	3	116	157	29	320
Experiment 2						
PKG-HA						
0 μM	55	3	6	125	64	253
0.5 μM	0	11	49	9	181	250
T619Q-HA						
0 μM	27	3	18	126	98	272
0.5 μM	22	19	55	117	35	248

C) % invaded sporozoites (mean ± SD)			Number of LS, 24h p.i (mean ± SD)		Number of LS, 48h p.i (mean ± SD)		
Experiment	PKG-HA	T619Q-HA	PKG-HA	T619Q-HA	PKG-HA	T619Q-HA	
Experiment 1 (n=4)	71 ± 7	31 ± 6	Experiment 1 (n=4)	456 ± 14	201 ± 21	Experiment 1 (n=4)	792 ± 18
Experiment 2 (n=4)	9 ± 0.6	4 ± 0.7	Experiment 2 (n=4)	26 ± 0.7	16 ± 3	Experiment 2 (n=4)	411 ± 10
Experiment 2 (n=3)	77 ± 2	39 ± 1.8				Experiment 3 (n=4)	157 ± 10

Supplementary Table 2

A) % invaded sporozoites (mean ± SD)			Number of LS, 24h p.i (mean ± SD)		Number of LS, 48h p.i (mean ± SD)		Number of merosomes, 65-72 h p.i (mean ± SD)				
	Control	CDPK4 cKO	Control	CDPK4 cKO	Control	CDPK4 cKO	Control	CDPK4 cKO			
Experiment 1 (n=4)	49 ± 7	17 ± 1	Experiment 1 (n=4)	84 ± 3	39 ± 2	Experiment 1 (n=4)	159 ± 4	86 ± 4	Experiment 1 (n=3)	3278 ± 1171	2833 ± 507
Experiment 2 (n=4)	53 ± 3	35 ± 2	Experiment 2 (n=4)	823 ± 17	459 ± 17	Experiment 2 (n=4)	696 ± 41	410 ± 10	Experiment 2 (n=3)	10227 ± 693	12000 ± 2327
Experiment 3 (n=4)	56 ± 9	25 ± 0.4	Experiment 3 (n=4)	746 ± 12	458 ± 20	Experiment 3 (n=4)	756 ± 23	418 ± 9	Experiment 3 (n=4)	2500 ± 833	3000 ± 1000
Experiment 4 (n=4)	41 ± 4	19 ± 1	Experiment 4 (n=4)	135 ± 3	54 ± 5	Experiment 4 (n=4)	143 ± 14	47 ± 3			

B) <i>In vivo</i> infection		C) Effect of Compound 1294 on sporozoite motility							
	Control	CDPK4 cKO	[1294]	Gliding	Drifting	Waving	Adherent	Complex	Total observed
Experiment 1	n = 5	n = 4	Experiment 1	# of sporozoites	# of sporozoites	# of sporozoites	# of sporozoites	# of sporozoites	
Day 4	3.20E-03 ± 2.21E-03	7.25E-04 ± 7.25E-04	0 µM	136	9	14	74	92	325
Day 6	5.84E-01 ± 2.66E-01	2.85E-01 ± 7.24E-02	2 µM	27	3	13	203	117	363
Experiment 2	n = 10	n = 10	Experiment 2						
Day 4	1.02E-02 ± 6.09E-03	3.00E-04 ± 3.37E-04	0 µM	134	119	13	169	9	444
Day 6	1.10E-01 ± 5.33E-02	3.95E-02 ± 1.92E-02	0.5µM	106	193	23	216	32	570
			Experiment 3						
			0 µM	36	4	13	68	8	129
			0.5µM	18	1	27	68	33	147