Supplementary Figure 1. (A) Sequence alignment of Fusion-F_{D12} and Fusion-R_{D12} chimeric proteins. The RON2L sequence in Fusion-F_{D12} and Fusion-R_{D12} are placed in the forward or reverse direction with respect to the AMA1 sequence and joined by linkers that connect them to the DII loop of AMA1. (B) Sequence alignment of HP41 and 3D7 AMA1-RON2L fusion proteins. Differences between the two proteins are highlighted red. The residues comprising the 1a, 1bcd, 1e, and 1f binding loops and the inserted RON2L sequence are highlighted.

Supplementary Figure 2. Biochemical and structural characterization of chimera Fusion-F_{D12}. (A) Coomassie stained SDS-PAGE gel of $AMA1_{D12}$ and Fusion-F_{D12} recombinant proteins. Red: reduced, NR: non-reduced. (B) Western blot analysis of $AMA1_{D12}$ and Fusion-F_{D12} proteins using conformation-dependent AMA1 mAbs 1F9 and 4G2 that bind AMA1 loop 1d and DII respectively. Removing DII loop of AMA1 to generate the fusion chimera negates mAb 4G2 binding while 1F9 epitope is still preserved. (C) Coomassie stained SDS-PAGE gel of $AMA1_{D12}$ and Fusion-R_{D12} recombinant proteins. Red: reduced, NR: non-reduced. (D) Western blot analysis of $AMA1_{D12}$ and Fusion-R_{D12} proteins using conformation-dependent AMA1 mAbs 1F9 and 4G2. Both antibodies did not bind Fusion-R_{D12}. (E) RON2L-Fc (1µg/mL) was used to test binding to recombinant 3D7$AMA1_{D12}$, Fusion-$F_{D12HP41}$ and Fusion-$R_{D12HP41}$ proteins. 3D7$AMA1_{D12}$ and Fusion-$R_{D12HP41}$ but not Fusion-$F_{D12HP41}$ bound to free RON2L. Data shown are mean±SEM of OD405 performed in three technical replicates. (F) RON2L-Fc binding to 3D7$AMA1_{D12}$ but not Fusion-F_{D123D7}. Data shown are mean±SEM of OD405 performed in three technical replicates. (G) Right: Secondary structure view of Fusion-F_{D12} with boxes indicating the regions where the chimeric PfRON2L peptide begins and terminates. Left: Zoomed in orthogonal views of the PfAMA1-PfRON2L contacts showing the successful fusion of the

PfAMA1 with PfRON2L. Few residues of both PfAMA1 and PfRON2L are indicated in ball and sticks for clarity.

Supplementary Figure 3. Antibody titer in purified IgG or serum of apoAMA1, AMA1+RON2L binary complex and Fusion-F_{D12} immunized animals. (A) Antibody titer against 3D7AMA1, Fusion-F_{D12}, or FVOAMA1 antibody in the purified IgG from animals immunized with the indicated antigens in AddaVax and Freund's adjuvants. Data are shown for individual animals (n=4-5 per group) and each data point is the average of three replicates. Horizontal lines show the mean titer within each group. (B) Antibody titer against 3D7AMA1, Fusion-F_{D12}, or FVOAMA1 in the serum of animals immunized with the indicated antigens in AddaVax and Freund's adjuvants. Data are shown for individual animals (n=5 per group) and each data point is the average of three replicates. Horizontal lines show the mean titer within each group. (C, D) Anti-3D7 AMA1 titer in the purified IgG from the three independent immunization studies using Freunds or AddaVax adjuvants. Log10 transformed titer was used to compare IgG between apoAMA1 $_{D12}$ (C) and Fusion-F $_{D12}$ (D) immunized animals. Brown-Forsythe and Welch ANOVA test was performed to compare differences between groups. Horizontal lines show the mean antibody titer in each group. (E, F) Anti-3D7 AMA1 titer in the serum from two independent immunization studies using Freunds or AddaVax adjuvants. Log10 transformed titer was used to compare antibody titer between apoAMA1 $_{D12}$ (E) and Fusion-F_{D12} (F) immunized animals. Brown-Forsythe and Welch ANOVA test was performed to compare differences between groups. Horizontal lines show the mean antibody titer in each group.

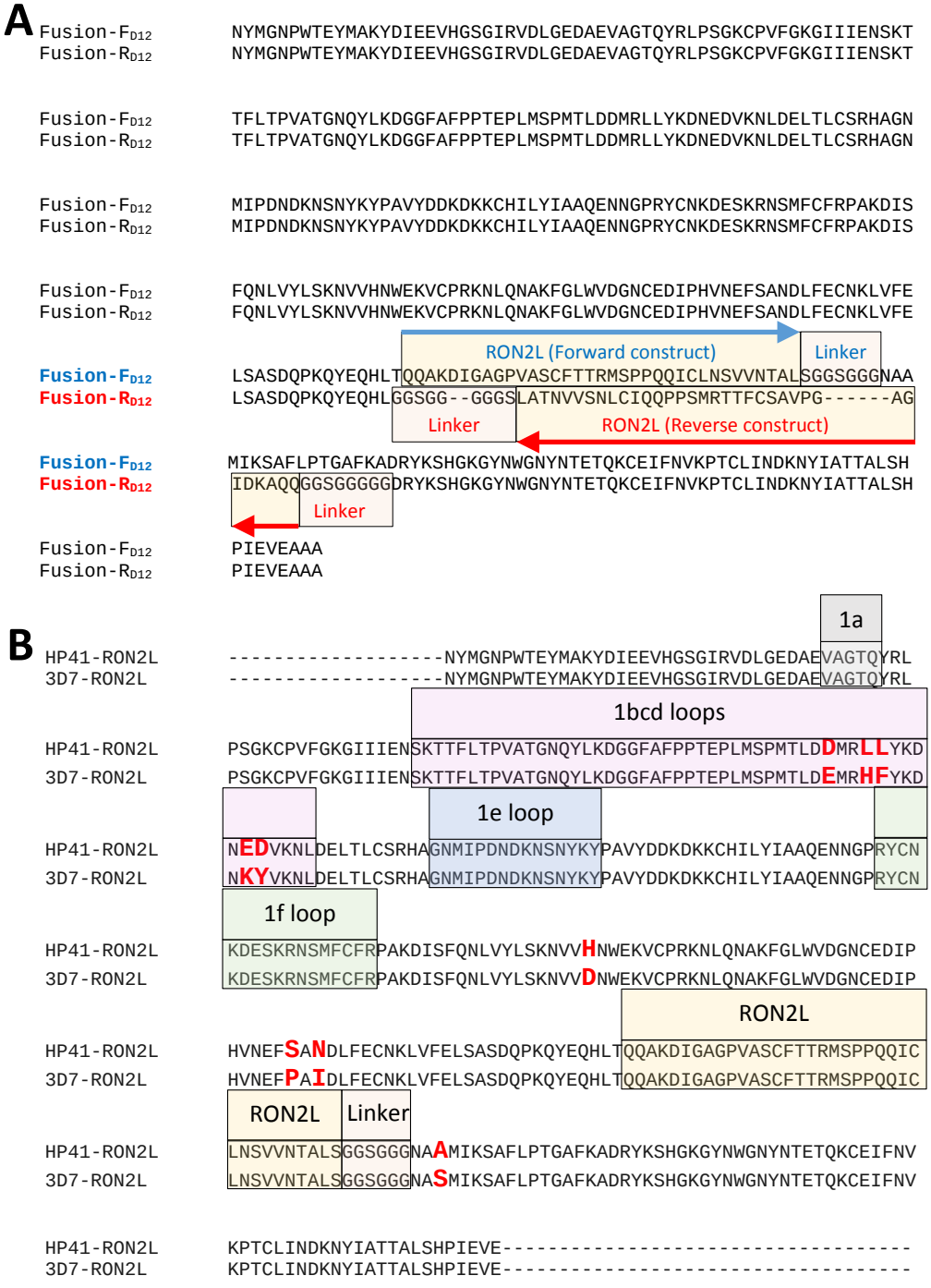
Supplementary Figure 4: Antibody titer and neutralizing activity of purified IgG from apoAMA1, binary complex (AMA1+RON2L) and Fusion-F_{D12} immunized animals. (A) *In vitro* neutralization (1-cycle) assay against vaccine-type Pf3D7 parasites using 2mg/mL of total IgG from each animal. Data are shown for individual animals (n=4 per group) and each data point is the average of three replicates. Horizontal lines show the mean neutralizing activity in each group. Brown-Forsythe and Welch ANOVA test was performed to compare differences between groups. (B) AMA1-specific antibody titers in purified IgG from animals immunized with 3D7apoAMA1$_{D12}$ (blue square), 3D7AMA1$_{D12}$+RON2L binary complex (green circle), and Fusion-$F_{D12HP41}$ (purple triangle) immunized rats in Freund's adjuvant. Data are shown for individual animals (n=4 per group) and each data point is the average of three replicates. Horizontal lines show the mean antibody titer in each group. Brown-Forsythe and Welch ANOVA test was performed to compare differences between groups. (C) Proportion of IgG binding to non-vaccine type FVOAMA1 to vaccine-type 3D7AMA1. Data are shown for individual animals (n=4 per group) and each data point is the average of three replicates. Horizontal line marks the mean for each group. One-way ANOVA was performed to compare differences between groups.

Supplementary Figure 5. Parasite neutralization assay using IgG and serum. (A) Relationship between anti-AMA1 antibody titer in serum (x-axis) and neutralizing (2-cycle) activity in 2% serum (y-axis) between Fusion-F_{D12} (purple triangle) and apoAMA1$_{D12}$ (blue square) antigens in Freund's adjuvant. Each data point is the average of two replicates. (B) Left panel: proportion of IgG1 to IgG2a AMA1-specific antibodies in rat sera of Fusion-F_{D12} (purple) relative to apoAMA1$_{D12}$ (blue) immunized animals in the AddaVax groups. Data shown are

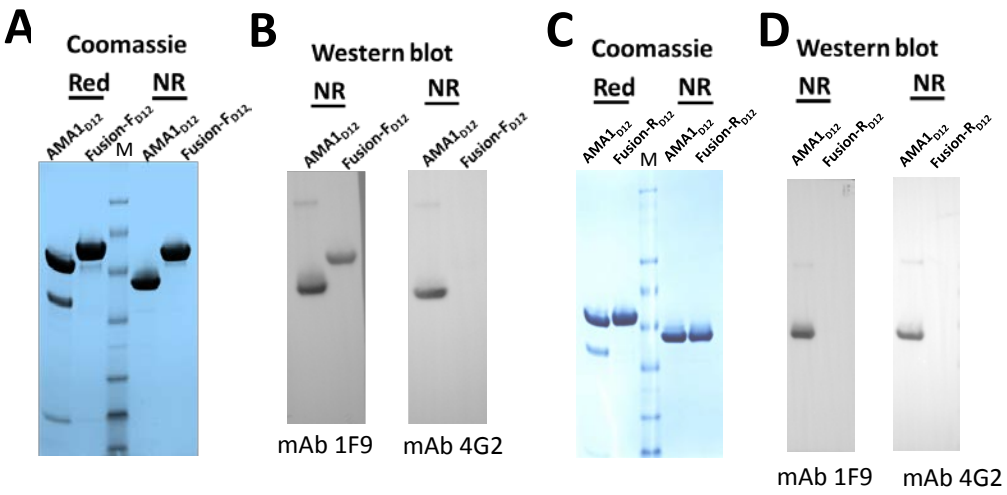
mean±SEM (n = 5 per group) performed in duplicate. Right panel: proportion of IgG1 to IgG2a Fusion-F_{D12}-specific antibodies in rat sera of Fusion-F_{D12} (purple) relative to apoAMA1$_{D12}$ (blue) immunized animals in the AddaVax group. Data are mean±SEM (n = 4 per group). Welch's t-test was performed to compare differences between groups. (C) Left panel: Proportion of IgG1 to IgG2a AMA1-specific antibodies in rat sera of Fusion-F_{D12} (purple) relative to apoAMA1$_{D12}$ (blue) immunized animals in the Freund's group. Data shown are mean±SEM (n = 5 per group). Right panel: Proportion of Fusion-F_{D12} vs apoAMA1$_{D12}$ specific IgM type antibodies in the sera of Fusion-F_{D12} (purple) relative to apoAMA1$_{D12}$ (blue) immunized animals in the Freund's group. Data are mean±SEM (n = 5 per group). Welch's t-test was performed to compare differences between groups. (D) Anti-RON2L-specific IgG titer in serum of apoAMA1$_{D12}$ (blue) or Fusion-F_{D12} (purple) antigen immunized animals in Freund's adjuvant. Data shown are mean±SEM (n = 5 per group. In all experiments the average of technical replicates for each animal was used for analysis. Welch's t-test was performed to compare differences between groups. (E) IgA titer in serum of apoAMA1$_{D12}$ (blue) or Fusion-F_{D12} (purple) antigen immunized animals in Freund's adjuvant. Data shown are mean±SEM (n = 5 per group. In all experiments the average of two technical replicates for each animal was used for analysis. Welch's t-test was performed to compare differences between groups.

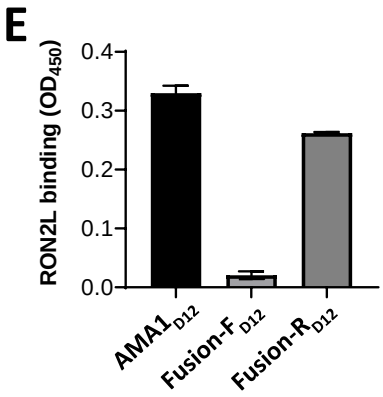
Supplementary Figure 6. Antibody type and isotype differences between apoAMA1$_{D12}$ and Fusion-F_{D12} immunized rat serum and parasite neutralizing activity. (A) ELISA OD_{450} after AMA1 titer normalization of IgG from apoAMA1$_{D12}$ and Fusion-F_{D12} groups (n=5 per group) used in Fig 3B, Fig 4A-4C, 4F. (B) Comparison of 1-cycle vs 2-cycle parasite neutralization assay. Spearman's rank test (rS) was performed to analyze correlation between the two assays.

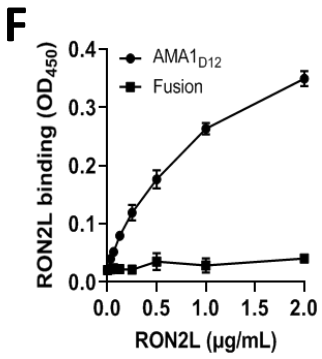
Suppl Fig 1

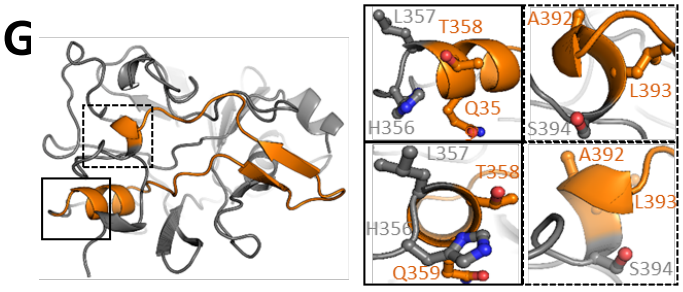

A
Fusion-FD12 NYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDAEVAGTQYRLPSGKCPVFGKGIIIENSKT
Fusion-RD12 NYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDAEVAGTQYRLPSGKCPVFGKGIIIENSKT
Fusion-FD12 TFLTPVATGNQYLKDGGFAFPPTEPLMSPMTLDDMRLLYKDNEDVKNLDELTLCSRHAGN
Fusion-RD12 TFLTPVATGNQYLKDGGFAFPPTEPLMSPMTLDDMRLLYKDNEDVKNLDELTLCSRHAGN
Fusion-FD12 MIPDNDKNSNYKYPAVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDIS
Fusion-RD12 MIPDNDKNSNYKYPAVYDDKDKKCHILYIAAQENNGPRYCNKDESKRNSMFCFRPAKDIS
Fusion-FD12 FQNLVYLSKNVVHNWEKVCPRKNLQNAKFGLWVDGNCEDIPHVNEFSANDLFECNKLVFE
Fusion-RD12 FQNLVYLSKNVVHNWEKVCPRKNLQNAKFGLWVDGNCEDIPHVNEFSANDLFECNKLVFE
RON2L (Forward construct)
Linker
Fusion-FD12 LSASDQPKQYEQHLTQQAKDIGAGPVASCFTTRMSPPQQICLNSVVNTALSGGSGGGNAA
Fusion-RD12 LSASDQPKQYEQHLGGSGG--GGGSLATNVVSNLCIQQPPSMRTTFCSAVPG------AG
Linker
RON2L (Reverse construct)
Fusion-FD12 MIKSAFLPTGAFKADRYKSHGKGYNWGNYNTETQKCEIFNVKPTCLINDKNYIATTALSH
Fusion-RD12 IDKAQQGGSGGGGGDRYKSHGKGYNWGNYNTETQKCEIFNVKPTCLINDKNYIATTALSH
Linker
Fusion-FD12 PIEVEAAA
Fusion-RD12 PIEVEAAA
B
1a
HP41-RON2L ----------NYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDAEVAGTQYRL
3D7-RON2L ----------NYMGNPWTEYMAKYDIEEVHGSGIRVDLGEDAEVAGTQYRL
1bcd loops
HP41-RON2L PSGKCPVFGKGIIIENSKTTFLTPVATGNQYLKDGGFAFPPTEPLMSPMTLDDMRLLYKD
3D7-RON2L PSGKCPVFGKGIIIENSKTTFLTPVATGNQYLKDGGFAFPPTEPLMSPMTLDEMRHFYKD
1e loop
HP41-RON2L NEDVKNLDELTLCSRHAGNMIPDNDKNSNYKYPAVYDDKDKKCHILYIAAQENNGPRYCN
3D7-RON2L NKYVKNLDELTLCSRHAGNMIPDNDKNSNYKYPAVYDDKDKKCHILYIAAQENNGPRYCN
1f loop
HP41-RON2L KDESKRNSMFCFRPAKDISFQNLVYLSKNVVHNWEKVCPRKNLQNAKFGLWVDGNCEDIP
3D7-RON2L KDESKRNSMFCFRPAKDISFQNLVYLSKNVVDNWEKVCPRKNLQNAKFGLWVDGNCEDIP
RON2L
HP41-RON2L HVNEFSANDLFECNKLVFELSASDQPKQYEQHLTQQAKDIGAGPVASCFTTRMSPPQQIC
3D7-RON2L HVNEFPAIDLFECNKLVFELSASDQPKQYEQHLTQQAKDIGAGPVASCFTTRMSPPQQIC
RON2L
Linker
HP41-RON2L LNSVVNTALSGGSGGGNAAMIKSAFLPTGAFKADRYKSHGKGYNWGNYNTETQKCEIFNV
3D7-RON2L LNSVVNTALSGGSGGGNASMIKSAFLPTGAFKADRYKSHGKGYNWGNYNTETQKCEIFNV
HP41-RON2L KPTCLINDKNYIATTALSHPIEVE----------
3D7-RON2L KPTCLINDKNYIATTALSHPIEVE----------

Suppl Fig 2

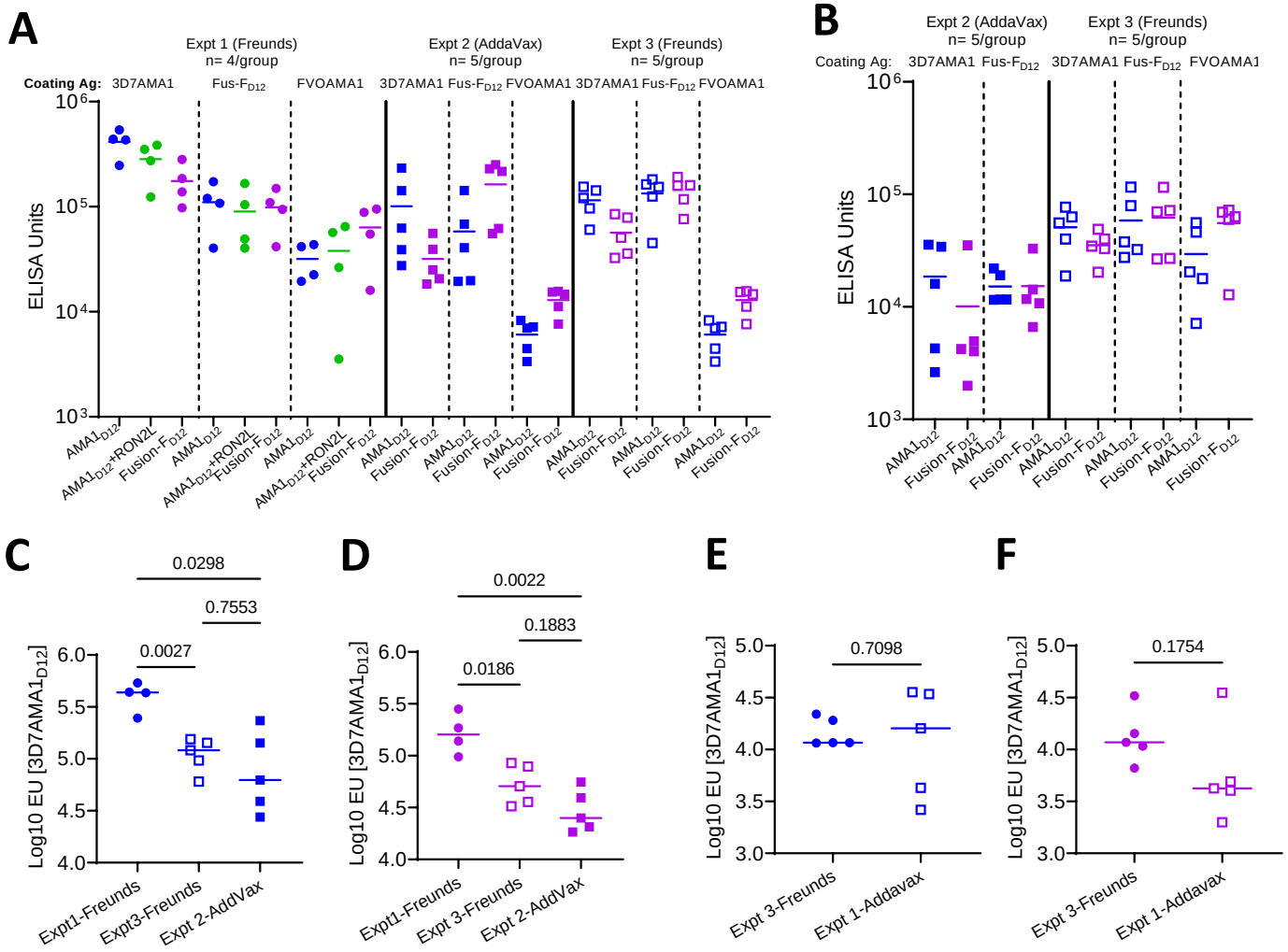

A
Coomassie
Red
NR
AMA1D12
Fusion-FD12
M
AMA1D12
Fusion-FD12
B
Western blot
NR
NR
AMA1D12
Fusion-FD12
AMA1D12
Fusion-FD12
mAb 1F9
mAb 4G2
C
Coomassie
Red
NR
AMA1D12
Fusion-RD12
M
AMA1D12
Fusion-RD12
D
Western blot
NR
NR
AMA1D12
Fusion-RD12
AMA1D12
Fusion-RD12
mAb 1F9
mAb 4G2


E
RON2L binding (OD450)
0.4
0.3
0.2
0.1
0.0
AMA1D12
Fusion-FD12
Fusion-RD12

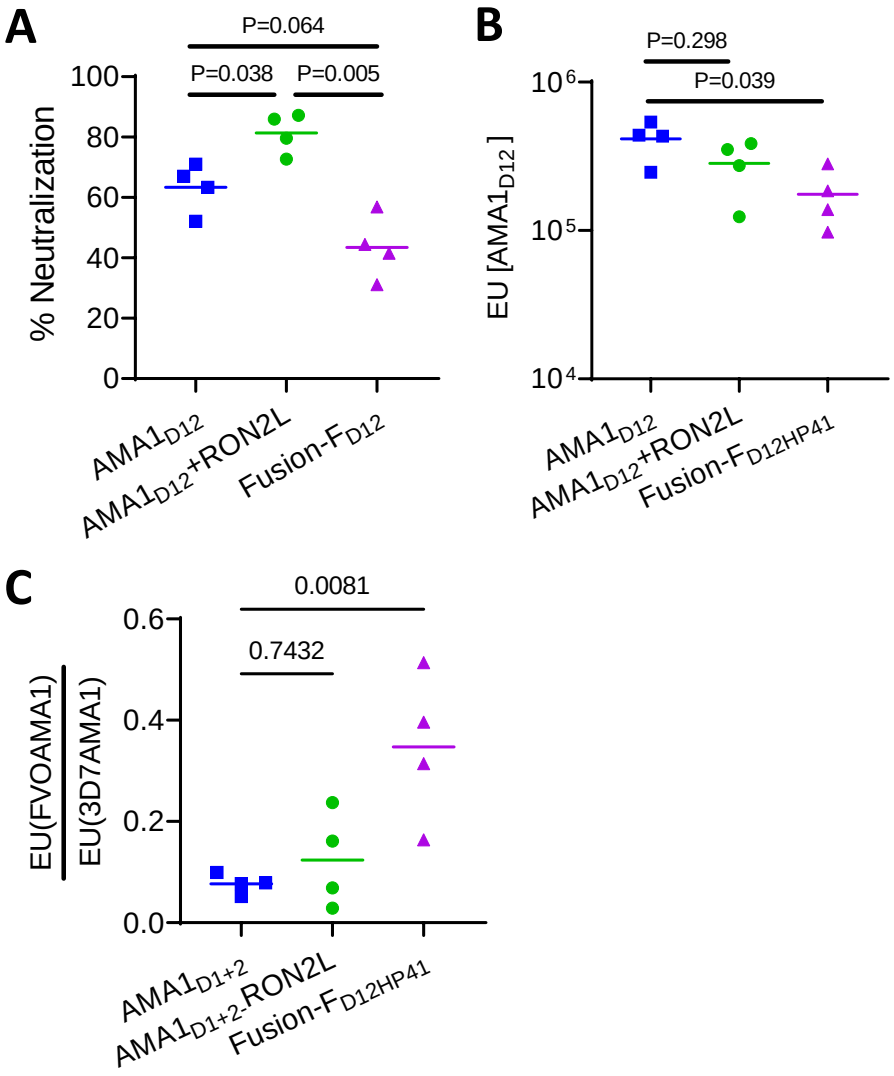

F
AMA1D12
Fusion
RON2L binding (OD450)
0.4
0.3
0.2
0.1
0.0
0.0
0.5
1.0
1.5
2.0
RON2L (μg/mL)


G
L357
T358
Q35
H356
A392
L393
S394
L357
T358
H356
Q359
A392
L393
S394

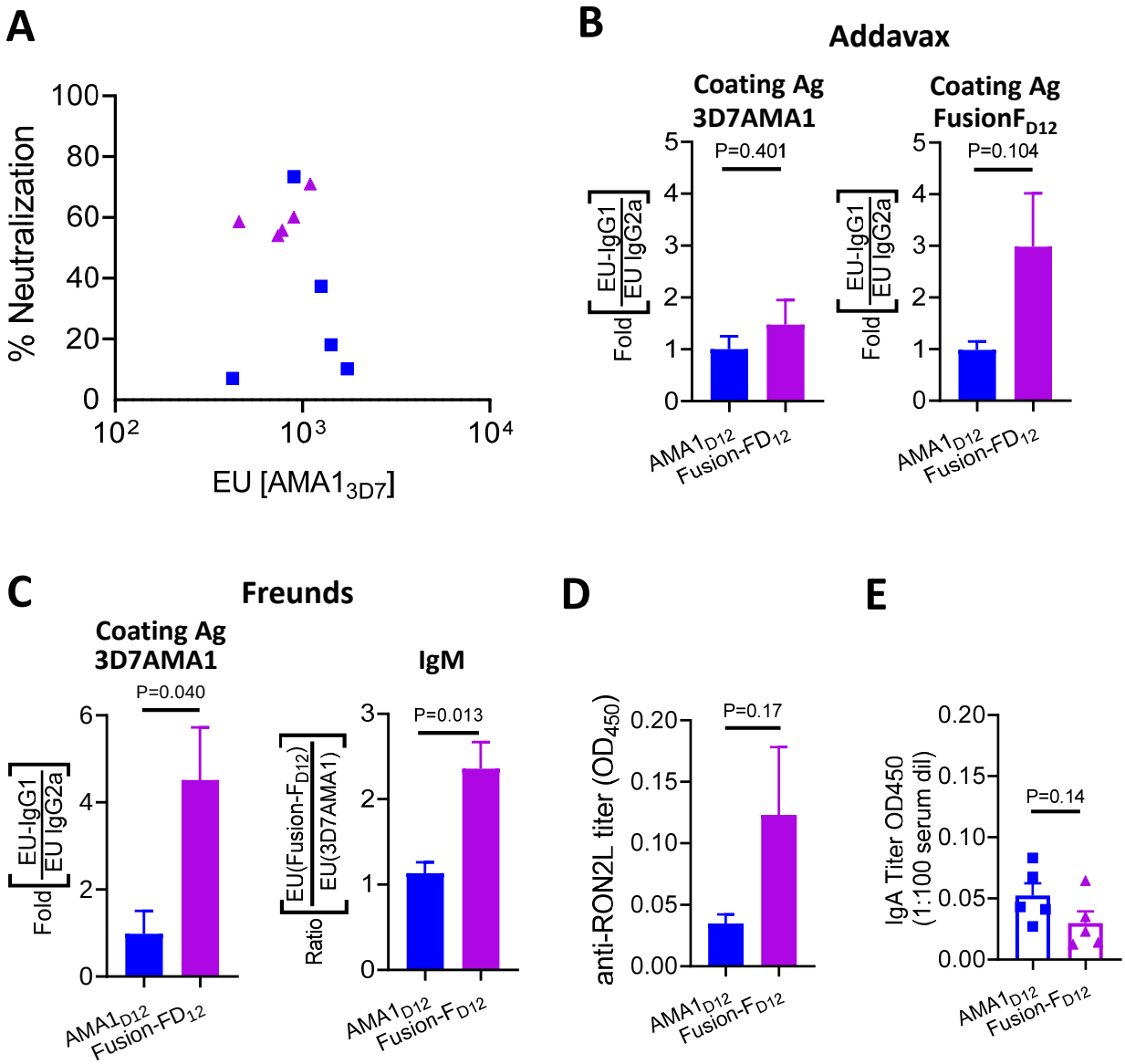
Suppl Fig 3


A
Expt 1 (Freunds)
n= 4/group
Expt 2 (AddaVax)
n= 5/group
Expt 3 (Freunds)
n= 5/group
Coating Ag: 3D7AMA1 Fus-FD12 FVOAMA1 3D7AMA1 Fus-FD12 FVOAMA1 3D7AMA1 Fus-FD12 FVOAMA1
ELISA Units
10^6
10^5
10^4
10^3
AMA1D12
AMA1D12+RON2L
Fusion-FD12
B
Expt 2 (AddaVax)
n= 5/group
Expt 3 (Freunds)
n= 5/group
Coating Ag: 3D7AMA1 Fus-FD12 3D7AMA1 Fus-FD12 FVOAMA1
ELISA Units
C
0.0298
0.7553
0.0027
Log10 EU [3D7AMA1D12]
Expt1-Freunds
Expt3-Freunds
Expt 2-AddVax
D
0.0022
0.1883
0.0186
Log10 EU [3D7AMA1D12]
Expt1-Freunds
Expt 3-Freunds
Expt 2-AddVax
E
0.7098
Log10 EU [3D7AMA1D12]
Expt 3-Freunds
Expt 1-Addavax
F
0.1754
Log10 EU [3D7AMA1D12]
Expt 3-Freunds
Expt 1-Addavax

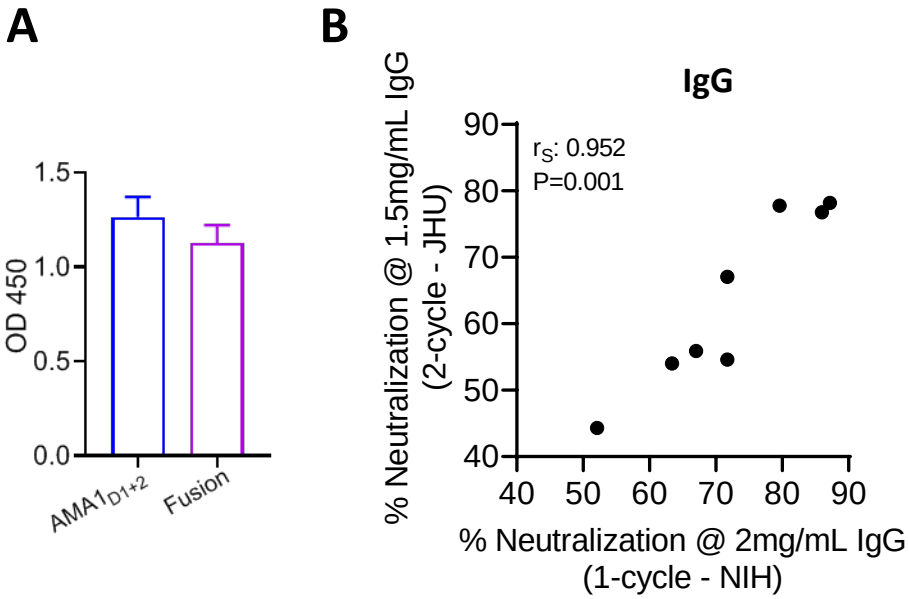
Suppl Fig 4


A
P=0.064
P=0.038
P=0.005
% Neutralization
100
80
60
40
20
0
AMA1D12
AMA1D12+RON2L
Fusion-FD12
B
P=0.298
P=0.039
EU [AMA1D12]
10^6
10^5
10^4
AMA1D12
AMA1D12+RON2L
Fusion-FD12HP41
C
0.0081
0.7432
EU(FVOAMA1) / EU(3D7AMA1)
0.6
0.4
0.2
0.0
AMA1D1+2
AMA1D1+2.RON2L
Fusion-FD12HP41

Suppl Fig 5


A
% Neutralization
EU [AMA1 3D7]
B
Addavax
Coating Ag 3D7AMA1
Coating Ag FusionF D12
P=0.401
P=0.104
Fold [EU-IgG1 / EU IgG2a]
AMA1 D12
Fusion-FD12
C
Freunds
Coating Ag 3D7AMA1
IgM
P=0.040
P=0.013
Ratio [EU(Fusion-F D12) / EU(3D7AMA1)]
D
anti-RON2L titer (OD450)
P=0.17
E
IgA Titer OD450 (1:100 serum dil)
P=0.14

Suppl Fig 6


A
B
OD 450
1.5
1.0
0.5
0.0
AMA1D1+2
Fusion
IgG
rS: 0.952
P=0.001
% Neutralization @ 1.5mg/mL IgG
(2-cycle - JHU)
90
80
70
60
50
40
40 50 60 70 80 90
% Neutralization @ 2mg/mL IgG
(1-cycle - NIH)

Supplementary Table 1. Data collection and refinement statistics

	Fusion-F_{D12}
A. Data collection statistics	
Spacegroup	$P3_12$
a, b, c (Å)	92.50, 92.50, 173.68
α, β, γ (deg.)	90, 90, 120
Wavelength	0.984
Resolution range (Å)	58.88 – 1.55 (1.58 – 1.55)
Measured reflections	855,225 (38,498)
Unique reflections	124,774 (6,114)
Redundancy	6.9 (6.3)
Completeness (%)	99.7 (100.0)
$I/\sigma(I)$	11.4 (2.1)
R_{merge}	0.075 (0.624)
B. Refinement statistics	
Resolution (Å)	57.89 – 1.55
R_{work} / R_{free}	0.190/0.219
No. of atoms	
Protein (A/B)	2703/2791
Solvent/Sulfate	700/5
Average B-values (Å^2)	
Protein (A/B)	25.5/28.9
Solvent/Sulfate	36.4/24.6
r.m.s. deviation from ideality	
Bond lengths (Å)	0.008
Bond angles (deg.)	1.14
Ramachandran statistics (%)	
Most favoured	98.0
Allowed	2.0
Disallowed	0.0

Values in parentheses are for the highest resolution shell