

## **Appendix**

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**Table S1: Distribution (%) of var transcripts by DBL $\alpha$  domain type**

	<b>Uncomplicated (n=8)</b>	<b>Severe malaria (n=36)</b>	<b>P§</b>	<b>Severe anaemia (n=21)</b>	<b>Cerebral malaria (n=9)</b>	<b>P§</b>
Domain subtypes	Median [IQR]	Median [IQR]		Median [IQR]	Median [IQR]	
DBL $\alpha$ 1	9 [0 - 49]	23 [11- 57]	0.32	20 [7 - 57]	57 [21- 77]	0.10
DBL $\alpha$ 1.[1/2/4/7]	4 [0 - 9]	19 [9 - 57]	0.01	18 [4- 44]	57 [14- 8]	0.12
DBL $\alpha$ 1.5	0 [0 - 0]	0 [0 - 0]	0.22	0 [0 - 0]	0 [0 - 0]	0.51
DBL $\alpha$ 1.6	0 [0 - 0]	0 [0 - 0]	0.22	0 [0 - 0]	0 [0 - 0]	0.13
DBL $\alpha$ 1.8	0 [0 - 0]	0 [0 - 0]	1.00	0 [0 - 0]	0 [0 - 0]	0.44
DBL $\alpha$ 2	0 [0 - 24]	13 [0 - 39]	0.24	16 [0 - 42]	0 [0 - 19]	0.21
DBL $\alpha$ 0	43.7 [21.9 - 84.7]	20.8 [0 - 35.1]	0.05	14.1 [0 - 32.1]	23.0 [5.4 - 25.8]	0.80
VAR1	0 [0 - 2]	0 [0 - 0]	1.00	0 [0 - 0]	0 [0 - 0]	0.83
DBL $\alpha$ 1.[1/2/4/7] +DBL $\alpha$ 2 + DBL $\alpha$ 2 prediction*	9 [4 - 42]	65 [28- 83]	0.005	68 [28- 84]	63 [38- 68]	0.74
DBL $\alpha$ 1.[1/2/4/7] : (DBL $\alpha$ 2+DBL $\alpha$ 2- prediction*) ratio <sup>\$</sup>	1.0 [0.2-8.9]	0.9 [0.3 - 13.6]	0.87	0.6 [0.2 - 3.2]	7.0 [0.7 - 66.6]	0.09

§ P values were calculated using Wilcoxon rank sum test

\* Transcripts annotated with a DBL $\alpha$ 1 or DBL $\alpha$ 2 domain or predicted by the DBL $\alpha$  tag sequence to encode a DBL $\alpha$ 2 domain.

<sup>\$</sup> The patient-wise ratio between the level of transcripts encoding group A type DBL $\alpha$  domains (DBL $\alpha$ 1.[1/2/4/7]) and group B type DBL $\alpha$  domains (DBL $\alpha$ 2) coupled to CIDR $\alpha$ 1.[4-7] and CIDR $\alpha$ 1.[1/8] types, respectively, was calculated as follows:  
 $(\sum \text{DBL}\alpha 1.[1/2/4/7] + 0.01) : (\sum \text{DBL}\alpha 2/\alpha 2\text{-prediction} + 0.01)$ .

**Table S2: Proportions (%) of *var* transcripts encoding the DBL domains listed**

	Uncomplicated (n=8)	Severe malaria (n=36)	P§
Domain subtypes#	Median [IQR]	Median [IQR]	
DBL2β	49 [20 - 65]	66 [40 - 79]	0.17
DBL2β1	0 [0 - 0]	0 [0 - 14]	0.04
DBL2β3	0 [0 - 34]	12 [0 - 26]	0.74
DBL2β5	0 [0 - 26]	0 [0 - 11]	0.57
DBL2β12	0 [0 - 4]	4 [0 - 30]	0.13
DBL2β A type*	19 [0 - 47]	48 [23 - 75]	0.05
DBL2β BC type\$	5 [0 - 41]	0 [0 - 16]	0.31
DBL2γ	0 [0 - 3]	0 [0 - 4]	1
DBL2δ	34 [6 - 41]	7 [0 - 22]	0.05
DBLε/ζ	11 [6 - 62]	4 [0 - 20]	0.13

# The number before the Greek letter reflects that the domain is the second DBL domain encoded by the gene.

§ P values were calculated using Wilcoxon rank sum test.

\* DBL2β A type: DBLβ [1/3/6/7/9/11/12]. DBLβ12 is part of the group B/A chimeric DC8 PfEMP1; DBLβ12 was here considered a group A domain.

\$DBLβBC types: DBLβ[2/4/5/8/10/13]

**Figure S1. Annotation of *var* genes expressed in patients suffering from malaria**

Patient ID and clinical symptoms/grouping cerebral malaria (CM), severe anaemia (SA), hyper-parasitaemia (hyper), respiratory distress (RD) or uncomplicated malaria (UM) is shown. Patients who died are also indicated (†). Domain composition of the six most abundant *var* transcripts in each patient is shown. For some genes the long range PCR amplification was not successful and these genes are annotated based on the DBLα-tag sequence (*Group A*, *Group B/C*, *DBLα2 prediction* or *VAR1*). Transcript proportions are here given as the un-normalized proportion of the total DBLα-tag sequence read count within the patient.



Patient	Syndrome(s)	Haemoglobin (g/dL)	Blantyre score	Gene	Proportion	D1	D2	D3	D4	D5	D6	D7	D8	D9
2114	SA	3.8	5	2114-1	25%	DBLo.11	CIDR $\alpha$ 2.4	DBL $\beta$ 1	CIDR $\beta$ 1	Intron				
				2114-2	19%	DBLo.11	CIDR $\alpha$ 1.7	DBL $\beta$ 11	DBLy2	DBLy9	DBL $\delta$ 1	CIDR $\beta$ 3	Intron	
				2114-3	16%	DBLo.9	...							
				2114-4	16%	Group A								
				2114-5	8%	DBLo.5	CIDR $\delta$ 1	DBL $\beta$ 3	DBLy12	DBLy2	Intron			
				2114-6	3%	Group A								
2120	SA	3.9	5	2120-1	89%	DBLo.2 prediction								
				2120-2	9%	DBLo.1.7	CIDR $\gamma$ 3	DBL $\beta$ 6	DBLy2	DBLy11	...			
				2120-3	2%	DBLo.1.4	CIDR $\alpha$ 1.3	DBL $\beta$ 1	DBLy15	DBLy1	DBLy8	DBLy2	DBLy5	Intron VAR1
2121	SA	4.5	5	2121-1	19%	Group A								
				2121-2	14%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 1	DBLy4	DBL $\delta$ 1	CIDR $\beta$ 6	Intron		
				2121-3	9%	Group A								
				2121-4	6%	DBLo.0.11	CIDR $\alpha$ 2.4	DBL $\beta$ 1	CIDR $\beta$ 1	Intron				
				2121-5	5%	Group A								
2132	SA RD	4.4	3	2132-1	23%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 3	DBL $\beta$ 7	DBL $\delta$ 1	CIDR $\gamma$ 9	Intron		
				2132-2	17%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 1	DBLy13	DBLy12	DBL $\delta$ 5	CIDR $\beta$ 4	DBL $\beta$ 9	DBLy9 Intron
				2132-3	10%	DBLo.1.1	CIDR $\alpha$ 1.7	DBL $\beta$ 1	DBLy2	DBLy4	DBLy2	DBL $\gamma$ 3	DBL $\epsilon$ 6	Intron
				2132-4	7%	DBLo.0.1	CIDR $\alpha$ 3.4	...						
				2132-5	5%	DBLo.1.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy11	DBLy6	Intron			
				2132-6	4%	Group B/C								
2142	SA	3.4	5	2142-1	42%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy4	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				2142-2	12%	DBLo.0.6	CIDR $\alpha$ 3.4	DBL $\beta$ 5	DBLy16	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				2142-3	11%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy4	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				2142-4	7%	DBLo.2	CIDR $\alpha$ 1.8a	DBL $\beta$ 12	DBLy4	DBL $\gamma$ 3	DBL $\epsilon$ 12	Intron		
				2142-5	5%	DBLo.1.4	CIDR $\gamma$ 3	DBL $\beta$ 6	DBLy2	DBLy12	DBL $\delta$ 1	CIDR $\beta$ 1	Intron	
				2142-6	5%	Group A								
2157	SA RD	3.8	3	2157-1	17%	DBLo.1.8	CIDR $\beta$ 2	DBL $\gamma$ 7	DBLy11	DBL $\gamma$ 2	DBL $\epsilon$ 6	Intron		
				2157-2	17%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 3	DBLy2	DBLy12	DBL $\delta$ 5	CIDR $\beta$ 5	Intron	
				2157-3	15%	DBLo.2	CIDR $\alpha$ 1.8b	DBL $\beta$ 12	DBL $\beta$ 5	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				2157-4	7%	Group A								
				2157-5	6%	DBLo2 prediction								
				2157-6	5%	Group B/C								
2268	SA	4.5	5	2268-1	59%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 3	DBL $\beta$ 14	DBL $\gamma$ 5	DBL $\epsilon$ 4	Intron		
				2268-2	21%	DBLo.1.4	CIDR $\alpha$ 1.3	DBL $\beta$ 1	DBLy15	DBLy1	DBLy8	DBLy2	DBLy5	Intron VAR1
				2268-3	4%	Group B/C								
				2268-4	3%	Group B/C								
				2268-5	3%	Group B/C								
				2268-6	3%	Group B/C								
1873	CM RD	N/A	0	1873-1	42%	Group B/C								
				1873-4	23%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy13	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				1873-2	19%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 3	DBLy16	DBL $\delta$ 5	CIDR $\beta$ 4	DBL $\beta$ 7	DBLy12	Intron
				1873-3	9%	DBLo.13	CIDR $\alpha$ 2.3	DBL $\beta$ 1	CIDR $\beta$ 7	Intron				
				1873-5	4%	VAR1								
				1873-6	2%	Group B/C								
1890	Hyper	6.8	5	1890-1	24%	DBLo.0.11	CIDR $\alpha$ 2.4	DBL $\beta$ 1	CIDR $\beta$ 1	Intron				
				1890-2	18%	DBLo.1.2	CIDR $\alpha$ 1.5a	DBL $\beta$ 3	DBLy12	DBL $\delta$ 5	CIDR $\beta$ 4	DBL $\beta$ 9	DBLy6	Intron
				1890-3	15%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 5	DBL $\delta$ 1	CIDR $\beta$ 1	Intron			
				1890-4	10%	DBLo2 prediction								
				1890-5	5%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 3	DBLy11	...				
				1890-6	3%	Group A								
1914	CM Hyper	6.9	2	1914-21	37%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 1	DBLy6	DBLy11	DBL $\delta$ 1	CIDR $\gamma$ 9	Intron	
				1914-14	26%	DBLo.1.7	CIDR $\alpha$ 1.7	DBL $\beta$ 6	DBLy2	DBLy6	DBL $\delta$ 4	CIDR $\beta$ 1	Intron	
				1914-12	22%	DBLo.1.8	CIDR $\gamma$ 3	DBL $\beta$ 7	DBL $\delta$ 5	CIDR $\beta$ 3	DBL $\beta$ 9	DBLy11	...	
				1914-20	4%	DBLo.10	CIDR $\alpha$ 2.2	DBL $\beta$ 1	CIDR $\beta$ 11	Intron				
				1914-16	2%	Group B/C								
				1914-27	2%	Group B/C								
1918	CM	N/A	0	1918-1	26%	DBLo.1.2	CIDR $\alpha$ 1.5a	DBL $\beta$ 7	DBLy10	DBLy11	DBL $\delta$ 4	DBL $\beta$ 4	...	
				1918-2	17%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 3	DBLy11	DBLy2	DBL $\delta$ 1	CIDR $\beta$ 1	Intron	
				1918-5	9%	DBLo.1.2	CIDR $\alpha$ 1.5b	DBL $\beta$ 17	DBL $\delta$ 5	CIDR $\beta$ 3	DBL $\beta$ 7	DBLy2	...	
				1918-3	9%	DBLo.1.2	CIDR $\alpha$ 1.4	DBL $\beta$ 1	DBLy7	DBLy13	DBL $\delta$ 6	CIDR $\beta$ 2	Intron	
				1918-4	8%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy11	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				1918-6	4%	Group A								
1919	CM SA RD Hyper	3.4	2	1919-1	59%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy6	DBL $\delta$ 4	...			
				1919-2	21%	DBLo.1.7	CIDR $\alpha$ 1.4	DBL $\beta$ 3	DBLy2	DBLy4	Intron			
				1919-3	10%	DBLo.1.7	CIDR $\alpha$ 1.7	DBL $\beta$ 1	DBLy11	DBL $\delta$ 1	CIDR $\gamma$ 9	Intron		
				1919-4	7%	DBLo.2	CIDR $\alpha$ 1.1	DBL $\beta$ 12	DBLy4	DBLy14	DBL $\gamma$ 5	DBL $\epsilon$ 4	Intron	
				1919-5	3%	Group A								
				1945-2	14%	Group A								
1945	CM	7.5	0	1945-1	12%	DBLo.1.2	CIDR $\alpha$ 1.7	DBL $\beta$ 3	DBLy2	DBLy11	DBL $\gamma$ 2	DBL $\delta$ 1	CIDR $\beta$ 1	Intron
				1945-4	8%	DBLo.1	...							
				1945-3	8%	DBLo.1.8	CIDR $\gamma$ 3	DBL $\beta$ 7	DBL $\delta$ 5	DBL $\epsilon$ 11	DBL $\epsilon$ 12	Intron		
				1945-5	8%	DBLo.2	CIDR $\alpha$ 1.8b	DBL $\beta$ 12	DBLy4	DBLy6	CIDR $\beta$ 5	Intron		
				1945-6	7%	Group A								
				1950-1	30%	DBLo.1.2	CIDR $\alpha$ 1.5a	DBL $\beta$ 6	DBLy11	...				
1950	CM +	6.1	2	1950-2	9%	DBLo.1.2	CIDR $\alpha$ 1.6b	DBL $\beta$ 3	DBLy14	DBL $\delta$ 1	CIDR $\beta$ 1	Intron		
				1950-3	6%	DBLo.1.2	CIDR $\alpha$ 6	DBL $\beta$ 1	DBLy6	CIDR $\beta$ 1	Intron			
				1950-4	5%	Group B/C								
				1950-5	5%	Group B/C								
				1950-6	4%	Group B/C								
				1974-1	14%	DBLo.1.6	CIDR $\gamma$ 3	DBLy11	DBL $\delta$ 5	CIDR $\beta$ 3	DBL $\beta$ 6	Intron		
1974	CM	8.8	2	1974-2	13%	DBLo.1.11	CIDR $\alpha$ 2.4	DBL $\beta$ 1	CIDR $\beta$ 1	DBLy				



**Figure S2**

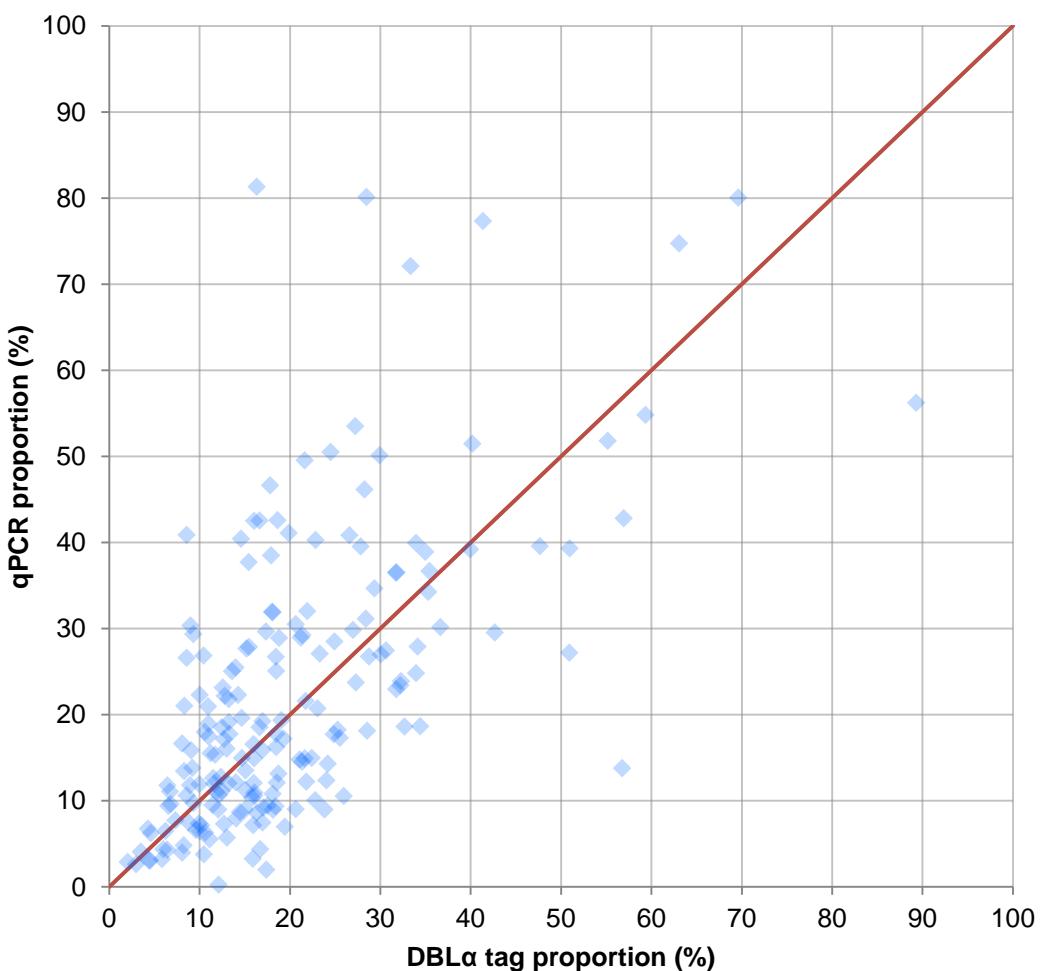


Figure S2: Plot of *var* transcript proportions determined by DBL $\alpha$ -tag analysis against qPCR reported transcript proportions. Red line added as a guide for perfect correlation.

**Figure S3**

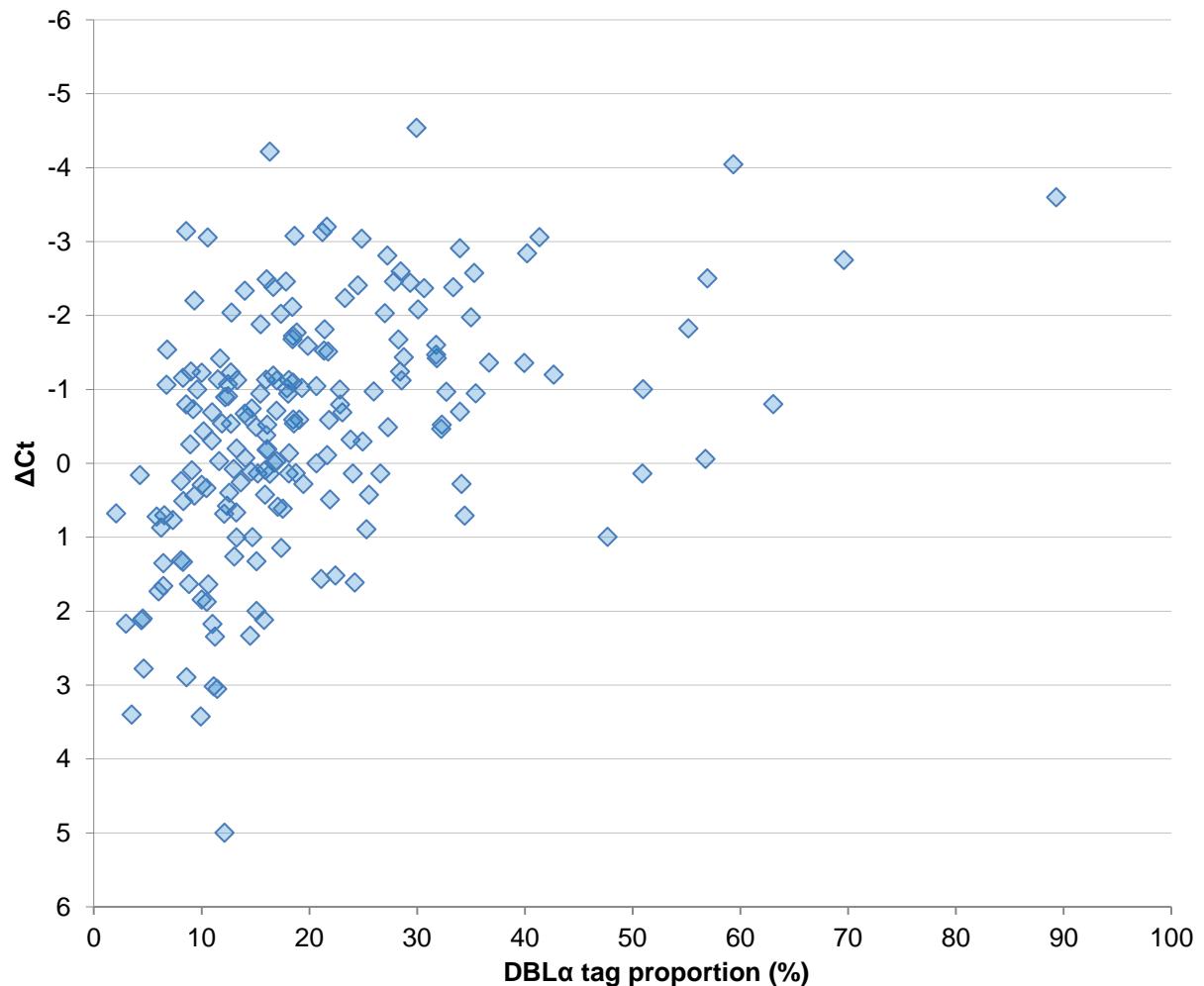


Figure S3: qPCR measured *var* transcript levels ( $\Delta Ct$  relative to endogenous control gene seryl-tRNA synthetase) plotted against the DBL $\alpha$ -tag determined transcript proportion.