SUPPLEMETARY DATA

Appendix Table S1: Global data concerning the gametocyte, oocyst, and sporozoite samples

Gametocyte Carrier	Blood samples (Gametocytes)				Gut samples (Oocysts)			Salivary gland samples (Sporozoites)				
	Gametocyte density*	MOI[†]	% *pfcrt* 76 alleles		N[‡]	Mean[§]	Prevalence[¶]	N[#]	MOI[‖]	N[**]	Mean % *pfcrt* 76 alleles	
			K76	***76T***							***K76***	***76T***
gc1	23	NA	31.3	68.7	30	0.2	13.3	NA	NA	16	51.0	49.0
gc3	88	NA	0.0	100.0	45	3.8	42.2	NA	NA	11	41.7	58.3
gc4	11	NA	0.0	100.0	28	0.0	0.0	NA	NA	3	56.3	43.7
gc6	24	NA	100.0	0.0	23	0.5	21.7	NA	NA	24	57.7	42.3
gc7	901	2	0.0	100.0	45	40.3	48.9	9	2	24	45.2	54.8
gc8	17	NA	32.1	67.9	60	4.2	51.7	NA	NA	16	30.8	69.2
gc9	107	NA	21.1	78.9	48	2.0	29.2	NA	NA	17	28.9	71.1
gc10	177	NA	0.0	100.0	29	17.5	69.0	NA	NA	12	13.6	86.4
gc11	33	NA	49.5	50.5	50	3.4	40.0	NA	NA	4	49.6	50.4
gc12	18	NA	0.0	100.0	46	0.9	21.7	NA	NA	5	14.9	85.1
gc13	31	3	0.0	100.0	39	6.0	51.3	4	3	23	29.5	70.5
gc14	16	3	32.1	67.9	47	1.9	34.0	3	3	15	27.1	72.9
gc16	91	NA	58.7	41.3	19	2.1	42.1	NA	NA	17	43.9	56.1
gc17	154	3	0.0	100.0	37	2.5	10.8	3	3	15	0.0	100.0
gc19	95	NA	0.0	100.0	31	74.9	87.1	NA	NA	12	24.7	75.3
gc20	83	NA	76.3	23.7	49	45.9	65.3	NA	NA	19	55.8	44.2
gc21	20	NA	21.3	78.9	79	2.3	60.8	NA	NA	11	16.8	83.2
gc22	23	NA	47.3	52.7	36	3.1	61.1	NA	NA	17	47.6	52.4
gc24	23	4	34.1	65.9	32	3.4	68.8	7	4	18	47.3	52.7

gc25	154	1	0.0	100.0	26	43.6	80.8	3	3	8	10.1	89.9
gc26	2304	1	0.0	100.0	38	185.1	81.5	6	2	3	28.9	71.1
gc27	11	NA	0.0	100.0	24	0.0	0.0	NA	NA	10	38.9	61.1
gc28	21	NA	0.0	100.0	25	5.5	56.0	NA	NA	6	21.6	78.4
gc35	36	5	34.9	65.1	47	6.2	85.1	6	5	4	45.4	54.6
gc36	18	NA	74.4	25.6	45	5.8	91.1	NA	NA	3	7.2	92.8
gc41	144	NA	22.2	77.8	46	5.3	56.5	NA	NA	5	31.2	68.8
gc47	245	2	74.7	25.3	41	6.3	58.5	11	5	10	97.1	2.9
gc48	49	2	0.0	100.0	32	2.3	75.0	19	5	7	0.0	100.0
gc49	524	5	20.4	79.6	48	15.7	80.9	5	3	5	14.0	86.0
gc50	21	4	65.9	34.1	74	3.5	70.3	34	6	10	18.3	81.7
gc52	55	3	NA	NA	43	1.4	53.5	5	3	NA	NA	NA
gc53	64	5	NA	NA	21	7.1	76.2	3	4	NA	NA	NA
gc56	173	3	75.5	24.5	65	13.9	81.5	15	4	5	91.8	8.2
gc60	46	2	100.0	0.0	50	4.0	84.0	15	5	10	84.4	15.6
gc63	57	5	75.6	24.4	54	8.3	90.7	10	4	9	78.6	21.4
gc64	647	1	100.0	0.0	23	53.7	78.3	12	3	10	96.1	3.9
gc66	296	1	0.0	100.0	51	36.4	84.3	15	4	17	1.4	98.6
gc67	165	6	31.8	68.2	33	20.2	93.9	12	7	9	38.0	62.0
gc68	56	2	29.2	70.8	28	9.6	82.1	16	5	10	34.3	65.7
gc69	103	2	59.0	41.0	38	1.8	52.6	30	8	13	48.7	59.3
gc70	48	3	100.0	0.0	46	1.2	52.2	19	7	8	63.9	36.1
gc71	62	1	0.0	100.0	49	3.0	71.4	3	1	4	0.0	100.0
gc72	33	2	0.0	100.0	35	2.3	48.6	13	3	8	87.5	12.5
gc76	266	2	56.5	43.5	50	14.4	84.0	28	4	11	67.0	33.0
gc78	23	4	66.1	33.9	50	5.9	90.0	10	4	9	56.5	43.5
gc79	32	2	0.0	100.0	23	5.1	91.3	9	3	9	32.0	68.0
gc80	33	7	50.4	40.6	45	10.5	93.3	8	3	18	0.0	100.0

gc81	19	6	72.0	28.0	40	1.8	60.0	14	7	10	34.0	66.0
gc83	370	6	56.5	43.5	54	26.0	88.9	6	6	6	56.8	43.2
gc85	107	3	68.3	31.7	30	21.2	83.3	12	4	10	62.8	37.2
gc86	53	5	35.2	64.8	69	23.9	95.7	12	5	12	29.9	70.1
gc88	44	6	28.7	71.3	38	3.0	79.0	12	6	19	49.6	50.4
gc89	29	4	40.2	59.8	34	2.8	73.5	7	3	7	7.4	92.6
gc90	68	1	0.0	100.0	64	3.2	81.3	12	2	14	0.0	100.0
gc93	257	1	100.0	0.0	27	44.6	96.3	10	1	6	75.0	25.0
gc95	23	6	37.0	63.0	57	3.0	75.4	12	6	10	9.9	90.1
gc96	98	6	29.0	71.0	70	8.8	78.6	12	3	11	0.0	100.0
gc97	13	10	75.7	24.3	47	0.4	31.9	12	6	10	7.0	93.0
gc99	48	5	86.2	13.8	24	1.1	45.8	12	3	18	88.0	12.0
gc102	39	4	0.0	100.0	58	0.6	36.2	12	4	10	77.0	23.0
gc105	219	2	100.0	0.0	44	55.9	88.6	12	2	15	100.0	0.0
gc106	257	2	100.0	0.0	36	35.4	94.4	12	1	16	100.0	0.0
gc108	122	5	0.0	100.0	40	2.5	67.5	12	5	20	81.5	18.5
gc111	74	1	NA	NA	36	5.4	83.3	10	2	NA	NA	NA
gc112	47	7	100.0	0.0	41	8.5	85.4	12	6	9	100.0	0.0
gc113	40	1	0.0	100.0	48	2.2	77.1	12	2	10	96.1	3.9
gc115	49	3	0.0	100.0	44	3.6	70.5	12	4	10	3.0	97.0

NA: not available

* Gametocyte density was expressed as the number of gametocytes per microliter

[†] Multiplicity of the infection (MOI) in the gametocyte population

[‡] Number of gut samples analyzed for oocyst content and prevalence of infection

[§] Mean number of oocysts per gut sample

[¶] Prevalence of infected mosquitoes

[#] Number of salivary gland samples analysed for microsatellite profile

[‖] Multiplicity of the infection (MOI) in sporozoite population

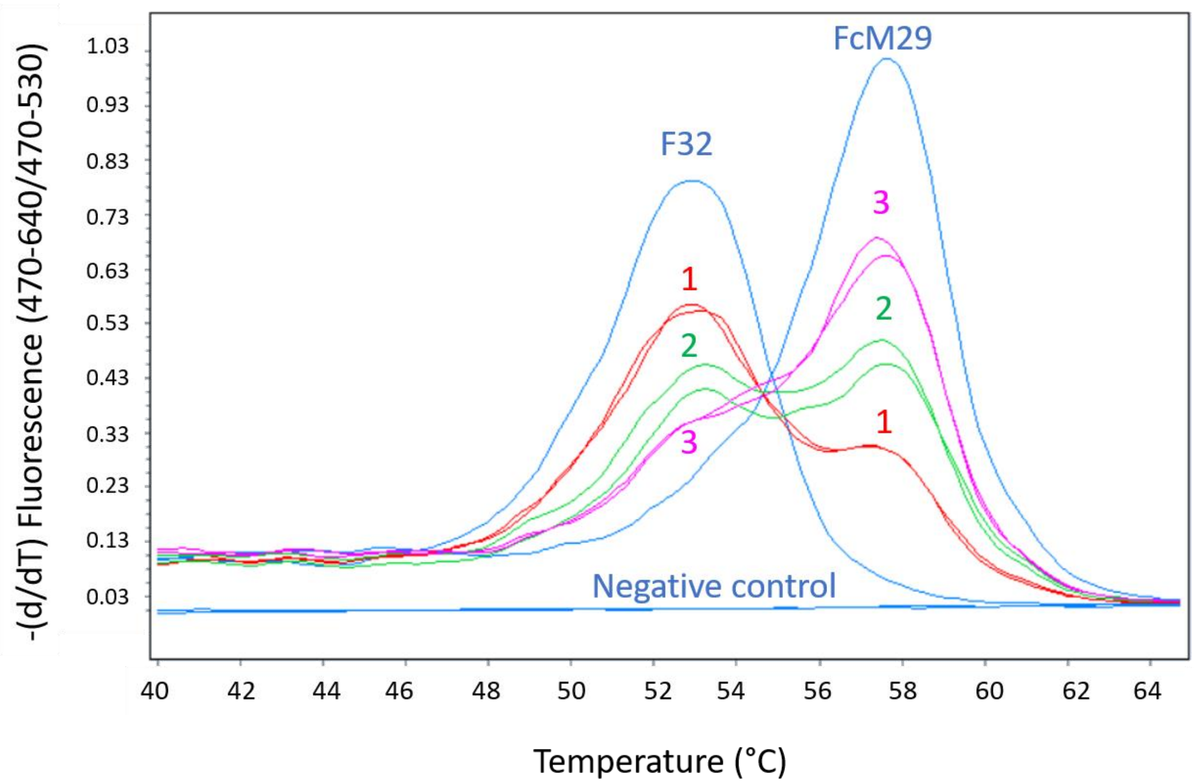
**Number of salivary gland samples analysed for *pfcrt* 76 genotyping

Appendix Figure S1: Quantification of mutant and wild-type *pfcrt* 76 alleles using FRET-based DNA hybridization probes. DNA from the FcM29/Cameroon clone carrying mutant *pfcrt* 76T allele was used as mutant control, whereas DNA from the F32/Tanzania strain carrying wild-type *pfcrt* K76 allele was used as wild-type control. We produced standards with a known proportion of mutant and wild-type *pfcrt* 76 alleles to quantify the proportion of *pfcrt* 76 alleles through the real-time PCR method and validate the reproducibility of the quantification method. Numbers 1–3 represent a F32/Tanzania: FcM29/Cameroon ratio of 7.5:2.5, 5:5, and 2.5:7.5, respectively. The figure shows the negative first derivative of fluorescence with respect to the temperature (-dF/dT) plotted against the temperature for the different mixtures of mutant and wild-type *pfcrt* 76 alleles. The two melting peaks of the mutant (*pfcrt* 76T) and the wild-type (*pfcrt* K76) alleles are clearly separated by ~5°C. In the mixtures of wild-type and mutant alleles, two peaks are obtained according to the proportion of each allele. The relative quantification of mutant or wild-type alleles was determined by calculating the ratio of melting peak heights of the high (mutant) or low (wild-type) temperature melting curves against the sum of heights of both mutant and wild-type melting peaks, respectively (22, 23):

$$\text{Mutant \%} = \frac{\text{Mutant Tm peak height}}{\text{Mutant Tm peak height} + \text{wild}-\text{type Tm peak height}} \text{ x } 100$$

$$\text{Wild-type \%} = \frac{\text{Wild}-\text{typeTm peak height}}{\text{Mutant Tm peak height} + \text{wild}-\text{type Tm peak height}} \text{ x } 100$$

These mixed samples (1-3) tested in duplicate showed the good reproducibility of the quantification method.

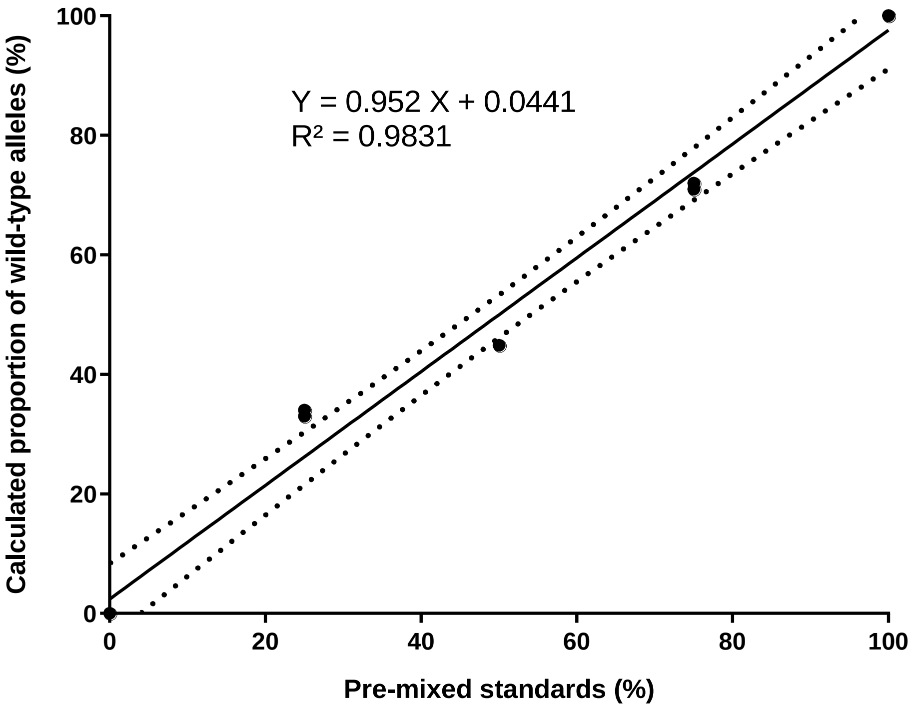

-(d/dT) Fluorescence (470-640/470-530)
1.03
0.93
0.83
0.73
0.63
0.53
0.43
0.33
0.23
0.13
0.03
FcM29
F32
3
1
2
2
1
3
Negative control
40
42
44
46
48
50
52
54
56
58
60
62
64
Temperature (°C)

34

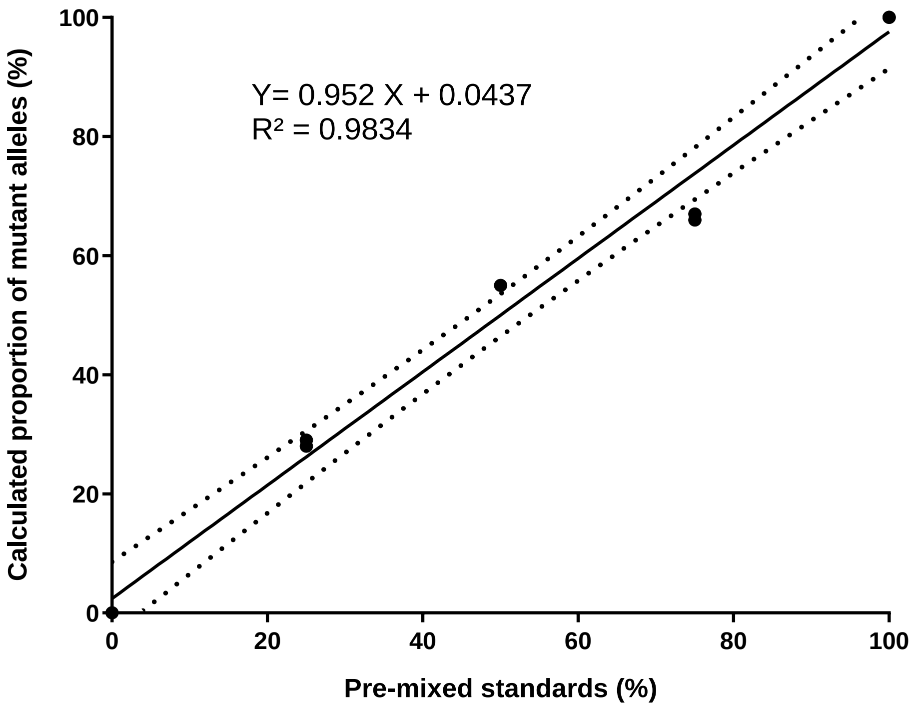
Appendix Figure S2: Validation of the method for the quantification of the proportion of mutant *pfcrt* 76T and wild-type *pfcrt* K76 alleles.

The relative amount of mutant and wild-type *pfcrt* 76 alleles was calculated for each sample as described in the material and method section and plotted against the theoretical amount of mutant and wild-type alleles present in the pre-mixed standards. A standard curve was plotted using the pre-mixed DNA standards in the 0–100% range and the calculated proportion of wild-type (A) or mutant (B) alleles present in samples. Linear regressions provided good fitting curves ($R^2 = 0.9831$ and $R^2 = 0.9834$ for the wild-type allele and for the mutant allele, respectively), validating the method for the accurate quantification of the mutant and wild-type *pfcrt* 76 alleles in biological samples.

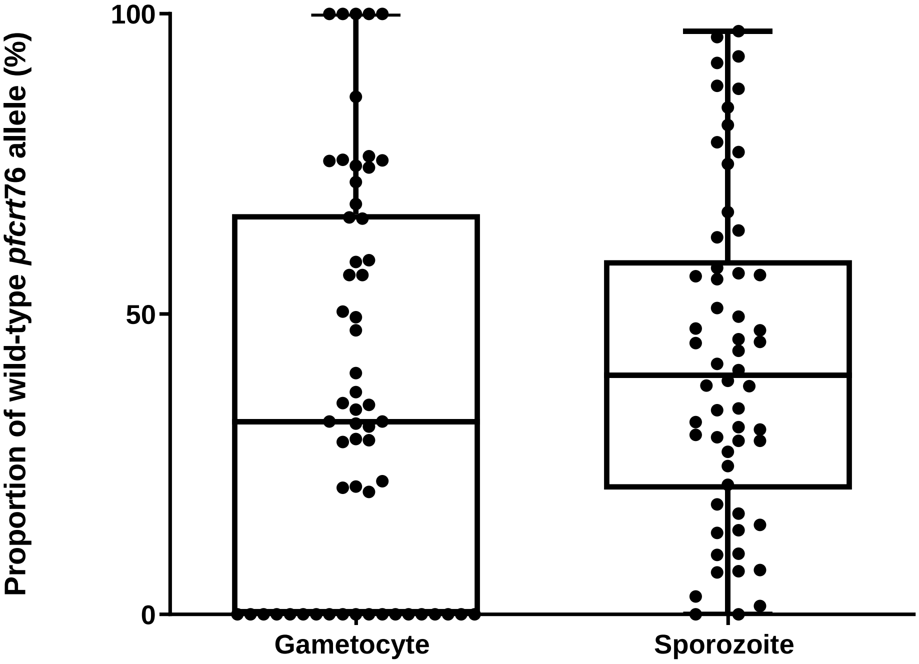
(A)



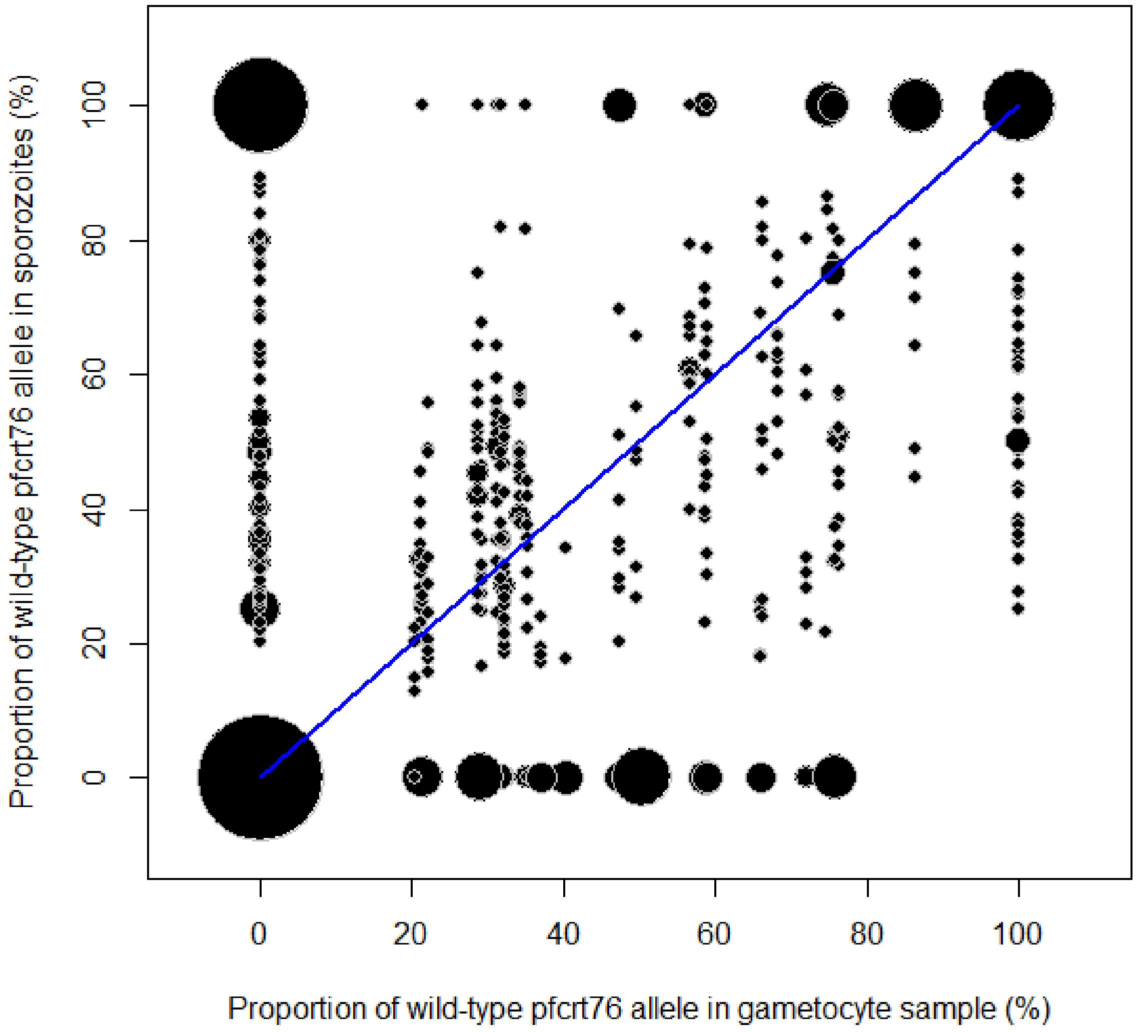
(B)


Y= 0.952 X + 0.0437
R² = 0.9834
Calculated proportion of mutant alleles (%)
Pre-mixed standards (%)
0
20
40
60
80
100
0
20
40
60
80
100

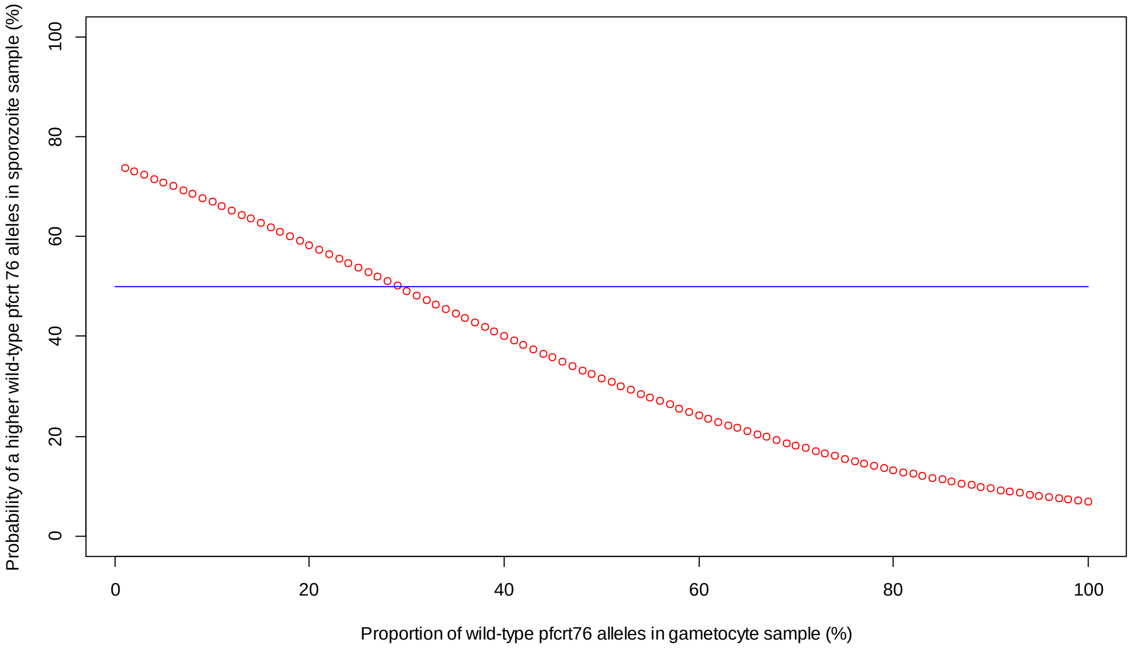
Appendix Figure S3: Box plot of wild-type *pfcrt* 76 alleles in gametocyte and sporozoite samples. The dots represent the observed proportion of wild-type *pfcrt* 76 alleles in the gametocyte samples and the observed average proportion of wild-type *pfcrt* 76 alleles in the sporozoite samples. Box plots represent the median, 25th and 75th percentiles, and the minimum and the maximum.



Appendix Figure S4: Dispersion of the proportion of wild-type *pfcrt* 76 alleles in gametocyte samples and in paired sporozoite samples. The dots represent the observed proportion of wild-type *pfcrt* 76 alleles in each sporozoite sample against the observed proportion of wild-type *pfcrt* 76 alleles in the paired gametocyte sample. The blue line represents the expected and theoretical distribution of dots if the vector has no influence in the transmission of *pfcrt* 76 alleles. The dot size is weighted by the number of samples



Appendix Figure S5: Logistic regression of the probability of a higher proportion of wild-type *pfcrt* 76 alleles in sporozoite samples as a function of the proportion of wild-type *pfcrt* 76 alleles in gametocyte samples. The proportion of wild-type *pfcrt* 76 alleles in sporozoite samples was compared to that in the paired gametocyte samples. The probability of having a higher proportion of wild-type *pfcrt* 76 alleles in sporozoite samples was calculated for each gametocyte sample using a logistic regression; the donor effect was taken into account. This probability (expressed in percentage) as a function of the proportion of wild-type *pfcrt* 76 alleles in gametocyte samples was plotted (red line). The blue line represents the expected probability if the variation of the proportion of wild-type *pfcrt* 76 alleles in sporozoite samples is randomly distributed (50% of a higher proportion and 50% of a lower proportion).



Appendix Figure S6: Distribution of the increase in the frequency of parasites with wild-type *pfcrt* 76 alleles between the gametocyte and sporozoite stages for experimental infections where the proportion of parasites harboring the considered allele does not exceed 20% at the gametocyte stage. Dashed line represents the average of this increase.