Supplementary material

Supplementary figure 1 A)
In silico characterization of *var* gene primers used in the present study

Supplementary figure 1 B)
In silico comparison of new DBLα targeting primers to old versions published by Lavstsen et al. PNAS, 2012

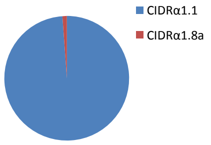
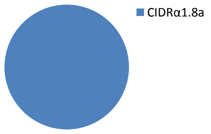
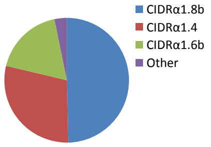
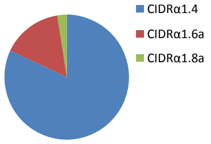
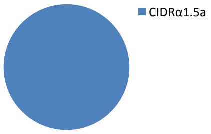
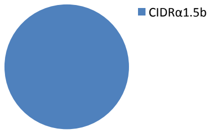
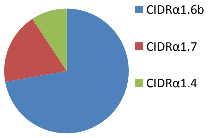
Supplementary Figure 2.
Overview of PfEMP1 domain classes, the estimated proportion of *var* genes encoding these, and predicted coverage of applied primer sets.

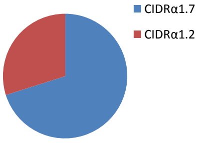
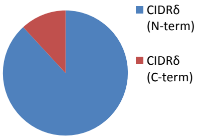
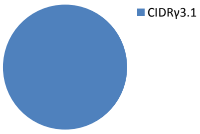
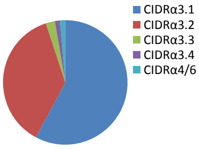
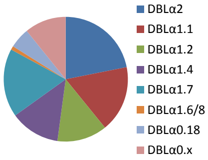
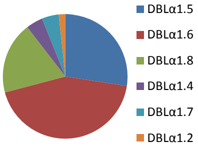
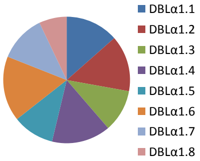
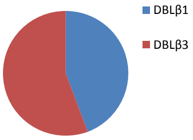
Supplementary Figure 3:
Transcript levels by primer sets reporting statistically significant different transcript levels between hospitalized patients with uncomplicated and severe malaria.

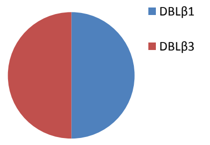
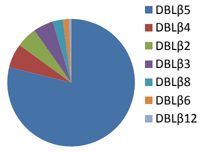
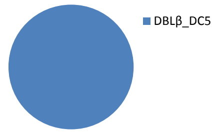
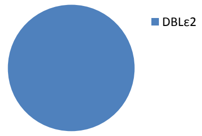
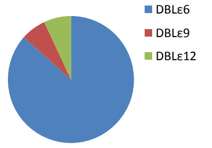
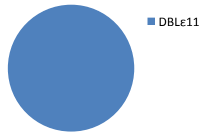
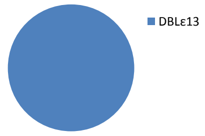
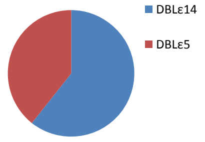
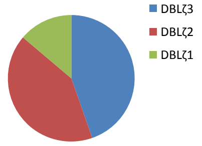
Supplementary Figure 4:
Median (25 and 75 percentiles, upper and lower adjacent values and outliers) *var* transcript levels (Tu) reported in children with severe malarial anaemia (N=44) or cerebral malaria (N=29) with the primer set targeting DBL1/3-1.

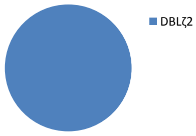
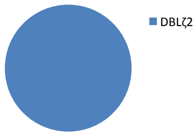
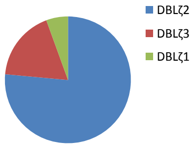
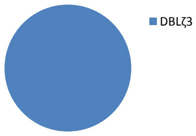
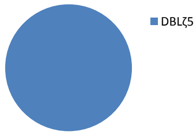
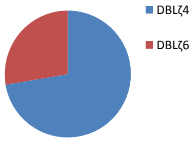
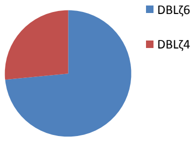
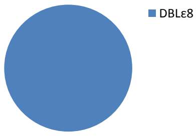
Supplementary Table 1.
Percentage of individuals of each patient group with *var* transcript levels higher than 16 Tu

Supplementary Figure 1A - In silico characterization of *var* gene primers used in the present study

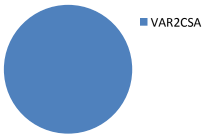
	Forward primers	Reverse primers	Amplicon size	Coverage	Specificity	
Primer name (Target domain type)	Mixed to a final total primer concentration of 20 µM	Mixed to a final total primer concentration of 20 µM	Median (Min - Max) bp	Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	Specificity graphic
CIDRa1.1 (CIDRα1.1)	TGGGAACATCAACTTAAGGATTGCATA TGGGAACATCAACTTAAGAATTGCATA TGGGAACATGAACTTAAGGATTGCATA	TAAATCTTYCNTAAATTGATHCCAT	270 (270-273)	274/290 (95%)	274/277 (99%)	
CIDRa1.8a (CIDRα1.8a)	ATAATTGTGAAATGAAAGGTTCA	TATGCAMTTCTTAAGTTTGGTTTCC	140 (140-140)	104/104 (100%)	104/104 (100%)	
CIDRa1.8b (CIDRα1.8b)	AATAGACAGTATAATGTGGGAA AAAGGATACTATAAAGTGGGAA	CAAAACATWTACAATTTTCGTTACA	101 (98-101)	61/61 (100%)	61/122 (50%) [Off target sequences also targeted by CIDRa1.4 and CIDRa1.6b primers, albeit all with 2 mismatches]	
CIDRa1.8 (CIDRα1.8)	Comment: A summarized transcript level for CIDRα1.8 domains (CIDRα1.8a+8b). There is no overlap between targeted sequences.					
CIDRa1.DC8	Comment: A summarized transcript level for DC8 domains (CIDRa1.1+CIDRα1.8a+8b). There is no overlap between targeted sequences.					
CIDRa1.4/6a (CIDRα1.4/6a)	AACTATCAAAAATGGGAATGCTATTA AACTATGAACAATGGAAATGCTATTA AACTATCAAAAATGGAATTGCTATTA AACTATGAAAACTGGCAATGCTATTA AACAATCAAATATGGAAATGCTATTA	AATGGGACAAACAAAAAACAAAATATG	163 (163-172)	CIDRα1.4: 281/345 (81%) CIDRα1.6a: 31/54 (58%)	312/319 (98%)	
CIDRa1.5a (CIDRα1.5a)	GATTTATGGATTAAGAATTTATTAAG GATTTGTGGGTTACGAATTTATTAAG GATTTGTGGGTTACATATTTATTAAG	TAATTCATCCGTAAATTTCTTCCA CAAATCTTCCTTAAGTTTTTTCCA TAATTCATCCGTAAATTGATTCCA CAAATCTTCTTTAAGTTTTTTCCA	306 (306-306)	98/104 (94%)	98/98 (100%)	
CIDRa1.5b (CIDRα1.5b)	ACGATACTATAGACTGGAAATACG ATTGGGAAWATAAACTTAAGACCTG TGGATACTACAGATTGGGATCGTA	AACCCATTGTTCAAAACATTTACA AACCCATTTATCAAAACACGTACA AACCCATTTATCAAAACACATACA	110 (98-113)	119/124 (96%)	114/114 (100%)	
CIDRa1.5	Comment: A summarized transcript level for CIDRα1.5 (CIDRa1.5a+CIDRa1.5b). There is no overlap between targeted sequences.					
CIDRa1.6b (CIDRα1.6b)	ATAATACTAATGTSACGGATTGT	CAGTTTCTTTATACTATCCCATTC ACATCCTTTATACTACCCCATTCC AATTCCTTTATACTCTTCCATTCTG	107 (104-107)	81/81 (100%)	81/112 (72%) [50% of the targeted genes are also targeted by 3 other primer sets: CIDRa1.4/7/8b]	

Primer name (Target domain type)	Forward primers	Reverse primers	Amplicon size	Coverage	Specificity	
	Mixed to a final total primer concentration of 20 µM	Mixed to a final total primer concentration of 20 µM	Median (Min - Max) bp	Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	Specificity graphic
CIDRa1.7 (CIDRα1.7)	CGGAAACTATAACGTGGAACGATAA CGGAAACTATAAGGTGGAACGATAA CGGAAACTATAACGTGGAAAGATAA GGATACTATAATGTGGAATGATAAA	TAGTTTCTTTATACTATTCCATTC TAGTTCCTTTATACTATTCCATTC TAGTTTCTTTATATTATTCCATTC TAGTTTCTTTATACTACTCCATTC TAATTCCTTTATATTATTCCATTC	148 (145-149)	277/282 (99%)	277/395 (70%) [unintended hits are all *var1* CIDRα1.2]	
CIDRa1.A (CIDRα1 of group A)	Comment: A summarized transcript level for all group A CIDRα1 domains (CIDRa1.4-7 primers). There is an estimated 2% of genes targeted twice by these primer sets.					
CIDRa1.all (All CIDRα1)	Comment: A summarized transcript level for all CIDRα1 domain primers. There is an estimated 5% of genes targeted twice by these primer sets.					
CIDRd (N-terminal CIDRδ)	TAAATGTAACTTAGATGTATGTGAAC TAAATGTAACTTACATGTATGTGAAC TAAATGTAACTTAGACGTATGTGAAC TAAATGTTACTTAGATGTATGTGAAC TAAATGTAACTTAGATATATGTGAAC TAAATGTAAGTTAGATGTATGTGAAC	AATACTTTAACCAACGTTTAATCAATAC AATACTTTAACCAACGCTTAATCAATAC AATACTGCAACCAACGTTTAATCAATAC AATGCTCTAACCAACGTTTAATGAATAC AATACATCAACCAACGCTTAATCAATAC	104 (104-104)	308/410 (75%)	308/350 (88%)	
CIDRg3.1 (CIDRγ3.1)	TATGTATATGCTGATGAACGTATTAC	TTCTATCCATTTTTCTAAACATTC	174 (174-174)	81/81 (100%)	81/81 (100%) [The N-terminal group A CIDRγ domains (ie. CIDRγ3 domains) are diversified into several small subgroups of which the CIDRγ3.1 is the largest (~20% of CIDRγ3)	
CIDRa3.1/2 (CIDRα3.1/2)	AHWWVCAAAAGACRTWCHATRATTT AHWWVCAAAAGACRTTCAATCCT ARAAAGTAAAGGATTATGTWGRTTT	TTTTTGTTCTCCAATRTATRGAATC	80 (80-81)	1479/1970 (75%) CIDRα2-6: 1536/9142 (17%)	1479/1536 (96%) CIDRα2-6: 1536/1536 (100%)	
DBLa2/1.1/2/4/7 (DBLα2/1.1/2/4/7)	GATTAYGTBCCTCAATTTTTAMGWTGGT	TACAATCATATCCATTAWGACTACAA TCACAATCGCATCCATTATGACTACAA	145 (145-157)	1422/1736 (82%)	1422/1489 (82%)	
DBLa1.5/6/8 (DBLα1.5/6/8)	GATTAYGTBCCTCAATTTTTAMGWTGGT	TTTTAGTACAATCATAACCATCACCA GATTTGTTTTWTTACAATCGTAACCCTC ACAATCCTCACCATCACCACTACAAT CGTGATATATCTGTTTKAGTACAATC GATCTGTTCGTTTACAATCGTAACCCTC	157 (144-173)	596/812 (73%)	596/666 (90%)	
DBLa1all (DBLα1.1-8)	TTGGGAAATGTRTTRGTTACAGCAAA TTGGGAAATGTGTTAGTTATGGCAAA TTGGGGAATTTGTTAGTTATGGCAAG TTGGGGAACCTATTAGTTATGGCAA TTAGGAAATATATTGGTAGCAGCAA TTAGGAAATATCTTGGTCACAGCAA	CCTATATCNGCAAAACTKCKWGC	116 (116-128)	2211/2404 (92%)	2211/2211 (100%)	
DBLb1/3-1 (Group A DBLβ1/3, subdomain 3)	TATACAAASAAGCAGAAATTTATGC TACGCAAAAGCACGAATTGTTGCTA	TTATAATACCCAGGACCRCCATT	52 (50-52)	N/A (8% of DBLβ3) (25% of DBLβ1 not *var1*)	N/A	

Primer name (Target domain type)	Forward primers	Reverse primers	Amplicon size	Coverage	Specificity	
	Mixed to a final total primer concentration of 20 µM	Mixed to a final total primer concentration of 20 µM	Median (Min - Max) bp	Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	Specificity graphic
DBLb1/3-2 (Group A DBLβ1/3, subdomain 3)	AACATGCACKAGTCGATATTGCT	CAGTAGAAGTWTTARGACCACCAT	51 (51-51)	N/A (5% of DBLβ3) (18% of DBLβ1 not *var1*)	N/A	
DBLb5-S1 (DBLβ5 subdomain 1)	AACAAAGTAGCGTATCAAATGCAT AACAAARYRGCACAMCWAATGCATVA	CCTTKTGWTGCATYRSCCYTYAA	92 (89-95)	521/1428 (37%)	521/661 (79%) [No target overlap with other DBLβ primers]	
DC5 (DBLβ domains in DC5)	GTTGCTCCYMCTTTTTGTAATGT CCCCCHCCYTTTTGTAAYGTNCC TTGCACCCATTTTTTGTAATATGCC	ACCACRTTGGTCGCATCTTTGTC CACTCACTATGTTGGTGYCATTTTT GCCASCACTTCWACTCCNACCAC YACAYTWACCACATTNGTSGM	69 (62-83)	388/500 (78%)	388/388 (100%) [No target overlap with other DBLβ primers]	
DBLe2 (DBLε2)	AAAWTTAATWGGTTTGGRAGCAC AATTTRATWGGTTTAAATGCAYACA	GACATAATTGTTGYACTCTAGGAGRMA	86 (85-89)	335/416 (80%)	335/335 (100%)	
DBLe6 (DBLε6)	GTTGCATAYAATGAAGGTTATTTCCT AAAACTRRACTTATGAATTSTGCCTACA ATTTCTTCAATATGYCTAYACTSAAGGA	ATCHGTWCCTTTAACTATATCTCCATA ATCTYTACCTTTTACTATATCAGCAAT	127 (117-135)	335/442 (76%)	335/387 (87%)	
DBLe11 (DBLε11)	AAAGHATTACAAAAAGAYGCATAT CATTACAAATATGTGCATATAATSAAG AAATACAGGAATCAGCATACAACGAAG	TTCCTTTAATTAAATYASCATAATCWGCA TCAGCATAACTYCTTTTCATATTTTCA	177 (151-178)	287/315 (91%)	287/287 (100%)	
DBLe13 (DBLε13)	CTGTTGCTGCAAATSAAGGATATAAT CTGCTGYTGCAAATGATGCATATAAT	ATAATCRTAAAAACTRTATTTCAATGCAT	107 (107-107)	185/196 (85%)	185/185 (100%)	
DBLe14 (DBLε14)	TTTAAYCAAGGAATWCTTTTAGGAA	ATATCTSCATARTCAGCAAAACTAT	95 (95-101)	117/137 (85%)	117/193 (61%)	
DBLe_all (DBLε2/6/11/13/14)	Comment: A summarized transcript level for all DBLe primers. There is no overlap between the primer sets summarized.					
DBLz2a (DBLζ2)	CCTCAACGTTTRAGATGGATGAAG	ATTTTGCAAACATAYTCTCCCCATT	50 (50-50)	64/336 (19%)	64/142 (45%)	

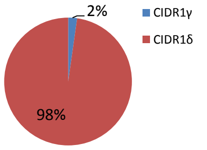
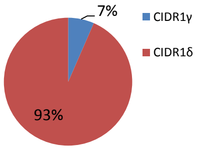
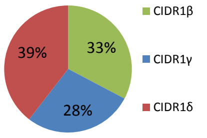
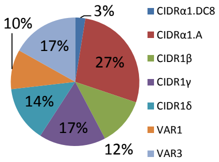
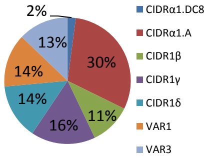
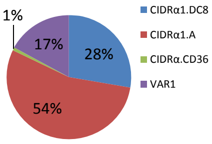
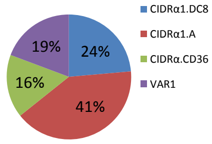
Primer name (Target domain type)	Forward primers	Reverse primers	Amplicon size	Coverage	Specificity	
	Mixed to a final total primer concentration of 20 µM	Mixed to a final total primer concentration of 20 µM	Median (Min - Max) bp	Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	Specificity graphic
DBLz2b (DBLζ2)	AGTGACCCTCCTGTGGATGATTA	TGCAATAATTTTCACTCCATTCCTT	73 (73-73)	114/336 (34%)	114/114 (100%)	
DBLz2c (DBLζ2)	ATGTRCCTCAAATACTTAGATGGATWA	TTCTTGCAATTCACAAAAATGTTC	62 (62-62)	104/336 (31%)	104/104 (100%)	
DBLz2 (DBLζ2)	Comment: A summarized transcript level for DBLζ2 domains (DBLz2a+DBLz2b+DBLz2c). There is no overlap between the primer sets summarized.			282/336 (84%)	282/371 (76%)	
DBLz3 (DBLζ3)	AACCTCCTTATGTTGATTACATTCCACA ATCCTCCTTATGATGATTATATWCCWCA	TTCTGACCATTCAGTCATCCATCT	59 (59-59)	90/264 (34%)	90/90 (100%)	
DBLz5 (DBLζ5)	GATTATGATTATATYCCTCAACCTT	TTCRCYCCATTCACTTAKCCAKCG	51 (51-51)	233/248 (94%)	233/233 (100%)	
DBLz4 (DBLζ4)	AACCTCCTGATTATGATTATATACCT	CAATAATATTCACTCCATTCTTGC	67 (67-67)	281/302 (93%)	281/390 (72%) [55% of targets also targeted by DBLz6]	
DBLz6 (DBLζ6)	CCTGATTATGATTATATWCCYCAACCTT	TTCACTCCATTCTTGCATSMAACG	54 (54-54)	324/400 (81%)	324/444 (73%)	
DBLz4/6 (DBLζ4/6)	Comment: Due to the considerable overlap between DBLz4 and DBLz6 primer sets, the transcript level for these domains were analysed using only the data from the primer reporting highest transcript level, *i.e* . either DBLz4 or the DBLz6 primer.					
DBLz_all (DBLζ)	Comment: The summarized transcript level for all DBLζ domains calculated as sum of DBLz2+z3+z4/6. There is no overlap between the primer sets summarized.					
var3 (DBLε8 of DC3 aka. DBLζ3-DBLε8)	AAGAGGATCTACTTAATGCTGCTTTTAG	AACTGAACTTCATAGCCTCATATGC	99 (99-99)	164/164 (100%)	164/164 (100%)	

↑ "DBLe8" from Lavstsen et al. PNAS, 2012

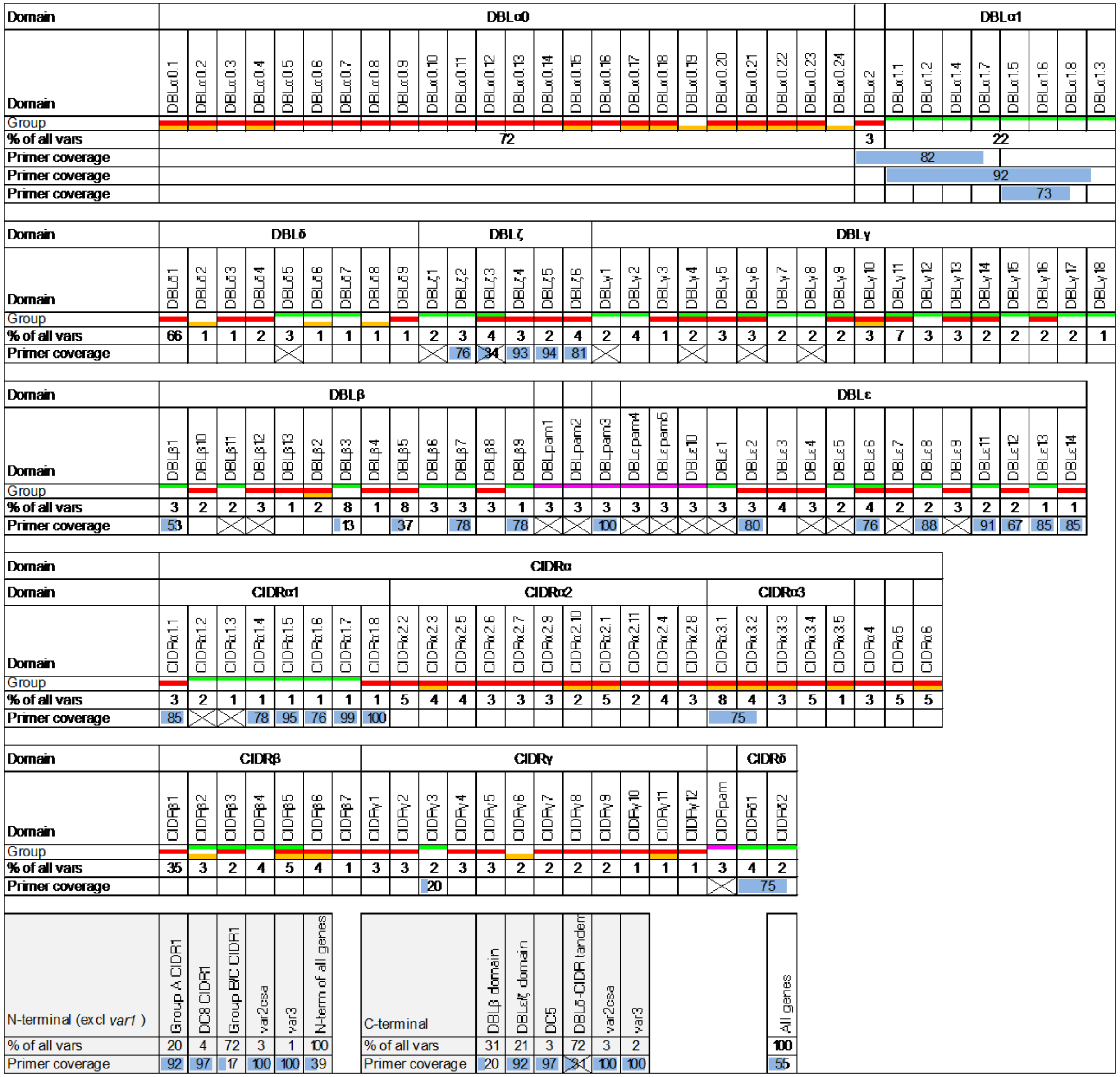
Primer name (Target domain type)	Forward primers Mixed to a final total primer concentration of 20 µM	Reverse primers Mixed to a final total primer concentration of 20 µM	Amplicon size Median (Min - Max) bp	Coverage Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Specificity	
					Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	Specificity graphic
var2csa (DBLpam3 aka. DBL3x)	AATGGGACAAACAAAAAACAAAATATG	GCTGATATACATTCAGGATAATTTTC	94 (94-94)	265/265 (100%)	265/265 (100%)	

↑ "T12/T13" from Sander et al. PLoS One, 2009

Supplementary Figure 1B - In silico comparison of new DBLa targeting primers to old versions published by Lavstsen et al. PNAS, 2012

Primer name (Target)	Forward primers Mixed to a final total primer concentration of 20 µM	Reverse primers Mixed to a final total primer concentration of 20 µM	Amplicon size Median (Min - Max) bp	Coverage Number of sequences amplified with intended domain class / number of sequences with intended domain class (%)	Specificity Number of sequences amplified with intended domain class / number of domain sequences amplified (%)	CIDR1 linkage Distribution of the CIDR1 subclasses linked to targeted DBLα sequences
DBLa1.5/6a Lavstsen et al PNAS, 2012	GATTAYGTCCCTCAATTTTTAMGWTGGT	GATTTGTTTTATTACAATCGTAACCCTC	157 (157-160)	26%	100%	
DBLa1.5/6b Lavstsen et al PNAS, 2012	ATTGTAGTMRKGAGGGTTACGATTGTA	CWTYTKCACAAYKTGGACAWTCTARATC	90 (90-90)	48%	97%	
DBLa1.5/6/8 (DBLα1.5/6/8) Present study	GATTAYGTBCCTCAATTTTTAMGWTGGT	GATTTGTTTTWTTACAATCGTAACCCTC GATCTGTTCGTTTACAATCGTAACCCTC TTTTAGTACAATCATAACCATCACCA ACAATCCTCACCATCACCACTACAAT CGTGATATATCTGTTTKAGTACAATC	157 (144-173)	73%	90%	
DBLa1 not var3 Lavstsen et al PNAS, 2012	TTGGGRAATBTGTTAGTTAYRGCAA	CTGCAAAACTKCGWGCAAG	109 (109-121)	72%	89%	
DBLa1all (DBLα1.1-8) Present study	TTGGGAAATGTRTTRGTTACAGCAAA TTGGGAAATGTGTTAGTTATGGCAAA TTGGGGAATTTGTTAGTTATGGCAAG TTGGGGAACCTATTAGTTATGGCAA TTAGGAAATATATTGGTAGCAGCAA TTAGGAAATATCTTGGTCACAGCAA	CCTATATCNGCAAAACTKCKWGC	116 (116-128)	92%	100%	
DBLa2/a1.1/2/4/7 Lavstsen et al PNAS, 2012	TCCTACCAATTTAGATTACGTCCCTCA	CGTACAATCATATCCATTAAGACTAC	160 (160-169)	33%	97%	
DBLa2/1.1/2/4/7 (DBLα2/1.1/2/4/7) Present study	GATTAYGTBCCTCAATTTTTAMGWTGGT	TACAATCATATCCATTAWGACTACAA TCACAATCGCATCCATTATGACTACAA	145 (145-157)	82%	82%	

Supplementary figure 1 **A)** In silico characterization of *var* gene primers used in the present study. **B)** In silico comparison of new DBLα targeting primers to old versions published by Lavstsen et al. PNAS, 2012. The primer sets DBLa1all, DBLa1.5/6/8 and DBLa2/1.1/2/4/7 target loci encoding DBLα domains, and replaced previously published primer sets designed for identical *var* subsets. A comparison of predicted coverage and specificity of new and old primer sets is given, as is a distribution of the C-terminally linked CIDR types of the targeted genes. The linkages to CIDR types help assess data reported by DBLa primers in relation to known binding phenotypes mediated by the CIDR domains.



Domain	DBLα0																									DBLα1							
Domain	DBLα0.1	DBLα0.2	DBLα0.3	DBLα0.4	DBLα0.5	DBLα0.6	DBLα0.7	DBLα0.8	DBLα0.9	DBLα0.10	DBLα0.11	DBLα0.12	DBLα0.13	DBLα0.14	DBLα0.15	DBLα0.16	DBLα0.17	DBLα0.18	DBLα0.19	DBLα0.20	DBLα0.21	DBLα0.22	DBLα0.23	DBLα0.24	DBLα2	DBLα1.1	DBLα1.2	DBLα1.4	DBLα1.7	DBLα1.5	DBLα1.6	DBLα1.8	DBLα1.3
Group	🟥🟧	🟥🟧	🟥	🟥🟧	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥🟧	🟥	🟥🟧	🟥🟧	🟧	🟥🟧	🟥🟧	🟥🟧	🟥🟧	🟧	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
% of all vars	72																								3	22							
Primer coverage																									82								
Primer coverage																										92							
Primer coverage																														73			

Domain	DBLδ									DBLζ						DBLγ																	
Domain	DBLδ1	DBLδ2	DBLδ3	DBLδ4	DBLδ5	DBLδ6	DBLδ7	DBLδ8	DBLδ9	DBLζ1	DBLζ2	DBLζ3	DBLζ4	DBLζ5	DBLζ6	DBLγ1	DBLγ2	DBLγ3	DBLγ4	DBLγ5	DBLγ6	DBLγ7	DBLγ8	DBLγ9	DBLγ10	DBLγ11	DBLγ12	DBLγ13	DBLγ14	DBLγ15	DBLγ16	DBLγ17	DBLγ18
Group	🟥	🟧	🟥	🟥	🟩	🟩🟧	🟩	🟧	🟥	🟩	🟩	🟩🟥	🟥	🟥	🟥	🟩	🟩	🟥	🟩🟥	🟥	🟩🟥	🟩	🟩	🟩🟥	🟥🟧	🟩🟥	🟩	🟩🟥	🟩🟥	🟩	🟩🟥	🟩	🟩
% of all vars	66	1	1	2	3	1	1	1	1	2	3	4	3	2	4	2	4	1	2	3	3	2	2	2	3	7	3	3	2	2	2	2	1
Primer coverage					X					X	76	X 34	93	94	81	X			X		X		X										

Domain	DBLβ																DBLε															
Domain	DBLβ1	DBLβ10	DBLβ11	DBLβ12	DBLβ13	DBLβ2	DBLβ3	DBLβ4	DBLβ5	DBLβ6	DBLβ7	DBLβ8	DBLβ9	DBLpam1	DBLpam2	DBLpam3	DBLεpam4	DBLεpam5	DBLε10	DBLε1	DBLε2	DBLε3	DBLε4	DBLε5	DBLε6	DBLε7	DBLε8	DBLε9	DBLε11	DBLε12	DBLε13	DBLε14
Group	🟩	🟥	🟩	🟥	🟥	🟥🟧	🟩	🟥	🟥	🟩	🟩	🟥	🟩	🟪	🟪	🟪	🟪	🟪	🟪	🟩	🟥	🟥	🟥	🟩	🟩🟥	🟥	🟩	🟥	🟩	🟥	🟩	🟥
% of all vars	3	2	2	3	1	2	8	1	8	3	3	3	1	3	3	3	3	3	3	3	3	4	3	2	4	2	2	3	2	2	1	1
Primer coverage	53		X	X			13		37		78		78	X	X	100	X	X	X	X	80		X	X	76	X	88	X	91	67	85	85

Domain	CIDRα																										
Domain	CIDRα1								CIDRα2												CIDRα3							
Domain	CIDRα1.1	CIDRα1.2	CIDRα1.3	CIDRα1.4	CIDRα1.5	CIDRα1.6	CIDRα1.7	CIDRα1.8	CIDRα2.2	CIDRα2.3	CIDRα2.5	CIDRα2.6	CIDRα2.7	CIDRα2.9	CIDRα2.10	CIDRα2.1	CIDRα2.11	CIDRα2.4	CIDRα2.8	CIDRα3.1	CIDRα3.2	CIDRα3.3	CIDRα3.4	CIDRα3.5	CIDRα4	CIDRα5	CIDRα6
Group	🟥	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟥🟧	🟥	🟥	🟥	🟥	🟥🟧	🟥🟧	🟥	🟥	🟥	🟥🟧	🟥🟧	🟥🟧	🟥	🟥🟧	🟥	🟥	🟥🟧
% of all vars	3	2	1	1	1	1	1	1	5	4	4	3	3	3	2	5	2	4	3	8	4	3	5	1	3	5	5
Primer coverage	85	X	X	78	95	76	99	100												75							

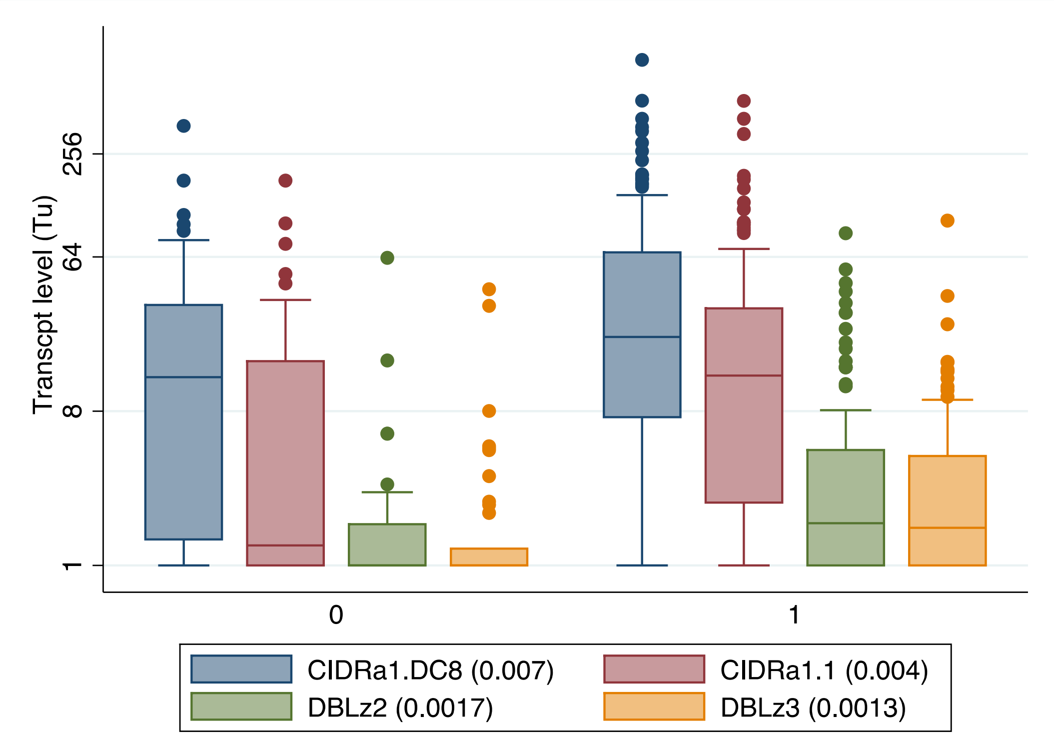
Domain	CIDRβ							CIDRγ													CIDRδ	
Domain	CIDRβ1	CIDRβ2	CIDRβ3	CIDRβ4	CIDRβ5	CIDRβ6	CIDRβ7	CIDRγ1	CIDRγ2	CIDRγ3	CIDRγ4	CIDRγ5	CIDRγ6	CIDRγ7	CIDRγ8	CIDRγ9	CIDRγ10	CIDRγ11	CIDRγ12	CIDRpam	CIDRδ1	CIDRδ2
Group	🟥	🟩🟧	🟩🟥	🟩	🟩🟧	🟥🟧	🟥	🟥	🟥	🟩	🟥	🟥	🟧	🟥	🟥	🟥	🟥	🟥🟧	🟥	🟪	🟩	🟩
% of all vars	35	3	2	4	5	4	1	3	3	2	3	3	2	2	2	2	1	1	1	3	4	2
Primer coverage										20										X	75	

N-terminal (excl *var1*)	Group A CIDR1	DC8 CIDR1	Group B/C CIDR1	var2csa	var3	N-term of all genes
% of all vars	20	4	72	3	1	100
Primer coverage	92	97	17	100	100	39

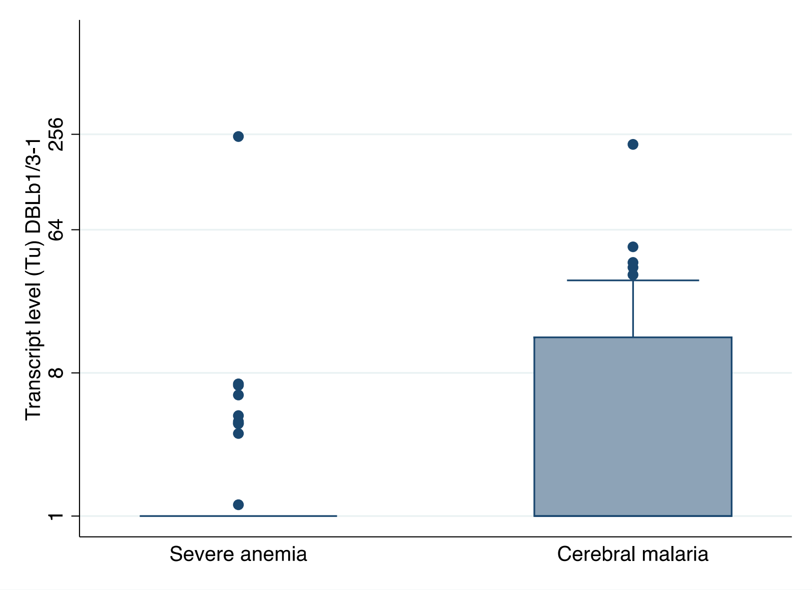
C-terminal	DBLβ domain	DBLε/ζ domain	DC5	DBLδ-CIDR tandem	var2csa	var3
% of all vars	31	21	3	72	3	2
Primer coverage	20	92	97	X 31	100	100

	All genes
% of all vars	100
Primer coverage	55

Supplementary Figure 2. Overview of PfEMP1 domain classes, the estimated proportion of *var* genes encoding these, and coverage of the primer set. X indicate domain classes indirectly accounted for by primers targeting linked domain classes.



Supplementary Figure 3: Transcript levels by primer sets reporting statistically significant different transcript levels between hospitalized patients with uncomplicated and severe malaria. Median (25 and 75 percentiles, upper and lower adjacent values and outliers) *var* transcript levels (Tu) reported in children hospitalized with uncomplicated (N=42) or severe malaria (N=123). P values are for comparison of levels in the two groups of patients (uncomplicated and severe malaria patients) (Wilcoxon rank sum test).



Supplementary Figure 4: Median (25 and 75 percentiles, upper and lower adjacent values and outliers) *var* transcript levels (Tu) reported in children with severe malarial anaemia (N=44) or cerebral malaria (N=29) with the primer set targeting DBL1/3-1.

Supplementary Table 1. Percentage of individuals of each patient group with *var* transcript levels higher than 16 Tu

Primer(s)	UM (%) (95% C.I.)	SM (%) (95% C.I.)	UM vs. SM P	SA (%) (95% C.I.)	CM (%) (95% C.I.)	SA vs. CM P
CIDRa1.all > 16 Tu	86	86		84	83	
CIDRa1.DC8 > 16 Tu	43 (28-58)	64 (56-73)	0.015	55	62	
CIDRa1.A > 16 Tu	67	61		52	59	
CIDRd > 16 Tu	14	12		13	24	
DBLb1/3-1 > 16 Tu	10	8		2 (0-12)	24 (12-42)	0.01
DBLz_all > 16 Tu	40	58		55	72	

P values calculated using Fishers Exact test. 95% confidence intervals only shown for P values < 0.05 shown.