

Cryo-electron microscopy of IgM-VAR2CSA complex reveals IgM inhibits binding of
Plasmodium falciparum to Chondroitin Sulfate A

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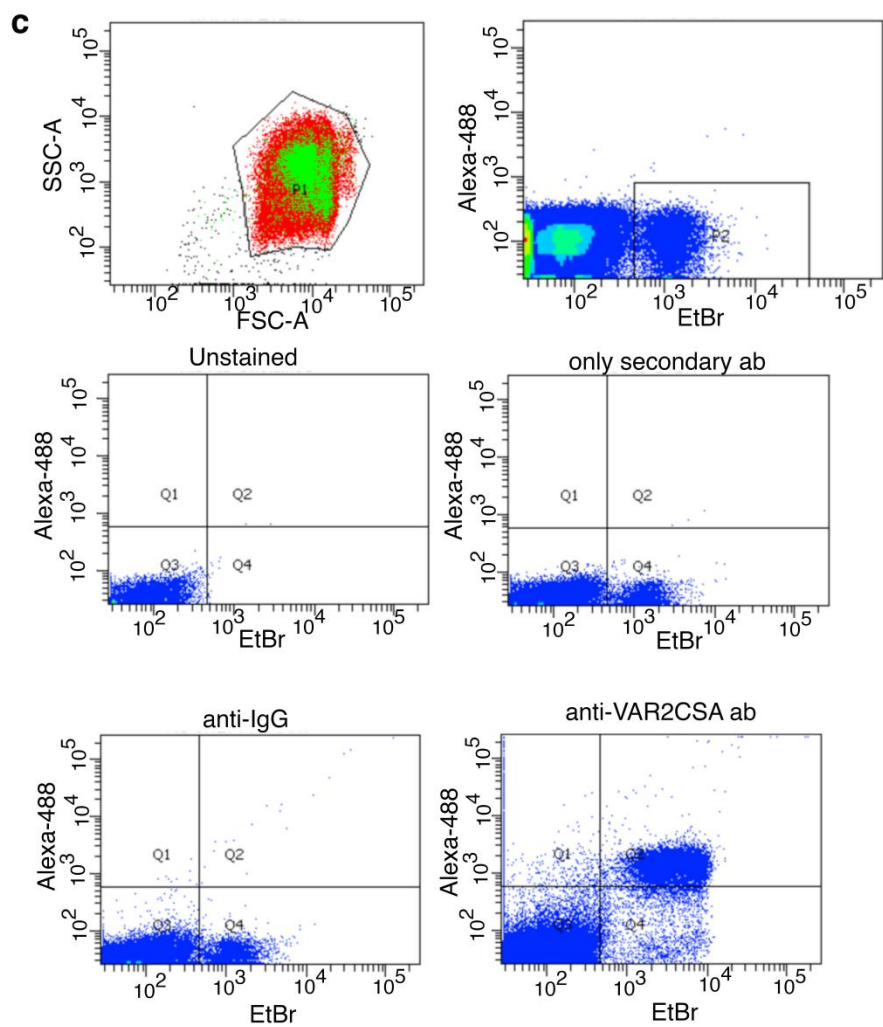
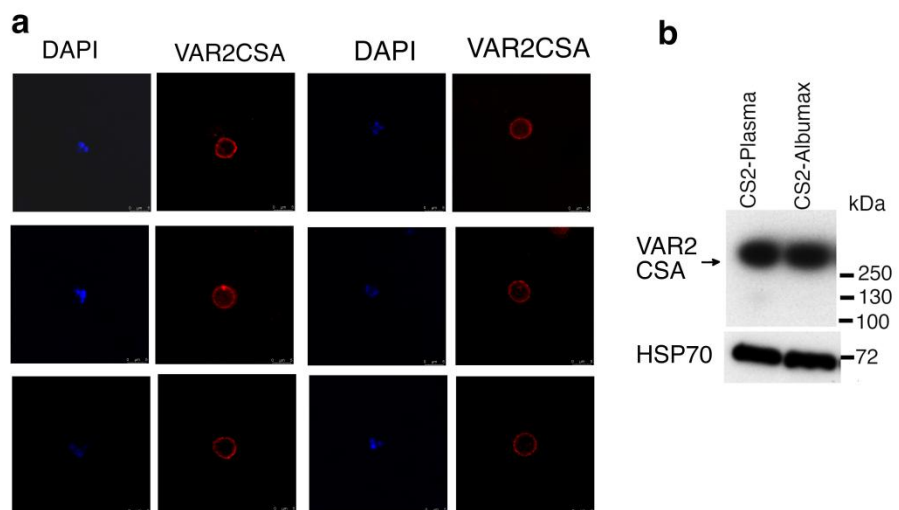
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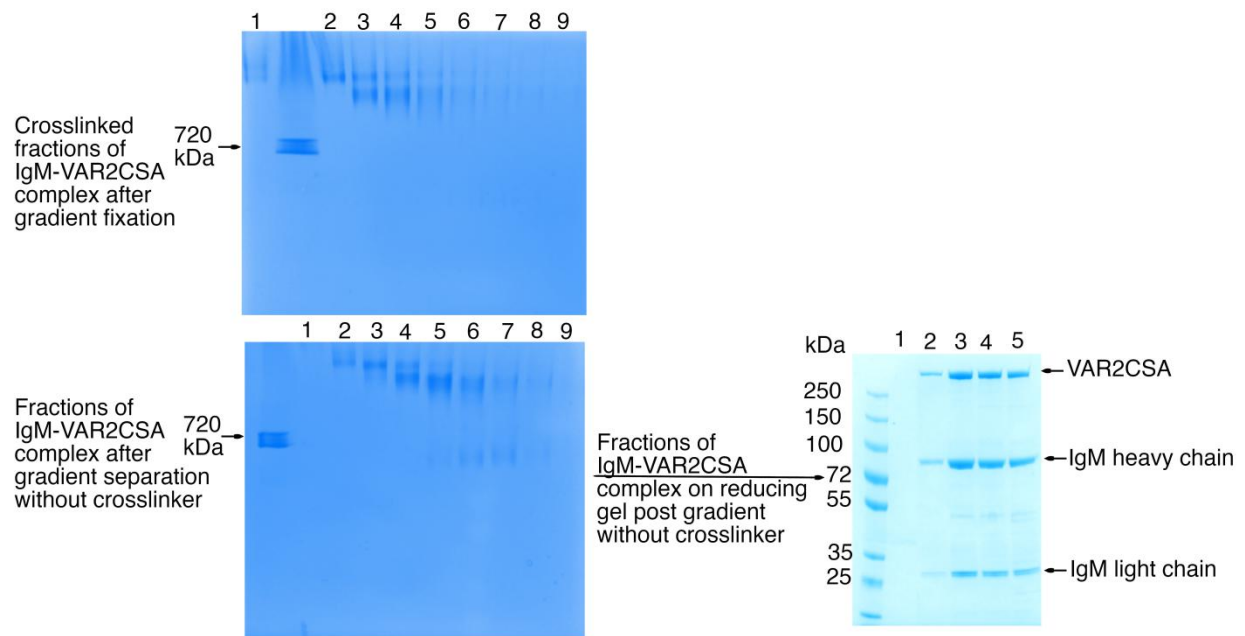
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³ Present address

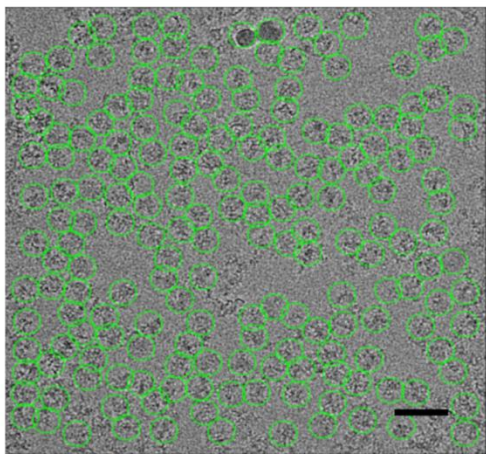
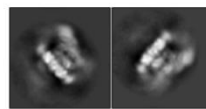
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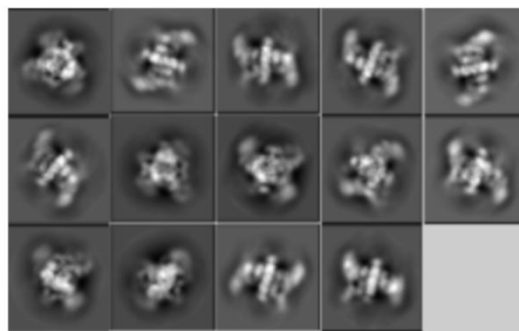
Supplementary Fig. 1. **a** Representative images of three experiments of VAR2CSA staining on IEs of CS2 using anti-VAR2CSA antibodies visualized using confocal microscope with LASX software at 63X objective lens. Scale bar-5 μ m. **b** Representative image of three experiments of western blot using anti-VAR2CSA and anti-PfHSP70 antibodies comparing the expression of VAR2CSA in CS2 grown in media containing normal plasma and albumax with HSP70 as loading control. **c** FACS analysis of CS2 IEs using anti-VAR2CSA antibodies. The RBCs were gated using FSC and SSC plots from the unstained cultures (P1 gate, top left) and IEs were gated after EtBr staining (top right, P2 gate). For analysis of staining, 10,000 IEs were counted and analyzed for alexa fluor 488 fluorescence after staining with anti-VAR2CSA antibodies, pre-immune rabbit IgG alone followed by anti-rabbit IgG alexa fluor 488, only secondary antibody were used as controls. Q1 represents population positive for only alexa fluor 488 signal, Q2 positive for both alexa fluor 488 and EtBr fluorescence, Q3 for unstained population and Q4 positive for only EtBr fluorescence. VAR2CSA expressing IEs are present in Q2 area.



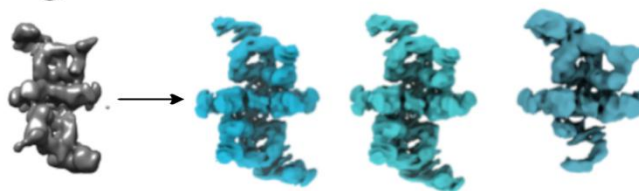
Supplementary Fig. 2. Fractions of gradient fixation (GraFix) of IgM-VAR2CSA complex in presence of crosslinker (top) and absence of crosslinker (bottom) analyzed after ultracentrifugation and fractionation on Native-PAGE. The non-crosslinked fractions 1 to 5 were also resolved on reducing SDS-PAGE(bottom, right) to show the presence of VAR2CSA as well as IgM in the complex.

a**b**

1:1 IgM:VAR2CSA ratio-
4590 particles



1:2 IgM:VAR2CSA ratio-
1.28 million particles

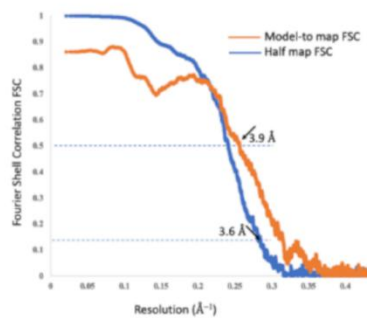
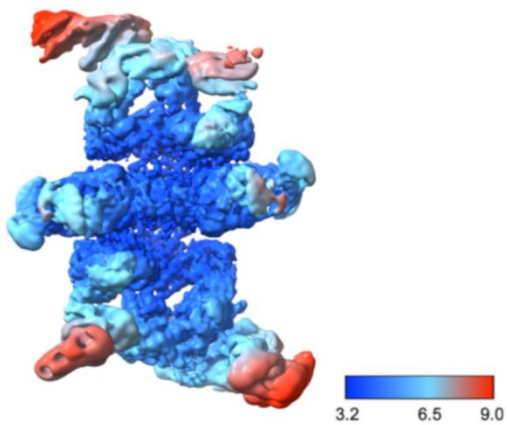
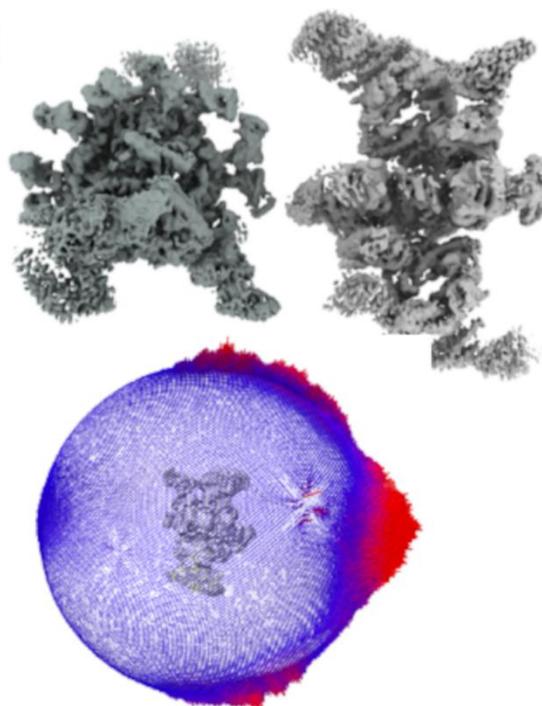
c

1289909 particles

401284 particles

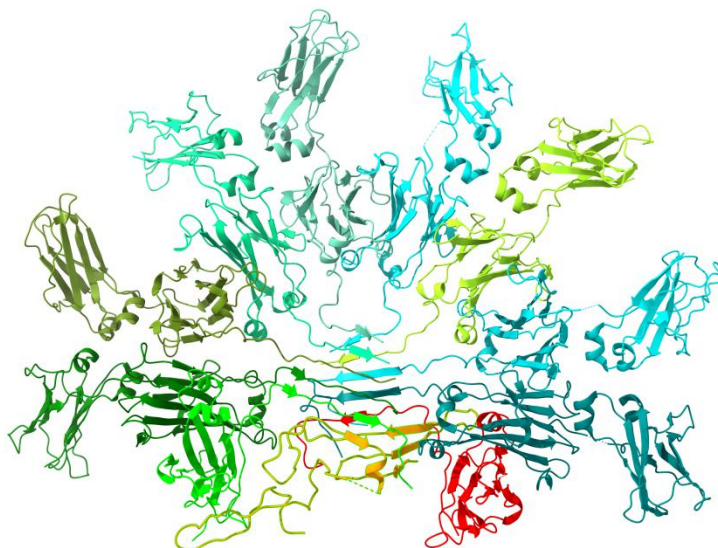
371049 particles

248220 particles

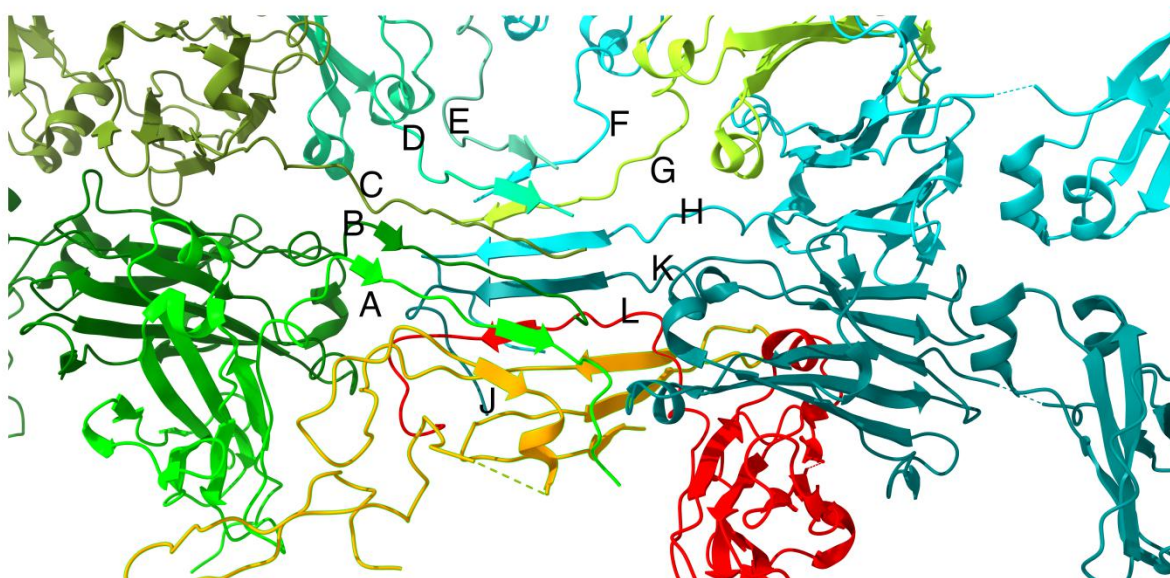
e**f****d**

Supplementary Fig. 3. Workflow for Cryo-EM image collection, processing and structure determination. **a** Representative raw micrograph of IgM-VAR2CSA complex collected on 300KeV Titan Krios microscope using falcon3 camera. The green circles shows picked particles. **b** 2D class averages of IgM-VAR2CSA complex. **c** Initial model generated from the 2D-class averages. 3D class averages along with number of particles in each class averages. **d** 3D-Autorefined cryo-EM map and Eulerian angle distribution of particles in angular build. **e** Gold standard Fourier Shell Correlation (FSC) of the half maps (blue) and of model-to-map FSC (orange). **f** Local resolution filtered map of IgM-VAR2CSA complex using RELION Localres.

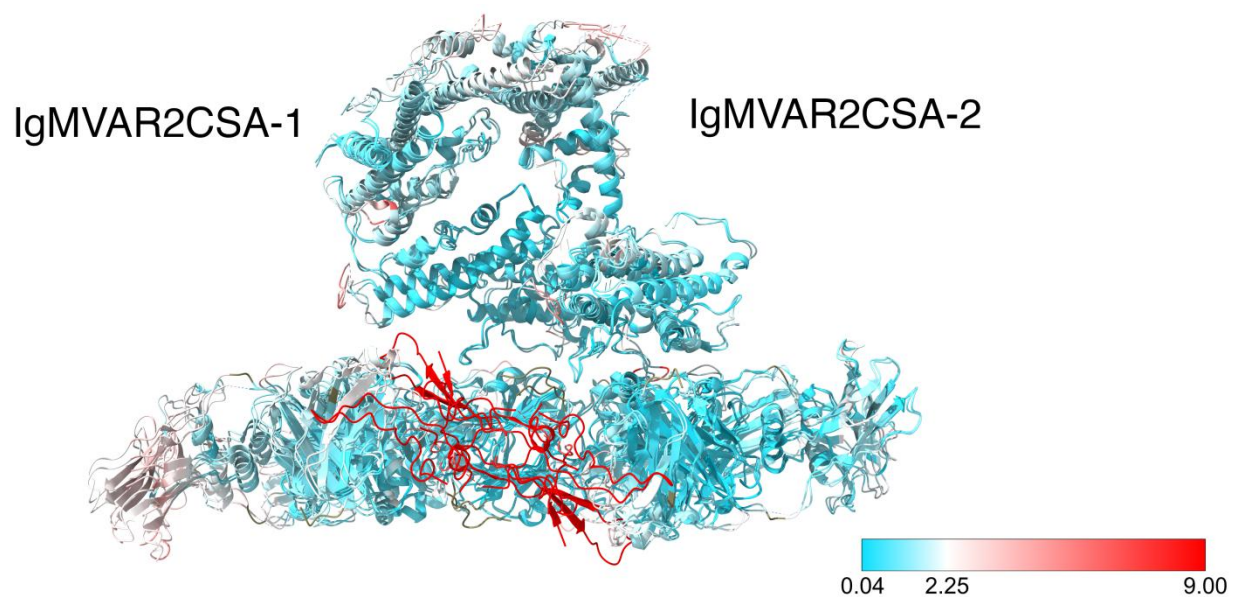
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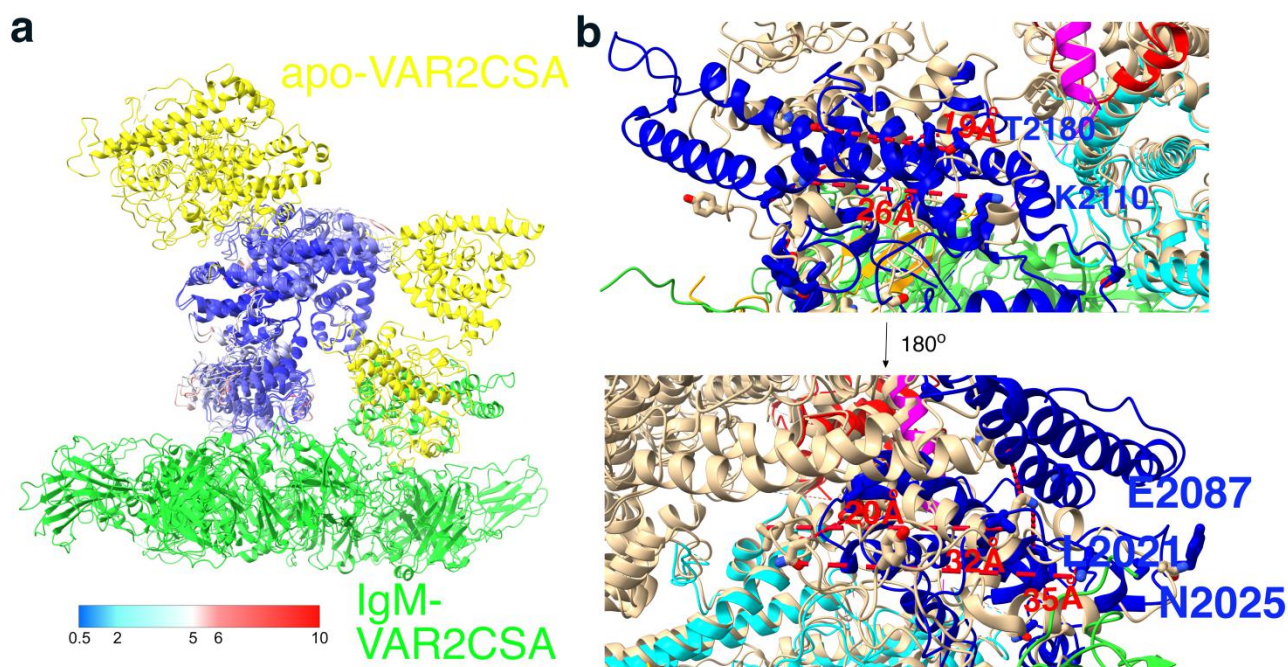
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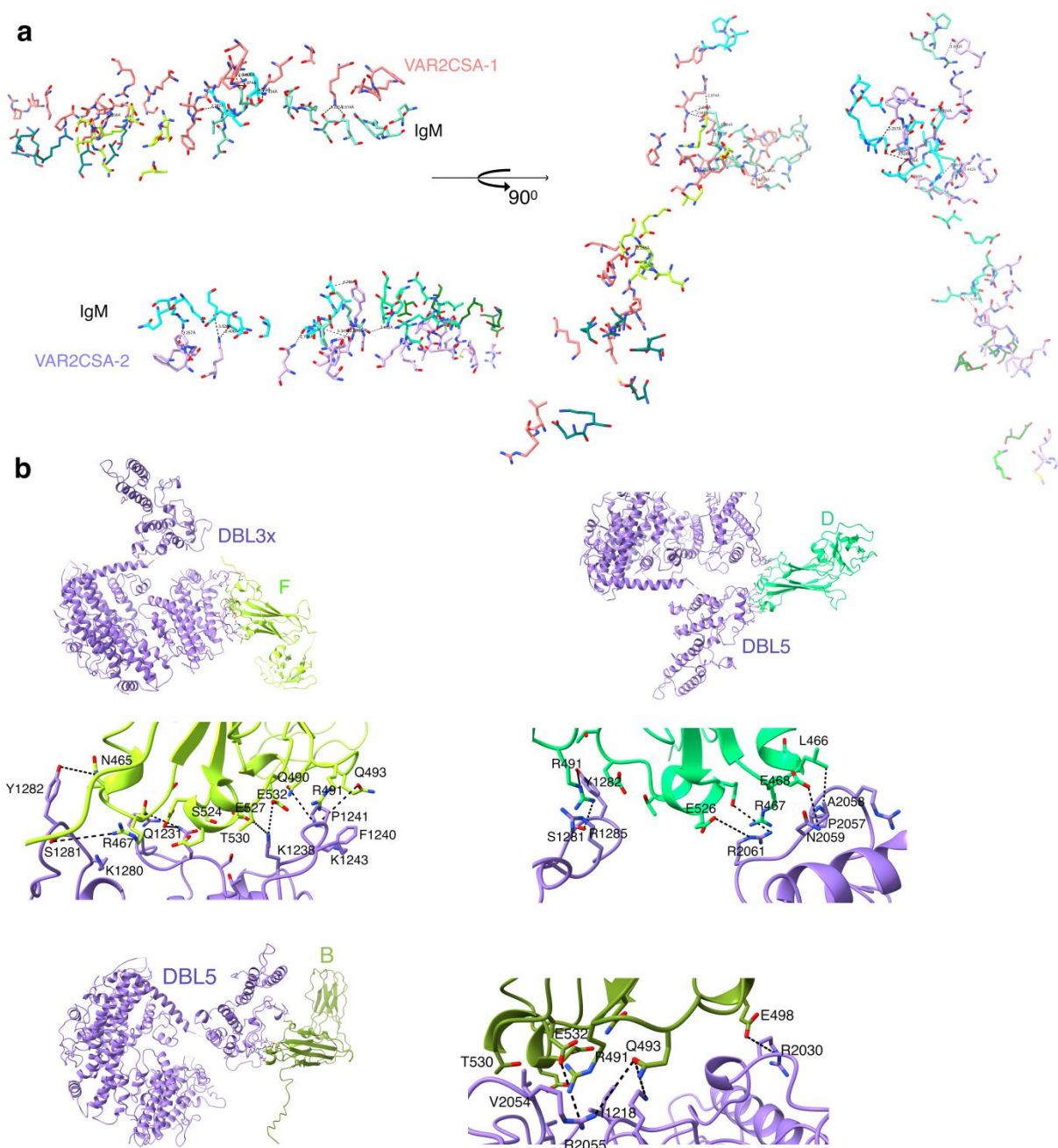
Supplementary Fig. 4. a The overall organization of IgM tailpieces depicted in different colors
b Five parallel β -sheets of A-E and F-L tailpieces; A (lime), B (green), C (olive drab), D (spring green), E (aquamarine), F (Cyan), G (green yellow), H (dark turquoise) and K (Teal), L (red) and J (orange) stack anti-parallel to stabilize the IgM core and facilitate formation of two identical faces.



Supplementary Fig. 5. Sequence RMSD based comparison of IgMVAR2CSA-1 and IgMVAR2CSA-2. The gradient from cyan to red show the increasing RMSD. The RMSD for 2696 residues is 0.99 Å and across 3261 residues, RMSD is 6.98 Å, due to J chain.



Supplementary Fig. 6. Comparison of apo-VAR2CSA(pdb7B52+pdb7NNH) and VAR2CSA in the complex. **a** Sequence RMSD based comparison of apo-VAR2CSA(yellow) and IgM-VAR2CSA (green). The gradient from blue to red show the increasing RMSD. The RMSD for 580 residues is 1.36 Å and across 865 residues, RMSD is 2.070 Å. Due to large conformational shift, Matchmaker did not include DBL5ε. **b** apo-VAR2CSA (beige). For the complex, IgM is colored green, VAR2CSA domains from Fig. 2b: ID2- red, DBL3x-cyan, ID3-magenta, DBL4ε- brown, DBL5ε-blue. The right panel shows shift of DBL5ε domain upon binding to IgM. The dashed red line shows the distances between corresponding residues of DBL5ε in apo-VAR2CSA and IgM-VAR2CSA.



Supplementary Fig. 7. a The orthogonal views showing the interfacing residues of VAR2CSA and IgM in the complex where 2 VAR2CSA (VAR2CSA-1; salmon, VAR2CSA-2 (medium purple) are bound by different chains of an IgM **b** Interacting interfaces along with respective atomic view of the interfacing residues between chain M (VAR2CSA) and chain F, chain D and chain B of IgM.

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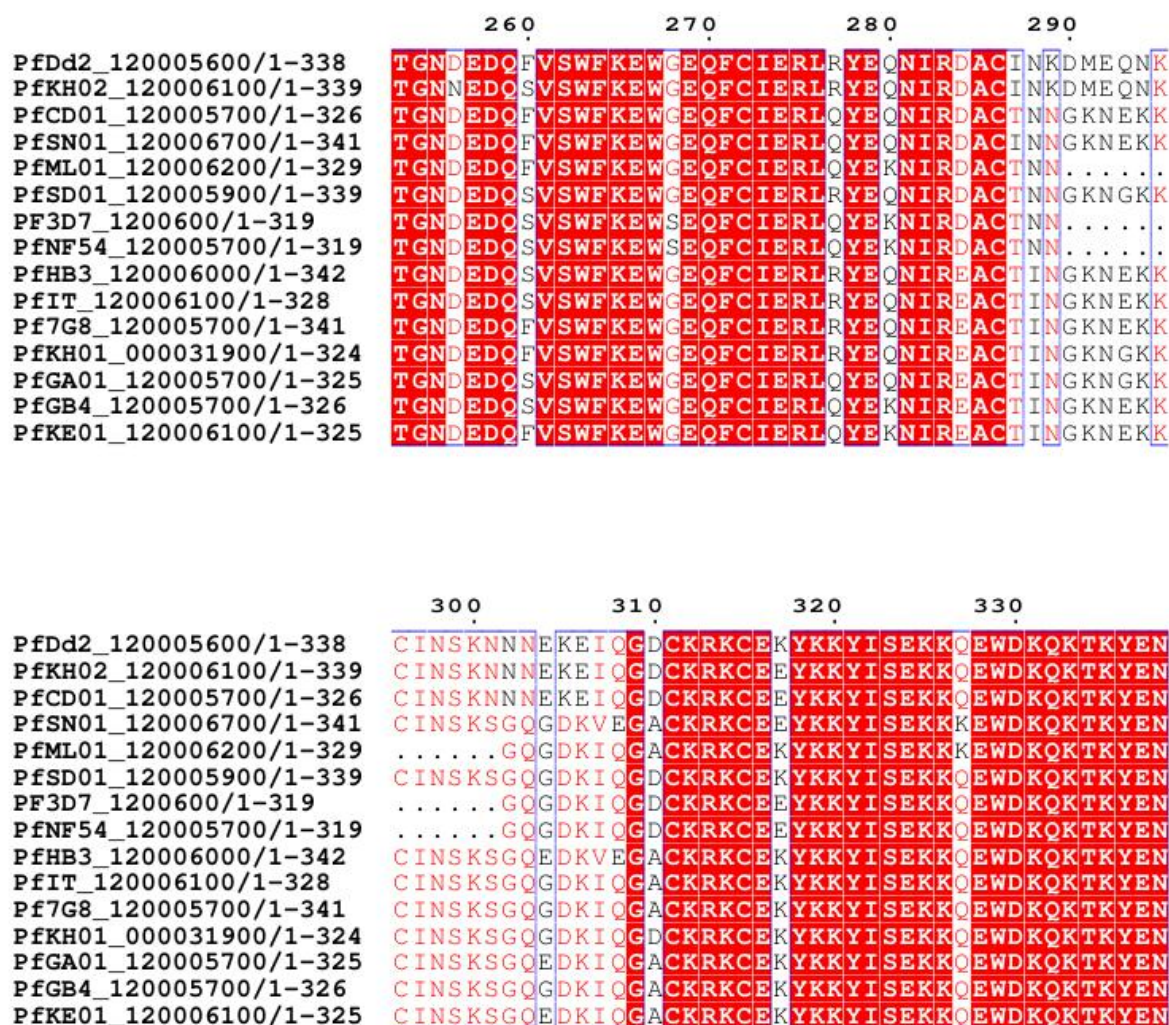
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Pf7G8_120005700/1-341	ILGTSVNTYEYIGKLQEDIKKIIE	KGTPQKDKIGG	VGSSTEN	
PfKH01_000031900/1-324	ILGTSVNIYEYIGKLQEDIKKIIE	QERTTK..E	KRVGSGAEN	
PfGA01_120005700/1-325	ILGTSVNIYEYIGKLQEDIKKIIE	QETTKQN..G	KT	VGSGADK
PfGB4_120005700/1-326	ILGTSVNIYEYIGKLQEDIKKIIE	QERTTK..E	KTVGSGADK	
PfKE01_120006100/1-325	ILGTSVNTYEYIGKLQEDIKKIIE	QGR.KPK..E	KRVGSGADK	

	220	230	240	250
PfDd2_120005600/1-338	VNDWWKEIEKDMWGAVKCGIKT	IKKQKK..NG	TFNGDECGV	SPP
PfKH02_120006100/1-339	VNDWWKEIEKDMWGAVKCGIKT	IKKQKK..NG	TYTGNECGV	SPP
PfCD01_120005700/1-326	VNDWWKEIEKDMWGAVKSGIKT	IKKQ..NNKC	TYTGNECGV	SPP
PfSN01_120006700/1-341	VNDWWKEIEKDMWGAVKCGIKT	IKKQ..NNKC	TYTGDECGIFPP	
PfML01_120006200/1-329	VNDWWKEIEKDMWGAVKCGITK	INKKQKKNG	TYTGDECGV	SPP
PfSD01_120005900/1-339	VNDWWKEIEKDMWGAVKCAITK	INKKNN..KC	TYTGNECGV	SPP
Pf3D7_1200600/1-319	VNAWWKGIEGEMWDVRCAITK	INKKQKKNG	TFSIDECGI	FPP
PfNF54_120005700/1-319	VNAWWKGIEGEMWDVRCAITK	INKKQKKNG	TFSIDECGI	FPP
PfHB3_120006000/1-342	VNDWWKGIEGEMWGAVKCGIKT	INKKQKKNG	TYTGNECGV	SPP
PfIT_120006100/1-328	VNAWWKGIEREMWDVRCAITK	INK..KNNNS	IFNGDECGV	SPP
Pf7G8_120005700/1-341	VNAWWKGIEGEMWDVRCAITK	IKK..QKKNG	TFNGDECGV	SPP
PfKH01_000031900/1-324	VNAWWKEIEKDMWGAVKCGIKT	IKK..QKKNG	TFNGDECGV	SPP
PfGA01_120005700/1-325	VNAWWKGIEGEMWGAVRCAITK	INK..KKKNG	TFSIDECGI	FPP
PfGB4_120005700/1-326	VNDWWKEIEGEMWDVRCGITK	INK..KNNNS	IFNGNECGN	SPS
PfKE01_120006100/1-325	VNDWWKEIEKDMWGAVKCGIKT	IKK..QKKNG	TFSIDECGV	SPP



Supplementary Fig. 8. ESPrIPT multiple sequence alignment of DBL3x domains of *P. falciparum* isolates.

	1	10	20	30	40
PfDd2_120005600/1-392	KYSA	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfSD01_120005900/1-381	KYSE	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDK
PfIT_120006100/1-378	KYSA	HKMKCTEVY	LEHV	EEQLNEIDNAIKDY	QLYPLDRCFDDQ
PfKH02_120006100/1-382	KYSA	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
Pf7G8_120005700/1-391	KYSK	LNLCCTEVY	LEHV	EEQLNEIDNAIKDY	QLYPLDRCFDDQ
PfGB4_120005700/1-386	KYSK	LNLCCTEVY	LDH	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfHB3_120006000/1-372	KYSA	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDK
PfCD01_120005700/1-392	KYSA	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfML01_120006200/1-388	KYSK	LNLCCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfSN01_120006700/1-371	KYSE	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
Pf3D7_1200600/1-387	KYSE	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDK
PfNF54_120005700/1-387	KYSE	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDK
PfGA01_120005700/1-377	KYSE	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfKE01_120006100/1-365	KYSA	HKMKCTEVY	LDH	EEQLKEIDNAIKDY	QLYPLDRCFDDQ
PfKH01_000031900/1-379	KYSA	HKMKCTEVY	LEHV	EEQLKEIDNAIKDY	QLYPLDRCFDDQ

	50	60	70	80
PfDd2_120005600/1-392	TKMKICDLIGDAIGCK	HKINLDELDEWNDMD	MDRDPY	YNKYKGV
PfSD01_120005900/1-381	SKMKVCDLIGDAIGCK	DKTNLEELDEWNDMD	DLRDPY	YNKYKGV
PfIT_120006100/1-378	TKMKVCDLIGDAIGCK	DKTKLDELDEWNDMD	DLRGT	YNKHKGV
PfKH02_120006100/1-382	TKMKICDLIGDAIGCK	HKINLDELDEWNDMD	MDRDPY	YNKYKGV
Pf7G8_120005700/1-391	TKMKICDLIGDAIGCK	DKTKLDELDEWNDMD	MDRDPY	YNKYKGV
PfGB4_120005700/1-386	TKMKICDLIGDAIGCK	HKINLDELDEWNDV	DMRGT	YNKHKGV
PfHB3_120006000/1-372	SKMKVCDLIGDAIGCK	DKTKLDELDEWNDV	DMRGT	YNKHKGV
PfCD01_120005700/1-392	TKMKVCDLIGDAIGCK	DKTNLEELDEWNDMD	DLRDPY	YNKYKGV
PfML01_120006200/1-388	TKMKICDLIGDAIGCK	DKTKLDELDEWNDV	DMRDPY	YNKHKGV
PfSN01_120006700/1-371	TKMKICDLIGDAIGCK	DKTKLDELDEWNDV	DMRDPY	YNKHKGV
Pf3D7_1200600/1-387	SKMKVCDLIGDAIGCK	HKTKLDELDEWNDV	DMRDPY	YNKYKGV
PfNF54_120005700/1-387	SKMKVCDLIGDAIGCK	HKTKLDELDEWNDV	DMRDPY	YNKYKGV
PfGA01_120005700/1-377	TKMKICDLIRDAIGCK	DKTKLDELDEWNDMD	DLRDPY	YNKYKGV
PfKE01_120006100/1-365	TKMKVCDLIGDAIGCK	DKTKLDELDEWNDV	DMRDPY	YNKYKGV
PfKH01_000031900/1-379	TKMKVCNLI	IGDAIGCKDKTKL	DELDEWNDMD	DLRDPY

	90	100	110	120
PfDd2_120005600/1-392	IPPRRRQLCF	SRIVRG	PANLRS	LNEFKEEILKGAO
PfSD01_120005900/1-381	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfIT_120006100/1-378	IPPRRRQLCF	SRIVRG	PANLRS	LNEFKEEILKGAO
PfKH02_120006100/1-382	IPPRRRQLCF	SRIVRG	PANLRS	LNEFKEEILKGAO
Pf7G8_120005700/1-391	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfGB4_120005700/1-386	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfHB3_120006000/1-372	IPPRRRQLCF	SRIVRG	PANLRS	LNEFKEEILKGAO
PfCD01_120005700/1-392	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfML01_120006200/1-388	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfSN01_120006700/1-371	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
Pf3D7_1200600/1-387	IPPRRRQLCF	SRIVRG	PANLRNLKEFKEEILKGAO	SEGKFLGN
PfNF54_120005700/1-387	IPPRRRQLCF	SRIVRG	PANLRNLKEFKEEILKGAO	SEGKFLGN
PfGA01_120005700/1-377	IPPRRRQLCF	SRIVRG	PANLRS	LNEFKEEILKGAO
PfKE01_120006100/1-365	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGAO	SEGKFLGN
PfKH01_000031900/1-379	IPPRRRQLCF	SRIVRG	PANLRNLNEFKEEILKGA	SEGKFLGN

	130	140	150	160
PfDd2_120005600/1-392	YYKEH.....	KDKEKALEAMKNSFYDYEY	TIKGS	SDILENI
PfSD01_120005900/1-381	YYNEDKGKEKKE.	DRKEKALEAMKNSFYDYED	IIKGT	DMLANI
PfIT_120006100/1-378	YYKEH.....	KDKEKALEAMKNSFYDYED	IIKGT	DMLTNI
PfKH02_120006100/1-382	YYKEH.....	KDKEKALEAMKNSFYDYEY	IIKGS	DILENI
Pf7G8_120005700/1-391	YYNEDDKDKEKKE.	DRKEKALEAMKNSFYDYED	IIKGS	DMLANI
PfGB4_120005700/1-386	YYNEDDKDKEKKE.	DRKEKALEAMKNSFYDYEY	IIKGS	DILENI
PfHB3_120006000/1-372	YYNEDKGKEKKE.	DRKEKALEAMKNSFYDYED	IIKGT	DMLTNI
PfCD01_120005700/1-392	YYKEHKGNYKEH	KDKEKALEAMKNSFYDYEY	IIKGT	DMLANI
PfML01_120006200/1-388	YYKEHKGNYKEH	KDKEKALEAMKNSFYDYEY	IIKGS	DILENI
PfSN01_120006700/1-371	YYKEKKDKKEKKEH	KDKEKALEAMKNSFYDYEY	IIKGT	DILENI
Pf3D7_1200600/1-387	YY.....NED	KDKEKALEAMKNSFYDYEY	IIKGS	DMLTNI
PfNF54_120005700/1-387	YY.....NED	KDKEKALEAMKNSFYDYEY	IIKGS	DMLTNI
PfGA01_120005700/1-377	YYKEHKGNYNED	KDKEKALEAMKNSFYDYEY	IIKGS	DILENI
PfKE01_120006100/1-365	YYKEH.....	KDKEKALEAMKNSFYDYEY	IIKGS	DMLTNI
PfKH01_000031900/1-379	YYKEH.....	KDKEKALEAMKNSFYDYED	IIKGS	DMLANI

	170	180	190	200
PfDd2_120005600/1-392	EFKDIKRKLDKLLT	KETNN	TKKAE	DWWE
PfSD01_120005900/1-381	QFKDIKI	KLDKLLT	KETN.	TKKVD
PfIT_120006100/1-378	EFKDIKI	KLDRLLE	KETN.	TKKAE
PfKH02_120006100/1-382	EFKDIKRKLDKLLT	KETNN	TKKAE	DWWE
Pf7G8_120005700/1-391	QFKDIKR	KLDKLLT	TKTNN	IRNAE
PfGB4_120005700/1-386	EFKDIKR	KLDKLLT	KETN.	TKKAE
PfHB3_120006000/1-372	EFKDIKM	KLDKLLT	KETN.	TKKAE
PfCD01_120005700/1-392	QFKDIKI	KLDKLLT	KETNN	TKKAE
PfML01_120006200/1-388	QFKDIKR	KLDKLLLE	KETNN	IQNAE
PfSN01_120006700/1-371	QFKDIKR	KLDKLLT	KETNN	IQNAE
Pf3D7_1200600/1-387	QFKDIKR	KLDRLLE	KETNN.	TEKVD
PfNF54_120005700/1-387	QFKDIKR	KLDRLLE	KETNN.	TEKVD
PfGA01_120005700/1-377	QFKDIKR	KLDRLLE	KETNN.	TKKVD
PfKE01_120006100/1-365	EFKDIKR	KLDKLLT	KETNN.	TKKVD
PfKH01_000031900/1-379	KFKDIKI	KLDKLLT	KETNN.	TKKAE

	210	220	230	240	250
PfDd2_120005600/1-392	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfSD01_120005900/1-381	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
PfIT_120006100/1-378	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfKH02_120006100/1-382	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
Pf7G8_120005700/1-391	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
PfGB4_120005700/1-386	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfHB3_120006000/1-372	KKSGNKI	IDRSWC	KIPTTE	TPQFLRW	KEWGNV
PfCD01_120005700/1-392	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
PfML01_120006200/1-388	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
PfSN01_120006700/1-371	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
Pf3D7_1200600/1-387	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfNF54_120005700/1-387	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfGA01_120005700/1-377	KKSGNKI	IDPSWC	KIPTTE	KTPQFLRW	KEWGT
PfKE01_120006100/1-365	KKSGNKI	IDPSWC	KIPTTE	TPQFLRW	KEWGT
PfKH01_000031900/1-379	KKSGNKI	IDPSWC	KIITTE	KTPQFLRW	KEWGT

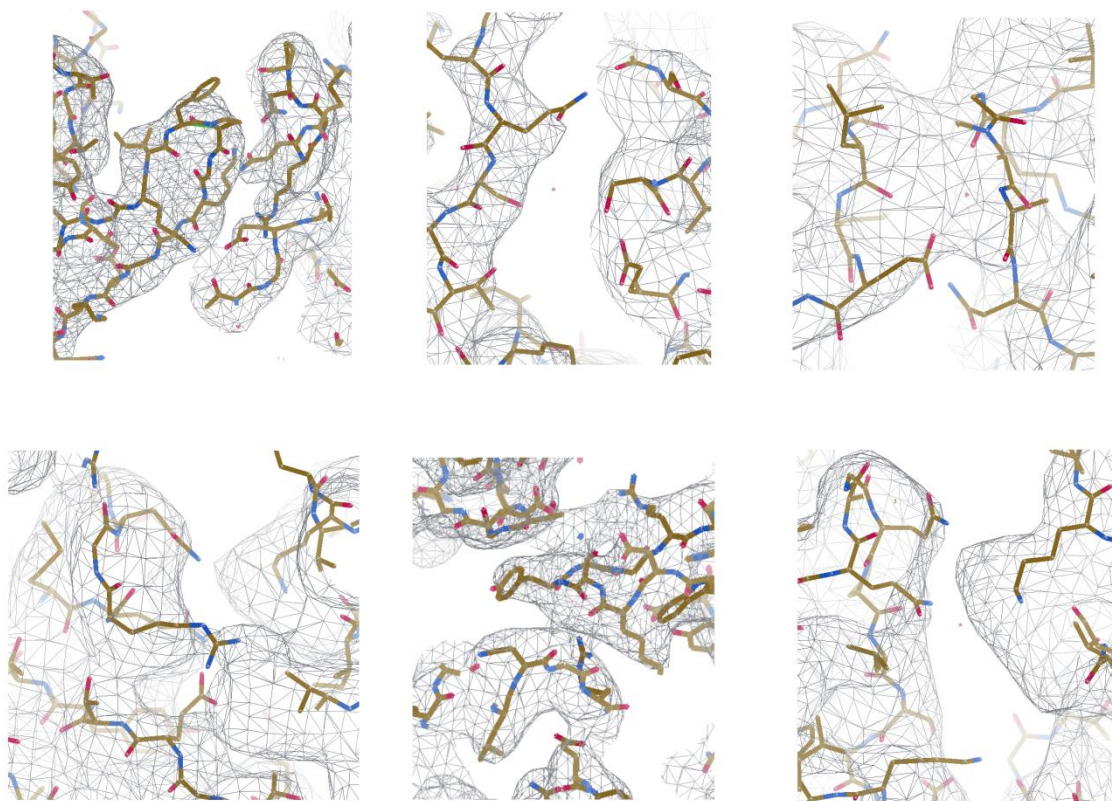
	260	270	280	290
PfDd2_120005600/1-392	NIVNIRKCTDS	IIIGKSIKASEKLE	CKLATGNYEQWNTNR	RLKE
PfSD01_120005900/1-381	EYVKS	CSNVTN...LG	QAASESNNCI	PEIRKYYQEW
PfIT_120006100/1-378	EYVKS	CSNVTN...LG	QAASESNNCI	TEIKKYYQEW
PfKH02_120006100/1-382	EYVKS	CSNVTNI.DLS	LQASESKNCT	SEIRKYYQEW
Pf7G8_120005700/1-391	QNVKLE	CSNVTNI.DLS	LQASESTKCI	PEIRKYYQEW
PfGB4_120005700/1-386	QNVKLE	CSNVSNI.DLS	LQALESKNCT	SEIRKYYQEW
PfHB3_120006000/1-372	QNVKSE	CSNVSNI.DLD	POASESNNCI	TEIRKYYQEL
PfCD01_120005700/1-392	EYVKS	CSNVTNI.DLD	POASESKNCT	SEIRKYYQEW
PfML01_120006200/1-388	EYVKS	CSNVTNN.NLGS	QESSESKNCT	SEIRKYYQEW
PfSN01_120006700/1-371	EYVKS	CSNVTNI.DLD	POASESKNCT	SEIRKYYQEW
Pf3D7_1200600/1-387	EYVKS	CSNVT...NLGA	QESSESKNCT	SEIKKYYQEW
PfNF54_120005700/1-387	EYVKS	CSNVT...NLGA	QESSESKNCT	SEIKKYYQEW
PfGA01_120005700/1-377	QNVKSE	CSNVPNN.NLGS	QESSESNNCI	TEIRKYYQEW
PfKE01_120006100/1-365	QNVKSK	CSNVPNN.NLGS	QESSESKNCT	SEIRKYYQEW
PfKH01_000031900/1-379	KNVKS	CSNVPNN.NLGS	QESSESKNCT	SEIRKYYQEW

	300	310	320
PfDd2_120005600/1-392	WKLLSQOYKTNKESN	KDID	MSELSAEQY
PfSD01_120005900/1-381	WETISKRYKR...	MDILK...DVK...	EPDANEY
PfIT_120006100/1-378	WETISKRYKRYKRMD	ILKD...VKEP...	DANTY
PfKH02_120006100/1-382	WEAISERYRKYKGM	VLKN...VKEP...	DANEY
Pf7G8_120005700/1-391	WEAISERYRKYKRM	DEFKNVFNNANEPN	ANEY
PfGB4_120005700/1-386	WEAISERYRKYKGM	DEFKNV...KEPD...	ANEY
PfHB3_120006000/1-372	WEAISERYRKYKGM	VLKNV...KEPD...	ANEY
PfCD01_120005700/1-392	WEAISERYKRYKRM	DEFKNTFKNIKEHD	ANEY
PfML01_120006200/1-388	WEAISERYKRYKGM	IL...KDVKEPD...	ANTY
PfSN01_120006700/1-371	WEAISERYK...RMD	IL...KDVKESD...	ANEY
Pf3D7_1200600/1-387	WEAISERYKRYKGM	DEFKNTFKNIKEPD	ANEPNANEY
PfNF54_120005700/1-387	WEAISERYKRYKGM	DEFKNTFKNIKEPD	ANEPNANEY
PfGA01_120005700/1-377	WEAISERYKRYKGM	DEFKNTFKNIKEPD	ANTY
PfKE01_120006100/1-365	WEAISERYKRYKRM	DEF...KNVKEPD...	ANEY
PfKH01_000031900/1-379	WEAISERYKRYKGM	DEF...KNVKEPD...	ANTY

	330	340	350	360	370
PfDd2_120005600/1-392	GCS	CRFSDMDELYIT	KLKNE	NLHEEILKNA	QIPIYFEEVYH
PfSD01_120005900/1-381	KPC	CGFNDMQEITND	NDKVEI	IFNRIIEKV	KIPAELEDVIYRI
PfIT_120006100/1-378	KPC	CGFNDMEEMNN	NEDNEKEAFKQ	IKEQVKI	PAELEDVIYRI
PfKH02_120006100/1-382	KPC	CGYNDMEEMNN	NEDNEKEGFKQ	IKEQVDI	PAELEDVIYRI
Pf7G8_120005700/1-391	KPC	CGFNDMEETND	TENKQDIFKQI	IEKVKI	PAELEDVIYRL
PfGB4_120005700/1-386	KPC	CGFNDMKEIAND	TENKQDIFKQI	IEQVDI	PAELEDVIYRL
PfHB3_120006000/1-372	KPC	CGYNDMQEIAND	TENKQDIFKQI	IEQVDI	PAELEDVI...
PfCD01_120005700/1-392	KPC	CGFNDMQEITKY	TNIGNEAFKQI	IEQVDI	PAELEDVIYRL
PfML01_120006200/1-388	KPC	CGFNDMKEISN	NEDNEKETFNRIIEKV	KIPAELEDVIYRL	
PfSN01_120006700/1-371	KPC	CGFNDMKEIAND	TENKQDIFKQI	IEQVDI	PAELEDVI...
Pf3D7_1200600/1-387	KPC	CGFNDMQEITKY	TNIGNEAFKQI	IEQVDI	PAELEDVIYRL
PfNF54_120005700/1-387	KPC	CGFNDMQEITKY	TNIGNEAFKQI	IEQVDI	PAELEDVIYRL
PfGA01_120005700/1-377	KPC	CGFNDMKEIAND	TENKQDIFNRIIEKV	KIPAELEDVI...	
PfKE01_120006100/1-365	KPC	CGYNDMEETND	TENKQDIFKQI	IEKVKI	PAELEDVI...
PfKH01_000031900/1-379	KPC	CGFNDMEETND	TENKQDIFKQI	IEQVDI	PAELEDVIYRI

	380	390
PfDd2_120005600/1-392	DRSDVQCPDNN	CNLYKSIPCK
PfSD01_120005900/1-381	KHHEYNSN.DY	
PfIT_120006100/1-378	KHHEYDKGNDY	
PfKH02_120006100/1-382	KHHKYDNDNNY	Y.....
Pf7G8_120005700/1-391	KHHEYNSN.DY	
PfGB4_120005700/1-386	KHHKYNSN.DY	
PfHB3_120006000/1-372		
PfCD01_120005700/1-392	KHHKYNSN.DY	
PfML01_120006200/1-388	KHHKYNSN.DY	
PfSN01_120006700/1-371		
Pf3D7_1200600/1-387	KHHEYDKGNDY	
PfNF54_120005700/1-387	KHHEYDKGNDY	
PfGA01_120005700/1-377		
PfKE01_120006100/1-365		
PfKH01_000031900/1-379	KNRKYNSN.DY	

Supplementary Fig. 9. ESPript multiple sequence alignment of DBL5ε domains of *P. falciparum* isolates.



Supplementary Fig. 10. Representative fit of the model side chains of the map

Data Collection and Processing

IgM-VAR2CSA complex EMD-34399, PDB 8GZN

Voltage	300(keV)
Microscope	FEI Titan Krios
Detector	falcon3
Total Electron exposure (e/Å ²)	51.2
Exposure Rate (e/Å ² /S)	0.98
Exposure Rate (e/Å ² /S)	0.81
Number of Frames collected per micrograph	50
Data collection Software	EPU
Defocus range (um)	-0.8 to -2.2
Pixel size	1.10
Micrographs used	15668
Symmetry imposed	C1
Final particle in Refine	371049
Resolution of map at 0.143 FSC (masked)	3.6
Map Sharpening	None
Initial Models used	6KXS, 7NNH, 7B52
Refinement software	PHENIX
CC mask	0.82
CC Box	0.82
CC Peaks	0.64
CC Volume	0.81
Model composition (non-hydrogen)	35402
Protein Residues	4409
Bonds RMSD	
Length (Å)	0.003
Angles	0.693
B factor (Å ²)	244
Validation	
MolProbity score	2.24
Clashscore	15.38
Ramachandran Plot (%)	
Favored	90.53
Allowed	9.45
Outliers	0.02
Rama-Z	
Whole (N= 4327)	-1.11
Helix (N = 1389)	0.69
Sheet (N= 753)	-0.47
Loop (N=2203)	-1.82
Cbeta outliers	0.00
CaBLAM outliers	5.80

Supplementary Table 1 Cryo-EM data collection, processing and real space refinement validation statistics