

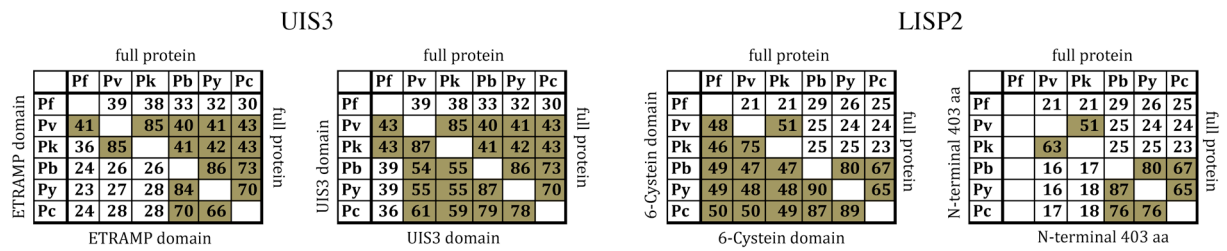
Supplementary material for

**Protective efficacy and safety of liver stage attenuated malaria parasites**

Hirdesh Kumar, Julia Magdalena Sattler, Mirko Singer, Kirsten Heiss,  
Miriam Reinig, Christiane Hammerschmidt-Kamper, Volker Heussler,  
Ann-Kristin Mueller and Friedrich Frischknecht

**Contents:**

- Supplementary Fig. S1**
- Supplementary Fig. S2**
- Supplementary Fig. S3**
- Supplementary Fig. S4**
- Supplementary Table S1**
- Supplementary Table S2**



**Figure S1: Percentage identity matrices of UIS3 and LISP2 in different *Plasmodium* species.**

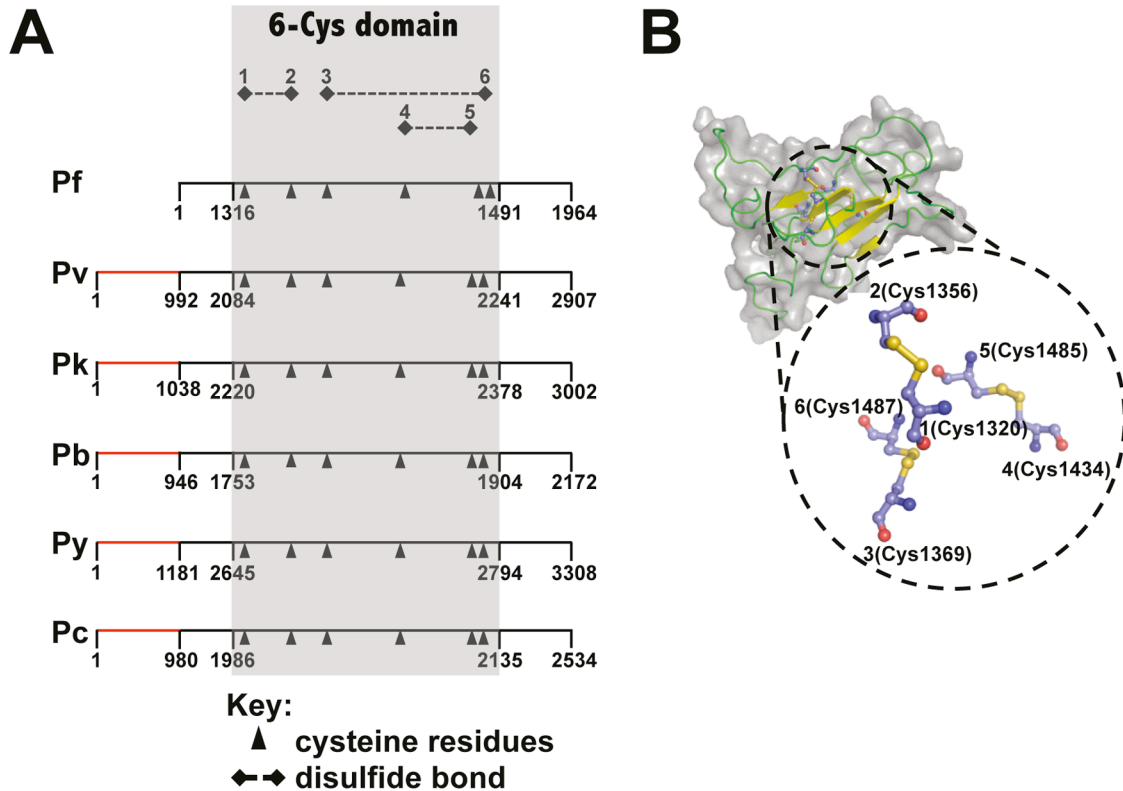
Pf: *P. falciparum* 3D7; Pv: *P. vivax* Sal1; Pk: *P. knowlesii*; Pb: *P. berghei* ANKA; Py: *P.*

*yoelii* 17X; Pc: *P. chabaudi chabaudi*. Dark cells represent sequences with >40% amino acid

identity. Upper half matrix: full length proteins; lower half matrix: ETRAMP and UIS3

domains as well as 6-cystein domain and 403 amino acid N-terminal region. An N-terminal

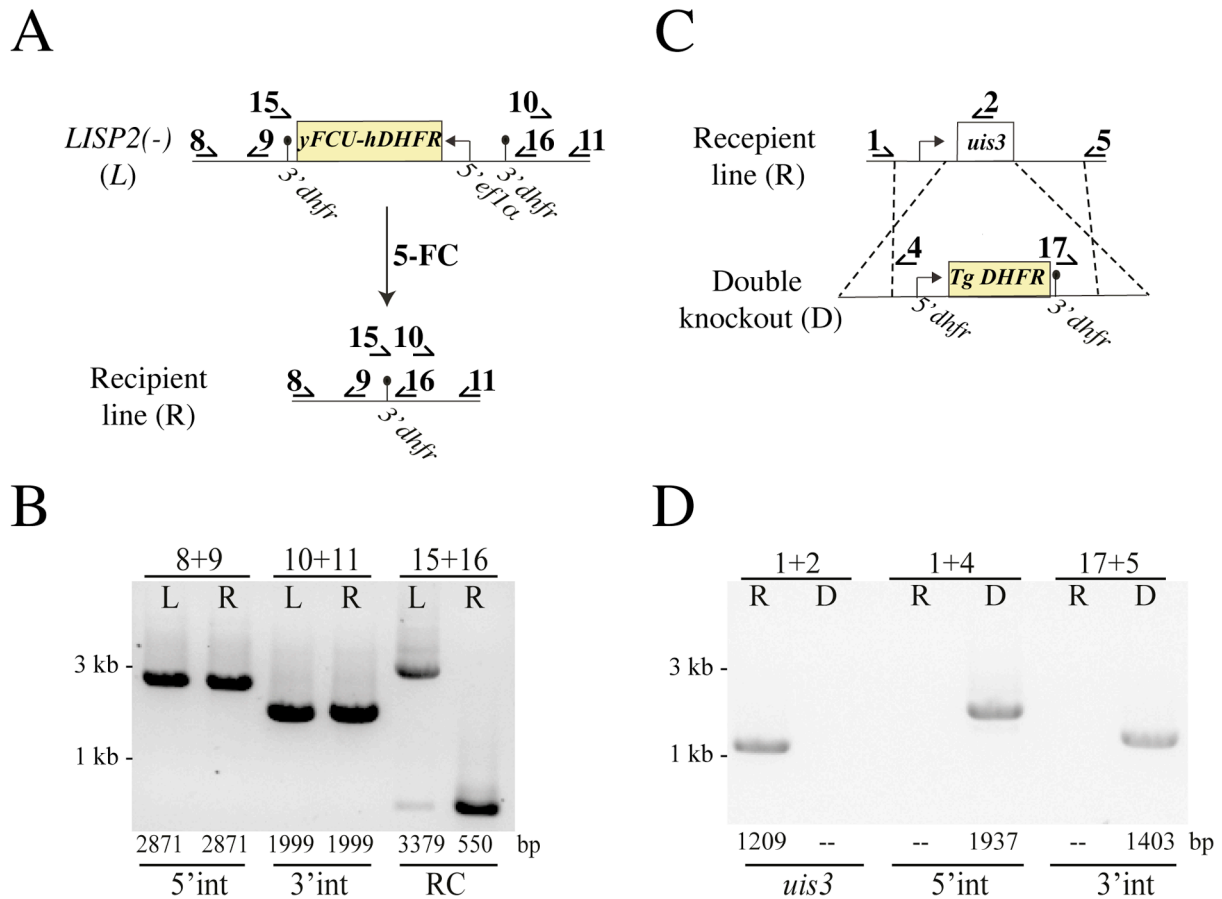
region in *P. falciparum* could not be identified.



**Figure S2: Predicted structure of the 6-cystein domain of LISP2.**

(A) Representative layout of LISP2 amino acid sequences in different *Plasmodium* species (Pf: *P. falciparum*, Pv: *P. vivax*, Pk: *P. knowlesii*, Pb: *P. berghei*, Py: *P. yoelii*, Pc: *P. chabaudi*). The 6-Cys domains predicted using SMART server, is highlighted in grey. Numbers below the gene models indicate amino acid positions.

(B) Homology model of the 6-Cys domain in *PfLISP2* (corresponding to the grey region in B) predicted by BhageerathH server. The characteristic  $\beta$ -sandwich is shown in a ribbon diagram, overlapped with the surface view (grey). The predicted loop region is hidden for clarity. The enlarged view depicts 6-conserved cysteine residues (arrow head in B), forming 3 disulfide bonds.



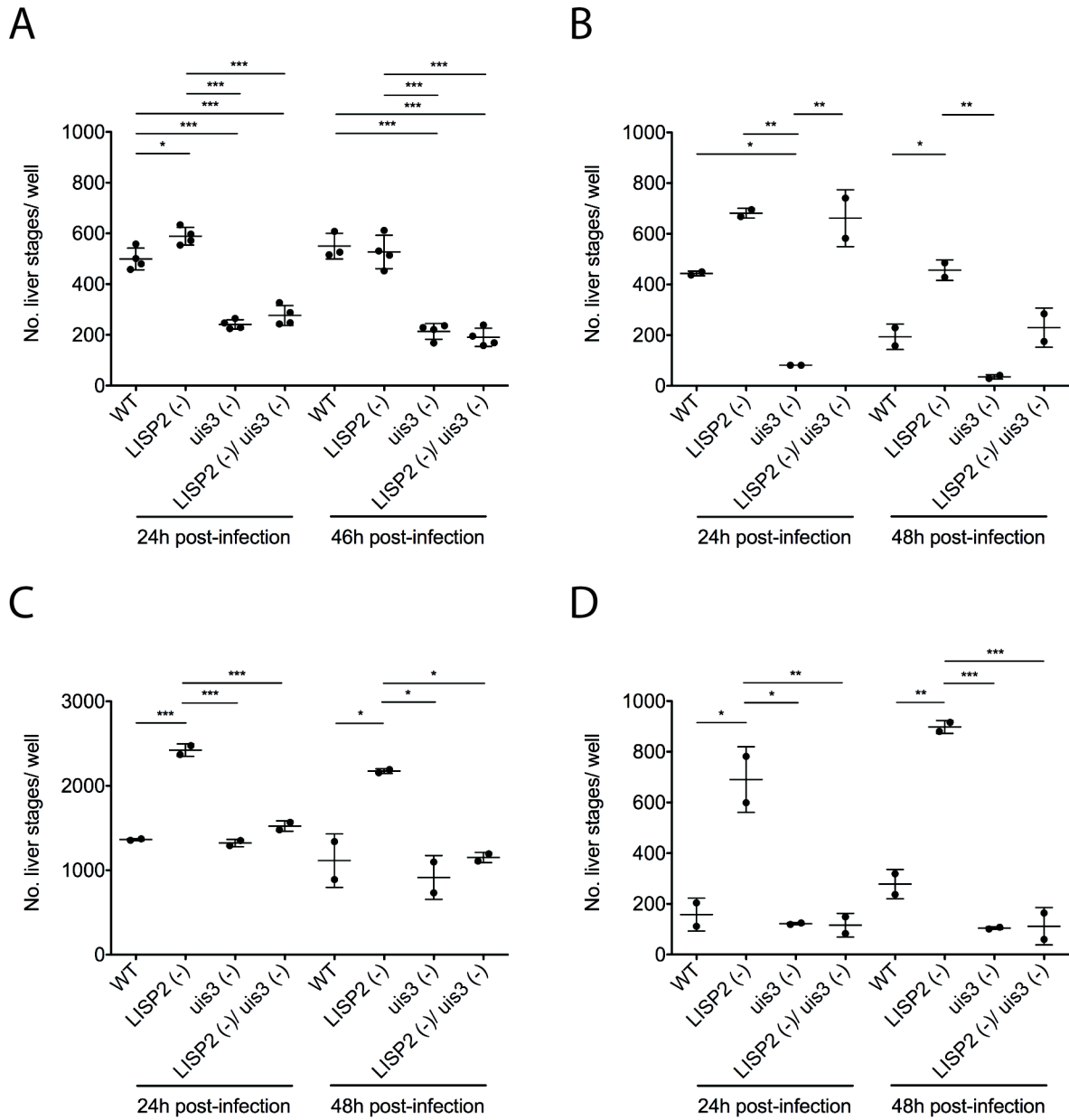
**Figure S3: Negative selection of *LISP2(-)* parasites to remove resistance marker and generation of the *LISP2(-)/uis3(-)* double knockout parasite line.**

(A) Schematic of the removal of the selection cassette by negative selection using 5-Fluorocytosine (5-FC). Primers used for diagnostic PCR reactions in B are indicated.

(B) Diagnostic PCR reactions and expected amplicon sizes.

(C) Schematic of the *uis3* deletion strategy using the 5' and 3'UTRs of *uis3* to integrate a resistance cassette by double crossover into the *LISP2(-)* parasite genome. The locations of primers used for PCR in D are indicated.

(D) Diagnostic PCR reactions and expected amplicon sizes.



**Figure S4: Characterization of a ANKA *LISP2*(-)/*uis3*(-) double knockout parasite line and comparison with single knockout lines in four individual liver stage assays.**

(A) Number of liver stages per well 24 and 46 hours post infection (hpi) of HepG2 cells with WT, *LISP2*(-), *uis3*(-) and *LISP2*(-)/*uis3*(-) sporozoites. Four individual wells were counted. Mean numbers ( $\pm$  SD) are shown.

(B-D) Number of liver stages per well 24 and 48 hours post infection (hpi) of HepG2 cells with WT, *LISP2*(-), *uis3*(-) and *LISP2*(-)/*uis3*(-) sporozoites. Two individual wells per assay were counted. Mean numbers ( $\pm$  SD) are shown.

**Supplementary Table 1: Primers used in this study.**

Used for	No.		Restriction Enzymes	Sequence
<b><i>PbA uis3</i> (-)</b>				
5' UTR	uis3-5'f		<i>EcoRI</i>	<b>CGGAATTC</b> GCTAGCATTAGCATAACATCTCATTGGC
	uis3-5'r		<i>KpnI</i>	GGGGT <b>ACC</b> GCATGCAACCACAACATAAAACAAAATTAATG
3' UTR	uis3-3'f-		<i>PstI</i>	AA <b>CTGCAGG</b> CCTGTACACTATATTGTATATTAGC
	uis3-3'r		<i>HindIII</i>	CCCA <b>AGCTT</b> CCCATCTACGCCTTTTATGCTT
Test-WT	1	Test-5'f	--	TGCTATAAAAGCGTATTATGAATTAATATAAAAGG
	2	uis3- r	--	GTCCTGATGATAACAAAGCAATTGCAAC
Test-5'Int	1	Test-uis3-5'f	--	TGCTATAAAAGCGTATTATGAATTAATATAAAAGG
	3	Test-uis3-5'r	--	GGCCCCGTTTTTCTTACTTATATATTTATACCAATTG
Test-3'Int	4	Test-uis3-3'f	--	GTCCATACAACATATATCCGAAC
	5	Test-uis3-3'r	--	GTATTTATGCCAAAAAAGCTAAATTGC
<b><i>PbA lisp2</i> (-)</b>				
5'UTR	lisp2-5'f		<i>Sall</i>	AT <b>CGTCTGACT</b> GGTATCCATTATAGCAGCAAACAC
	lisp2-5'r		<i>EcoRI</i>	GTCAC <b>GAATTC</b> AATTTGTTTGCACGCTTTATCG
3'UTR	lisp2-3'f		<i>HindIII</i>	ACCG <b>AAGCTT</b> TGTTTGGACATAATGAATACGATGG
	lisp2-3'r		<i>XhoI</i>	ACCG <b>CTCGAG</b> CCACGGATGCACTAATAGAAGC
Test-gene	6	lisp2-gene-f	<i>NheI</i>	AT <b>GCTAGCAT</b> GAAAGATCATATAAAAAATGTATGCTTTCCG
	7	lisp2-gene-r	<i>BamHI</i>	AC <b>GGATCCC</b> GTGTTTCTTTCTTTTTCTCG
Test-5'Int	8	Test-lisp2-5'f	--	TTGTGCACCCTCGTATACTG
	9	Test-lisp2-5'r	--	ACGCAACCTTATATTCCTCAATTAC
Test-3'Int	10	Test-lisp2-3'f	--	CATACTAGCCATTTTATGTG
	11	Test-lisp2-3'r	--	GAAGCAACTATGAAAAAACTAATGGG
RC	15	Test hdfr-r	--	GCCGATATCCAAGAAAGCTGGGTGGTACCC
	16	Test hdfr-f	--	CCCAAGCTTCAAAAAAGCAGGCTTGCCGC
<b><i>N-LISP2</i></b>				
Gene-insert	6	N-lisp2-f	<i>NheI</i>	AT <b>GCTAGCAT</b> GAAAGATCATATAAAAAATGTATGCTTTCCG
	7	N-lisp2-r	<i>BamHI</i>	AC <b>GGATCCC</b> GTGTTTCTTTCTTTTTCTCG
Promoter-insert		lisp2-Prom-f	<i>XhoI, NotI, SacII</i>	AA <b>ACTCGAGGCGGCCG</b> CAA <b>ACC</b> GCGGGAGTTGCATTATC GTCAAAAAGTG
3'insert	lisp2-3-f		<i>HindIII</i>	AAAA <b>AAGCTT</b> AGAGCTACCAAAATTTAAAGAAAATCC
	lisp2-3'r		<i>XhoI</i>	ACCG <b>CTCGAG</b> CCACGGATGCACTAATAGAAGC
Test-gene	6	N-lisp2-f	<i>NheI</i>	AT <b>GCTAGCAT</b> GAAAGATCATATAAAAAATGTATGCTTTCCG
	7	N-lisp2-r	<i>BamHI</i>	AC <b>GGATCCC</b> GTGTTTCTTTCTTTTTCTCG
Test-5'Int	8	Test-Nlisp2-5'f	--	TTGTGCACCCTCGTATACTG
	12	Test-N-lisp2-5'r	--	CGCAATTTGTTGTACATAAAATAGGC
Test-3'Int	6	Test-N-lisp2-3'f	<i>NheI</i>	AT <b>GCTAGCAT</b> GAAAGATCATATAAAAAATGTATGCTTTCCG
	11	Test-lisp2-3'r	--	GAAGCAACTATGAAAAAACTAATGGG
RC	13	Test hdfr-r	--	GCCGATAT <b>CCA</b> AGAAAGCTGGGTGGTACCC
	14	Test hdfr-f	--	CCCA <b>AGCTT</b> CAAAAAAGCAGGCTTGCCGC
<b><i>PbA LISP2(-)/uis3(-)</i></b>				
Test-gene	1		--	TGCTATAAAAGCGTATTATGAATTAATATAAAAGG
	2		--	GTATTTATGCCAAAAAAGCTAAATTGC
Test-5'Int	1		--	TGCTATAAAAGCGTATTATGAATTAATATAAAAGG
	4		--	GTCCATACAACATATATCCGAAC
Test-3'Int	17		--	AAAGCTAGCTTTGATCCCCGTTTTTC
	5		--	GTATTTATGCCAAAAAAGCTAAATTGC

**Supplementary Table 2:** Total number of i.v. injected sporozoites and mice.

<b>Attenuated parasite line (<i>P. berghei</i> ANKA)</b>	<b>Mice*</b>	<b>Injected sporozoites</b>	<b>Break-through infections</b>	<b>Total number of injected sporozoites</b>
<i>LISP2(-)</i>	15	10.000	-	2.230.000
	14	20.000	-	
	17	30.000	1	
	12	50.000	-	
	1	220.000	-	
	1	470.000	-	
<i>uis3(-)</i>	14	20.000	3	2.470.000
	16	30.000	-	
	12	50.000	3	
	3	220.000	-	
	1	450.000	-	
<i>LISP2(-)</i> <i>/uis3(-)</i>	14	20.000	-	4.870.000
	16	30.000	-	
	9	50.000	-	
	1	100.000	-	
	7	200.000	-	
	3	220.000	-	
	6	250.000	-	
<i>N-LISP2</i>	4	10.000	4	160.000
	4	30.000	4	

\*all mice injected with sporozoites were naïve