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Supplementary Materials for

Versatile transgenic multistage effector-gene combinations for *Plasmodium falciparum* suppression in *Anopheles*

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Supplementary Materials

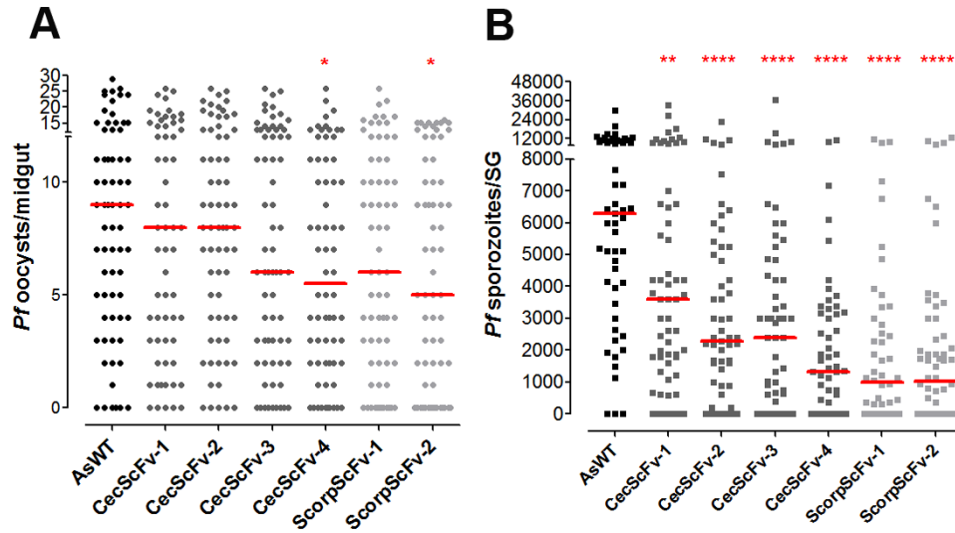


Fig. S1. *P. falciparum* infection oocyst and sporozoite loads of the heterozygous transgenic ScFv lines. (A-B) *P. falciparum* (NF54) oocyst or sporozoite infection intensities of individual CecScFv and ScorpScFv transgenic lines at 8 dpi in the gut (A) or 14 days dpi in the salivary glands (SG) (B) with additional naïve blood meals at day 5 and 9 post-infectious blood meal (PIBM). Assays were performed with at least two biological replicates. Each dot represents the number of parasites in an individual midgut or salivary glands, and the horizontal lines (red) indicate the median values. Mann-Whitney test was used to calculate *p*-values and determine the significance of parasite numbers.

Table S1. Primers used for the generation of constructs for embryo microinjections to generate transgenic mosquitoes, verification of transgenes on the transgenic mosquito chromosome, and qRT-PCR expression analysis primers for the transgenes, bacterial ribosomal gene *16S* primers to measure the bacterial loads, and the primers for expression profiling of endogenous effector genes.

Name	Primer Name	Primer sequence	RE sites, Notes
AgCp promoter	AgCp-Pro-F	GATC <u>GGCCGGCC</u> AGGAGGCATGTCATA AGC	<u>FseI</u>
	AgCp-Pro-R	TGGATCCGCTCGGCCGCTTC	
AsVg promoter	AsVg-Pro-F	gg <u>GGCGGCCT</u> GTACATGGTAAAGCAAT	<u>AscI</u>
	AsVg-Pro-R	GGTTCGGTTGTTCTCAGATGC	
AsVg-3'UTR full	AsVg-5'UTR-F	TGTACATGGTAAAGCAAT	
	AsVg-3'UTR-R	AGCCGGAGGAGGTCACCTTA	
Trypsin Terminator	Tryp-Ter-F	TGAATACTAGTTAGGTAGCTGAGCGCAT GCGATCTC	<u>SpeI</u>
	Tryp-Ter-R	TAAGTGCGGCCGCG <u>GGCCGGCC</u> GGTTCGG CGCGCCACCCTTGAG	<u>NotI, FseI</u>
MultiEff-veri	MultiEff-F	GCCATCCTGGCGGTGGCGCTC	
	MultiEff-R	ACCCACCGCACGCGCATCGCC	
CecC-ScFv-veri	CecCScFv-F	ATGAACTTCACCAAGCTGTTC	
	CecCScFv-R	TCGTTCTCAGGTTGTTGATCT	
PLA2-ScFv-veri	PLA2ScFv-F	GTTTAAACATGCAAGTCGTTT	
	PLA2ScFv-R	ACTAGTTTGGTACACTTTTCG	
Scorp-ScFv-veri	ScorpScFv-F	ATGAACTTCTCCAAGATCTTC	
	ScorpScFv-R	ATCCAACCCATCCACTTCAGAC	
GFP-veri	GFP-F	ATGGTGAGCAAGGGCGAGGAGCTGT	
	GFP-R	TTACTTGTACAGCTCGTCCATGCCG	
DsRed-veri	DsRed-F	ACCGTGAAGCTGAAGGTGACCA	
	DsRed-R	AGGCCTCCCAGCCCATGGTCT	
AsS7	AsS7-F	TCGGTTCCAAGGTGATCAAAGC	qRT-PCR
	AsS7-R	AGCGCGGTCTCTTCTGCTTGT	qRT-PCR
MultiEff	RT-MultiEff-F	GGCATCGGCGCCGTGCTGAAG	qRT-PCR
	RT-MultiEff-R	GATCTTGCCCAGCAGGTAGCC	qRT-PCR
CecC-ScFv	RT-CecCScFv-F	ACAGCCGGTAGATGGTGCGCC	qRT-PCR
	RT-CecCScFv-R	ACGGCCTATGTTTCGTGAACC	qRT-PCR
PLA2-ScFv	RT-PLA2ScFv-F	GGAGGAACGGATAATATATCC	qRT-PCR
	RT-PLA2ScFv-R	TGCATCCGTGTGCTTGAACCG	qRT-PCR
Scorp-ScFv	RT-ScorpScFv-F	GATTCAGAAAAAGATTGACGA	qRT-PCR

	RT-ScorpScFv-R	GCACTGAAATTCGTTCTTGGC	qRT-PCR
16S-rRNA	16S-qPCR-F	TCCTACGGGAGGCAGCAGT	qRT-PCR
	16S-qPCR-R	GGACTACCAGGGTATCTAATCCTGTT	qRT-PCR
CEC1	AsCEC1-qPCR-F	GGAAGCGGGACGCCTGAA	qRT-PCR
	AsCEC1-qPCR-R	CCTTGACACCTGCCACCACC	qRT-PCR
DEF1	AsDEF1-qPCR-F	AGTCGTGGTCCTGGCGGCTCT	qRT-PCR
	AsDEF1-qPCR-R	ACGAGCGATGCAATGCGCGGCA	qRT-PCR
GAM1	AsGAM1-qPCR-F	GTACGTCAGCCGGAAGGGAG	qRT-PCR
	AsGAM1-qPCR-R	CGTAATGAACGAGGACGAACAGC	qRT-PCR
FBN9	AsFBN9-qPCR-F	AACAATCTGACCGCACTGC	qRT-PCR
	AsFBN9-qPCR-R	TGTGACGCATTCCCTGTAG	qRT-PCR

Table S2. *P. falciparum* infection data for the MultiEff, PLAScFv, CecScFv, and ScorpScFv transgenic mosquitoes. Additional statistical analysis of *P. falciparum* parasite numbers for **Figs. 1 and 2** (Mann-Whitney test, chi-squared and fisher's exact test).

Fig. 1F <i>Pf</i> oocysts (medium)	AsWT	MultiEff		
n=	54	63		
range	0-89	0-53		
prevalence	88%	73%		
Fisher's exact test p-value		0.0118 (*)		
median	19.0	7.5		
Mann-Whitney test p-value		0.0034 (**)		
Fig. 1G <i>Pf</i> oocysts (low)	AsWT	MultiEff		
n=	72	57		
range	0-19	0-13		
prevalence	83.0%	45.0%		
Fisher's exact test p-value		<0.0001 (****)		
median	3.5	0.0		
Mann-Whitney test p-value		0.001 (***)		
Fig. 1H <i>Pf</i> sporozoites (low)	AsWT	MultiEff		
n=	54	54		
range	0-11206	0-11800		
prevalence	75.0%	46.0%		
Fisher's exact test p-value		<0.0001 (****)		
median	2300	0		
Mann-Whitney test p-value		<0.0001 (****)		
Fig. 1J <i>Pf</i> oocysts (Aseptic)	AsWT	MultiEff		
n=	65	65		
range	1-65	0-38		
prevalence	100%	83.0%		
Fisher's exact test p-value		<0.0001 (****)		
median	25.0	5.0		
Mann-Whitney test p-value		<0.0001 (****)		
Fig. 1K <i>Pf</i> oocysts (Septic)	AsWT	MultiEff		
n=	69	78		
range	0-23	0-11		
prevalence	77.0%	49.0%		
Fisher's exact test p-value		<0.0001 (****)		
median	2.0	0.0		
Mann-Whitney test p-value		<0.0001 (****)		
Fig. 2F <i>Pf</i> oocysts (no additional blood meals)	AsWT	PLAScFv	CecScFv	ScorpScFv

n=	69	75	60	57
range	0-45	0-27	0-35	0-32
prevalence	88.0%	84.0%	82.0%	83.0%
Chi-square test p-value		ns	ns	ns
median	3.0	3.0	2.0	2.0
Mann-Whitney test p-value		0.2032 (ns)	0.2235 (ns)	0.0690 (ns)
<i>Fig. 2G Pf sporozoites (no additional blood meals)</i>	AsWT	PLAScFv	CecScFv	ScorpScFv
n=	63	72	72	72
range	0-33563	0-33200	0-38438	0-35000
prevalence	84.0%	82.0%	72.0%	73.0%
Chi-square test p-value		ns	ns	ns
median	2719.0	2600.0	2400.0	1875.0
Mann-Whitney test p-value		0.4045 (ns)	0.1382 (ns)	0.0484 (*)
<i>Fig. 2H Pf oocysts (Additional naïve blood meals)</i>	AsWT	PLAScFv	CecScFv	ScorpScFv
n=	72	72	72	72
range	0-28	0-28	0-29	0-21
prevalence	83.0%	79.0%	77.0%	75.0%
Chi-square test p-value		ns	ns	ns
median	8.0	7.0	7.0	6.0
Mann-Whitney test p-value		0.7282 (ns)	0.4747 (ns)	0.0557 (ns)
<i>Fig. 2I Pf sporozoites (Additional naïve blood meals)</i>	AsWT	PLAScFv	CecScFv	ScorpScFv
n=	54	54	54	54
range	0-21000	0-24570	0-15600	0-18500
prevalence	85.0%	81.0%	63.0%	50.0%
Chi-square test p-value		0.5727 (ns)	0.0004 (***)	<0.0001 (****)
median	6300.0	5400.0	1230.0	967.5
Mann-Whitney test p-value		0.5591 (ns)	<0.0001 (****)	<0.0001 (****)

Table S3. *P. falciparum* infection data for the MultiEff, CpRel2, ScorpScFv, and hybrid transgenic lines (Rel2ScFv, MultiEffScFv). Additional statistical analysis of *P. falciparum* parasite numbers for **Fig. 3** (Mann-Whitney test, chi-squared test).

Fig. 3A (<i>Pf</i> oocysts)	AsWT	ScorpScFv	CpRel2	Hybrid (Rel2ScFv)
n=	66	66	66	66
range	0-33	0-31	0-22	0-14
prevalence	87.9%	86.4%	66.7%	66.7%
Chi-square test p-value		0.8339 (ns)	0.0006 (***)	0.0006 (***)
median	9.0	7.0	3.5	3.0
Mann-Whitney test p-value		0.0113 (*)	0.0001 (***)	<0.0001 (****)
Fig. 3B (<i>Pf</i> sporozoites)	AsWT	ScorpScFv	CpRel2	Hybrid (Rel2ScFv)
n=	80	80	80	80
range	0-18000	0-13800	0-18000	0-15000
prevalence	87.5%	55.0%	66.3%	45.0%
Chi-square test p-value		<0.0001 (****)	0.0007 (***)	<0.0001 (****)
median	6200.0	1770.0	2050.0	0.0
Mann-Whitney test p-value		<0.0001 (****)	<0.0001 (****)	<0.0001 (****)
p-value (ScorpScFv vs Hybrid)		0.0186 (*)		
p-value (CpRel2 vs Hybrid)			0.0021 (**)	
Fig. 3C (<i>Pf</i> oocysts)	AsWT	ScorpScFv	MultiEff	Hybrid (MultiEffScFv)
n=	68	68	68	68
range	0-17	0-17	0-12	0-12
prevalence	76.5%	66.2%	48.5%	51.5%
Chi-square test p-value		0.2753 (ns)	<0.0001 (****)	0.0004 (***)
median	3.5	2.0	0.0	1.0
Mann-Whitney test p-value		0.1168 (ns)	<0.0001 (****)	0.0002 (***)
Fig. 3D (<i>Pf</i> sporozoites)	AsWT	ScorpScFv	MultiEff	Hybrid (MultiEffScFv)
n=	72	72	72	72
range	0-12000	0-12000	0-12000	0-13800
prevalence	75.0%	40.3%	47.2%	36.1%
Chi-square test p-value		<0.0001 (****)	<0.0001 (****)	<0.0001 (****)
median	3505.0	0.0	0.0	0.0
Mann-Whitney test p-value		<0.0001 (****)	<0.0001 (****)	<0.0001 (****)
p-value (ScorpScFv vs Hybrid)		0.0215(*)		
p-value (MultiEff vs Hybrid)			0.0223 (*)	

Supporting text

Sequences of the synthetic transgenes on the plasmid constructs for the transformation of *Anopheles* mosquito embryos.

Synthesized gene fragment *MultiEff* at GenScript (Inc.) for cloning into the *pUC57-Kan* vector

*Bam*HI ggGGATCC
AgCp sp ATGGCCATCCTGGCGGTGGCGCTCAGTGTGCGAAGCGGCCGAGCGGATCCA
Melittin ggcacgogcogcgtgctgaagtgctgacgacgggocctgcccggccctgatctcgtggatcaagcgcaagcgccagcag
P2A GCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCC
(**TP10**) 2 gccggctacctgctgggcaagatcaacctgaaggccctggccgocctggccaagaagatcctgggocggcgccggctacctgct
ggcaagatcaacctgaaggccctggccgocctggccaagaagatcctg

P2A GCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCC
(**EPIP**) 4 Gat aaa tcc ctg gtt aag ggt ccg ggc ccg Gac aaa tgc ctg gtc aaa ggt ccg ggc ccc gat
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P2A GCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCC
Shival Atg ccg cgt tgg cgt ctg ttc cgc cgt atc gac cgc gtt ggc aaa cag att aag cag ggt Att
ctg cgt gcg ggt ccg gcg att gcg ctg gtt ggc gat gcg cgt gcg gtc ggt

P2A GCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCC
Scorpine Ggc tgg att aac gaa gag aag att cag aaa aag att gac gaa cgg atg ggc aac acg gtc Ctg
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ggc acc aag tgt aaa tgc ggc acc ccc ctg tct tat

*Xho*I CTAGCGgg

> *AsVg-Scorp-ScFv-3'UTR*

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AscI: GGCGGCC; PmeI: GTTTAAAC SpeI: ACTAGT XhoI: CTCGAG AsVg promoter: tgtaca...ccacc
AsVg 5'-UTR: agaagg.....aacc CecA-sp: ATGAAC.....CAGACGGAAGCG Scorpine: GGCTGGATT...TCTTAT
15aa: ggtggtggtggttccgggtggtggtggttccgggtggtggttccg; 5aa: ggtggtggtggttccg
V_H starts: aagcag V_L starts: gatatc
AsVg 3'UTR: aaagccaa.....ttataataa AsVg 3'300 bp termination seq: aatgaa.....ccggtc
CecA: also named as Cec1, AGAP000693-RA

> *AsVg-CecC-ScFv-3'UTR*

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CecC: also called Cec3, AGAP000694-RA
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> *AsVg-PLA2-ScFv-3'UTR*

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