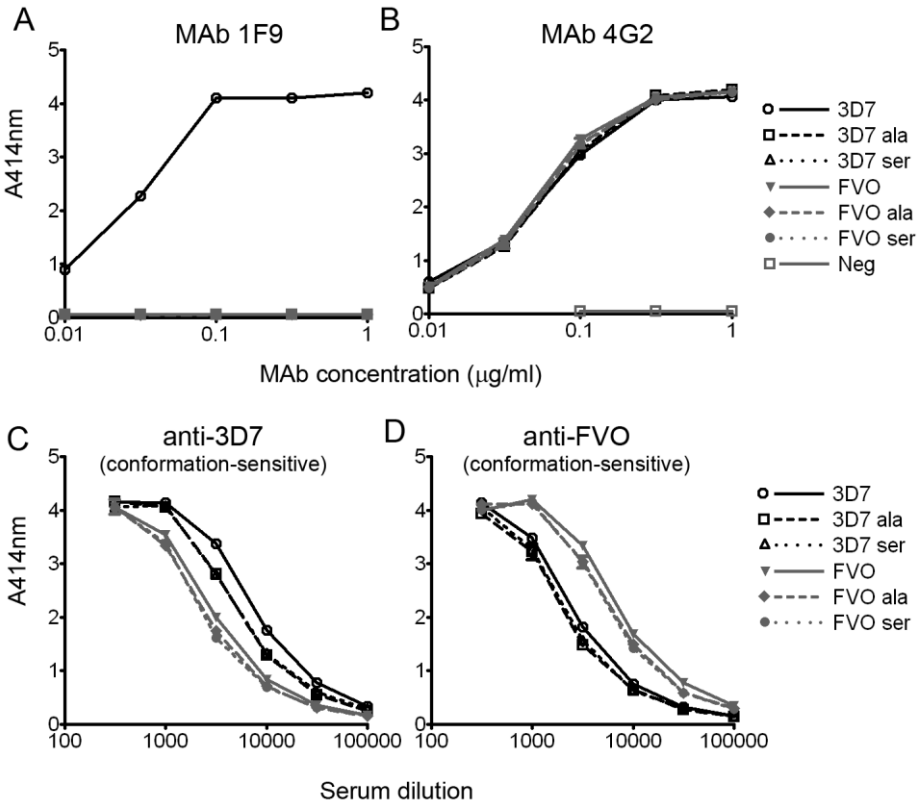

Fig. S1. *AMA1 ala and ser mutants do not bind MAb 1F9 but reactivity with other conformationally sensitive antibodies is retained.* Titration curves showing binding of antibodies to AMA1. MAbs (A) 1F9 and (B) 4G2, and conformation-sensitive antisera raised against (C) 3D7 or (D) FVO AMA1, were allowed to interact with recombinant AMA1 immobilized on a microtiter plate. The average of duplicate wells is reported and error bars represent the range.

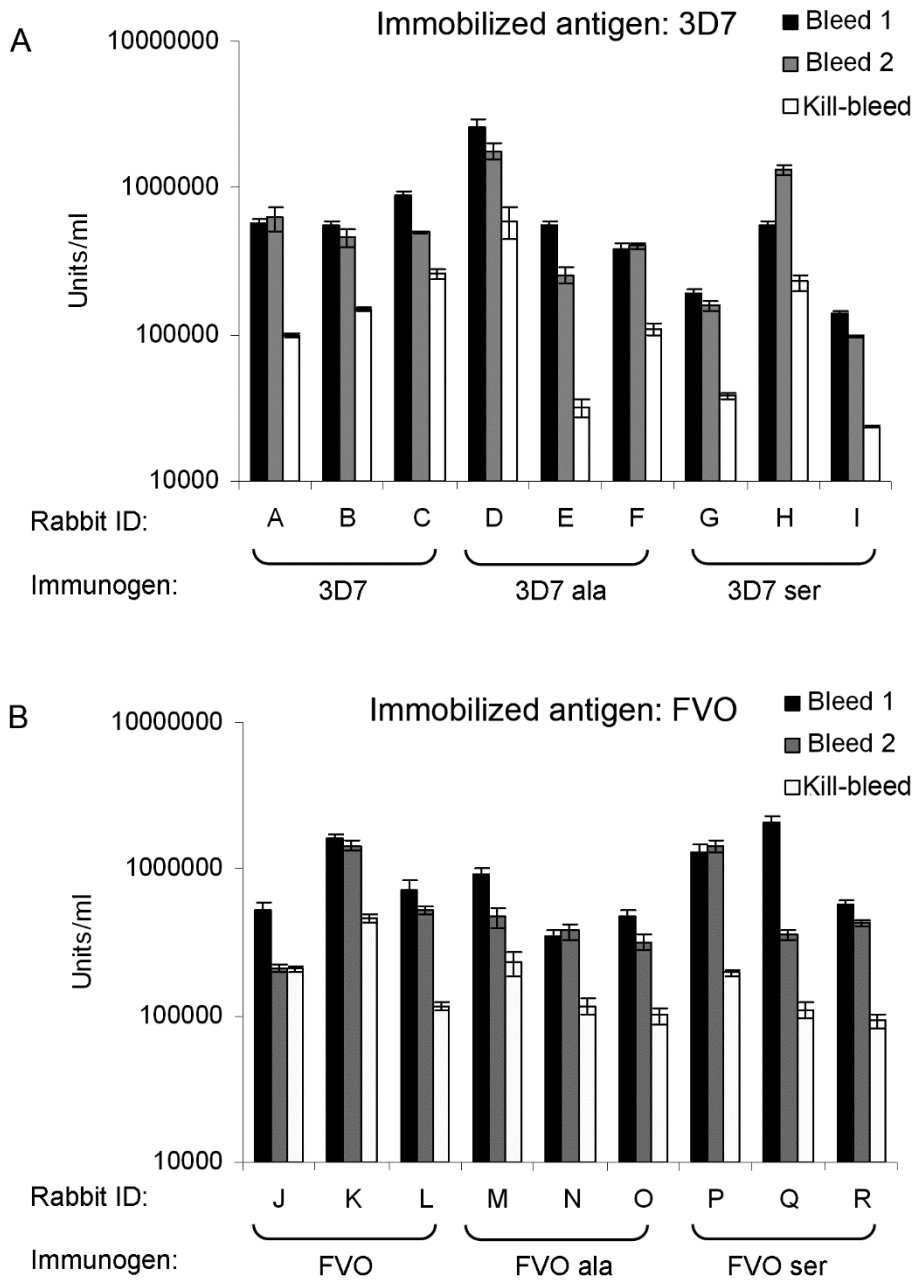

Fig. S2. *Immune response to the wt antigen of rabbits immunized with wt or mutant AMA1.* Serological analyses were conducted using a direct binding ELISA to measure the total IgG antibodies in a single dilution of each serum relative to a standard. Each serum was tested on the appropriate wt antigen only. (A) Antibody responses of rabbits immunized with wt and mutant forms of 3D7 AMA1. (B) Antibody responses of rabbits immunized with wt and mutant forms of FVO AMA1. The average of at least three independent experiments is reported ± SEM.

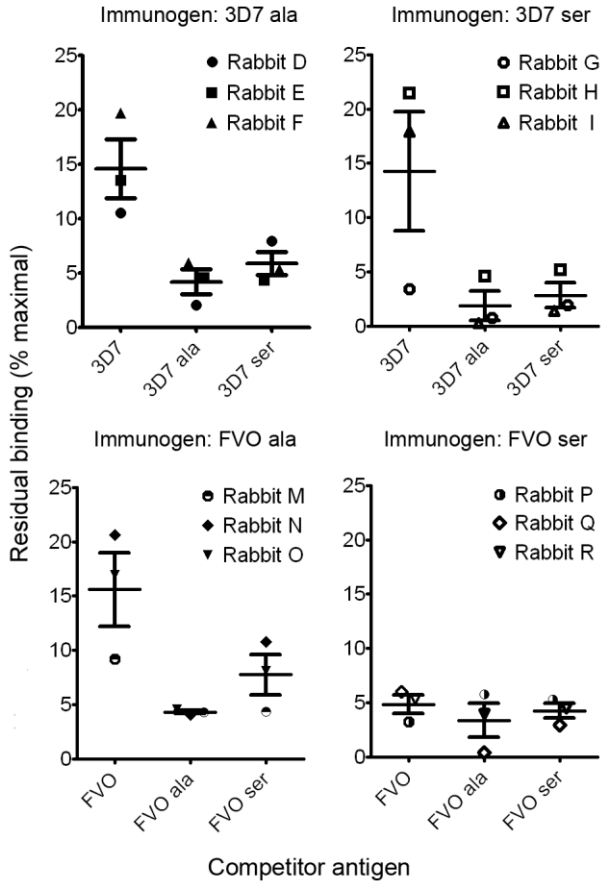

Fig. S3. *Loop Id mutants induce a mutant-specific antibody response.* Constant concentrations of sera raised against mutant forms of AMA1 were allowed to interact with the corresponding mutant antigens (eg. sera raised against 3D7 ala was tested on immobilized 3D7 ala) in the presence of increasing concentrations of soluble competitor antigens. The average residual binding determined from two independent experiments is reported for each rabbit. The mean ± SEM of each immunization group is also shown.

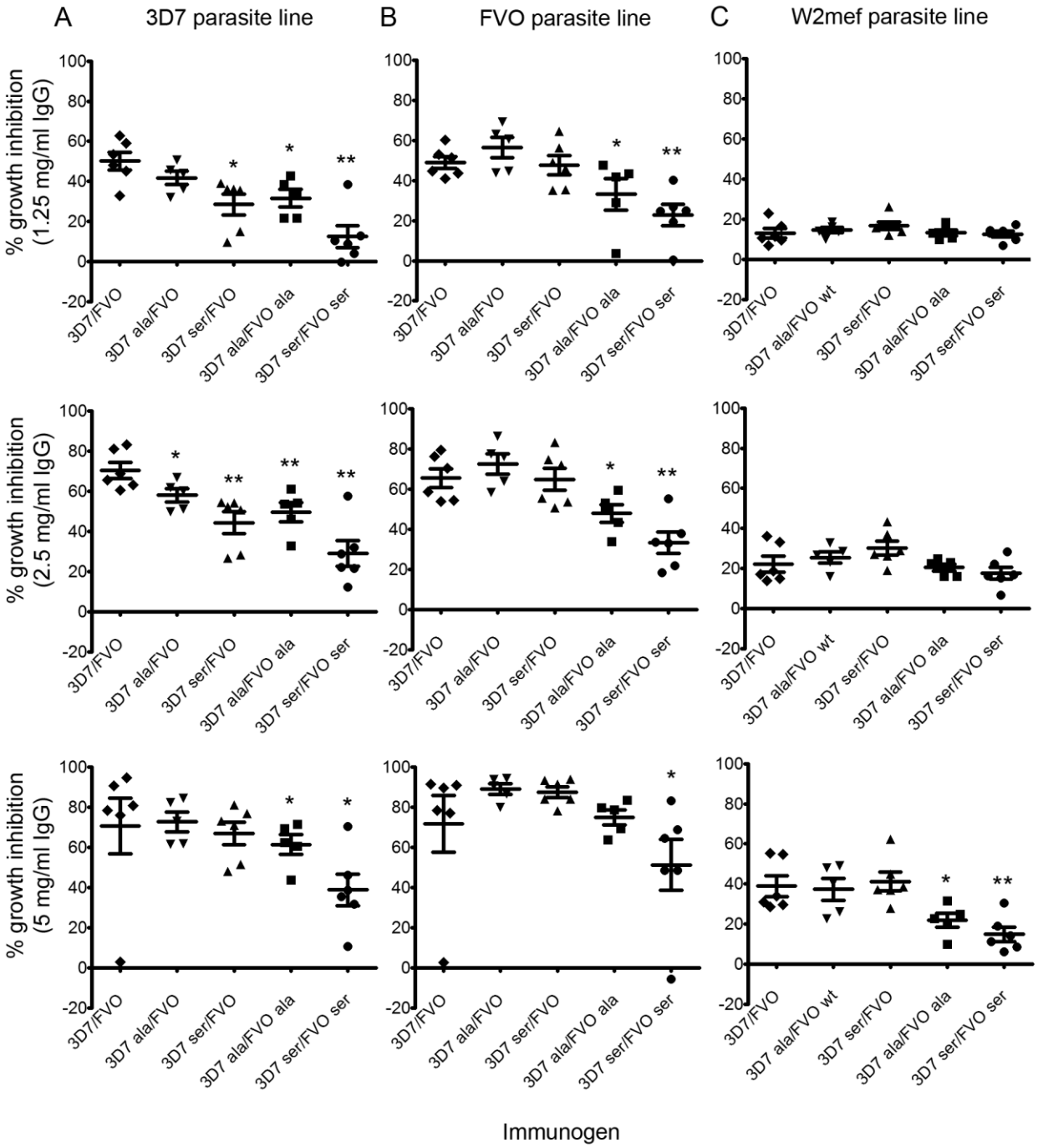

Fig. S4. *Effect of purified IgG from rabbits immunized with individual antigens on the growth of 3D7 and FVO parasite lines.* Inhibition of growth of the (A) 3D7 and (B) FVO parasite lines mediated by different concentrations of IgG rasied against wt and mutant forms of AMA1. (C) Inhibition of the FVO parasite line expressed as a percentage of inhibition of the 3D7 parasite line. (D) Inhibition of the 3D7 parasite line expressed as a percentage of inhibition of the FVO parasite line. For calculation of relative inhibition levels (C & D), an IgG concentration resulting in 40-90% inhibition of the homologous parasite line and >20% inhibition of the heterologous parasite line was used. Two rabbits in the 3D7 ser group and one rabbit in the 3D7 ala group were excluded from this analysis due to low overall inhibition levels (<40%). In all cases, the average of duplicate wells from a single representative experiment is reported for each rabbit. The mean ± SEM of each immunization group is shown where appropriate.

Table S1. *IgG concentrations and percent parasite inhibition used to calculate relative growth inhibition of heterologous versus homologous parasite lines.*

Rabbit ID	Immunogen	mg/ml IgG	% inhibition 3D7[a]	% inhibition FVO[a]
A	3D7 wt	4	88.3	29.4
B	3D7 wt	2	78	21.7
C	3D7 wt	4	77.7	34.5
D	3D7 ala	4	88.6	52.6
E	3D7 ala	-	-	-
F	3D7 ala	4	67.8	36.6
G	3D7 ser	4	72.5	44.8
H	3D7 ser	-	-	-
I	3D7 ser	-	-	-
J	FVO wt	2	28.8	58.6
K	FVO wt	2	54.7	69.2
L	FVO wt	2	33.7	64.3
M	FVO ala	4	34.5	64.4
N	FVO ala	4	42.3	87.1
O	FVO ala	4	24.6	69.6
P	FVO ser	4	36.7	88.1
Q	FVO ser	4	22.3	84.5
R	FVO ser	4	41.3	74.2

[a]The values used to calculate the data displayed in Fig. 6 C & D are reported. Parasite inhibition levels are the average of duplicate wells.

Table S2. *IgG concentrations and percent parasite inhibition used to calculate relative growth inhibition of heterologous versus homologous parasite lines.*

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Rabbit ID	Immunogen	IgG (mg/ml)	% inhibition 3D7[a]	% inhibition FVO[a]	% inhibition W2mef[a]
1	3D7/FVO	-	-	-	-
2	3D7/FVO	2.5	83.3	76.4	36.2
3	3D7/FVO	2.5	81.2	79.6	33.3
4	3D7/FVO	-	-	-	-
5	3D7/FVO	5	76.0	78.5	29.8
6	3D7/FVO	5	80.8	77.2	31.0
7	3D7 ala/FVO	2.5	62.0	76.4	28.5
8	3D7 ala/FVO	2.5	67.0	86.4	26.7
9	3D7 ala/FVO	2.5	60.3	77.6	33.0
10	3D7 ala/FVO	5	61.9	80.2	26.1
11	3D7 ala/FVO	5	61.8	86.8	22.7
12	3D7 ser/FVO	5	51.6	84.2	37.1
13	3D7 ala/FVO	2.5	54.4	72.0	36.8
14	3D7 ala/FVO	2.5	54.2	83.2	43.4
15	3D7 ala/FVO	2.5	50.7	74.8	27
16	3D7 ala/FVO	5	48.2	78.2	27.8
17	3D7 ala/FVO	5	71.0	84.2	38.5
18	3D7 ala/FVO ala	5	62.4	69.3	20.3
19	3D7 ala/FVO ala	5	60.5	79.9	24.8
20	3D7 ala/FVO ala	5	69.3	83.5	22.7
21	3D7 ala/FVO ala	-	-	-	-
22	3D7 ala/FVO ala	5	71.5	78.9	31.6
23	3D7 ser/FVO ser	-	-	-	-
24	3D7 ser/FVO ser	-	-	-	-
25	3D7 ser/FVO ser	5	70.5	83.2	30.5
26	3D7 ser/FVO ser	-	-	-	-
27	3D7 ser/FVO ser	-	-	-	-
28	3D7 ser/FVO ser	-	-	-	-

a The values used to calculate the data displayed in Fig. 9 are reported. Parasite inhibition levels are the average of duplicate wells