High proportion of genome-wide homology and increased pre-treatment *pvcrt* levels in *Plasmodium vivax* late recurrences: a chloroquine therapeutic efficacy study

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Supplemental Material

Table S1. PCR reaction mixes and thermocycling conditions.

	DOD	·	•	1.1	1.	1	C	•	/ 11*/		•
A) P('k	reaction	mixes a	nd therm	ocycling	conditions	tor	micros	atellite	genoty	nino
1 1		c reaction	mixes u	na unerm	ocycning	conditions	101	meros	utenne	Sonory	pins

Gene	Primer sequences	qPCR mix (µl)		Ther	mocycling
pvmsp1F3	msp1F3-fwdp:	Hotstart 10x Buffer	2	1x	95°C, 10min
	5'-GGAGAACATAAGCTACCTGTCC-3'	dNTP mix (20mM)	2		95°C, 15sec
	msp1F3-revp:	MgCl2 (25 mM)	1.6	25x	59°C, 30sec
	5'-GTTGTTACTTGGTCTTCCTCCC-3'	Forward Primer (10µM)	0.5		72°C, 30sec
		Reverse Primer (10µM)	0.5	Thermocy conditions 1x 95°C 25x 59°C 25x 59°C 1x 72°C 1x 72°C 1x 95°C 25x 59°C 25x 59°C 1x 95°C 25x 59°C 1x 72°C 1x 72°C 1x 95°C 40x 60°C 1x 72°C 1x 95°C 40x 58°C 72°C 1x 1x 95°C 40x 58°C 72°C 1x 1x 95°C 40x 58°C 72°C 1x 1x 95°C 40x 54°C 95°C 95°C 40x 54°C 72°C 1x 1x 72°C 1x 72°C 1x 72°C 1x 72°C 1x 72°C <t< td=""><td>72°C, 10min</td></t<>	72°C, 10min
		ces qPCR mix (µ) rAAGCTACCTGTCC-3' Hotstart 10x Buffer 2 GGTCTTCCTCCC-3' MTP mix (20mM) 0.5 rGGTCTTCCTCCC-3' Forward Primer (10µM) 0.5 rGGTCTTCCTCCCC-3' Forward Primer (10µM) 0.5 rGGTCTTCCTCCC-3' Forward Primer (10µM) 0.5 rGGTCTTCCTCCCC-3' Hotstart Taq Polymerase (Qiagen) 0.2 H ₂ O Molecular Biology grade 8.2 0NA 5 CTACCAAGAATTGATCCCCCAA-3' MTP mix (20mM) 2 0.5 CGTAGTCCTCGGCGTAGTCC-3' + tail Forward Primer (10µM) 0.5 0.5 Reverse Primer (10µM) 0.5 1.6 0.5 0.2 rTACCAAGAATGCTCGGCGTAGTCC-3' + tail Forward Primer (10µM) 0.5 0.2 HQC Molecular Biology grade 8.2 0.2 1.6 0.5 TACTGTTGACGCTGAA-3' MTP mix (20mM) 0.5 0.5 0.5 0.5 AAGGAACATGCTCGATGA-3' Reverse Primer (10µM) 2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 </td <td></td> <td></td>			
			8.2		
		DNA	5		
	msp1F3-fwdn:	Hotstart 10x Buffer	2	1x	95°C, 10min
Gene F pvmsp1F3 n S n S n S n S n MS4 N MS10 N F F C S PvSal1814 F F C	VIC-5'-CAAGCCTACCAAGAATTGATCCCCAA-3'	dNTP mix (20mM)	2		95°C, 15sec
	msp1F3-revn:	qPCR mix (μl) Hotstart 10x Buffer 2 MTP mix (20mM) 2 MgCl2 (25 mM) 1.6 Forward Primer (10µM) 0.5 Reverse Primer (10µM) 0.5 Hotstart Taq Polymerase (Qiagen) 0.2 H ₂ O Molecular Biology grade 8.2 DNA 5 Hotstart 10x Buffer 2 dNTP mix (20mM) 0.5 Forward Primer (10µM) 0.5 Reverse Primer (10µM) 0.5 Reverse Primer (10µM) 0.5 Hotstart Taq Polymerase (Qiagen) 0.2 H ₂ O Molecular Biology grade 8.2 Primary PCR product 5 Hotstart 10x Buffer 2.5 dNTP mix (20mM) 0.5 Forward Primer (10µM) 2 Reverse Primer (10µM) 2 Reverse Primer (10µM) 2 Hotstart 10x Buffer 2.5 DNA 5 Hotstart 10x Buffer 2.5 MNP mix (20mM) 0.5 Forward Primer (10µM) 2	1.6	25x	59°C, 30sec
	5'-ATTACTTTGTCGTAGTCCTCGGCGTAGTCC-3' + tail	Forward Primer (10µM)	$\begin{array}{c cccc} & condit 2 & 1x & 6 2 & 1x & 7 2 & 1x & 7 2 & 1x & 7 1.6 & 25x & 7 0.5 & 1x & 7 (Qiagen) & 0.2 ade & 8.2 & - 1.6 & 25x & 7 0.5 & 1x & 7 (Qiagen) & 0.2 ade & 8.2 & - 1.6 & 25x & 7 0.5 & 1x & 7 (Qiagen) & 0.2 ade & 8.2 & - 2.5 & 1x & 7 (Qiagen) & 0.2 ade & 12.55 & - 2 & 40x & 7 (Qiagen) & 0.2 ade & 12.55 & - 2 & 40x & 7 (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 1 x & 7 (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & 12.55 & - 5 & - (Qiagen) & 0.2 ade & - 1 & 40x & 7 (Qiagen) & 0.2 ade & - 1 & - 1 & - 0 & - 1 & - 1 & - 1 & - 0 & - 1 & - $	72°C, 30sec	
		Reverse Primer (10µM)		72°C, 10min	
		qPCR mx (μ) conv Hotstart 10x Buffer 2 1x dNTP mix (20mM) 2 1x MgCl2 (25 mM) 1.6 25x Forward Primer (10µM) 0.5 1x Hotstart Taq Polymerase (Qiagen) 0.2 1x Hotstart Taq Polymerase (Qiagen) 0.2 1x Hotstart 10x Buffer 2 1x dNTP mix (20mM) 2 1x MgCl2 (25 mM) 1.6 25x Forward Primer (10µM) 0.5 1x Hotstart 10x Buffer 2 1x Hotstart Taq Polymerase (Qiagen) 0.2 1x Hotstart 10x Buffer 2.5 1x Hotstart 10x Buffer 2.5 1x dNTP mix (20mM) 0.5 1x Hotstart 10x Buffer 2.5 1x dNTP mix (20mM) 0.5 1x Hotstart 10x Buffer 2.5 1x Hotstart 10x Buffer 2.5 1x Hotstart 10x Buffer 2.5 1x Hotst			
		H ₂ O Molecular Biology grade	$\begin{array}{c cccc} & condit 2 & 1x & 5 2 & 1x & 5 2 & 1x & 5 0.5 & 1x & 5 0.5 & 1x & 5 0.5 & 1x & 5 1 & 2 & 1x & 5 2 & 1x & 5 2 & 1x & 5 1 & 5 & 1x & 5 0.5 & 1x & 5 0.5 & 1x & 5 0.5 & 1x & 5 2 & 40x & 6 2 & 40x & 6 2 & 40x & 6 2 & 1x & 5 1 & 5 & 1x & 5 2 & 40x & 6 2 & 40x & 6 2 & 1x & 5 1 & 5 & 1x & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 5 1 & 1 & 1 0.2 & 1x & 5 1 & 1 & 1 0.2 & 1x & 5 1 & 1 & 1 0.2 & 1x & 5 1 & 1 & 1 0.2 & 1x & 5 1 & 1 & 1 0.1 & 1x & 5 1 & 1 & 1 0 & 1x & 5 1 & 1 & 1 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1\\ 0 & 1x & 5\\ 1 & 1 & 1\\ 1 &$		
		Primary PCR product	5		
MS4	MS4-fwd:	Hotstart 10x Buffer	2.5	1x	95°C, 10min
	PET-5'- CGATTTACTGTTGACGCTGAA-3'	dNTP mix (20mM)	0.5	conditions 1x 95°C, 10 95°C, 11 95°C, 11 25x 59°C, 30 72°C, 30 1x 72°C, 10 95°C, 11 25x 59°C, 10 95°C, 11 25x 59°C, 30 72°C, 30 1x 95°C, 11 25x 59°C, 30 1x 72°C, 10 1x 95°C, 11 95°C, 11 95°C, 11 1x 95°C, 10 1x 95°C, 10 95°C, 30 40x 40x 60°C, 40 72°C, 30 40x 40x 58°C, 10 95°C, 30 40x 40x 58°C, 40 72°C, 40 55 1x 72°C, 50 55 1x 55 1x 55 1x 55 1x 54°C, 40 72°C, 40 54°C, 41 72°C, 40 <tr td=""></tr>	95°C, 30sec
	MS4-rev:	Forward Primer (10µM)	2	40x	60ºC, 40sec
MS4 MS10	5'-CTGTCTTCAAAGGAACATGCTCGATGA-3'	Reverse Primer (10µM)	2		72°C, 40sec
		Hotstart 10x Buffer dNTP mix (20mM) MgCl2 (25 mM) Forward Primer (10µM) Reverse Primer (10µM) Hotstart Taq Polymerase (Qiagen) H ₂ O Molecular Biology grade DNA Hotstart 10x Buffer A-3' dNTP mix (20mM) MgCl2 (25 mM) C-3' + tail Forward Primer (10µM) Reverse Primer (10µM) Reverse Primer (10µM) Hotstart Taq Polymerase (Qiagen) H ₂ O Molecular Biology grade Primary PCR product Hotstart 10x Buffer dNTP mix (20mM) Forward Primer (10µM) Reverse Primer (10µM) Reverse Primer (10µM) Reverse Primer (10µM) Acetylated BSA (0,1µg/µl) Hotstart 10x Buffer dNTP mix (20mM) Forward Primer (10µM) Reverse Primer (10µM) Reverse Primer (10µM) Reverse Primer (10µM) Acetylated BSA (0,1µg/µl) Hotstart 10x Buffer dNTP mix (20mM) Forward Primer (10µM) Reverse Primer (10µM)	0.25	1x	72°C, 10min
pvmsp1F3 r r f r f MS4 f MS10 f f <		Hotstart Taq Polymerase (Qiagen)	0.2		
		H ₂ O Molecular Biology grade	12.55	conditions 1x 95°C, 1 95°C, 1 25x 1x 72°C, 3 1x 72°C, 1 25x 59°C, 1 95°C, 1 95°C, 1 1x 72°C, 3 1x 72°C, 1 25x 59°C, 1 95°C, 1 95°C, 1 95°C, 3 72°C, 3 1x 72°C, 1 95°C, 3 40x 40x 60°C, 4 72°C, 1 95°C, 1 95°C, 3 40x 40x 58°C, 1 95°C, 3 40x 40x 58°C, 4 72°C, 4 1x 1x 95°C, 5 95°C, 3 40x 40x 58°C, 4 72°C, 4 1x 1x 72°C, 4 1x 72°C, 5 95°C, 3 40x 40x 54°C, 4 72°C, 4 1x 1x 72°C, 8 40x 54°C, 4 72°C, 4 1x	
		DNA	5		
MS10	MS10fwd:	Hotstart 10x Buffer	2.5	1x	95°C, 10min
	FAM-5'-TTATCCCTGCTGGATGTGAA-3'	dNTP mix (20mM)	0.5		95°C, 30sec
	MS10rev:	Forward Primer (10µM)	2	40x	58°C, 40sec
	5'-CTGTCTTTCCTTCAGGTGGGACTTGTT-3'	Reverse Primer (10µM)	2		72°C, 40sec
		Acetylated BSA (0,1µg/µl)	0.25	1x	72°C, 10min
		Hotstart Taq Polymerase (Qiagen)	0.2		
		H ₂ O Molecular Biology grade	12.55		
		DNA	5		
PvSal1814	PvSal1814-fwd:	Hotstart 10x Buffer	5	1x	95°C, 5min
	FAM-5'-AAACAGGCATTAGGTTTAAGAGTG-3'	dNTP mix (20mM)	0.5		95°C, 30sec
	PvSal1814-rev:	Forward Primer (10µM)	1	40x	54ºC, 40sec
	CAGTGGCTTCTTCTTTAGTGG	Reverse Primer (10µM)	1		72°C, 40sec
		acetylated BSA (0,1µg/µl)	0	1x	72°C, 8min
		Hotstart Taq Polymerase (Qiagen)	0.2		
		H ₂ O Molecular Biology grade	32.3		
		DNA	10		

B) PCR reaction mixes and thermocycling conditions for *pvmdr1* genotyping.

Gene	Primer sequences	qPCR mix (μl)		Ther conc	mocycling litions
pvmdr1	GolPvmdr-1 OF	5X Buffer	10	1x	95°C, 5min
	5'-CGCCATTATAGCCCTGAGCA-3'	MgCl2 (25 mM)	5		95°C, 15sec
	GolPvmdr-1 OR	dNTP mix (20mM)	0.5	33x	55°C, 30sec
	5'-TCTCACGTCGATGAGGGACT-3'	Forward Primer (10µM)	1		72°C, 1min
		Reverse Primer (10µM)	1	1x	72°C, 7min
		GoTaq Polymerase (Promega)	0.25		
		H ₂ O Molecular Biology grade	27.25		
		DNA	5		
	Lin-mdr1_Fw	Buffer	10	1x	95°C, 5min
	5'-ATAGTCATGCCCCAGGATTG-3'	MgCl2 (25 mM)	5		95°C, 15sec
	Lin-mdr1_Rev	dNTP mix (20mM)	0.5	33x	55°C, 30sec
	5'-CCTTTCGAAGGACAGCTTTG-3'	Forward Primer (10µM)	1		72°C, 1min
		Reverse Primer (10µM)	1	1x	72°C, 7min
		GoTaq Polymerase (Promega)	0.25		
		H ₂ O Molecular Biology grade	31.25		
		Primary Product	1		

Data S1. (Excel [Data_S1_GRC.xlsx]). Genetic Report Card (SpotMalaria). The dataset contains drug-resistance genotypes as well as the SNP barcode (Sheet A). An explanatory table with detail of sequenced positions and reference/mutant codon is provided in Sheet B.

Table S2. Whole-genome sequencing coverage.

		Day 0		Day of recurrence						
Patient code	parasites/µl	coverage (mean X ± SD)	% missing positions	parasites/µl	coverage (mean X ± SD)	% missing positions				
007	41506	41.2 ± 52	0.8%	769	21.4 ± 33	4.4%				
008	6068	27.6 ± 51	13.8%	3428	45.0 ± 43	0.4%				
010	5431	6.8 ± 11	0.9%	212	1.6 ± 15	88.7%				
018	2408	25.5 ± 37	2.9%	103	3.3 ± 22	81.9%				
022	13430	38.9 ± 54	1.6%	444	44.0 ± 46	0.4%				
027	5517	37.1 ± 57	4.6%	3268	42.2 ± 42	0.4%				
034	4010	42.1 ± 56	2.1%	1536	45.5 ± 47	0.4%				
037	11401	38.5 ± 55	2.5%	310	37.5 ± 41	0.6%				
043	55892	42.7 ± 39	0.3%	7477	34.2 ± 40	0.8%				
044	274	46.4 ± 51	0.6%	68	2.5 ± 9	48.6%				
047	4927	25.0 ± 55	27.8%	91	35.0 ± 39	0.6%				
048	13965	50.1 ± 47	0.3%	856	16.9 ± 41	35.5%				
053	11262	8.6 ± 40	81.0%	2469	21.8 ± 52	34.4%				
054	14096	32.3 ± 50	4.0%	440	35.7 ± 55	3.9%				
All		33.1 ± 47	10.2%		27.6 ± 38	21.5%				



Fig S1. Distribution of discordant SNPs by whole-genome sequencing. The proportion of discordant SNPs was determined by calculating the Prevosti distance between all pair-wise comparisons (*i.e.* number of allelic differences/number of possible differences) using the R-package *poppr* (https://cran.r-project.org/web/packages/poppr/index.html). Due to the high likelihood of multiclonal infections in *P. vivax*, distances were fitted as the sum of four Gaussian distributions using the R-package *mixtools* (https://cran.r-project.org/web/packages/mixtools/index.html). The distribution with the lowest proportion of discordant SNPs (red line) was used to define the threshold (mean plus 3x the standard deviation for that distribution) of homologous recurrence for identify-by-state (IBS) analysis.



Fig S2. qPCR standard curves for *pvcrt*, *pvmdr1* and β -tubulin. Standard curves were prepared using 10-fold serial dilutions of dsDNA fragments covering the targeted region in each gene of interest (range 4000000-4 copies/µl). Amplification efficiencies calculated from cycle threshold (Ct) *versus* dsDNA copy number (log transformed) plots were 1.88, 1.90 and 1.84 for *pvcrt*, *pvmdr1* and β -tubulin, respectively.



Fig S3. Time to parasite clearance by treatment outcome. Kaplan-Meier survival curves for total parasite clearance (A) and gametocyte clearance (B) separated by non-recurrent (n=49) or recurrent (n=18) courses of infection. Curves are stratified by light microscopy (blue line) and qPCR (A; red line) or RTqPCR (B; red line), respectively. Colored areas indicate 95% confidence intervals. P-values were calculated using log-rank test. One patient with no Day 7 sample by qPCR/RTqPCR was excluded from this analysis in the non-recurrent group.



Fig S4. CQ dose received by patients. Graph shows the exact CQ dose administered in mg per kg of body weight, stratified by treatment outcome.

 Table S3. Microsatellites genotyping. Results of allele calling using Genetools software. Shared alleles between

 sample pairs at Day 0 and Day of recurrence (DRec) are indicated in bold.

Patient	D		Р	vMSP1.	F3		MS4		MS10		PvSal1814						
code	Day	Allele1	Allele2	Allele3	Allele4	Allele5	Allele1	Allele2	Allele1	Allele2	Allele1	Allele2	Allele3	Allele4	Allele5	Allele6	
	Dav0	264	313	i incico	r more r	r mores	195	198	216	r merez	528	543	558	1 more 1	r menes	rinereo	
003	DRec	264						198			528	543		_			
	Day0	229		264	278	313	198	201	201	207	599	614	629				
007	DRec		259			313		201	201		599	614	629				
000	Day0	276					195		201		563	578					
008	DRec	276	325				195		201		563	578					
010	Day0	259	306				204		213								
010	DRec																
019	Day0	259	264	316			198	201	204	213	510	525	540				
018	DRec	259							204								
021	Day0	253		300				204	195					573	588	603	
021	DRec		259				201			207	528	543	558				
022	Day0	219	278	327			198	_	195		599	614	629				
022	DRec		278				198		195		599						
027	Day0			325	374		201		198		555	584	599				
027	DRec	147	197		374		201		198		555	584	599				
028	Day0	147	197	325	374		201		198								
028	DRec																
024	Day0	325		•			201		198			581		596			
034	DRec	325					201		198		555		584				
027	Day0	259					201		201								
037	DRec																
0/3	Day0	264	272	ļ					204		555	569	584				
043	DRec		272				198		204				584	614			
044	Day0	264	-				204		210		584	599	614	_			
044	DRec	264							210		584	599	614				
047	Day0	253	264				195	201	207	4	599						
047	DRec	253	264					201	204								
048	Day0	264	4				223		201	-							
010	DRec	264					223		201		599		1				
050	Day0	264	4				198	_	213	-	620	635	_				
	DRec	264							213		620			1	1		
053	Day0	258					201		201	-		573	588		_		
	DRec	258					201		201		558	573	588	603			
054	Day0	250					213		204	-	620	635					
	DRec	250					213		204		620	635					

 Table S4. Genotyping and categorization of recurrent infections.

	Para densi	isite ty by		Ν	Aicrosatellites	1		SN	NP barco	de			Whole	Whole-genome sequencing				
Patient code	LM (p/µl)	C	OI	Discordont		C	OI	Dice		COI		IBS		IBD			
	Day0	DRec	Day0	DRec	MS	Туре	Day0	DRec	SNP	Туре	Day0	DRec	Disc. SNP	Туре	% IBD	Туре		
003	3470	0	3	2	0/3	homologous	1	1	1/38	heterologous	-	-	-	-	-	-		
007	41506	769	4	3	0/4	homologous	2	1	2/38	heterologous	>1	>1	14.3%	heterologous	35.2%	heterologous		
008	6068	3428	2	2	0/4	homologous	1	1	0/37	homologous	1	1	2.2%	homologous	99.9%	homologous		
010	5431	212	2	-	-	-	1	2	1/38	heterologous	-	-	-	-	-	-		
018	2408	103	3	1	0/2	homologous	2	-	-	-	>1	-	-	-	-	-		
021	3369	1225	3	3	4/4	heterologous	2	-	-	-	-	-	-	-	-	-		
022	13430	444	3	1	0/4	homologous	1	2	0/37	homologous	1	>1	6.8%	heterologous	98.1%	homologous		
027	5517	3268	3	3	0/4	homologous	1	-	-	-	1	1	2.3%	homologous	99.5%	homologous		
028	6849	24	4	-	-	-	1	-	-	-	-	-	-	-	-	-		
034	4010	1536	2	2	1/4	heterologous	1	2	0/38	homologous	1	1	1.6%	homologous	99.8%	homologous		
037	11401	310	1	-	-	-	1	-	-	-	1	1	2.0%	homologous	99.7%	homologous		
043	55892	7477	3	2	0/3	homologous	1	2	1/38	heterologous	>1	1	12.6%	heterologous	99.8%	homologous		
044	274	68	3	3	0/3	homologous	-	2	-	-	>1	-	-	-	-	-		
047	4927	91	2	2	1/3	heterologous	2	2	0/22	homologous	>1	>1	8.0%	heterologous	32.5%	heterologous		
048	13965	856	1	1	0/3	homologous	1	2	0/20	homologous	1	1	1.7%	homologous	99.5%	homologous		
050	5852	0	2	1	0/3	homologous	1	-	-	-	-	-	-	-	-	-		
053	11262	2469	2	4	0/4	homologous	1	2	0/38	homologous	-	1	-	-	-	-		
054	14096	440	2	2	0/4	homologous	1	2	5/38	heterologous	1	1	2.4%	homologous	100%	homologous		
Homolog rate:	jous recu	rrence		·		12/15 (80%)				6/11 (55%)				6/10 (60%)	b) 8/10 (80%)			
LM, light identity-b	microsco v-descent	py; DRe t.	ec, samp	les at da	y of recurrence	e; COI, comple	xity of ii	ıfection;	MS, micr	osatellites (Pvm	nsp1F3+	MS4+M	S10+PvS	Sal1814); IBS, id	dentity-by	v-state; IBD,		



Fig S5. Gene expression of *pvcrt* and *pvmdr1* and proportion of ring-stages in infections at **Day 0.** Gene expression ratios (R) of *pvcrt* (A) and *pvmdr1* (B) relative to reference gene β -*tubulin* are displayed in log-scale (y axis). Linear regression was fitted to data (black line with standard error bounds in grey) did not show an association between R and ring-stage percentage (*pvcrt*: coef.=-0.0003, p=0.886; *pvmdr1*: coef.=-0.001, p=0.655).



Fig S6. Gene expression of *pvcrt* **and** *pvmdr1* **in Day 0/DRec paired samples.** Differences in gene expression ratio R between infections at DRec and paired infections at Day 0 are shown for both *pvcrt* (A) and *pvmdr1* (B). Type of recurrences based on identity-by-descent (IBD) analysis is shown with coloured bars (black, IBD-homologous recurrence; grey, IBD-heterologous recurrence; white, undetermined by WGS).