Supplemental Table 1. LRIM9 is a novel antagonist of *P. berghei* in susceptible mosquitoes

Expt	Knock-down	*N*	Live oocysts				Knockdown efficiency (%)
			Prevalence		Infection intensity		
			%	P-value	Median (Range)	P-value	
1	*GFP*	7	86	N/A	6 (0-19)	N/A	N/A
	LRIM1	4	100	NS	519 (307-751)	< 0.001	72
	LRIM9	10	100	NS	26 (4-608)	< 0.05	81
2	*GFP*	16	75	N/A	3.5 (0-22)	N/A	N/A
	LRIM1	13	100	NS	326 (225-748)	< 0.001	95
	LRIM9	17	59	NS	1 (0-183)	NS	86
3	*GFP*	33	88	N/A	13 (0-298)	N/A	N/A
	TEP1	20	95	NS	240 (0-700)	< 0.001	ND
	LRIM9	42	95	NS	43 (0-293)	< 0.05	ND
Pooled	*GFP*	56	84	N/A	8 (0-298)	N/A	N/A
	LRIM1	17	100	NS	357 (225-751)	< 0.001	81
	TEP1	20	95	NS	240 (0-700)	< 0.001	ND
	LRIM9	69	87	NS	23 (0-608)	< 0.05	84

LRIM9 was silenced using RNAi, susceptible N'gousso strain mosquitoes were infected with fluorescent *P. berghei* and parasite load was monitored after 7 days Non-specific *GFP* dsRNA was used as a negative control and *LRIM1* or *TEP1* as positive controls. Three independent biological replicates and pooled data ar shown. *N* is the number of individual mosquito midguts. The Kruskal-Wallis test with Dunn's post-test was used for infection intensity, comparing to ds*GFP* Fisher's exact test was used for prevalence. Significant P-values (< 0.05) are shown in red. Knockdown efficiency was calculated by qRT-PCR. NS, ND and N/A are not significant, not determined and not applicable, respectively.

Supplemental Table 2. *LRIM9* silencing reduces melanization of *P. berghei* parasites in refractory L3-5 mosquitoes

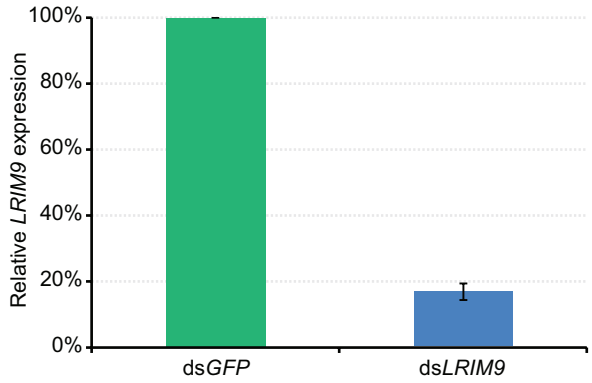
Expt	Knock-down	*N*	Live oocysts				Melanized ookinetes				Knockdown efficiency (%)
			Prevalence		Infection intensity		Prevalence		Infection intensity		
			%	P-value	Median (Range)	P-value	%	P-value	Median (Range)	P-value	
1	*GFP*	43	0	N/A	0	N/A	74	N/A	7 (0-65)	N/A	N/A
	LRIM9	45	4	NS	0 (0-1)	NS	66	NS	1 (0-28)	< 0.01	89
2	*GFP*	57	2	N/A	0 (0-1)	N/A	33	N/A	0 (0-68)	N/A	N/A
	LRIM9	58	0	NS	0	NS	19	NS	0 (0-11)	NS	ND

LRIM9 was silenced using RNAi, L3-5 refractory mosquitoes were infected with fluorescent *P. berghei* and parasite load was monitored after 7 days. Two independent biological replicates are shown. Non-specific *GFP* dsRNA was used as a negative control. *N* is the number of individual mosquito midguts. The Mann-Whitney test was used for infection intensity, comparing to ds*GFP*. Fisher's exact test was used for prevalence. Significant P-values (< 0.01) are shown in red. Knockdown efficiency was calculated by qRT-PCR. NS, ND and N/A are not significant, not determined and not applicable, respectively.

Supplemental Table 3. *LRIM9* and *CTL4* silencing in susceptible mosquitoes

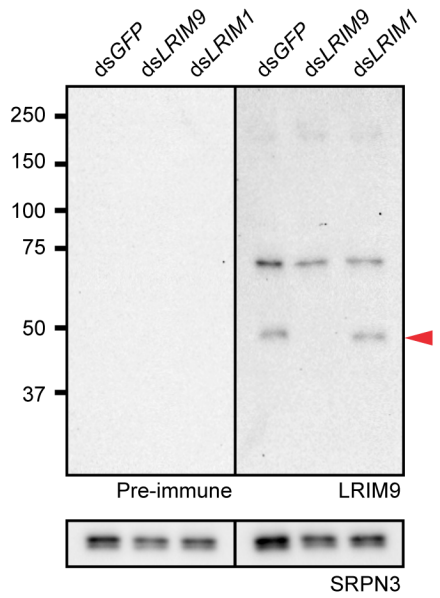
Expt	Knock-down	*N*	Live oocysts				Melanized ookinetes				Knockdown efficiency (%)
			Prevalence		Infection intensity		Prevalence		Infection intensity		
			%	P-value	Median (Range)	P-value	%	P-value	Median (Range)	P-value	
1	*GFP*	8	88	N/A	6.5 (0-23)	N/A	0	N/A	0	N/A	N/A
	CTL4	9	33	< 0.05	0 (0-3)	NS	56	< 0.05	1 (0-17)	NS	ND
	CTL4/TEP1	9	78	NS	43 (0-66)	< 0.05	0	< 0.05	0	< 0.05	66
	CTL4/LRIM9	10	70	NS	1.5 (0-26)	N/A	30	NS	0 (0-18)	NS	76
2	*GFP*	37	49	N/A	0 (0-168)	N/A	14	N/A	0 (0-6)	N/A	N/A
	CTL4	32	19	< 0.05	0 (0-37)	NS	69	< 0.0001	0 (8-583)	< 0.001	ND
	CTL4/TEP1	27	93	< 0.0001	130 (0-497)	< 0.001	11	< 0.0001	0 (0-2)	< 0.001	ND
	CTL4/LRIM9	23	39	NS	0 (0-19)	NS	65	NS	4 (0-371)	NS	ND

Genes were silenced using RNAi, susceptible N'gousso strain mosquitoes were infected with fluorescent *P. berghei* and parasite load was monitored after 7 days. Two independent biological replicates and pooled data are shown. Non-specific *GFP* dsRNA was used as a negative control and *LRIM1* or *TEP1* as positive controls. *N* is the number of individual mosquito midguts. The Kruskal-Wallis test with Dunn's post-test was used for infection intensity, comparing to ds*CTL4* (for ds*CTL4/TEP1* and ds*CTL4/LRIM9*) and ds*GFP* (for ds*CTL4)*. Fisher's exact test was used for prevalence. Significant P-values (< 0.05) are shown in red. Knockdown efficiency was calculated by qRT-PCR. NS, ND and N/A are not significant, not determined and not applicable, respectively.



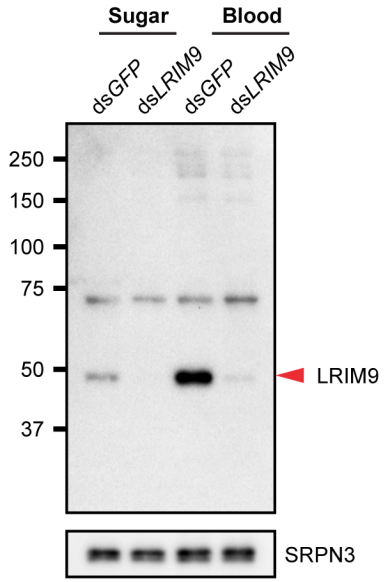
Supplemental Fig. 1

Fig. S1. Knockdown efficiency for *LRIM9* transcript. RNA was extracted from 10 mosquitoes after ds*GFP* or ds*LRIM9* injection and cDNA synthesized. *LRIM9* expression was determined using qRT-PCR and normalized to ribosomal *S7*. *LRIM9* expression in ds*LRIM9*-treated mosquitoes was calculated relative to expression in ds*GFP*-treated mosquitoes. Mean efficiency from two independent experiments is shown with standard error bars.



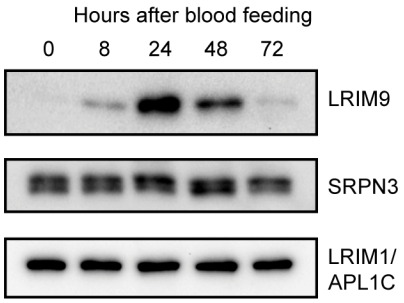
Supplemental Fig. 2

Fig. S2. Development of an LRIM9 antibody. Mosquitoes were injected with ds*GFP*, ds*LRIM9* or ds*LRIM1* and hemolymph was collected after 4 days. Non-reduced hemolymph was analyzed by western blot and probed with pre-immune serum or the LRIM9 antibody. A unique band at approximately 50 kDa was detected in ds*GFP*- and ds*LRIM1*-treated mosquitoes but was absent in ds*LRIM9* and the preimmune serum (see red arrowhead). Blots were re-probed with a SRPN3 antibody as a loading control.



Supplemental Fig. 3

Fig. 3. Induction of LRIM9 protein by blood feeding is prevented by gene silencing. Three days after *GFP* or *LRIM9* silencing, mosquitoes were fed either sugar or murine blood. Hemolymph collected 24 h later was analyzed by western blot using antibodies against LRIM9 and SRPN3 (as a loading control). LRIM9 protein is shown at approximately 50 kDa (red arrowhead). A band at approximately 70 kDa and another faint cluster of bands between 180 and 250 kDa were unaffected by *LRIM9* silencing and therefore considered non-specific.



Supplemental Fig. 4

Fig. S4. LRIM9 protein is induced after feeding on human blood. Mosquitoes were allowed to feed on human blood and hemolymph was collected after 8, 24, 48 and 72 hours. Hemolymph was analyzed by western blot under non-reducing conditions and probed with antibodies against LRIM9, SRPN3 (as a loading control) and APL1C (to detect the LRIM1/APL1C complex).