

**Supplementary Information for:**

**Structure-based design of a strain transcending AMA1-RON2L malaria vaccine**

Palak N. Patel<sup>1</sup>, Thayne H. Dickey<sup>1</sup>, Ababacar Diouf<sup>2</sup>, Nichole D. Salinas<sup>1</sup>, Holly McAleese<sup>3</sup>, Tarik Ouahes<sup>3</sup>, Carole A. Long<sup>2</sup>, Kazutoyo Miura<sup>2</sup>, Lynn E. Lambert<sup>3</sup>, Niraj H. Tolia<sup>1\*</sup>

<sup>1</sup>Host-Pathogen Interactions and Structural Vaccinology Section, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

<sup>2</sup>Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

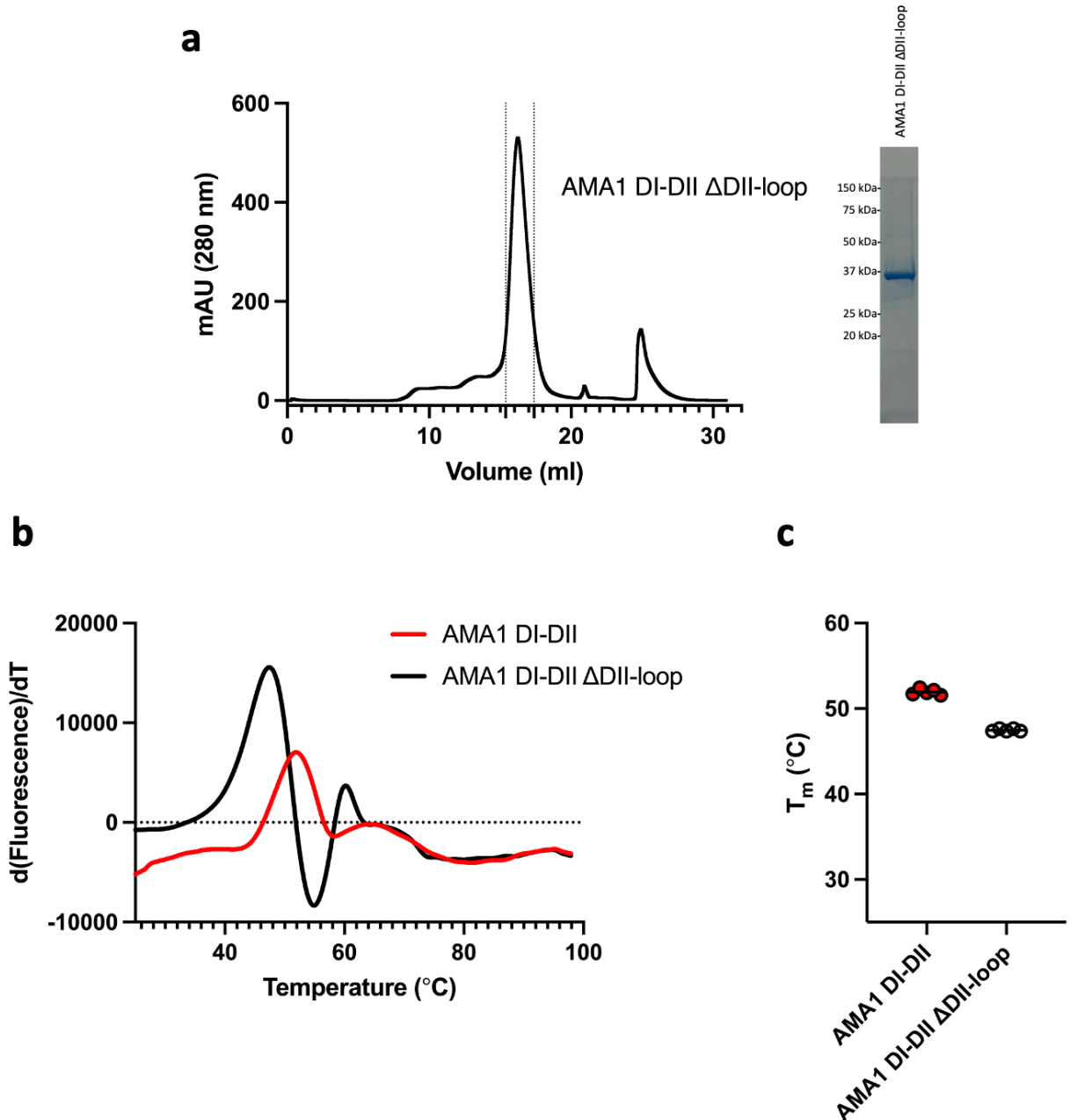
<sup>3</sup> Vaccine Development Unit, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

\*Corresponding author:

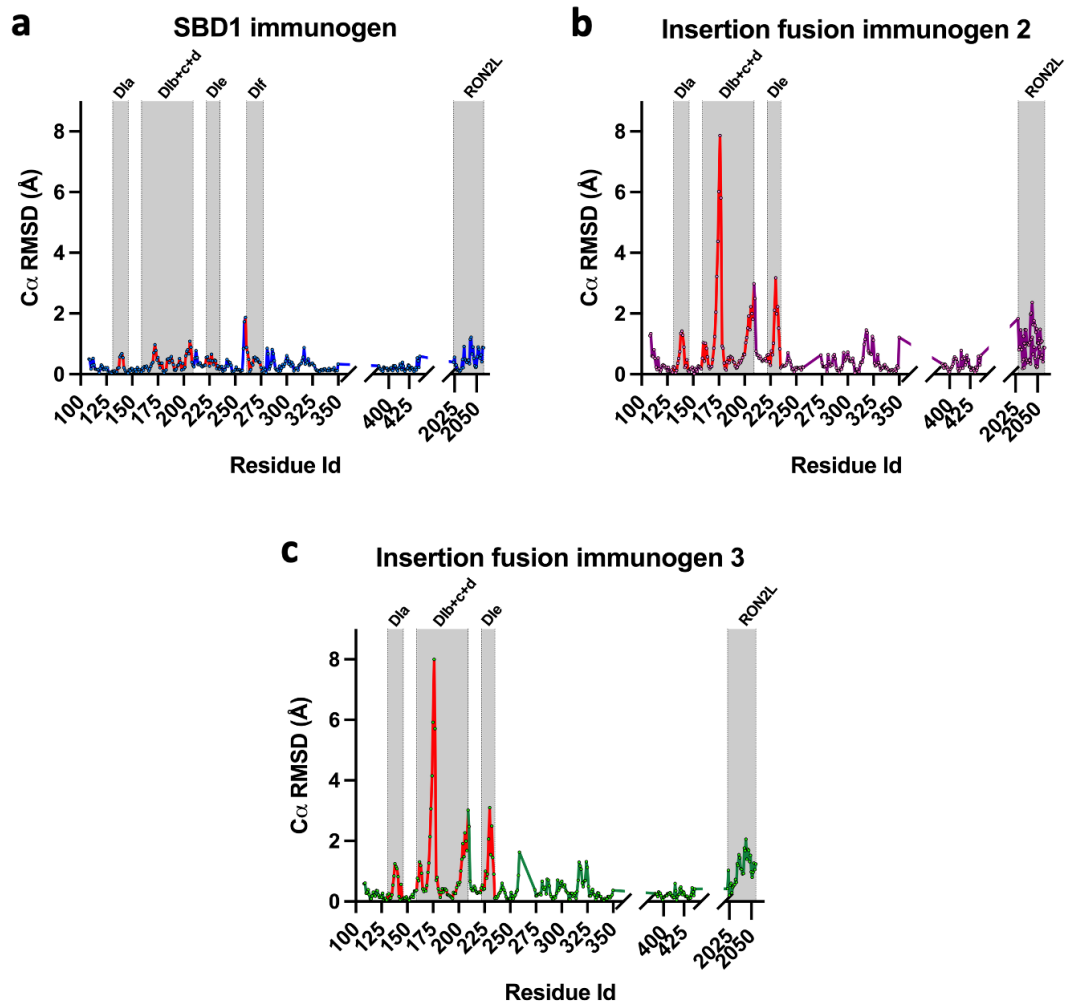
[niraj.tolia@nih.gov](mailto:niraj.tolia@nih.gov)

9000 Rockville Pike, Building 29B, 4NN08

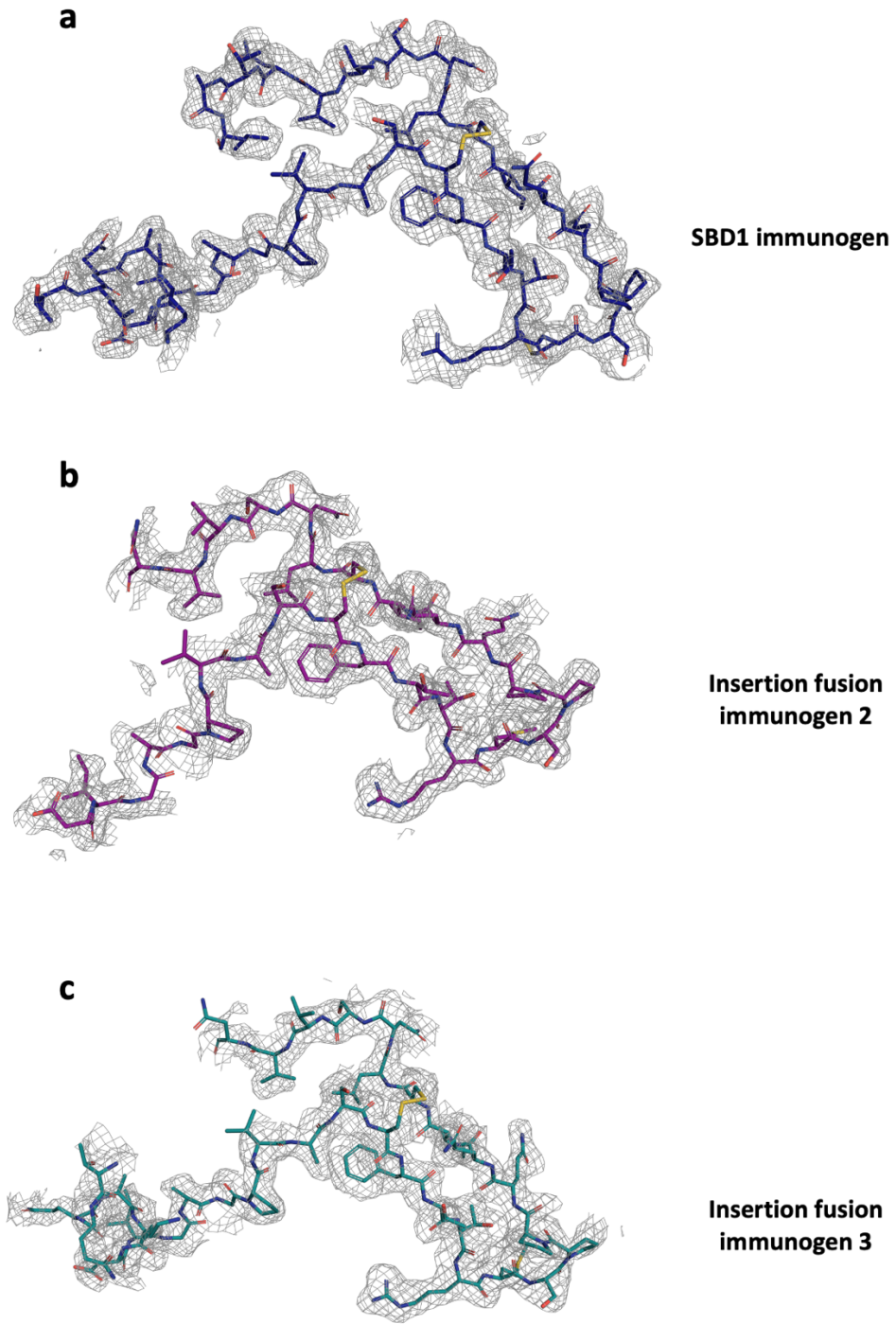
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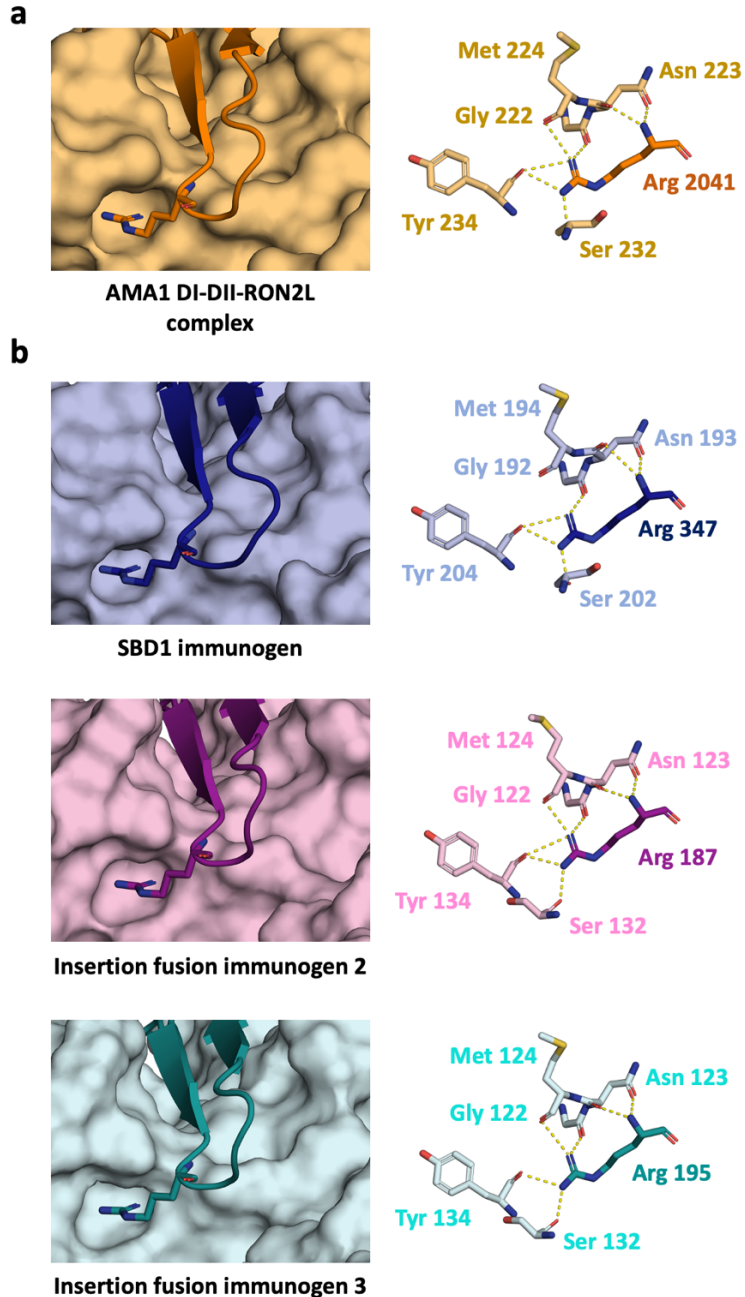
**Supplementary Fig. 1: Purification and characterization of the AMA1 DI-DII  $\Delta$ DII-loop.** **a** Size exclusion chromatography (SEC) profile of the AMA1 DI-DII  $\Delta$ DII-loop. The peak for the AMA1 DI-DII  $\Delta$ DII-loop is shown between two dotted lines. Inset shows Coomassie Brilliant Blue-stained SDS-PAGE gel for AMA1 DI-DII  $\Delta$ DII-loop (35.4-kDa) under reducing condition. **b** Differential scanning fluorimetry indicated that deletion of DII loop does not improve the stability of engineered AMA1 DI-DII. **c**  $T_m$  from five independent measurements. Bar represents mean. Source data are provided as a Source data file.



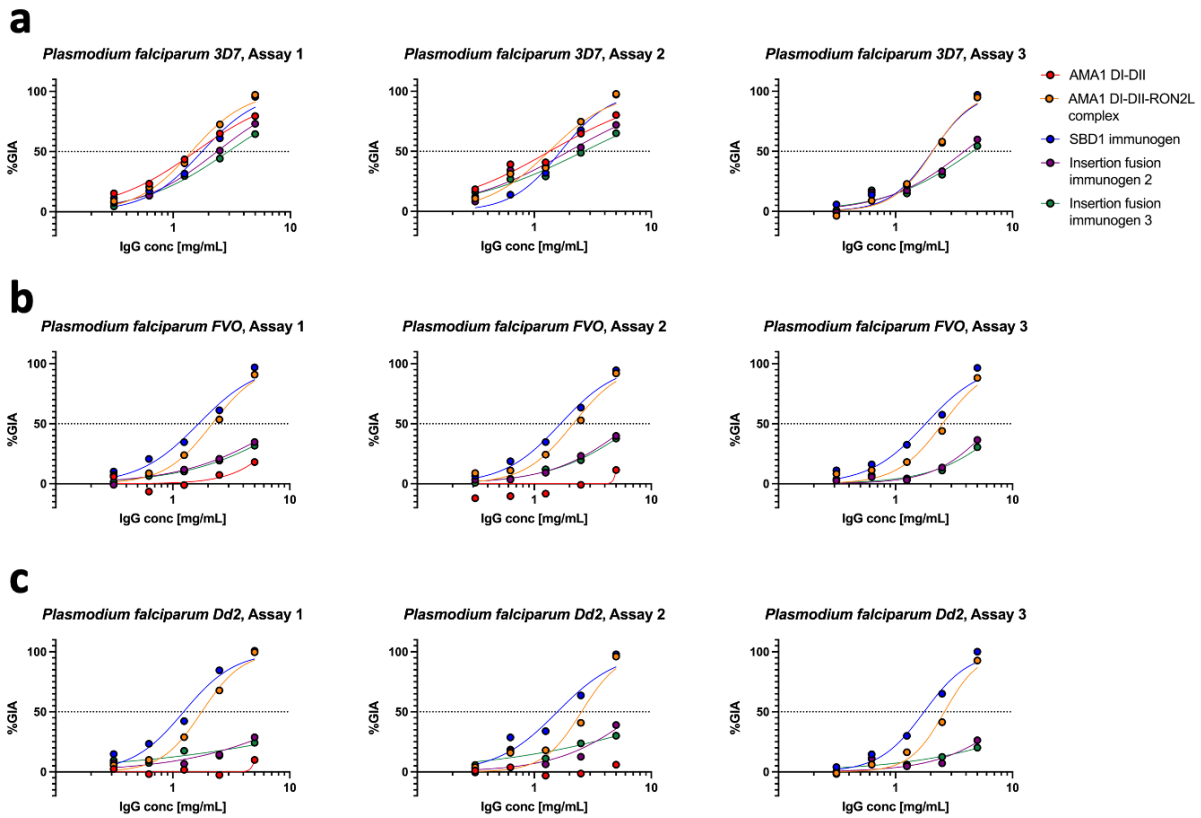
**Supplementary Fig. 2: SBD1 immunogen possesses the greatest structural similarity to the AMA1-RON2L complex.** The root-mean-square deviation (RMSD) for the  $C\alpha$  atom of each residue in the **a** SBD1 immunogen, **b** Insertion fusion immunogen 2, and **c** Insertion fusion immunogen 3, when aligned to the AMA1 DI-DII-RON2L complex (PDB ID: 3zwz, <https://www.rcsb.org/structure/3ZWZ>). The residues are labeled based on the wildtype AMA1 and RON2 sequence numbering. The domain I loops are indicated by red lines and silver shades beneath the corresponding data points. Source data are provided as a Source data file.



**Supplementary Fig. 3: RON2L of single-component immunogens shows a disulfide-anchored U-shaped conformation in the hydrophobic groove of AMA1. Electron density for the RON2L component of **a** SBD1 immunogen, **b** Insertion fusion immunogen 2, and **c** Insertion fusion immunogen 3 from a Polder map (gray mesh) contoured at 1.0  $\sigma$  level (1.43 rmsd).**



**Supplementary Fig. 4: An influential residue, Arg2041, is located at the tip of the  $\beta$ -hairpin and with its guanidyl group is adequately positioned within the preformed pocket of AMA1. As in **a** AMA1 DI-DII-RON2L complex structure (PDB ID: 3zwz, <https://www.rcsb.org/structure/3ZWZ>), the Arg residue of RON2L of **b** all three single-component immunogens fits snugly into a deep pocket in the surface of AMA1. The complex network of hydrogen bonds stabilizes this structure.**



**Supplementary Fig. 5: *In vitro* GIA dilution series of pooled purified IgG from each group at day 63 against *Plasmodium falciparum* a 3D7 b FVO c Dd2. Data from individual assays are shown. IC<sub>50</sub> values were determined by interpolation after fitting data to a four-parameter dose-response curve. IgG from AMA1 DI-DII IgG group was not tested in Assay 3 for each strain. Source data are provided as a Source data file.**

a

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                                D1a loop
                                (Leu131-Ser146)
                                D1b loop
                                (Glu159-Ala182)
AMA1DI-DII-Pf3D7  NYMGNPWTEYMAKYDIEEVHSGSIRVDLGEDAEVAGTQYRLPSGKCPVFGKGIIEENSNTTFLTPVATGNQYLKD
AMA1DI-DII-PfFVO  NYMGNPWTEYMAKYDIEEVHSGSIRVDLGEDAEVAGTQYRLPSGKCPVFGKGIIEENSNTTFLKPVATGNQDLKD
AMA1DI-DII-PfDd2  NYMGNPWTEYMAKYDIEEVHSGSIRVDLGEDAEVAGTQYRLPSGKCPVFGKGIIEENSNTTFLTPVATGNQYLKD
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

                                D1c loop
                                (Phe183-Pro192)
                                D1d loop
                                (Thr194-Lys209)
                                D1e loop
                                (Gly222-Lys235)
AMA1DI-DII-Pf3D7  GGFAPFPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMI PDNDKNSNYKYPVYDDKDKKCHILYIA
AMA1DI-DII-PfFVO  GGFAPFPPTNPLISPMTLNGMRDFYKNEYVKNLDELTLCSRHAGNMNPDNDKNSNYKYPVYDYNDKKCHILYIA
AMA1DI-DII-PfDd2  GGFAPFPPTKPLMSPMTLDDMRLLYKDNEDVKNLDELTLCSRHAGNMNPDNDKNSNYKYPVYDYNDKKCHILYIA
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

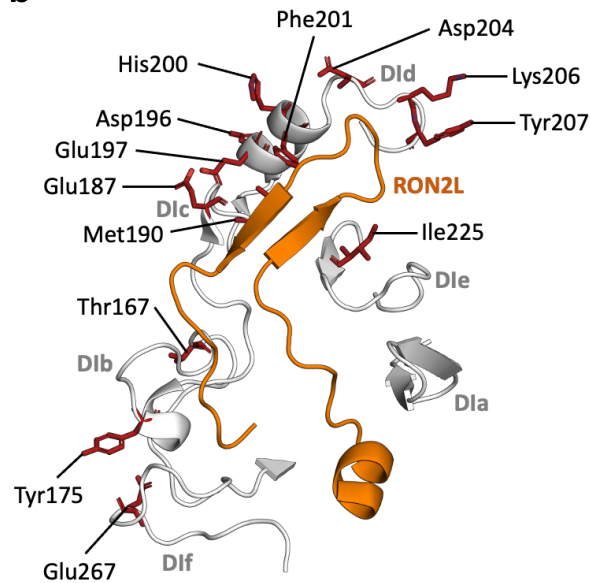
                                D1f loop
                                (Arg261-Arg277)
AMA1DI-DII-Pf3D7  AQENNGPRYCNKDESKRNSMFCFRPAKDISFQNYTYLSKNVVDNWEKVCPRKNLQNAKFLWVDGNCEDIPHVNE
AMA1DI-DII-PfFVO  AQENNGPRYCNKQSKRNSMFCFRPAKDLFENYTYLSKNVVDNWEVVCPRKNLENAKFLWVDGNCEDIPHVNE
AMA1DI-DII-PfDd2  AQENNGPRYCNKDESKRNSMFCFRPAKDSFQNYTYLSKNVVDNWEVVCPRKNLENAKFLWVDGNCEDIPHVNE
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

AMA1DI-DII-Pf3D7  FPAIDLFECNKLVEFELSASDQPKQYEQHLDTYEKIKEGFKNKNASMIKSAFLPTGAFKADRYKSHGKGYNWGNYN
AMA1DI-DII-PfFVO  FSANDLFECNKLVEFELSASDQPKQYEQHLDTYEKIKEGFKNKNASMIKSAFLPTGAFKADRYKSHGKGYNWGNYN
AMA1DI-DII-PfDd2  FSANDLFECNKLVEFELSASDQPKQYEQHLDTYEKIKEGFKNKNASMIKSAFLPTGAFKADRYKSHGKGYNWGNYN
*.* *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

AMA1DI-DII-Pf3D7  TETQKCEIFNVKPTCLINSSYIATTALSHPIEVE
AMA1DI-DII-PfFVO  RETQKCEIFNVKPTCLINSSYIATTALSHPIEVE
AMA1DI-DII-PfDd2  RKTQKCEIFNVKPTCLINSSYIATTALSHPIEVE
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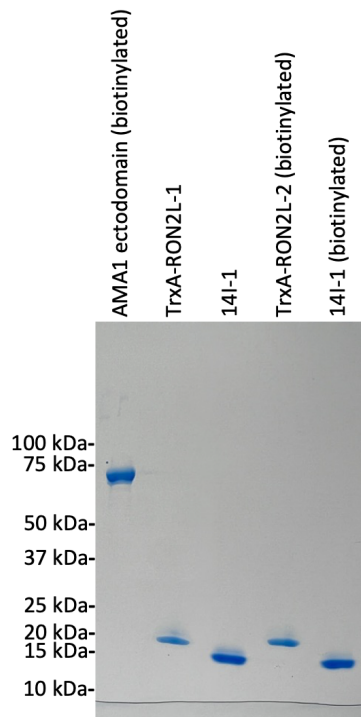
b



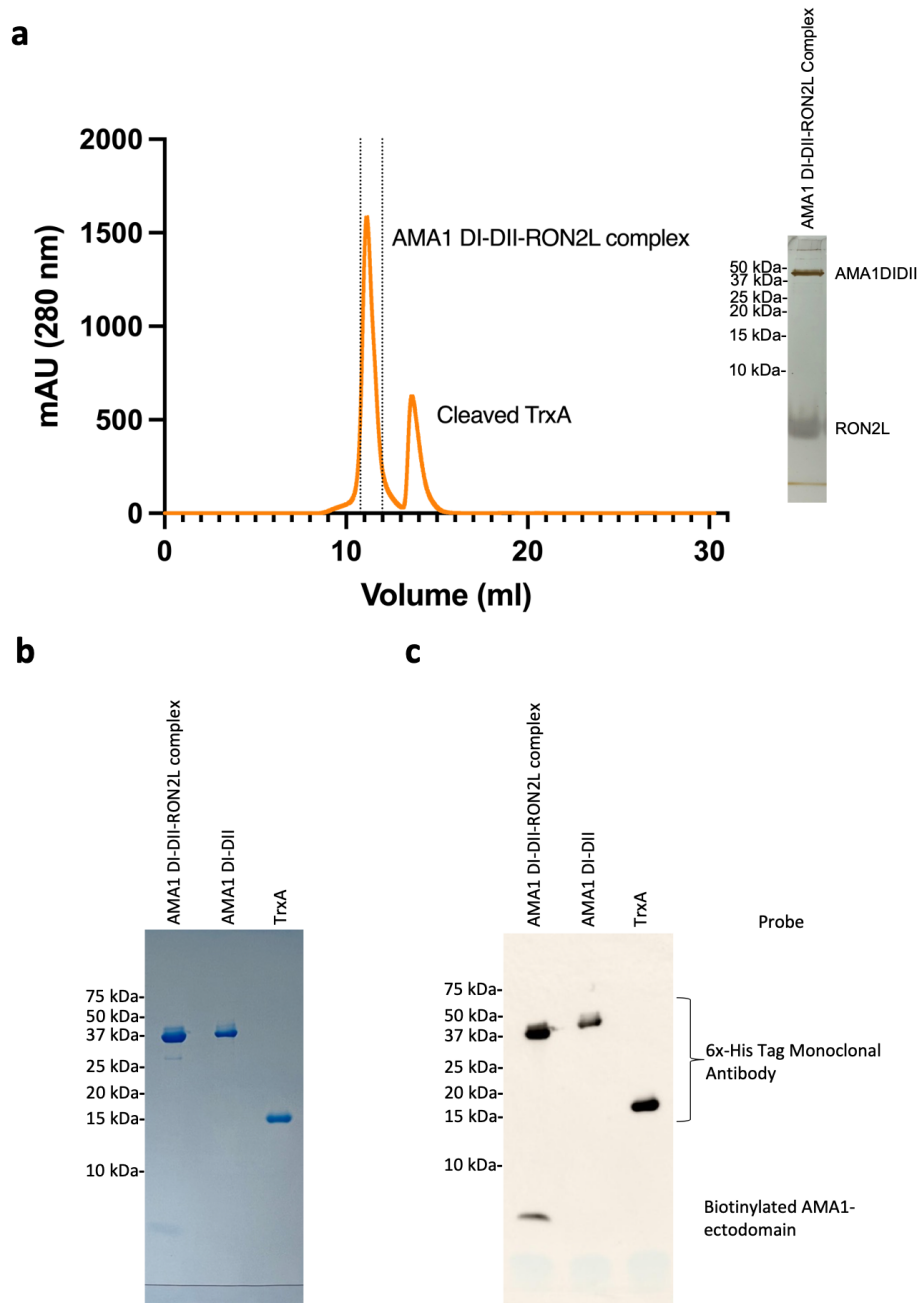
Supplementary Fig. 6: Immunization with the SBD1 immunogen induces higher levels of broadly neutralizing antibodies potentially by targeting new functional regions on AMA1 including conserved domain I loops. a Sequence alignment of AMA1 DI-DII from *Plasmodium falciparum* 3D7, FVO, and Dd2 strains. Polymorphic residues, which vary between the strains, are marked in bold red. Residues within the

AMA1 domain I loops surrounding the RON2L binding site, in the presence of RON2L, are marked in bold and labeled. The multiple sequence alignments were generated using the Clustal Omega/TCoffee. **b** Polymorphic residues mapped onto the AMA1 domain I loops surrounding the RON2L binding site in the presence of RON2L (PDB ID: 3zwz, <https://www.rcsb.org/structure/3ZWZ>). The polymorphic residues are depicted as red sticks, indicating their positions within the AMA1 domain I loops. The AMA1 loops are represented as a white cartoon (labeled DIa, DIb, DIc, DId, DIe, and DIf loops). The RON2L is depicted as an orange cartoon.





**Supplementary Fig. 7:** Coomassie Brilliant Blue-stained SDS-PAGE gel for AMA1 ectodomain (biotinylated, 63.8-kDa), TrxA-RON2L-1 (18.1-kDa), IgNAR 14I-1 (14.2-kDa), TrxA-RON2L-2 (biotinylated, 20.2-kDa) and IgNAR 14I-1 (biotinylated, 16.7-kDa) under reducing condition. Source data are provided as a Source data file.



**Supplementary Fig. 8: Purification and characterization of the AMA1 DI-DII-RON2L complex.** **a** Size exclusion chromatography (SEC) profile of the AMA1 DI-DII-RON2L complex. The peak for the AMA1 DI-DII-RON2L complex is shown between two dotted lines. A second peak corresponds to cleaved TrxA (6x-His tagged). Inset shows silver-stained SDS-PAGE gel for AMA1 DI-DII (39.2-kDa) and RON2L (4.2-kDa) components of complex under reducing condition. **b** Coomassie Brilliant Blue-stained SDS-PAGE gel for AMA1 DI-DII and RON2L components of complex under non-reducing condition. **c**

Western blot for the AMA1 DI-DII and RON2L components of complex is depicted, along with the primary probe used. AMA1 DI-DII (39.2-kDa) and TrxA (14.73-kDa) were used as controls under non-reducing condition. Source data are provided as a Source data file.

**Supplementary Table 1: Sequences of immunogens and constructs used in the study after signal peptide cleavage. Cloning scars, tags, and linkers are shown in lowercase.**

Name	Sequence
<b>Native AMA1 DI-DII</b>	etgNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIIIENSNTAFLTPVA TGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYP AVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDISFQNYAYLSKNVVDNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFPAILDFECNKL VFELSASDQPKQYE QHLTDYEKIKEGFKNKNA MIKSAFLPTGAFKADRYKSHGKGYNWGNYNTE TQKCEIFNVKPTCLINNAAYIATTALSHPIEVEgtkhhhh hh
<b>AMA1 DI-DII ΔDII-loop</b>	etgNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIIIENSNTAFLTPVA TGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYP AVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDISFQNYAYLSKNVVDNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFPAILDFECNKL VFELSASDQPGGSGYKSHGKGYNWGNYNTE TQ KCEIFNVKPTCLINNAAYIATTALSHPIEVEgtkhhhhhh
<b>SBD1 immunogen</b>	etgKADRYKSHGKGYNWGNYNTE TQKCEIFNVKPTCLINNAAYIATTALSHPIEVEggggsgggsgggsggg gsNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIIIENSNTAFLTPVA TGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYP AVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDISFQNYAYLSKNVVDNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFPAILDFECNKL VFELSASDQPKQYE QHLTTQAKDIGAGPVASC FTRMSPPQQICLNSVNTALSgtkhhhhhh
<b>Insertion fusion immunogen 2</b>	etgNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIIIENSNTAFLTPVA TGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYP AVYDDKDKKCHILYIAAQENNGggggsgsDITQQA KDIGAGPVASCFTTRMSPPQQICLNSVNTALSggsg MFCFRPAKDISFQNYAYLSKNVVDNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFPAILDFECNKL VFELSASDQPGggsgYKSHGKGYNWGNYNTE TQKCEIFNVKPTCLINNAAYIATTALSHPIEVEgtkhhhhhh
<b>Insertion fusion immunogen 3</b>	etgNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIIIENSNTAFLTPVA TGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYP AVYDDKDKKCHILYIAAQENNGPRYCN GggsgggsgsDITQQA KDIGAGPVASCFTTRMSPPQQICLNSV NTALSggsgMFCFRPAKDISFQNYAYLSKNVVDNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFP AILDFECNKL VFELSASDQPGggsgYKSHGKGYNWGNYNTE TQKCEIFNVKPTCLINNAAYIATTALSHPIEVE gtkhhhhhh
<b>AMA1 ectodomain</b>	etgQNYWEHPYQNSDVYRPIHEHREHPKEYEYPLHQEHTYQQEDSGEDENTLQHA YPIDHEGAEPAPQ EQNL FSSIEIVERSNYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDA EVAGTQYRLPSGKCPVFGKGIII NSNTTFLTPVATGNQYLKDGGFAPPTEPLMSPMTLDEMRHFYKDNKYVKNLDELTLCSRHAGNMIPD NDKNSNYKYP AVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDISFQNYTYLSKNV DNWEKVCPRK NLQNAKFGLWVDGNCE DIPHVNEFPAILDFECNKL VFELSASDQPKQYE QHLTDYEK IKEGFKNKNA MIKSAFLPTGAFKADRYKSHGKGYNWGNYNTE TQKCEIFNVKPTCLINNSSYIATTALS HPIEVENNFPCSLYKDEIMKEIERESKRILNDNDDEGNKKIAPRIFISDDKDSLKCPCDPEMVSNSTCR FFVCKCVERRAEVT SNNEVVVKEYKDEYADIPEHKPTgtggsgsgIndifeaqkiewhegrtkhhhhhh
<b>TrxA-ROn2L-1</b>	etghhhhhMSDKIHLTDDSFDTDLVKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL NIDQ NPGTAPKYGIRGIPTLLLFKNGEVAATKV GALSkgqlKEFLDANLAGSGSlevlfqgpDITQQA KDIGAGPVAS CFTTRMSPPQQICLNSVNTALS
<b>TrxA-ROn2L-2</b>	etghhhhhMSDKIHLTDDSFDTDLVKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL NIDQ NPGTAPKYGIRGIPTLLLFKNGEVAATKV GALSkgqlKEFLDANLAGSGSLEVLFQGPDITQQA KDIGAG PVASCFTTRMSPPQQICLNSVNTALSgsgsgIndifeaqkiewhe
<b>TrxA</b>	etghhhhhMSDKIHLTDDSFDTDLVKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL NIDQ NPGTAPKYGIRGIPTLLLFKNGEVAATKV GALSkgqlKEFLDANLAGSGSgIndifeaqkiewhe
<b>14I-1</b>	etgAWVDQTPRTATKETGESLTINCVL RDASLELKD TGWYRTKLGSTNEQSISIGGRYVETVNKGSKSF S LRISDLRVEDSGTYKCAFYS LPLRDYNYALLFRGEKGAGTALTVKAAAgtkhhhhhh
<b>14I-1 (biotinylated)</b>	etgAWVDQTPRTATKETGESLTINCVL RDASLELKD TGWYRTKLGSTNEQSISIGGRYVETVNKGSKSF S LRISDLRVEDSGTYKCAFYS LPLRDYNYALLFRGEKGAGTALTVKAAAgtgsgsgIndifeaqkiewhegrtk hhhh

**Supplementary Table 2: Contact residues between RON2L and AMA1 residues for SBD1 immunogen.**

Residues in RON2L	Residues in RON2L (Numbered according to PDB ID:3ZWZ)	Interface with AMA1 (PDB ID: 3ZWZ)	Residues in RON2L (SBD1 immunogen)	Interface with AMA1 (SBD1 immunogen)	Interface with AMA1 (SBD1 immunogen, numbered according to PDB ID:3ZWZ)
Thr3	Thr2023	Leu131	Thr329	Leu101	Leu131
Gln4	Gln2024	Val137	Gln330	Val107	Val137
Gln5	Gln2025	Thr140	Gln331	Thr110	Thr140
Ala6	Ala2026	Gln141	Ala332	Gln111	Gln141
Lys7	Lys2027	Tyr142	Lys333	Tyr112	Tyr142
Asp8	Asp2028	Arg143	Asp334	Arg113	Arg143
Ile9	Ile2029	Val169	Ile335	Val139	Val169
Gly10	Gly2030	Ala170	Gly336	Ala140	Ala170
Ala11	Ala2031	Thr171	Ala337	Thr141	Thr171
Gly12	Gly2032	Gly172	Gly338	Gly142	Gly172
Pro13	Pro2033	Gln174	Pro339	Gln144	Gln174
Val14	Val2034	Leu176	Val340	Tyr145	Tyr175
Ala15	Ala2035	Phe183	Ala341	Leu146	Leu176
Ser16	Ser2036	Pro184	Ser342	Phe153	Phe183
Cys17	Cys2037	Pro185	Cys343	Pro154	Pro184
Phe18	Phe2038	Thr186	Phe344	Pro155	Pro185
Thr19	Thr2039	Glu187	Thr345	Thr156	Thr186
Thr20	Thr2040	Pro188	Thr346	Glu157	Glu187
Arg21	Arg2041	Met190	Arg347	Pro158	Pro188
Met22	Met2042	Phe201	Met348	Met160	Met190
Ser23	Ser2043	Tyr202	Ser349	Phe171	Phe201
Pro24	Pro2044	Asn205	Pro350	Tyr172	Tyr202
Pro25	Pro2045	Val208	Pro351	Asn175	Asn205
Gln26	Gln2046	Arg219	Gln352	Val178	Val208
Gln27	Gln2047	Gly222	Gln353	Arg189	Arg219
Ile28	Ile2048	Asn223	Ile354	Gly192	Gly222
Cys29	Cys2049	Met224	Cys355	Asn193	Asn223
Leu30	Leu2050	Ile225	Leu356	Met194	Met224
Asn31	Asn2051	Pro226	Asn357	Ile195	Ile225
Ser32	Ser2052	Asp227	Ser358	Pro196	Pro226
Val33	Val2053	Asn228	Val359	Asp197	Asp227
Val34	Val2054	Ser232	Val360	Asn198	Asn228
Asn35	Asn2055	Tyr234	Asn361	Ser202	Ser232
Thr36	Thr2056	Lys235	Thr362	Tyr204	Tyr234
Ala37	Ala2057	Tyr236	Ala363	Lys205	Lys235
Leu38	Leu2058	Tyr251	Leu364	Tyr206	Tyr236
		Ile252		Tyr221	Tyr251
		Ala253		Ile222	Ile252
		Ala254		Ala223	Ala253
		Gln255		Ala224	Ala254
		Glu256		Gln225	Gln255
		Asn257		Glu226	Glu256
		Tyr262		Asn227	Asn257
		Asn271		Tyr232	Tyr262
		Met273		Asn241	Asn271
		Phe274		Met243	Met273
		Phe276		Phe244	Phe274
		Gln349		Phe246	Phe276
		Pro350		Gln319	Gln349
		Arg389		Pro320	Pro350
		Tyr390		Gln325	Gln355
				Leu327	Leu357
				Thr328	Thr358
				Arg4	Arg389
				Tyr5	Tyr390

A red box indicates residues present at the interface of the AMA1 DI-DII-RON2L complex (PDB: 3zwz, <https://www.rcsb.org/structure/3ZWZ>) but not in the SBD1 immunogen, while a green box indicates residues present at the interface of the SBD1 immunogen but absent in the complex.

**Supplementary Table 3: Contact residues between RON2L and AMA1 residues for Insertion fusion immunogen 2.**

Residues in RON2L	Residues in RON2L (Numbered according to PDB ID:3ZWZ)	Interface with AMA1 (PDB ID: 3ZWZ)	Residues in RON2L (Insertion fusion immunogen 2)	Interface with AMA1 (Insertion fusion immunogen 2)	Interface with AMA1 (Insertion fusion immunogen 2, numbered according to PDB ID:3ZWZ)
Asp8	Asp2028	Leu131	Asp174	Leu31	Leu131
Ile9	Ile2029	Val137	Ile175	Val37	Val137
Gly10	Gly2030	Tyr142	Gly176	Tyr42	Tyr142
Ala11	Ala2031	Arg143	Ala177	Arg43	Arg143
Gly12	Gly2032	Val169	Gly178	Val69	Val169
Pro13	Pro2033	Ala170	Pro179	Ala70	Ala170
Val14	Val2034	Thr171	Val180	Thr71	Thr171
Ala15	Ala2035	Gly172	Ala181	Gly72	Gly172
Ser16	Ser2036	Gln174	Ser182	Asn73	Asn173
Cys17	Cys2037	Leu176	Cys183	Lys77	Lys177
Phe18	Phe2038	Phe183	Phe184	Phe83	Phe183
Thr19	Thr2039	Pro184	Thr185	Pro84	Pro184
Thr20	Thr2040	Pro185	Thr186	Pro85	Pro185
Arg21	Arg2041	Thr186	Arg187	Thr86	Thr186
Met22	Met2042	Glu187	Met188	Glu87	Glu187
Ser23	Ser2043	Pro188	Ser189	Pro88	Pro188
Pro24	Pro2044	Met190	Pro190	Met90	Met190
Pro25	Pro2045	Phe201	Pro191	Phe101	Phe201
Gln26	Gln2046	Tyr202	Gln192	Tyr102	Tyr202
Gln27	Gln2047	Asn205	Gln193	Asp104	Asp204
Ile28	Ile2048	Val208	Ile194	Asn105	Asn205
Cys29	Cys2049	Arg219	Cys195	Gly122	Gly222
Leu30	Leu2050	Gly222	Leu196	Asn123	Asn223
Asn31	Asn2051	Asn223	Asn197	Met124	Met224
Ser32	Ser2052	Met224	Ser198	Ile125	Ile225
Val33	Val2053	Ile225	Val199	Pro126	Pro226
Val34	Val2054	Pro226	Val200	Asp127	Asp227
Asn35	Asn2055	Asp227	Asn201	Asn128	Asn228
		Asn228		Lys130	Lys230
		Ser232		Ser132	Ser232
		Tyr234		Asn133	Asn233
		Lys235		Tyr134	Tyr234
		Tyr236		Lys135	Lys235
		Tyr251		Tyr136	Tyr236
		Ile252		Tyr151	Tyr251
		Ala253		Ile152	Ile252
		Ala254		Ala153	Ala253
		Gln255		Ala154	Ala254
		Glu256		Gln155	Gln255
		Asn257		Glu156	Glu256
		Tyr262		Asn157	Asn257
		Asn271		Phe213	Phe276
		Phe276		Gln286	Gln349
		Gln349		Pro287	Pro350
		Pro350			
		Tyr390			

A red box indicates residues present at the interface of the AMA1 DI-DII-RON2L complex (PDB: 3zwz, <https://www.rcsb.org/structure/3ZWZ>) but not in the Insertion fusion immunogen 2, while a green box indicates residues present at the interface of the Insertion fusion immunogen 2 but absent in the complex.

**Supplementary Table 4: Contact residues between RON2L and AMA1 residues for Insertion fusion immunogen 3.**

Residues in RON2L	Residues in RON2L (Numbered according to PDB ID:3ZWZ)	Interface with AMA1 (PDB ID: 3ZWZ)	Residues in RON2L (Insertion fusion immunogen 3)	Interface with AMA1 (Insertion fusion immunogen 3)	Interface with AMA1 (Insertion fusion immunogen 3, numbered according to PDB ID:3ZWZ)
Thr3	Thr2023	Leu131	Thr177	Leu31	Leu131
Gln4	Gln2024	Val137	Gln178	Val37	Val137
Gln5	Gln2025	Thr140	Gln179	Thr40	Thr140
Ala6	Ala2026	Gln141	Ala180	Gln41	Gln141
Lys7	Lys2027	Tyr142	Lys181	Tyr42	Tyr142
Asp8	Asp2028	Arg143	Asp182	Arg43	Arg143
Ile9	Ile2029	Val169	Ile183	Val69	Val169
Gly10	Gly2030	Ala170	Gly184	Ala70	Ala170
Ala11	Ala2031	Thr171	Ala185	Thr71	Thr171
Gly12	Gly2032	Gly172	Gly186	Gly72	Gly172
Pro13	Pro2033	Gln174	Pro187	Asn73	Asn173
Val14	Val2034	Leu176	Val188	Lys77	Lys177
Ala15	Ala2035	Phe183	Ala189	Phe83	Phe183
Ser16	Ser2036	Pro184	Ser190	Pro84	Pro184
Cys17	Cys2037	Pro185	Cys191	Pro85	Pro185
Phe18	Phe2038	Thr186	Phe192	Thr86	Thr186
Thr19	Thr2039	Glu187	Thr193	Glu87	Glu187
Thr20	Thr2040	Pro188	Thr194	Pro88	Pro188
Arg21	Arg2041	Met190	Arg195	Met90	Met190
Met22	Met2042	Phe201	Met196	Phe101	Phe201
Ser23	Ser2043	Tyr202	Ser197	Tyr102	Tyr202
Pro24	Pro2044	Asn205	Pro198	Asp104	Asp204
Pro25	Pro2045	Val208	Pro199	Asn105	Asn205
Gln26	Gln2046	Arg219	Gln200	Gly122	Gly222
Gln27	Gln2047	Gly222	Gln201	Asn123	Asn223
Ile28	Ile2048	Asn223	Ile202	Met124	Met224
Cys29	Cys2049	Met224	Cys203	Ile125	Ile225
Leu30	Leu2050	Ile225	Leu204	Pro126	Pro226
Asn31	Asn2051	Pro226	Asn205	Asp127	Asp227
Ser32	Ser2052	Asp227	Ser206	Asn128	Asn228
Val33	Val2053	Asn228	Val207	Lys130	Lys230
Val34	Val2054	Ser232	Val208	Ser132	Ser232
Asn35	Asn2055	Tyr234	Asn209	Asn133	Asn233
		Lys235		Tyr134	Tyr234
		Tyr236		Lys135	Lys235
		Tyr251		Tyr136	Tyr236
		Ile252		Tyr151	Tyr251
		Ala253		Ile152	Ile252
		Ala254		Ala153	Ala253
		Gln255		Ala154	Ala254
		Glu256		Gln155	Gln255
		Asn257		Glu156	Glu256
		Tyr262		Asn157	Asn257
		Asn271		Phe221	Phe276
		Phe276		Gln294	Gln349
		Gln349		Pro295	Pro350
		Pro350		Tyr300	Tyr390
		Arg389			
		Tyr390			

A red box indicates residues present at the interface of the AMA1 DI-DII-RON2L complex (PDB: 3zwz, <https://www.rcsb.org/structure/3ZWZ>) but not in the fusion 3,

while a green box indicates residues present at the interface of the fusion 3 but absent in the complex.