

Table S1. Sporozoite numbers of *P. berghei* GFP tagged parasites in *A. coluzzii* infections.

Parasite	Mean	SEM	P-value
ANKA 2.34	5,934	2875	
<i>c01::gfp</i>	612	395	0.0048
<i>c57::gfp</i>	354	175	0.0042
<i>c22::gfp</i>	8,441	2422	0.4880

Mean oocyst sporozoite numbers from two biological replicates of *A. coluzzii* infections with transgenic *P. berghei* parasites carrying GFP tagged versions of the genes *PIMMS01*, *PIMMS57* and *PIMMS22*. For each biological replicate sporozoite numbers were determined from at least 30 homogenized mosquito midguts 15 dpbf. P values were calculated using the unpaired Student's t-test. SEM represents standard error of mean.

Table S2. Oocyst numbers of *P. berghei* HA-tagged parasites in *A. coluzzii* infections

Time	Parasite	Number of midguts	Prevalence (%)	Arithmetic mean	Median	Parasite Range	P value	Fold difference
8 dpbf	<i>c507</i>	20	75	47.9	39	0-164		
	<i>c01::3xha</i>	29	59	12.6	3	0-61	0.0076	3.8
	<i>c57::3xha</i>	35	49	3.5	0	0-22	0.0001	13.7

This table summarizes the oocyst data from two replicates of *A. coluzzii* infected with transgenic *P. berghei* parasites carrying 3xHA tagged versions of the genes *PIMMS01* and *PIMMS57*. P values were calculated using the Mann-Whitney t-test. Fold differences between the transgenic parasites and the *507 wt* oocyst densities were calculated using the arithmetic mean.

Table S3. Oocyst numbers of *c507*, $\Delta c01$, $\Delta c57$ and $\Delta c22$ parasites in *A. coluzzii* infections

Time	Parasite	Number of exp	Number of midguts	Prevalence (%)	Arithmetic mean	Median	Parasite Range	P value	Fold difference
8 dpbf	<i>c507</i>	Pool	120	77	27.6	8	0-203		
	$\Delta c01$		73	52	4.3	1	0-40	<0.0001	6.4
	$\Delta c57$		89	40	1.2	0	0-14	<0.0001	23.0
	$\Delta c22$		81	56	4.5	1	0-33	<0.0001	6.1
	<i>c507</i>	R1	40	85	29.7	6	0-203		
	$\Delta c01$		17	65	5.9	1	0-40	0.0222	5.0
	$\Delta c57$		39	33	0.7	0	0-6	<0.0001	42.4
	$\Delta c22$	20	55	4.6	1	0-28	0.0045	6.5	
	<i>c507</i>	R2	42	52	20.2	1	0-166		
	$\Delta c01$		26	19	1.9	0	0-20	0.0041	10.6
	$\Delta c57$		19	42	1.1	0	0-7	0.0939	18.4
	$\Delta c22$	30	30	1.2	0	0-12	0.0111	16.8	
	<i>c507</i>	R3	38	95	33.4	24	0-137		
	$\Delta c01$		30	73	5.5	3	0-29	<0.0001	6.1
	$\Delta c57$		31	48	1.9	0	0-14	<0.0001	17.6
$\Delta c22$	31	81	7.6	6	0-33	<0.0001	4.4		

This table summarizes the oocyst data from three biological replicates of *A. coluzzii* infected with *c507*, $\Delta c01$, $\Delta c57$ or $\Delta c22$ parasite lines. P values were calculated using the Mann-Whitney t-test. Fold differences between the mutant parasites and the *507 wt* oocyst densities were calculated using the arithmetic mean.

Table S4. Sporozoite numbers of *c507*, $\Delta c01$, $\Delta c57$ and $\Delta c22$ parasites in *A. coluzzii* infections

Parasite	Replicates	Midgut Sporozoites			Salivary gland Sporozoites			Bite-back
		Mean	SEM	P-value	Mean	SEM	P-value	
<i>c507</i>	3	3,556	970		4,344	1,086		6/6 (2/2;2/2;2/2)
$\Delta c01$	3	256	48	0.0488	110	68	0.0337	0/6 (0/2;0/2;0/2)
$\Delta c57$	3	197	113	0.0484	36	7	0.0317	0/6 (0/2;0/2;0/2)
$\Delta c22$	3	223	24	0.0486	211	144	0.0369	0/6 (0/2;0/2;0/2)

Mean oocyst and salivary gland derived sporozoite numbers from three biological replicates of *A. coluzzii* infections. For each biological replicate (shown in brackets), sporozoite numbers was determined from 25-30 homogenized mosquito midguts or salivary glands at days 15 and 21 dpbf respectively. Infectivity of sporozoites was assessed by infected mosquito bite back experiments with at least 30 mosquitoes on C57/BL6 mice at 21 dpi. Following this, parasitaemia was monitored until 14 days post mosquito bite. P values were calculated using the unpaired Student's t-test. SEM represents standard error of mean.

Table S5. Invasion assay in CTL4 knockdown *A. coluzzii*

Time	Parasite	Number of exp	Number of midguts	Prevalence (%)	Arithmetic mean	Median	Parasite range	P value
7 dpbf	<i>c507</i>	Pool	65	78	56.9	18	0-558	
	$\Delta c01$		66	70	45.2	10.5	0-419	0.3971
	$\Delta c57$		70	69	30.5	6	0-356	0.0337*
	$\Delta c22$	R1	79	71	30.5	7	0-581	0.0487*
	<i>c507</i>		35	83	49.7	13	0-558	
	$\Delta c01$		30	63	41.5	12.5	0-231	0.4145
	$\Delta c57$	R2	33	70	47.6	8	0-356	0.5952
	$\Delta c22$		48	75	38.9	7	0-581	0.2605
	<i>c507</i>		30	73	65.2	18.5	0-533	
	$\Delta c01$		36	75	48.3	10.5	0-419	0.6583
	$\Delta c57$		37	68	15.2	4	0-113	0.0299*
	$\Delta c22$		31	65	17.6	7	0-148	0.0708

Numbers of melanised parasites detected in the midguts of *c507* or $\Delta c01$ or $\Delta c57$ or $\Delta c22$ infected CTL4 kd *A. coluzzii* mosquitoes at 7days post feeding. The P value was calculated using the Mann-Whitney U test. Data from pooled and independent biological replicates are presented.

Table S6. Primers for qRT-PCR and generation of transgenic parasites

Primer name	Sequence (5' to 3')	Description
<i>Pbc01</i> qRT-PCR F	TCGCTTTATTGTTTACCATAGCATCC	
<i>Pbc01</i> qRT-PCR R	TGTATGTTGCTGCTGGATTTTGT	
<i>Pbc57</i> qRT-PCR F	GCAGGCTGTTTCGTATGTTGTATT	
<i>Pbc57</i> qRT-PCR R	TGTCTTTAAAAGCTTGTGCCATT	
<i>Pbc22</i> qRT-PCR F	TTCAGGATGTGAATGGGCATTTTT	
<i>Pbc22</i> qRT-PCR R	TAAGAGACCCTGTGGATGACTTTG	
<i>Pfc01</i> qRT-PCR F	AACCGTTTCGTTGTATTATGTCCCA	
<i>Pfc01</i> qRT-PCR R	GCCTTGGCTGCATTTTCTGTT	
<i>Pfc57</i> qRT-PCR F	AGAAAAGTAAATTTTCGCACCACG	
<i>Pfc57</i> qRT-PCR R	GTAAC TGGGACCAGCATCCA	
<i>Pfc22</i> qRT-PCR F	TGTTAGGTTCCAATTGTCCCAGT	
<i>Pfc22</i> qRT-PCR R	AAGACCTTGTGGGTGACTTAGG	
<i>GFP</i> qRT-PCR F	CCTGTCTTTTACCAGACAACCA	
<i>GFP</i> qRT-PCR R	GGTCTCTTTTTCGTTGGGATCT	
<i>Pf arginyl-tRNA synthetase</i> qRT-PCR F	AAGAGATGCATGTTGGTCATTT	
<i>Pf arginyl-tRNA synthetase</i> qRT-PCR R	GAGTACCCCAATCACCTACA	
01 P1 F	TTGGGCCCGCGTTTTGTCCAATACAATAGTATGAT	GFP Tagging upstream target Apal
01 P2 R	CCAAGCTTTAACCGTCTATTATTATTTTT	GFP Tagging upstream target HindIII
01 P3 F	TGAATTCGCACTTGCAATGAAAAAAGGAAACATAG	GFP Tagging downstream target EcoRI
01 P4 R	TTGGATCCTACTTTTTATTATTCTTATCAAT	GFP Tagging downstream target BamHI
57 P5 F	TTGGGCCCGAGTTATTAATAGTTCTAAACAATG	GFP Tagging upstream target Apal
57 P6 R	GGCCGCGGATAGGTTCTTTTATTGCTCATCA	GFP Tagging upstream target SacII
57 P7 F	TTCTCGAGATAATCCATGTATTACATAATGATAAT	GFP Tagging downstream target XhoI
57 P8 R	TTCCCGGGAATAAAATTTAATTTCTCTCTAAGAG	GFP Tagging downstream target XmaI
22 P9 F	TTGGGCCCGAAACAGCATTATTAATATGCATAAATG	GFP Tagging upstream target Apal
22 P10 R	CCAAGCTTAGCATTCTTCTACTGGATAAGACAGC	GFP Tagging upstream target HindIII
22 P11 F	TGAATTCGCCTTTTTATATATAAACGTTGGCG	GFP Tagging downstream target EcoRI
22 P12 R	TTGGATCCGGATCTTAAATTTGGGGTACTATTTTC	GFP Tagging downstream target BamHI
<i>GFP-DHFR 3'UTR</i> (P13 F)	TTAAGCTTATGAGTAAAGGAGAAGAACTTTT	GFP tag HindIII
<i>GFP-DHFR 3'UTR</i> (P14 R)	TTAAGCTTCCGAAATTGAAGGAAAAAACATCATTTG	GFP tag HindIII
<i>GFP-DHFR 3'UTR</i> (P15 F)	GGCCGCGGATGAGTAAAGGAGAAGAACTTTT	GFP tag SacII
<i>GFP-DHFR 3'UTR</i> (P16 R)	GGCCGCGGCCGAAATTGAAGGAAAAAACATCATTTG	GFP tag SacII
01 tag INT F (P17)	CATGAATGCTTTAAACTTTCTACATTTTAC	Diagnostic primer WT and GFP/HA tag
01 GFP tag WT R (P18)	CTAATAAACATTTCCCTAATTTTTAACTTA	Diagnostic primer WT
57 tag INT F (P19)	GAGCAATAGCAATGATAAAAATAATATCG	Diagnostic primer WT and GFP/HA tag
57 GFP tag WT R (P20)	GCATACATATGCATATTCATATTTCCG	Diagnostic primer WT
22 tag INT F (P21)	GTAAGTTTGTAAATGAGAACCTTACAG	Diagnostic primer WT and GFP tag
22 tag WT R (P22)	GTTTATATATAAAAAGGCAAAGTAGCG	Diagnostic primer WT
GFP INT R (P23)	CTAATTCACAAGAATTGGGACAACCTCC	Diagnostic primer GFP tag
01 P24 F	TTGGGCCCCCAATACAATAGTATGATAC	Disruption upstream target Apal
01 P25 R	CCAAGCTTCTCATTATTATAACTGGATGC	Disruption upstream target HindIII
01 P26 F	TGAATTCGAATTTGAGAATCGATTATGGAG	Disruption downstream target EcoRI

01 P27 R	TTGGATCCGATCACGCCTTTATTGAATTAATGAAG	Disruption downstream target BamHI
57 P28 F	TTGGGCCCTAATATATTATACATAGACAATTGCTC	Disruption upstream target Apal
57 P29 R	<u>CCAAGCTT</u> CATATATTGATATATGATTTTAC	Disruption upstream target HindIII
57 P30 F	<u>TGAATTCGGT</u> ACCATATAACTATTCTGAAGCATATG	Disruption downstream target EcoRI
57 P31 R	TTGGATCCGCTCATCATATAGAAAGTGGTATAATATATG	Disruption downstream target BamHI
22 P32 F	TTGGGCCCGATAATATATACATCCATTTTTTTTG	Disruption upstream target Apal
22 P33 R	<u>CCAAGCTT</u> CATATTTTCCACATAATACAGATTC	Disruption upstream target HindIII
22 P34 R	<u>TGAATTCCTT</u> CATGTTTGTATTTTTAAAAAGC	Disruption downstream target EcoRI
22 P35 R	TTGGATCCGGCATGTATTTAATAAAAATAGCATG	Disruption downstream target BamHI
01 INT F (P36)	GGATGTATTCCTATAACTATGTG	Diagnostic primer WT and KO/c507
01 WT R (P37)	CTCCTTGTATGTTGCTGCTGG	Diagnostic primer WT/c507
57 INT F (P38)	GATGTCTAGCTAATTTGGGAATTAGTG	Diagnostic primer WT and KO/c507
57 WT R (P39)	GCATTATCTCTATCTTCATAATTTG	Diagnostic primer WT/c507
22 INT F (P40)	GAAATGGAGCATATATGTAATAAACCCG	Diagnostic primer WT and KO/c507
22 WT R (P41)	AAATTTGAATAAGGATTGACGCATGC	Diagnostic primer WT/c507
<i>TgDHFR</i> 5'UTR R (P42)	GATGTGTTATGTGATTAATTCATACAC	Diagnostic primer KO/c507
01 P43 F	GATTACGCCAAGCTT <u>GGGCCCGCG</u> TTTTGTCCAATACAATAGTATGATACTTAATTGTA AGCTAGCT	HA Tagging upstream target Apal
01 P44 R	CTAGAGCGGCCGCCACCGCGGCTAAGCATAGTCTGGAACGTCATAAGGGTATGCGTA ATCTGGCACGTCGTATGGATATGCATAATCTGGTACATCGTATGGGTATAACCGTCTAT TATTATTTTTCCATGAATAA	HA Tagging upstream target SacII
01 P45 F	CTTCAATTTGGGTACCCTCGAGAATACCTCTAAGTAAAAAATTAGGAAATGTTTATTA G	HA Tagging downstream target XhoI
01 P46 R	GAATTCGCGGCCGCCCGCCGGGTTTTATAAGGTATAATGGAATATGTATGCTC	HA Tagging downstream target XmaI
57 P47 F	GATTACGCCAAGCTT <u>GGGCCCGAG</u> CAATAGCAATGATAAAAATAATATCGGTAACAGAA GAG	HA Tagging upstream target Apal
57 P48 R	CTAGAGCGGCCGCCACCGCGGCTAAGCATAGTCTGGAACGTCATAAGGGTATGCGTA <u>ATCTGGCACGTCGTATGGATATGCATAATCTGGTACATCGTATGGGTAATAGGTTCTTT</u> <u>TATTGCTCATCATATAGAAAG</u>	HA Tagging upstream target SacII
57 P49 F	CTTCAATTTGGGTACCCTCGAGATAATCCATGTATTACATAATGATAAT	HA Tagging downstream target XhoI
57 P50 R	GAATTCGCGGCCGCCCGCCGGGAATAAAATTTAATTTCTCTCTAAGAG	HA Tagging downstream target XmaI
01 3xHA tag WT R (P51)	GTTGCGTGATAATTCATAATTACATGG	Diagnostic primer WT
57 3xHA tag WT R (P52)	GCCTTATGATGAATTAATTGTAAGTGCG	Diagnostic primer WT
3xHA INT R (P53)	GTATGCGTAATCTGGCACGTCGTATG	Diagnostic primer HA tag

Where appropriate, target restriction sites are shown as underlined. The appropriate restriction enzyme is presented in the description column. F, forward; R, reverse; INT, integration; WT, wild-type; KO, knockout; UTR, untranslated region. All primers are listed in a 5' to 3' direction