Figure 5. Individual deletion of seven *etramps* in blood stages and passage through mosquito and liver stages reveals no critical function in any stage. (A) Generalized schematic representation of the strategy used to replace *P. yoelii etramp* ORFs with TgDHFR/TS. (B) Genotyping of *etramp* KO blood stage parasites before ("Input") and after ("Output") passage through mosquito and liver stages. The presence of knockout parasites in the output blood stages indicates that each individual etramp is not essential for completion of the parasite life cycle. Specific oligonucleotide primer amplicons indicated in (A) are used to test for integration of the KO construct from the 5' end or 3' end, and also to test for the presence of the endogenous *etramp* ORF. PY03652$^-$ and PY04799$^-$ parasites were analyzed as clones; the remaining strains were analyzed as mixed parental populations of gene deletion parasites and WT parasites, and therefore show evidence for both 5' and 3' integration, as well as the unmodified ORF, in different parasites.

Figure S1. Blood stage development of the PY03652$^-$ and PY04799$^-$ is comparable to wildtype. One million infected red blood cells isolated from infected Swiss Webster mice infected with either wildtype (WT), PY03652$^-$ or PY04799$^-$ parasites were injected intravenously into groups of five Swiss Webster mice. Parasitemia of the infected mice was determined by Giemsa-stained thin blood smear at successive days after the initial injection. The data show that there is no significant difference between the three parasite lines in their ability to propagate as blood stages in the mouse.

Figure S2. Development of late liver stage PY03652$^-$ and PY04799$^-$ parasites is not inhibited, based on size, when compared to WT. Liver sections were labeled with anti-Hep17 antibody, and DAPI (to label DNA). A differential interference contrast (DIC) image of the liver section is shown to the right. Scale bar = 5 micrometers.

Supporting information

Table S1. A list of all etramps genes identified from the PFAM database and edited to remove redundant or spurious entries.

Table S2. A list of all primer sequences used in this study.

Table S3. Numbers of sporozoites produced per mosquito in PY03652^{-} or PY04799^{-} clones compared to those produced by WT *P. yoelii*. The data are the average number of sporozoites per mosquito obtained by dissecting at least 25 mosquitoes.

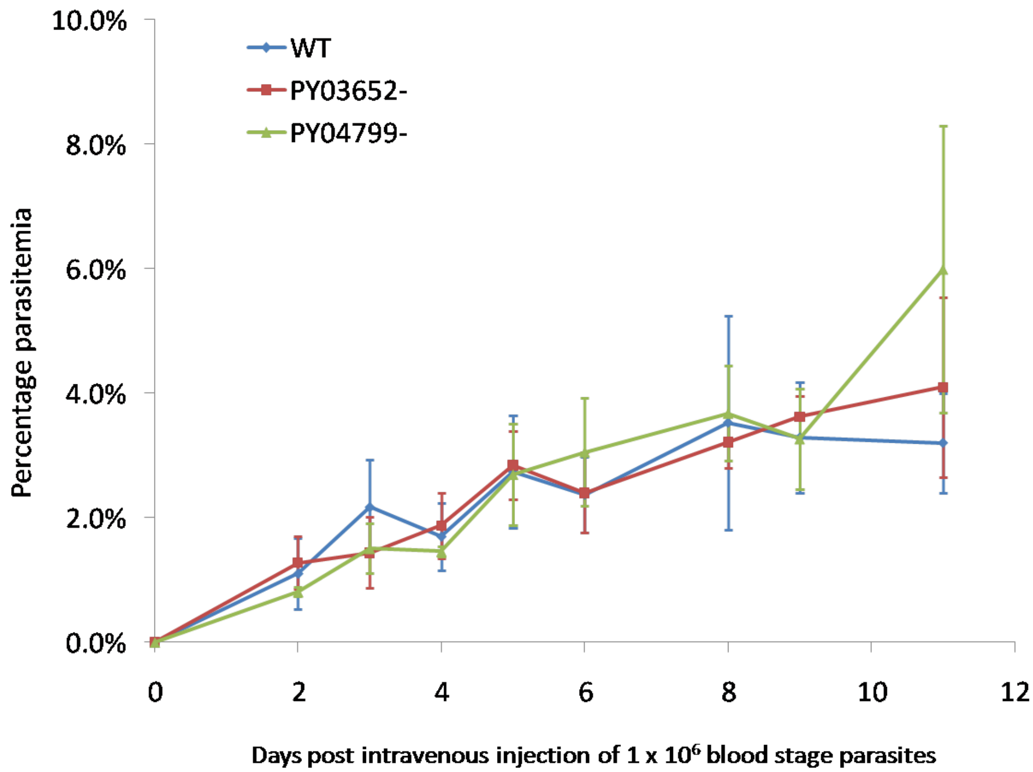
Supplementary Figure 1. Blood stage growth of PY03652^{-} or PY04799^{-} clone parasites is similar to the rate of blood stage growth for *P. yoelii* WT *in vivo*.

Supplementary Figure 2. Liver stage growth of PY03652^{-} or PY04799^{-} clone parasites is similar to the rate of liver stage growth for *P. yoelii* WT *in vivo*.

Supplementary Video 1. A three-dimensional reconstruction of a stack of images taken from an erythrocyte infected with a PY03652myc schizont. The stack is rotated 180 degrees through the Y axis, and back. The myc signal is in red, the MSP1 in green, and DAPI in blue. The myc signal appears as individual foci within the daughter merozoites, likely in secretory organelles; the protein is not forming a continuous network in three dimensions that could be misrepresented in a single cross-sectional image.

Type of file: figure
Label: Figure 01-05,Table 01-02&Supp Figure S1-S2,Table S1-S3
Filename: cmi_1656_figures and tables.pdf

Figure S1



10.0%
8.0%
6.0%
4.0%
2.0%
0.0%
Percentage parasitemia
WT
PY03652-
PY04799-
0
2
4
6
8
10
12
Days post intravenous injection of 1 x 10⁶ blood stage parasites

Figure S2

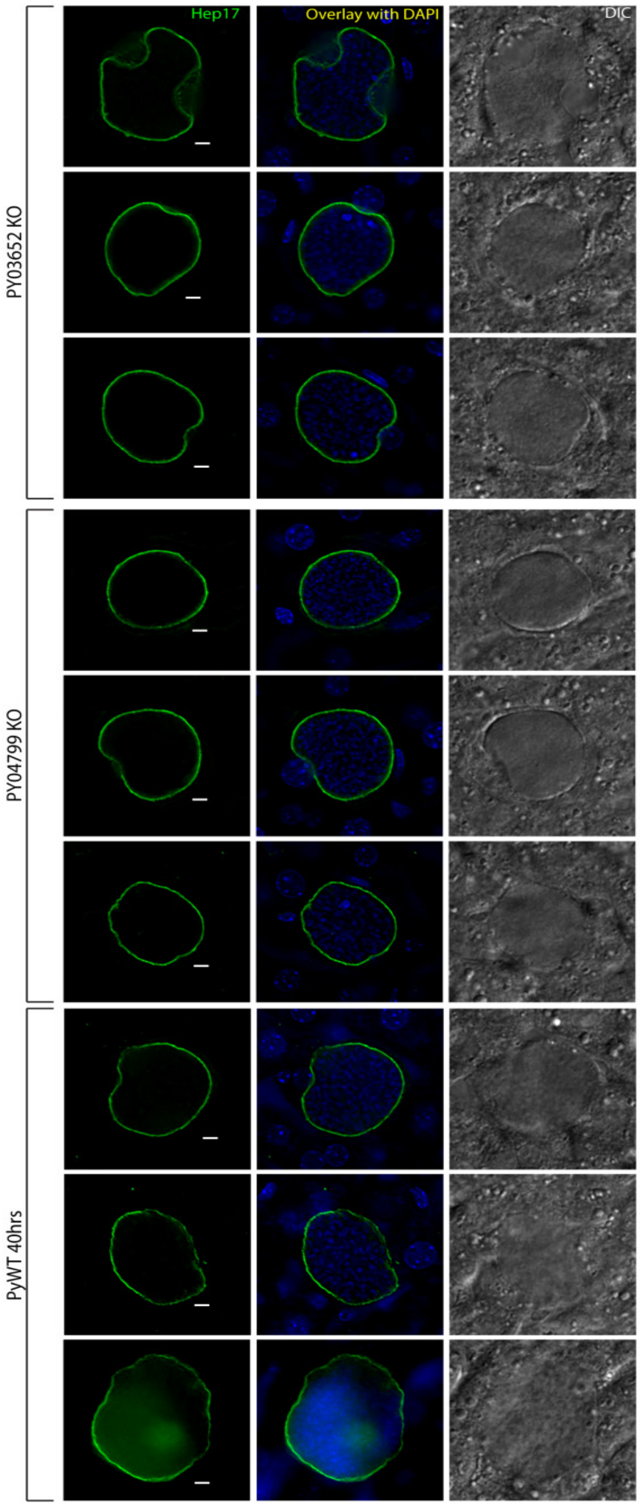

Hep17
Overlay with DAPI
DIC
PY03652 KO
PY04799 KO
PyWT 40hrs

Table S1. A list of all etramps genes identified from the PFAM database and edited to remove redundant or spurious entries.

Species	Name	NCBI	PlasmoDB	OrthoMCL	Uniprot
P. berghei	PbUIS4	XM_673549	PB100551.00.0	OG_84497	-
P. berghei	-	XM_668793	PB402471.00.0	OG4_50898	Q4YIJ4
P. berghei	-	XM_673321	PB105376.00.0	OG4_60733	Q4YK90
P. berghei	PbUIS3	XM_669589	PB000892.03.0	OG4_48314	Q4YNP1
P. berghei	-	XM_674919	PB106570.00.0	OG4_50898	Q4YT14
P. berghei	PbSEP3	AJ420907	PB_RP0267:2295-2759	-	Q8MNY3
P. berghei	PbSEP2	XM_670193	PB106367.00.0	OG4_64898	Q8MNY4
P. berghei	PbSEP1	XM_674012	PB100384.00.0	OG4_60511	Q8MNY5
P. chabaudi	-	-	PCHAS_146830	OG4_48457	-
P. chabaudi	-	-	PCHAS_093790	OG4_48457	-
P. chabaudi	-	-	PCHAS_000550	OG4_60733	Q25666
P. chabaudi	-	XM_740585	PC105586.00.0	-	Q4XVR0
P. chabaudi	-	-	PCHAS_050120	OG4_60511	Q4XXC4
P. chabaudi	-	XM_739639	PC104072.00.0	OG4_50898	Q4XZA0
P. chabaudi	PcUIS3	XM_732037	PCHAS_140270	OG4_49314	Q4Y4D5
P. chabaudi	-	XM_739810	PC100816.00.0	-	Q4Y781
P. chabaudi	-	-	PC10115w	-	Q7YZ77
P. chabaudi	-	AY149018	PCHAS_001130	OG4_48457	Q7Z1C7
P. chabaudi	-	AY149017	PCHAS_090040	OG4_48457	Q7Z1C8
P. chabaudi	-	AY149016	PCHAS_052450	OG4_48457	Q7Z1C9
P. falciparum	etramp8	XM_001349194	MAL8P1.6	OG4_84661	C0H4Z2
P. falciparum	etramp2	XM_001349503	PFB0120w	OG4_54905	O96128
P. falciparum	etramp11.1	XM_001347679	PF11_0039	OG4_60511	Q7KQK8
P. falciparum	etramp14.1	XM_001348153	PF14_0016	OG4_118692	Q7KQM5
P. falciparum	"etramp9"	XM_001352189	PFI1745c	OG4_84706	Q8I2F9
P. falciparum	etramp5	XM_001351833	PFE1590w	OG4_84523	Q8I3F3
P. falciparum	etramp12	XM_001350759	PFL1945c	-	Q8I522
P. falciparum	etramp13	XM_001349714	PF13_0012	OG4_48314	Q8IEU1
P. falciparum	etramp4	XM_001351504	PFD1120c	OG4_64898	Q8IFM9
P. falciparum	etramp11.2	XM_001347680	PF11_0040	OG4_54905	Q8IIX3
P. falciparum	etramp10.2	XM_001347571	PF10_0323	OG4_49514	Q8IJ76
P. falciparum	etramp10.3	XM_001347413	PF10_0164	-	Q8IJM9
P. falciparum	etramp10.1	XM_001347268	PF10_0019	OG4_118692	Q8IK21
P. falciparum	etramp14.2	XM_001348867	PF14_0729	OG4_84684	Q8IK77
P. knowlesi	-	XM_002260986	PKH_030840	OG4_84691	B3KZZ2
P. knowlesi	-	XM_002261140	PKH_031940	OG4_84523	B3L0E6
P. knowlesi	-	XM_002257972	PKH_041730	OG4_54905	B3L0X6
P. knowlesi	-	XM_002257989	PKH_050120	OG4_84661	B3L0Z3
P. knowlesi	-	XM_002258252	PKH_052730	OG4_64898	B3L1Q6
P. knowlesi	-	XM_002261648	PKH_060100	OG4_118797	B3L1S8
P. knowlesi	-	XM_002261815	PKH_061760	OG4_49514	B3L295
P. knowlesi	-	XM_002258601	PKH_073400	OG4_84706	B3L333
P. knowlesi	-	XM_002260476	PKH_127030	OG4_84684	B3LA56
P. knowlesi	-	XM_002261879	PKH_140080	OG4_48314	B3LBI4

P. reichenowi	-	GU329527	-	-	D3JXV2
P. vivax	-	XM_001616903	PVX_121950	OG4_48314	A5JZ15
P. vivax	-	XM_001615979	PVX_118680	OG4_84684	A5K448
P. vivax	-	XM_001614817	PVX_088870	OG4_84661	A5K5F5
P. vivax	-	XM_001615088	PVX_090230	OG4_48457	A5K676
P. vivax	-	XM_001612902	PVX_003565	OG4_54905	A5KBH5
P. vivax	-	XM_001612721	PVX_096070	OG4_84523	A5KC26
P. vivax	-	XM_001612652	PVX_001715	OG4_118797	A5KCB1
P. vivax	-	XM_001608388	PVX_111065	OG4_49514	A5KDQ5
P. vivax	-	XM_001608277	PVX_086915	OG4_84706	A5KE67
P. vivax	-	XM_001616438	PVX_084090	OG4_84708	A5KOB4
P. yoelii	7506	XM_723325	PY07506	OG4_50898	Q7R7S2
P. yoelii	6488	XM_722055	PY06488	OG4_85057	Q7RAL2
P. yoelii	5433	XM_720798	PY05433	OG4_85057	Q7RDI9
P. yoelii	4799	XM_720054	PY04799	OG4_60511	Q7RFA8
P. yoelii	3869	XM_718992	PY03869	OG4_64898	Q7RHW4
P. yoelii	3652	XM_718746	PY03652	-	Q7RIH1
P. yoelii	3365	XM_726274	PY03365	OG4_85057	Q7RJ98
P. yoelii	3011, UIS3	XM_725879	PY03011	OG4_48314	Q7RK90
P. yoelii	2667	XM_725486	PY02667	OG4_60733	Q7RL79
P. yoelii	0205	XM_724753	PY00205	OG4_54905	Q7RSZ6
P. yoelii	0204, UIS4	XM_724752	PY00204	OG4_84497	Q7RSZ7

Table S2. A list of all primer sequences used in this study.

Primer name	Sequence (5' to 3', endonuclease sites underlined)
0205 orfF	GGAACAATCGCATTAGTAATTGGTACTACGCTTGG
0205 orfR	CAAATAACGTAAGTTATATCGATATGGGTTTGTA
0205 Rep1F	GGGGTACCCCATAATTTTTTTGTATCCTTTAAAAAAC
0205 Rep2R	GCCCAAGCTTTCCGGCTAATGTAGATATAAATGCAACCTTG
0205 Rep3F	GGACTAGTCAAACCAATAAGATGGCAATAGTTCGAGGCA
0205 Rep4R	TCCCCGCGGCAGATAAAAATATATATCACGGTACTATTTGC
0205 TestF	GCGTGTAATTCAAAGAATGCG
0205 TestR	CAGTTTACCACAACCAAACAGTATTAATAGC
2667 orfF	CCTAATATTCCTAACCCAACTGCTAATGCTC
2667 orfR	GTGGGGTTTCAGTATTTTCGGTGGTTTCAGG
2667 Rep1F	GGGGTACCTGGAGCTATGCATGCAAAAAACATCGCT
2667 Rep2R	GCCCAAGCTTCATCTAAAATCGCTTGCTATTATTTGGAAAC
2667 Rep3F	GGACTAGTGGCGCCTTTAAAATTGTATTGTATTATATGGG
2667 Rep4R	TCCCCGCGGCAATATATTGGCGTATGATGTATGTATTTGCC
2667 TestF	GCCTAAGAAAGTTGAATGAAAGATGTGCTTT
2667 TestR	CCGGTTGCATATATAATTGCACGTGTGAAC
3365 orfF	GCCTTTTTATTGGCCATAAATGTATTAACTTTAGG
3365 orfR	TCAGTAACTACAGTGGGATATTGGTGGATACTCT
3365 Rep1F	GGGGTACCGTTACTATTCCTGGAATAACCACTACTCTTCG
3365 Rep2R	GCCCAAGCTTTTGTTCCACGGTCCTGGTTTATGAGTTTG
3365 Rep3F	GGACTAGTCCAAAAATTCAAGTATATCACACATTCTG
3365 Rep4R	TCCCCGCGGACTACATTTCCCAACGTTATGTTAATACGG
3365 TestF	CCCCAAAATTATCTTCCAAAATTGTGGTC
3365 TestR	TTCAAGGGTTCAGGGTTAGGGATTGGGT
3652 orfF	GAAATTAGCAAAAGCATTATATTTTGTTGCCTT
3652 orfR	CGAATGGGTTACCTTCATAATTAATTGGTTTGCC
3652Rep1F	GGGGTACCCGCTATAAATTCCATGAGAGATATTTATGAA
3652 Rep2R	GCCCAAGCTTTGTTTTGTGAAGCTGTGATATAGAAATATATCC
3652 Rep3F	GGACTAGTCCATAAATACCATAAAATAATGGAAAACCATCACCA
3652 Rep4R	TCCCCGCGGACATATCAATCCTGGTTCTTAACGCAAACACA
3652 TestF	GTTTAACGTTTTATACGAACTATGTCATT
3652 TestR	ATATTTTCCCAACGTCATTCTACAGCAGTAGC
3869 orfF	GGCCATAAAAGTGTTAATCCCAGGAGCTAACAA
3869 orfR	TGATAGTATATGGGGCAGTTGGGGTTCTGTTGCT
3869 Rep1F	GGGGTACCGTCCCGATAAAAATTGTTATAATAAAAGAACTA
3869 Rep2R	GCCCAAGCTTGGGGATTATTGAATTATAAGAGTTTGTTATAA
3869 Rep3F	GGACTAGTCTAATACCGAAGCTACATTAAAATAATTCTCT
3869 Rep4R	TCCCCGCGGGGAATCTGAGAATATATATAGCACAATTATAGTAC
3869 TestF	GGGCCTAATAATTTTATGAATATTTAACCATCGCTA
3869 TestR	TGCTAGTTTCATGTGTTACTGCTTTAAAAATGAG

4799 orfF	GCAACATTAGCATTAGCAATTGCTACTACATTTG
4799 orfR	GTGATGCTGGTGGATAAGCTCTTGAATTACCA
4799 Rep1F	GGGGTACCGACCCGATAAAAATTGTTATTATAAAAGACCT
4799 Rep2R	GCCCAAGCTTGCTTTTGCTAATTTCATTTTTGCACATGT
4799 Rep3F	GGACTAGTGACAGTAGACCAAAATTTCAAGTATATCCCACA
4799 Rep4R	TCCCCGCGGCACGGTACTACTTACAATAAACAATAAATCACGGT
4799 TestF	GTTGCTTGGGAAAGTATTTTTTCTAAATTATTTCT
4799 TestR	GTCTAACCATATATGTGAATTCGTAACATGT
5433 orfF	GTATTAACCCCAGAATCTAATAATTATGTTGAAG
5433 orfR	CATCAACGTAACATTTATATTGAATCATTCTGATC
5433 Rep1F	GGGGTACCCCACATTACAGCTTCACTAAAAAAAATATA
5433 Rep2R	GCCCAAGCTTTCTGGGGTTAATACATTTATAGCTAATAAAAAGGC
5433 Rep3F	GGACTAGTCAATTGTAACATCCCGTTCGGAAATTATAGT
5433 Rep4R	TCCCCGCGGGTGTGATGAAAATAATACCGAAATGTTGCA
5433 TestF	GCCTGAACCCTGGAAATTAAACCTGATATA
5433 TestR	GTTAGTATTTCGCATTTGGGCGGAGAAA
6488 orfF	GCCTTTTTATTGGGCATAAATGTATTAACCCCAGG
6488 orfR	CACTTACAAATAAAAATATATGTCATGATAC
6488 Rep1F	GGGGTACCCCCCCCAATAAATCACCACACTATAACAGT
6488 Rep2R	GCCCAAGCTTTCGTGAAGATGTGATGTGGAAATATATCATG
6488 Rep3F	GGACTAGTCCGTGATTTATTCTGTATTCCCAATAGTATC
6488 Rep4R	TCCCCGCGGGGGTTGGGTTATATTGAATTATATATATCA
6488 TestF	CTATGAATATGTAACATCAAATATATTCTTTC
6488 TestR	CATATCATATTCCAAAATTTCAAGGGTTC
7506 orfF	CAAGAAAGCCATCGCGATTGCATTAGGATC
7506 orfR	GATTCTACTTTAGAAGATGTATCTGGTACAG
7506 Rep1F	GGGGTACCTGGCGAATGCCTATTCTAAAAGAACTAGC
7506 Rep2R	GCCCAAGCTTCCGCATAGTATTTAAATGTAAGGACATGC
7506 Rep3F	GGACTAGTGGTTCTGCTTTTAGCTCTACTGGGTCAAGT
7506 Rep4R	TCCCCGCGGCGGCATGTCATCGACTATTTTAATACATAACC
7506 TestF	CCGGATAAAATTATATATATAATGGGGATTCC
7506 TestR	GCACACCATACATTATGAAAAGCGAATAAGCA
TgF	GGCTACGTCCCGCACGGACGAATCCAGATGG
TgR	CGCATTATATGAGTTCATTTTACACAATCC

Table S3. Numbers of salivary gland sporozoites obtained from PY03652KO and PY04799KO parasites are equivalent to wildtype.

Experiment	**PY03652⁻**	**PY04799⁻**	**PyWT**
1	60000	34000	8000
2	33000	19000	51000
3	42000	48000	47000
4	25000	18000	14000
Avg	40000	29750	30000
StDev	15033	14198	22136
Ttest vs WT	0.487	0.986	1