

Additional File 7: Supplementary forest plots and results for additional outcomes.

Anaemia

Four studies provided estimates for the association between antibody responses to pregnancy specific *P. falciparum* antigens and maternal anaemia at delivery [1-4], and one study examined antibody responses and anaemia during the second and third trimester [2]. Whilst the definitions of anaemia differed in each study, the overall trend was for increased odds or risk of anaemia in antibody responders compared to non-responders (Supplementary Figure 1, Additional file 7) [1-4]. Notably, Kenyan Ab responders to a placental isolate (EJ24) had a 2.6-fold increase in odds of severe anaemia (Hb <7.0g/dl; 95%CI, 1.81-3.68) compared to non-responders (Supplementary Figure 1, Additional file 7)[1]. Five studies included in narrative form found no association between antibodies to pregnancy-specific pRBC or VAR2CSA antigens and anaemia [5-9] or haematocrit [10]. In contrast, in studies of Malawian women, total IgG and opsonizing Abs to CS2 were positively associated with haemoglobin levels at 36 weeks [11] and decreased anaemia at delivery [12], suggesting antibodies may have a protective effect. Overall, the associations between antibody responses to pregnancy-specific *P. falciparum* antigens and anaemia were heterogeneous, and this is probably reflective of differences in the populations studied, in the specific outcome measurements, and the multiple (non-malarial) causes of anemia.

Severe malaria

A single Sudanese case-control study examined antibody responses in three groups of pregnant women: those with severe malaria, those with uncomplicated malaria, and uninfected controls. Women who were positive for total IgG or opsonic phagocytosis Abs to CS2 pRBC at T2/T3 had reduced odds of severe malaria, when uninfected controls were the reference group, compared to women who were negative for these antibodies (Supplementary Figure 2, Additional file 7); but this association was significant for phagocytic antibodies only [13]. In contrast, DBL5 responders had a non-significant increase in odds of severe malaria compared to non-responders, when uninfected controls were the reference group (Supplementary Figure 2, Additional file 7).

Preterm birth

Three studies provided estimates for the association between antibody responses to pregnancy-specific *P. falciparum* antigens at delivery and odds of preterm birth (PTB), with heterogeneous results (Supplementary Figures 3A, 11A, and 12A, Additional file 7). At delivery, Kenyan women positive for CSA adhesion inhibitory Abs [4] and Malian women positive for DBL3 Abs [14] had reduced odds of PTB, but Ab responders to FV2 had increased odds of PTB compared to non-responders [3]. Only two studies examined the association between antibody responses measured earlier in pregnancy and risk of PTB. Aitken et al found no association between antibodies to CS2, measured at T2 or T3, and PTB (Supplementary Figure 3B, Additional file 7)[2]. Fried et al found that antibody responders to ID1-ID2a at enrolment had increased odds of PTB compared to non-responders (Supplementary Figure 3B, Additional file 7), but this association was not observed for other VAR2CSA antigens, nor in secundigravidae/multigravidae (Supplementary Figure 12B,

Additional file 7) [14]. Conversely, among secundigravidae/multigravidae only, antibody responders to DBL3 at enrolment had decreased odds of PTB, but this association was not observed for other antigens (Supplementary Figure 12B, additional file 7).

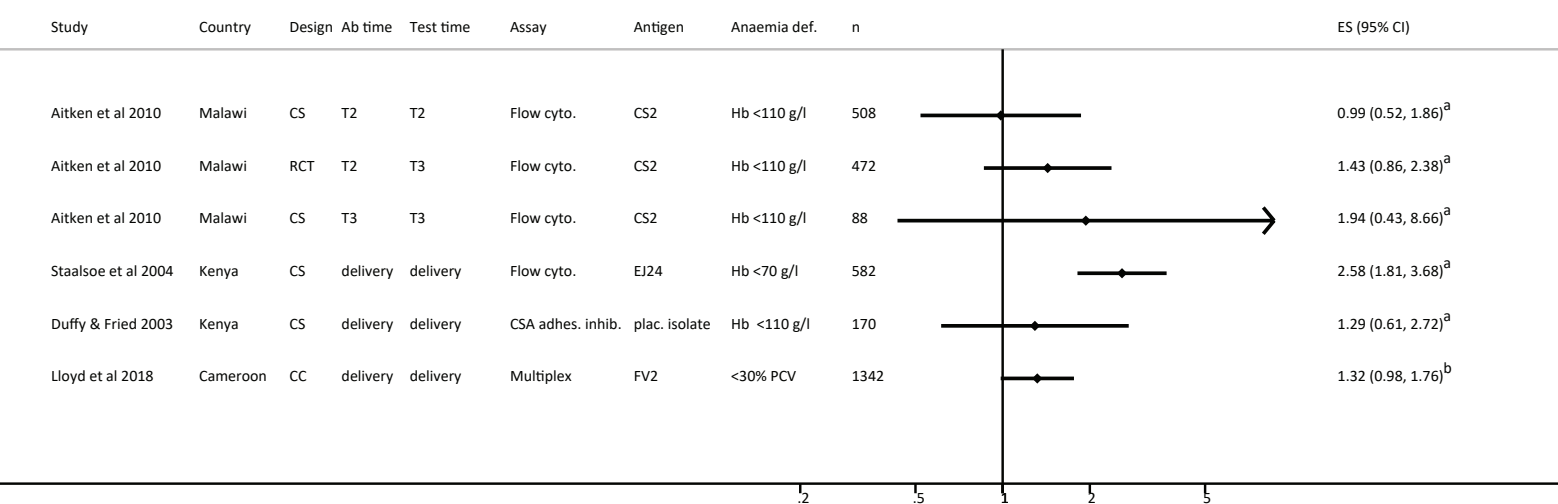
Of the studies included in narrative form, one examined PTB as an outcome and found that of those women negative for peripheral infection at enrolment, higher CSPG-binding inhibitory capacity, but not total IgG, was associated with a lower risk PTB [8]. In addition, one study examined the association between antibody responses to a range of pRBC and VAR2CSA antigens and found that higher Abs against DBL2X were associated with younger gestational age and among women with at least one malaria episode during pregnancy, high Abs to a placental isolate, DBL3X and DBL6E were associated with increased gestational age [10].

References

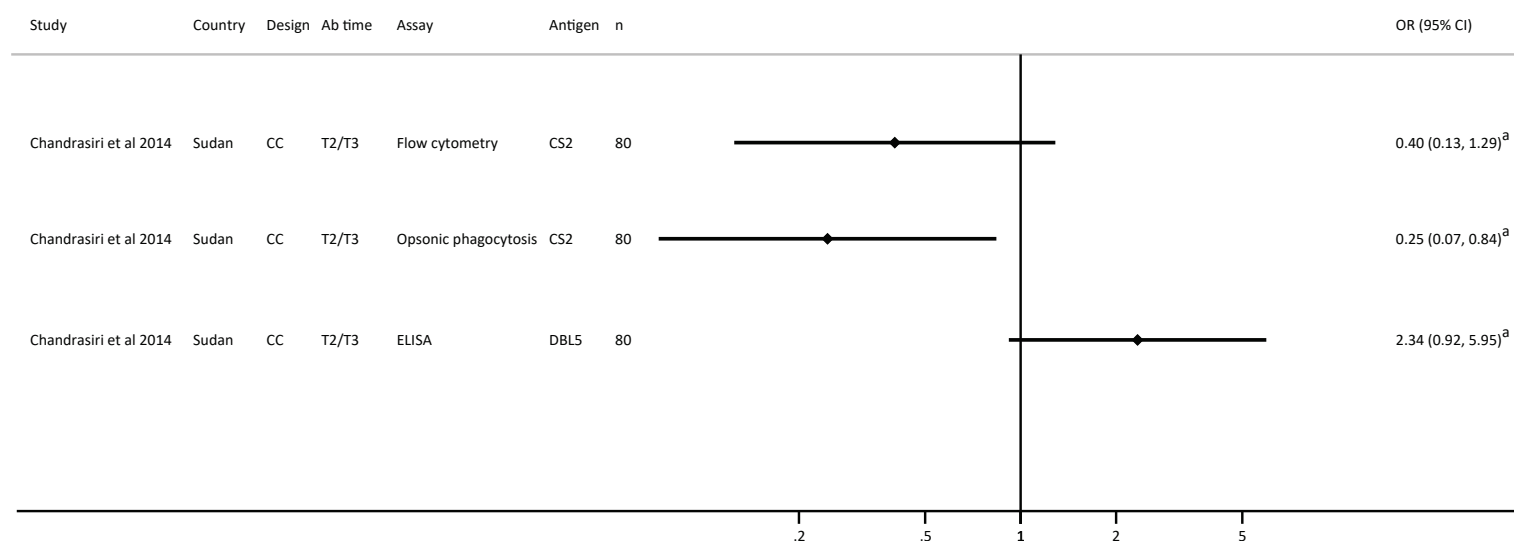
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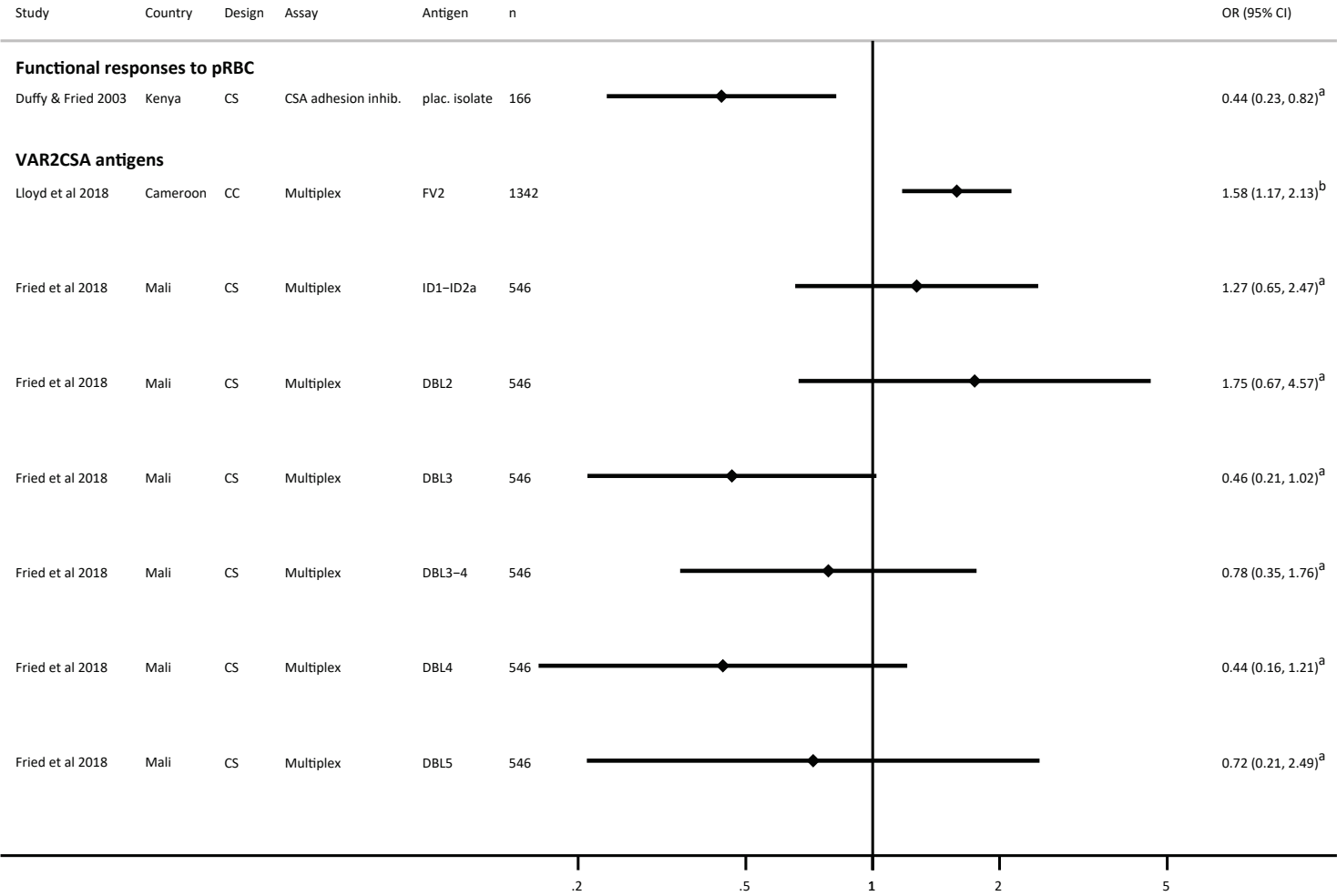


Supplementary Figure 1. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and anaemia. Estimates represent the odds (CS and CC studies) or risk (RCT) of anaemia or severe anaemia, as defined in individual publications, in Ab responders compared to Ab non-responders and are for women of all gravidities included in original studies. Timing of antibody determination (Ab time) and anaemia determination (Test time) are as indicated. ^aData supplied by original authors and estimate calculated by current authors; ^bEstimate calculated by current authors from data in original publication. CS, cross-sectional; CSA adhes. inhib., CSA adhesion inhibition assay; CC, case-control; ES, estimate; Flow cyto., flow cytometry; Hb, haemoglobin; n, number of participants included in estimate; plac. isolate, placental isolate; PCV, packed cell volume; RCT, randomized controlled trial; T2, second trimester; T3, third trimester.

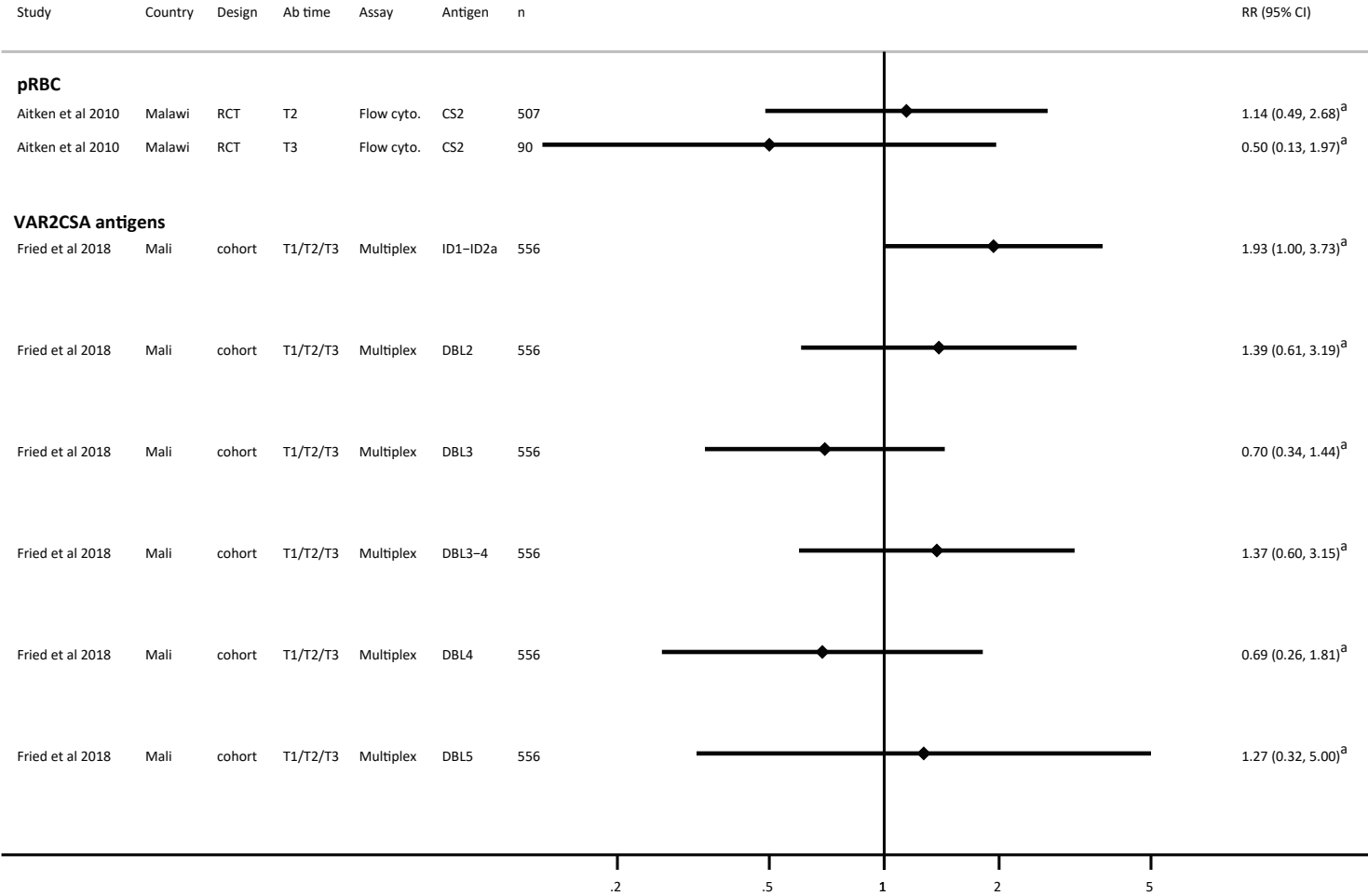


Supplementary Figure 2. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and severe malaria. Estimates represent the odds of severe malaria (compared to uninfected women) in Ab responders compared to Ab non-responders and are for women of all gravidities. ^aEstimate calculated by current authors from data in original publication. CC, case control; n, number of participants included in estimate; OR, odds ratio; T2, second trimester; T3, third trimester.

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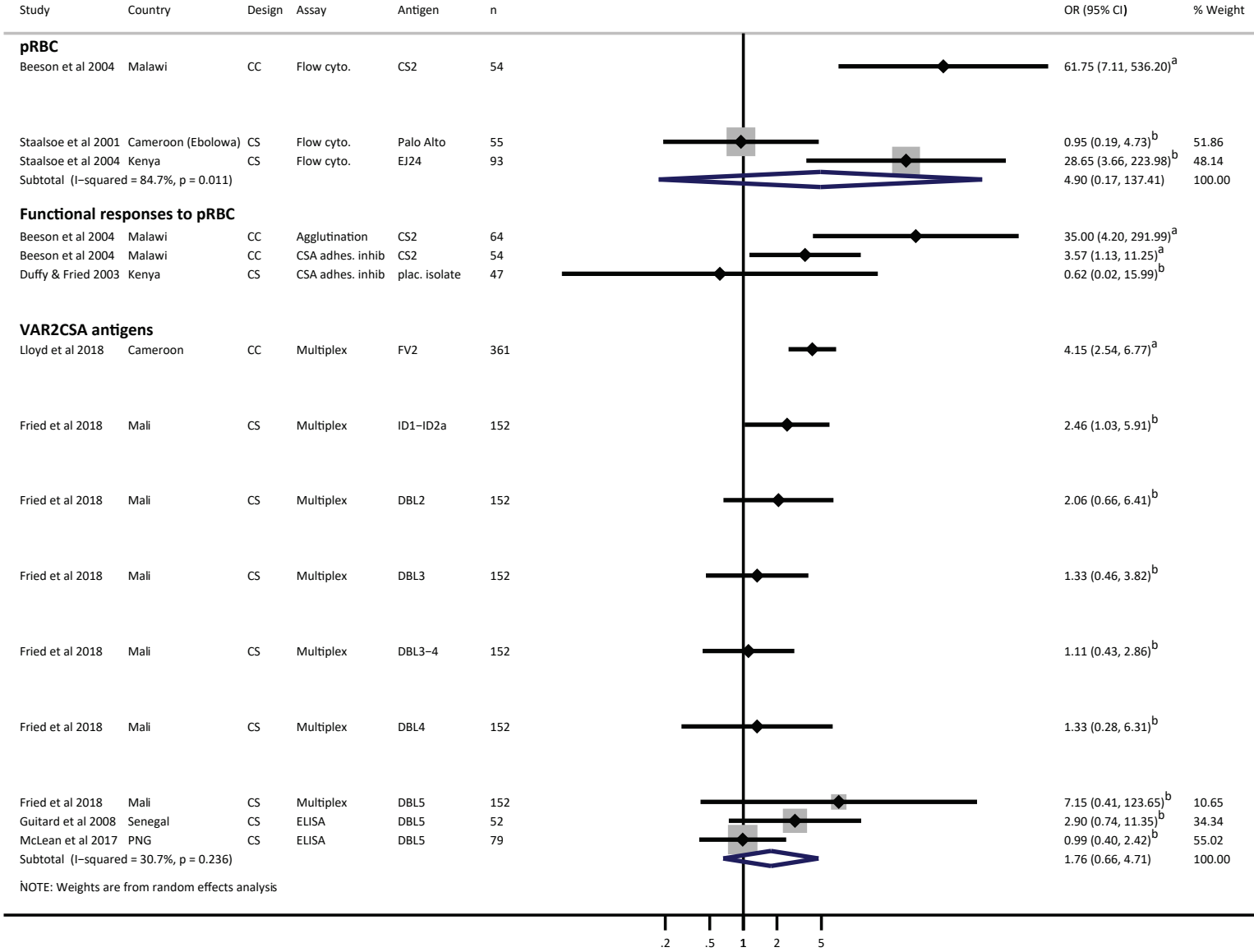


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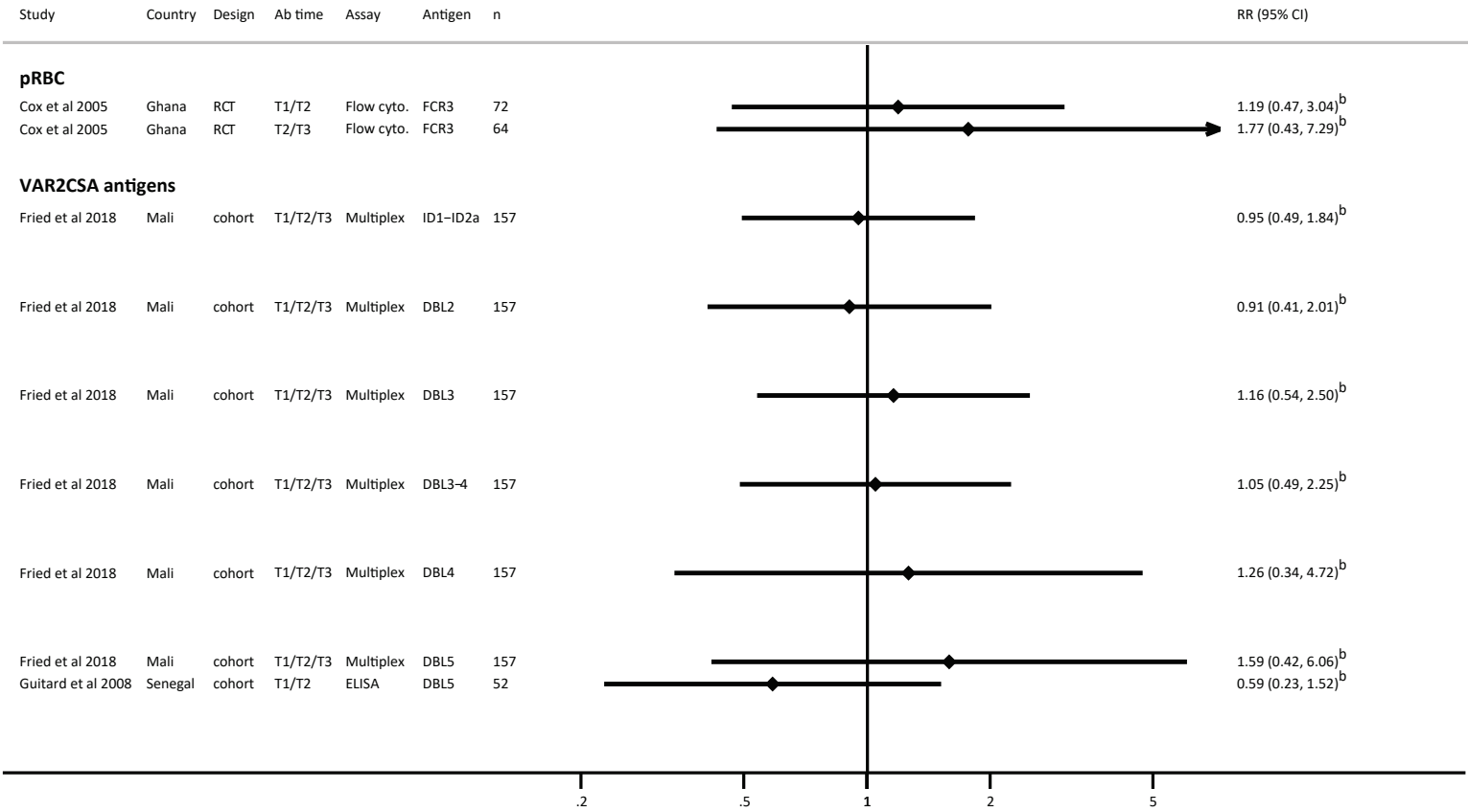


Supplementary Figure 3. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and preterm birth. A. Estimates represent the odds of preterm birth in Ab responders compared to Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of preterm birth in Ab responders compared to non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). Estimates are for women of all gravidities. Meta-analysis was not performed as antigens differed between studies ^aData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; CSA adhesion inhib., CSA adhesion inhibition assay; Flow cyto., flow cytometry; n, number of participants included in estimate; OR, odds ratio; plac. isolate, placental isolate; pRBC, parasitized red blood cells; RCT, randomized controlled trial; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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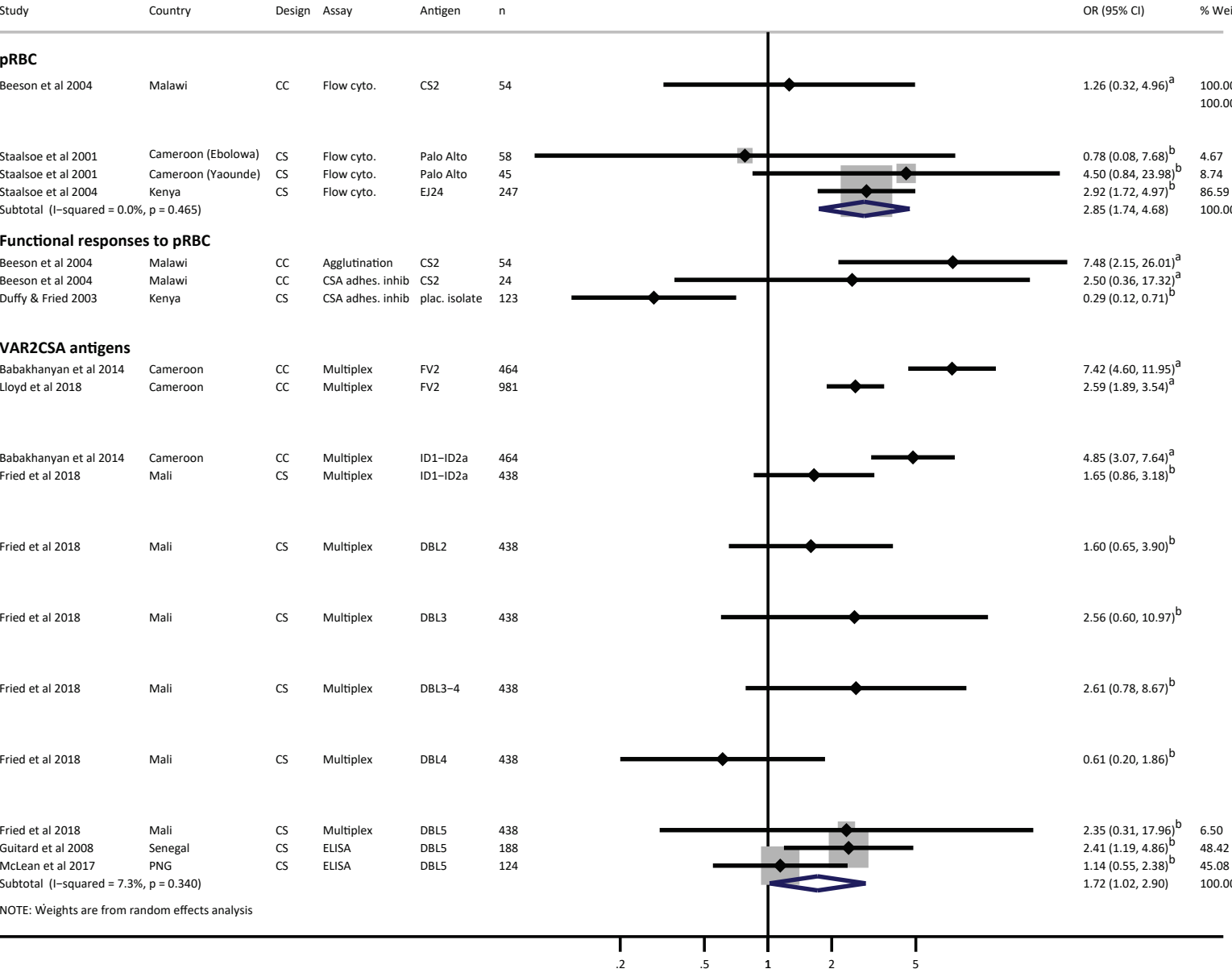


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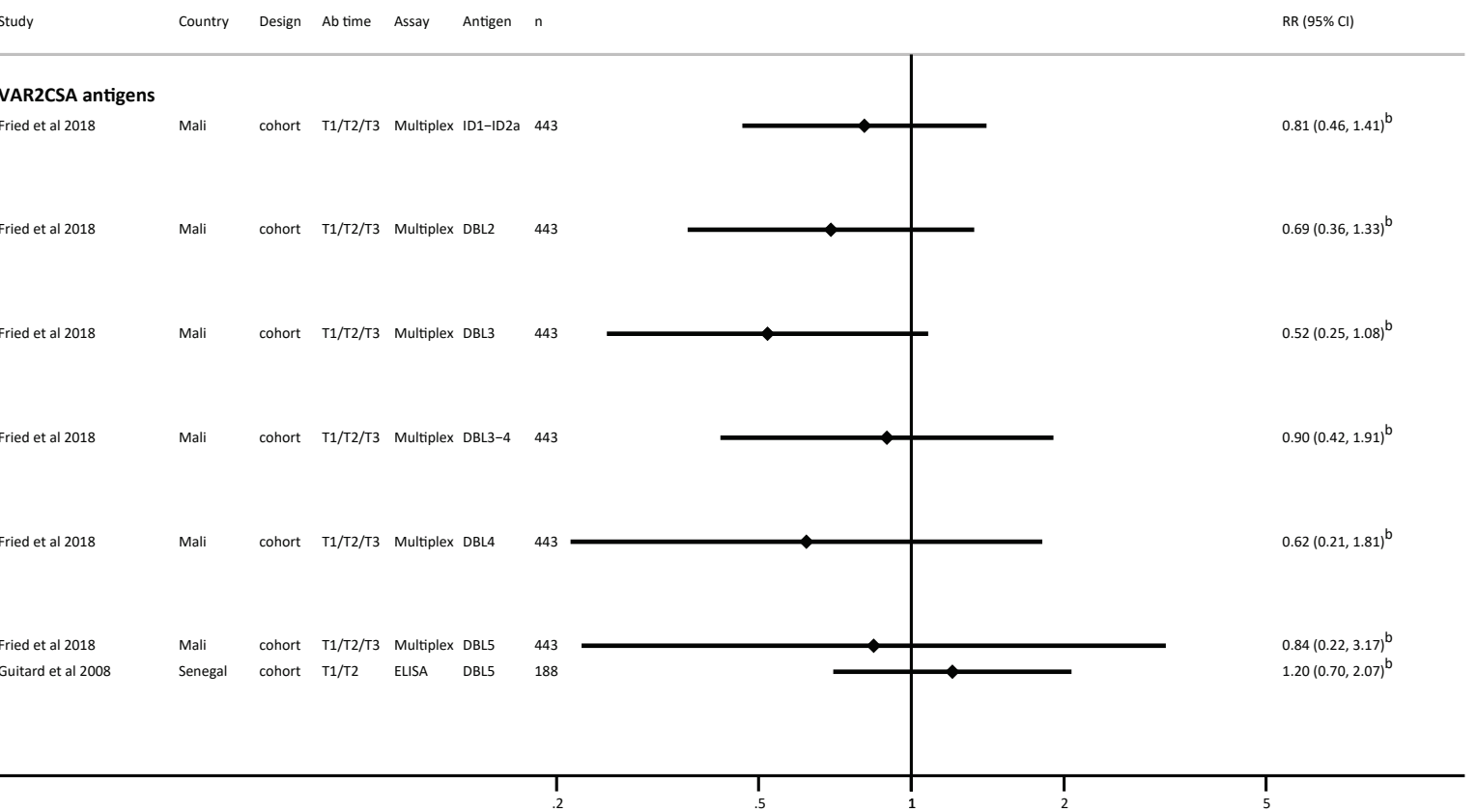


Supplementary Figure 4. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and placental malaria in primigravidae. A. Estimates represent the odds of placental malaria in Ab responders compared to Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of placental malaria in Ab responders compared to non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). Estimates for McLean et al 2017 represent IgG3 responses, as total IgG was not available. Meta-analysis was only performed on estimates where VAR2CSA antigen or functional assay (where applicable) and timing of antibody determination were the same. ^aEstimate calculated by current authors from data in original publication; ^bData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; CSA adhes. inhib., CSA adhesion inhibition assay; n, number of participants included in estimate; OR, odds ratio; plac. isolate, placental isolate; pRBC, parasitized red blood cells; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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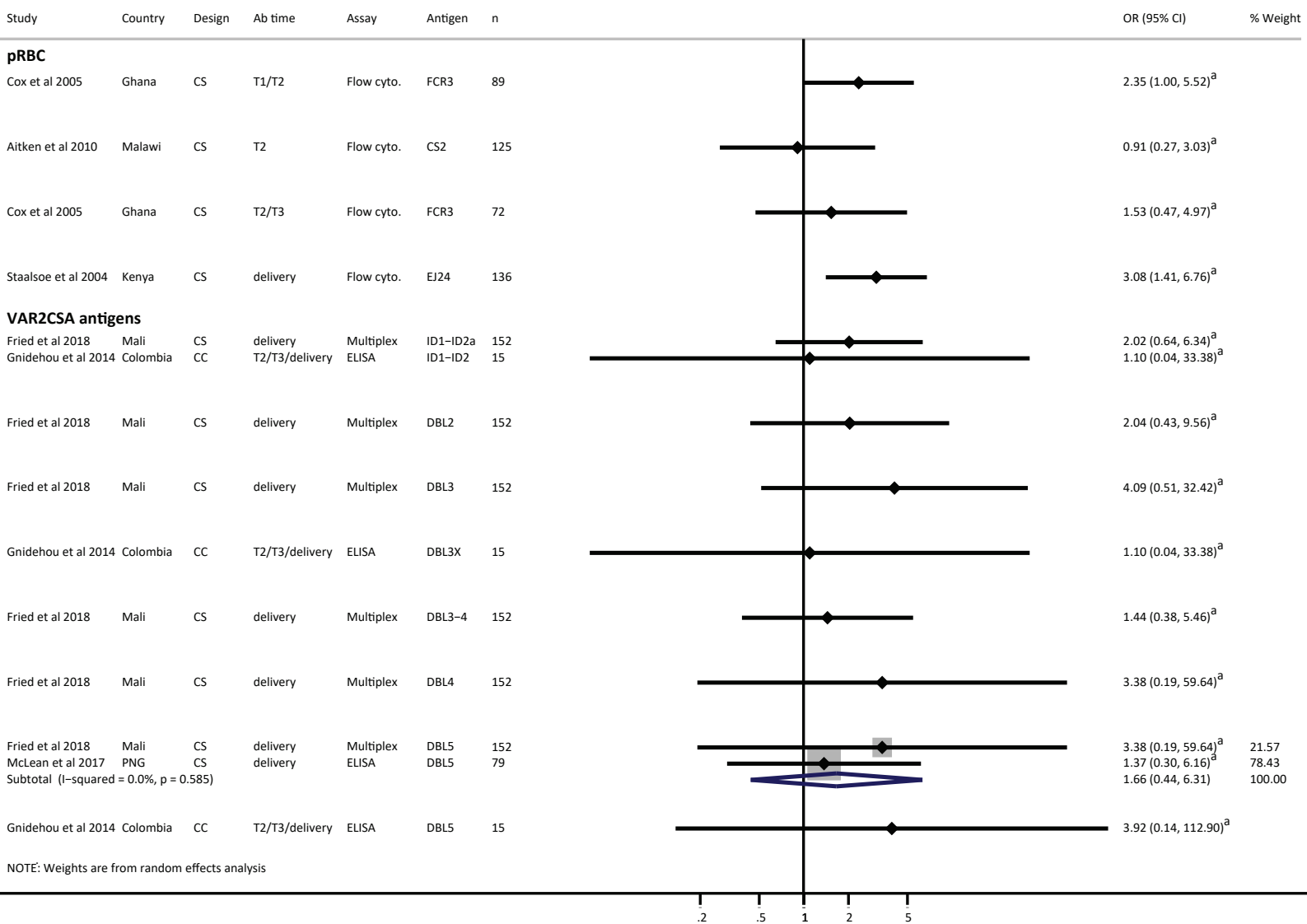


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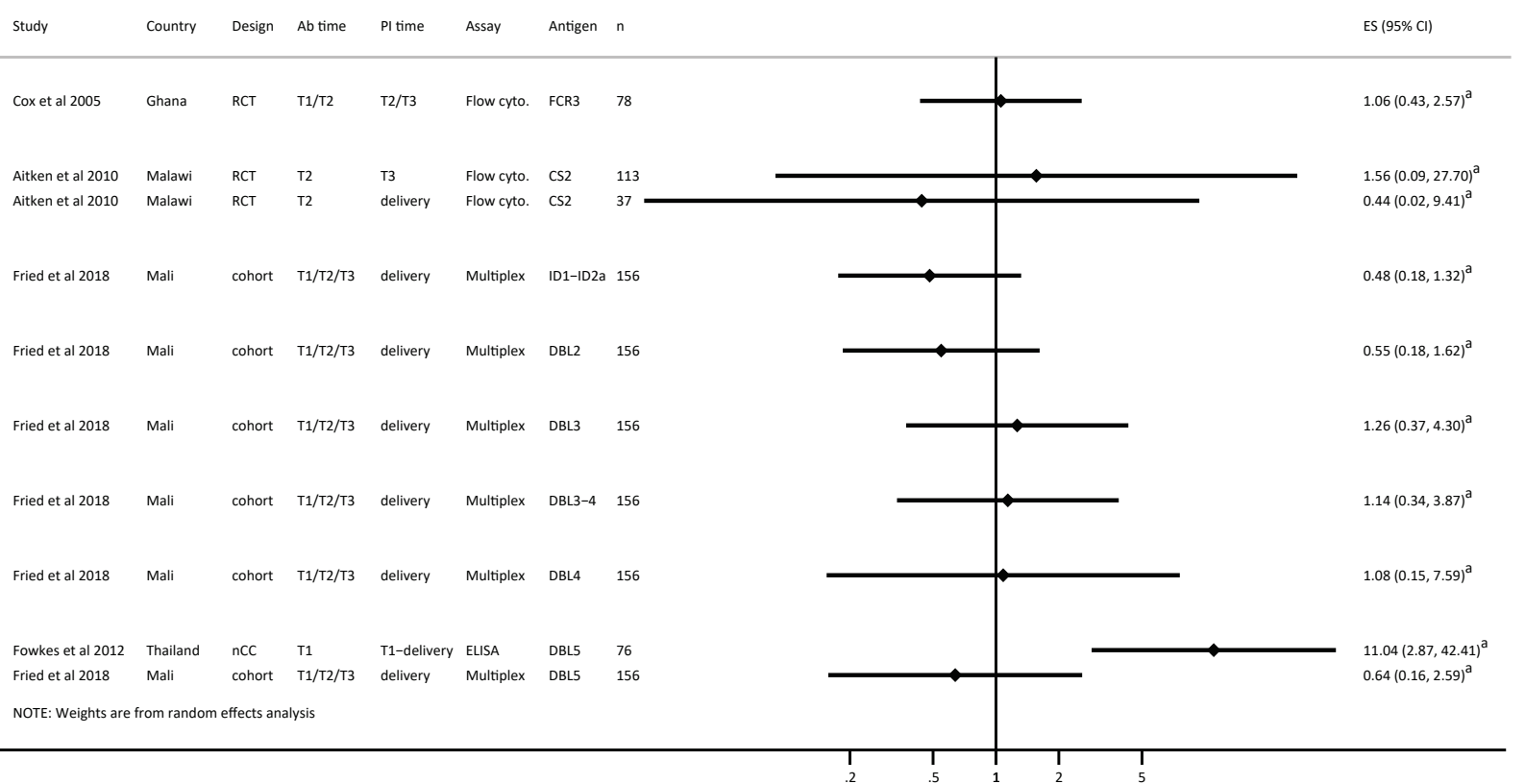


Supplementary Figure 5. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and placental malaria in secundigravidae and multigravidae. A. Estimates represent the odds of placental malaria in Ab responders compared to Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of placental malaria in Ab responders compared to non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). Estimates for McLean et al 2017 represent IgG3 responses, as total IgG was not available. Meta-analysis was only performed on estimates where VAR2CSA antigen or functional assay (where applicable) and timing of antibody determination were the same. ^aEstimate calculated by current authors from data in original publication; ^bData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; CSA adhes. inhib., CSA adhesion inhibition assay; n, number of participants included in estimate; OR, odds ratio; plac. isolate, placental isolate; pRBC, parasitized red blood cells; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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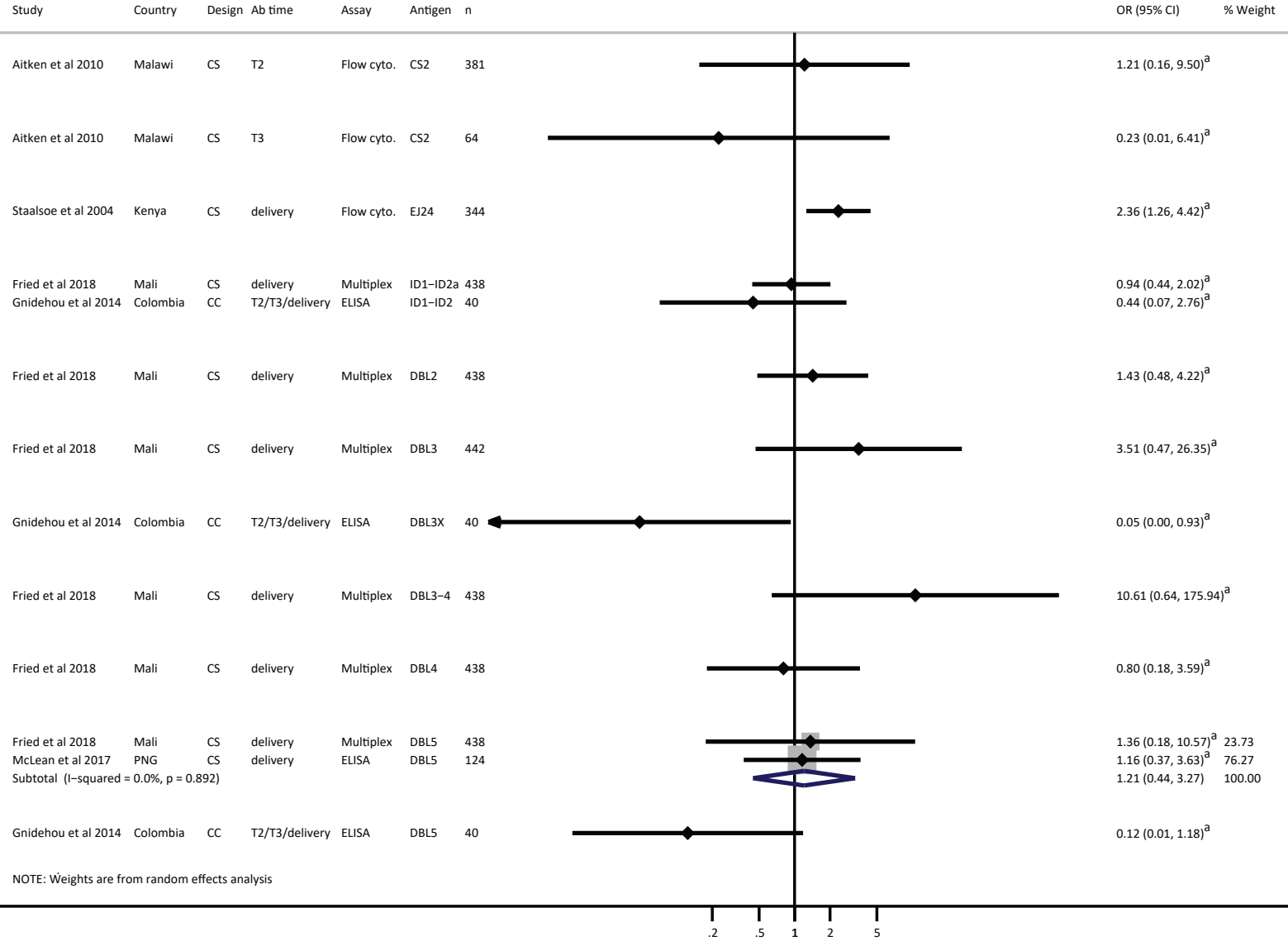


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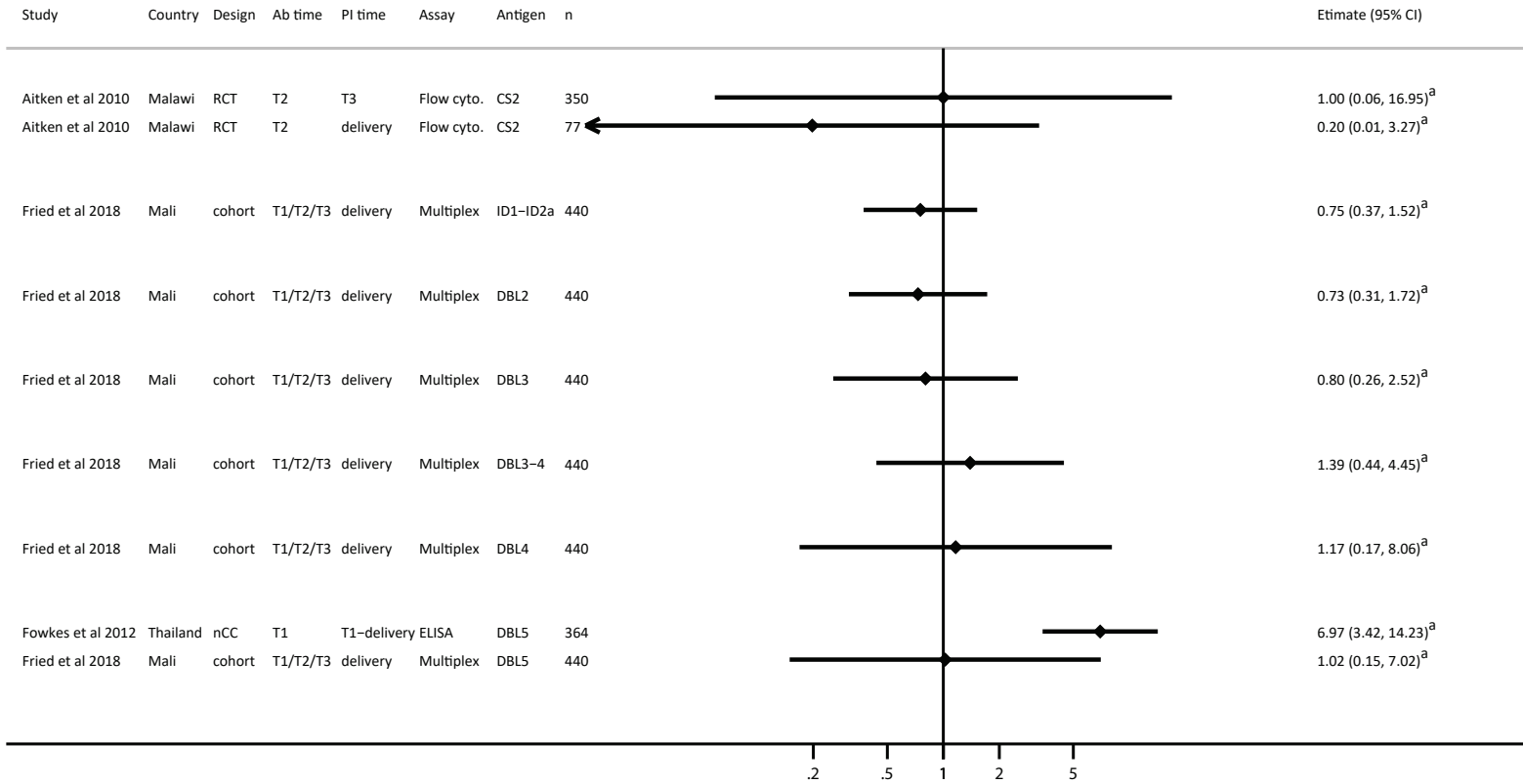


Supplementary Figure 6. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and peripheral parasitaemia in primigravidae. A. Estimates represent the odds of peripheral *P. falciparum* parasitaemia in Ab responders compared to Ab non-responders, where antibodies were measured at the same time point as parasitaemia (cross-sectional studies). B. Estimates of peripheral *P. falciparum* parasitaemia in Ab responders compared to Ab non-responders, where antibodies were measured at time-points prior to parasitaemia determination. Estimates for RCT and cohort studies are risk ratios and estimate for the nested case control study is an odds ratio. The timing of antibody and parasitaemia determination is indicated. Estimate for McLean et al 2017 represents IgG3 responders only as total IgG was not available. DBL5 estimates were only combined when study design was the same. ^aData supplied by original authors and estimate calculated by current authors. CS, cross-sectional; ES, estimate; n, number of participants included in estimate; Flow cyto., flow cytometry; nCC, nested case control; OR, odds ratio; PI time, timing of determination of parasitaemia; pRBC, parasitized red blood cells; RCT, randomized controlled trial; T1, first trimester; T2, second trimester; T3, third trimester.

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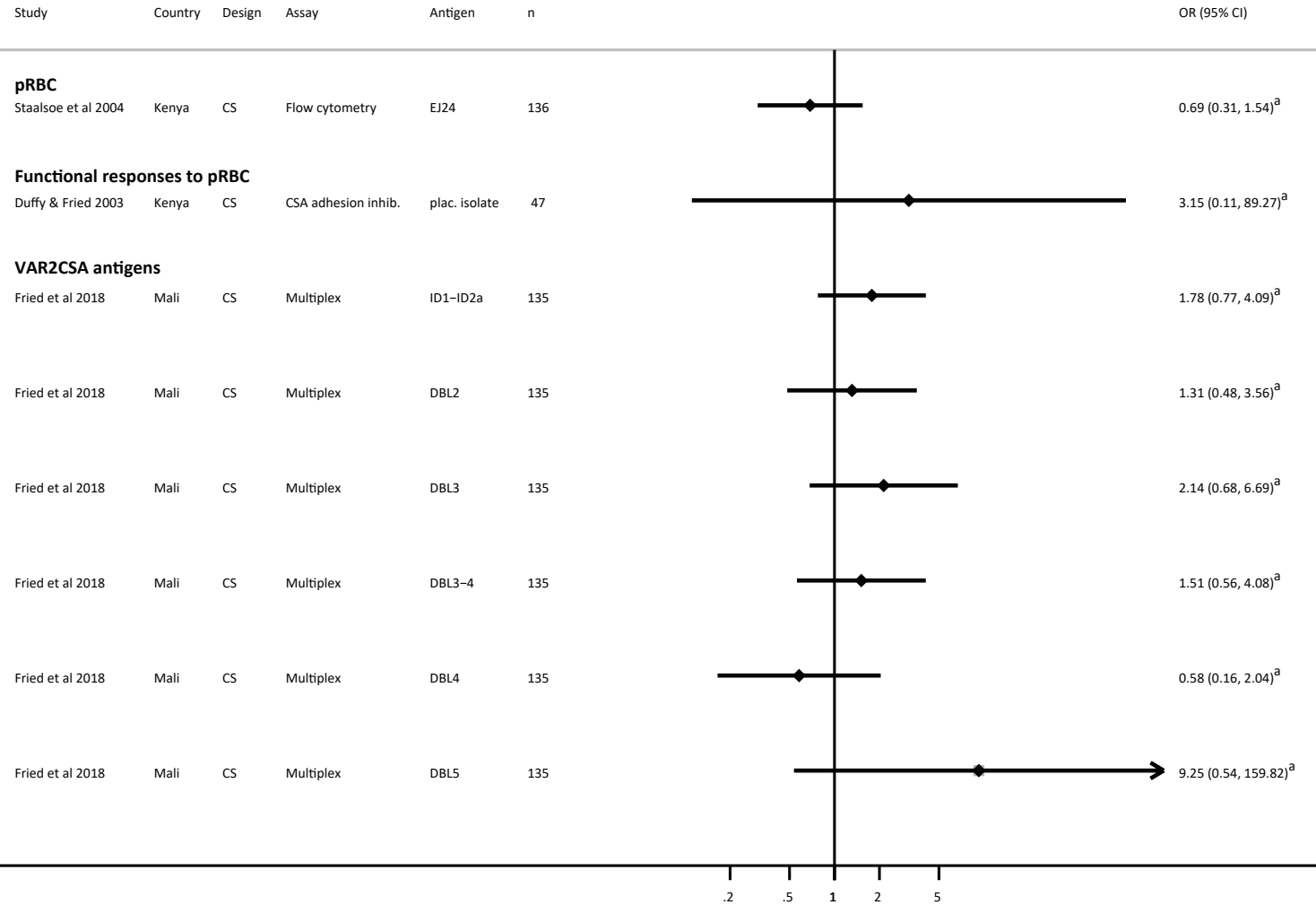
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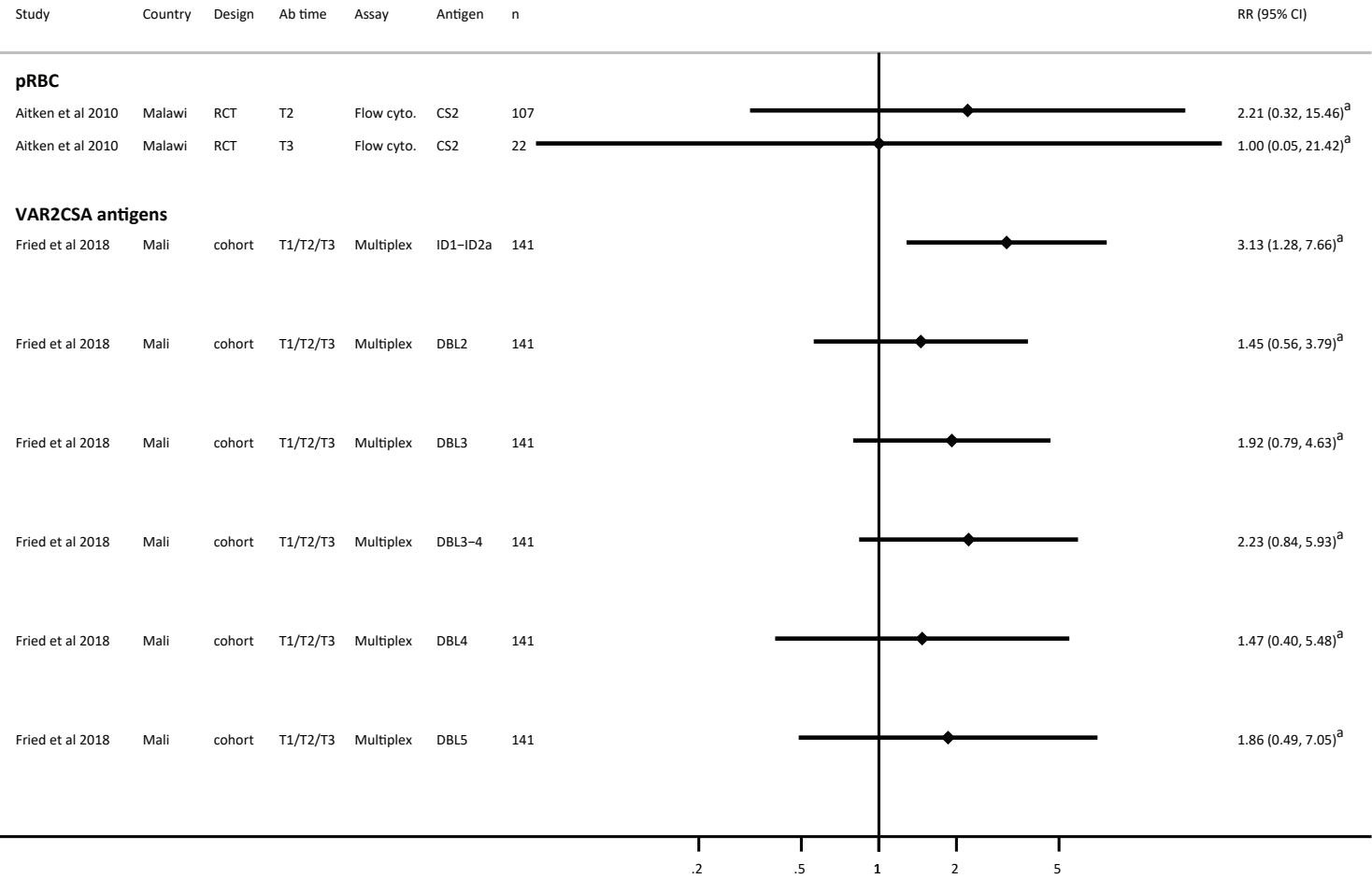
Supplementary Figure 7. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and peripheral parasitaemia in secundigravidae and multigravidae. A.

Estimates represent the odds of peripheral *P. falciparum* parasitaemia in Ab responders compared to Ab non-responders, where antibodies were measured at the same time point as parasitaemia (cross-sectional studies). B. Estimates of peripheral *P. falciparum* parasitaemia in Ab responders compared to Ab non-responders, where antibodies were measured at time-points prior to parasitaemia determination. Estimates for RCT and cohort studies are risk ratios and estimate for the nested case control study is an odds ratio. The timing of antibody and parasitaemia determination is indicated. Estimate for McLean et al 2017 represents IgG3 responders only as total IgG was not available. DBL5 estimates were only combined when study design was the same. ^aData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; ES, estimate; n, number of participants included in estimate; Flow cyto., flow cytometry; nCC, nested case control; OR, odds ratio; PI time, timing of determination of parasitaemia; pRBC, parasitized red blood cells; RCT, randomized controlled trial; T1, first trimester; T2, second trimester; T3, third trimester.

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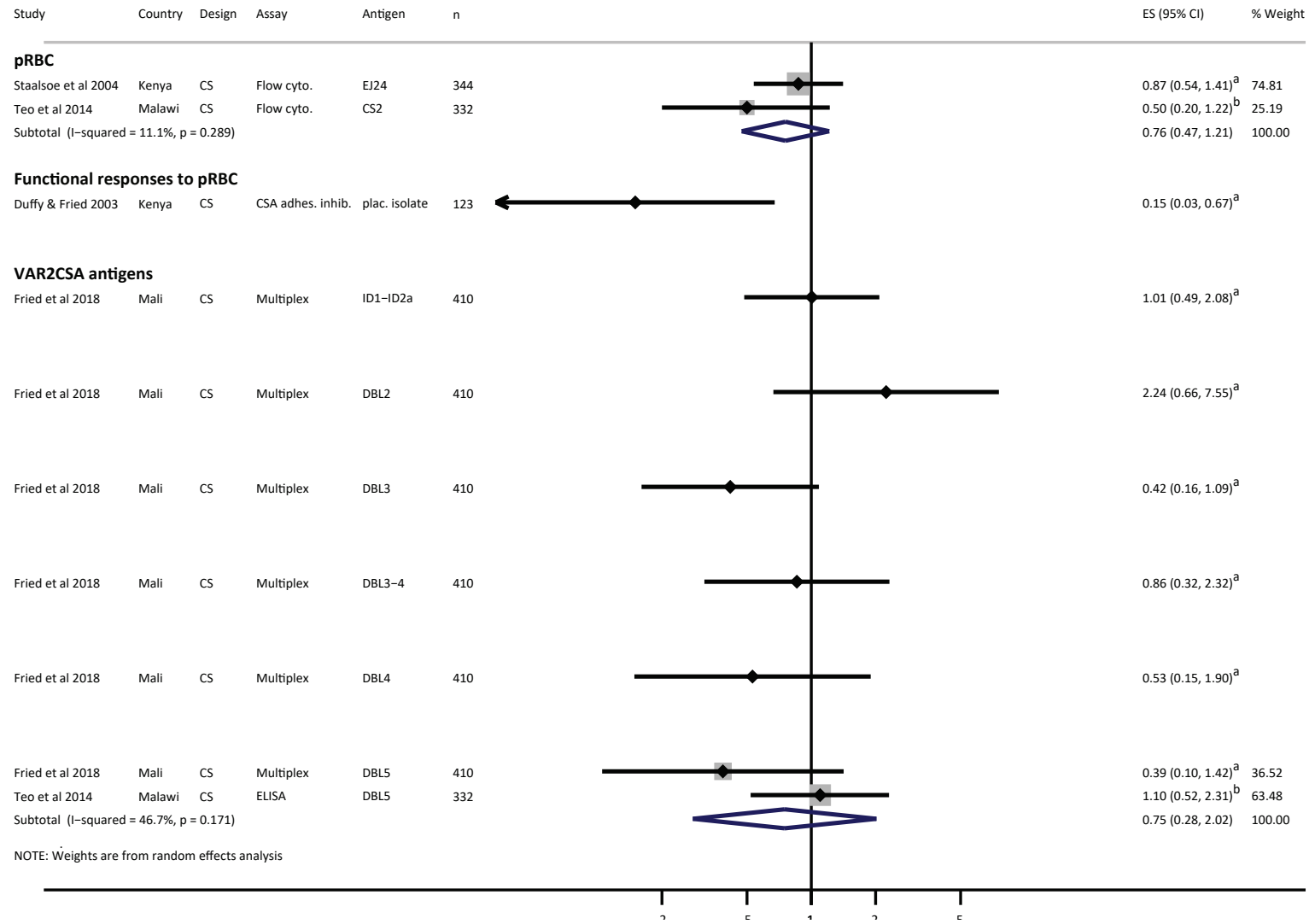


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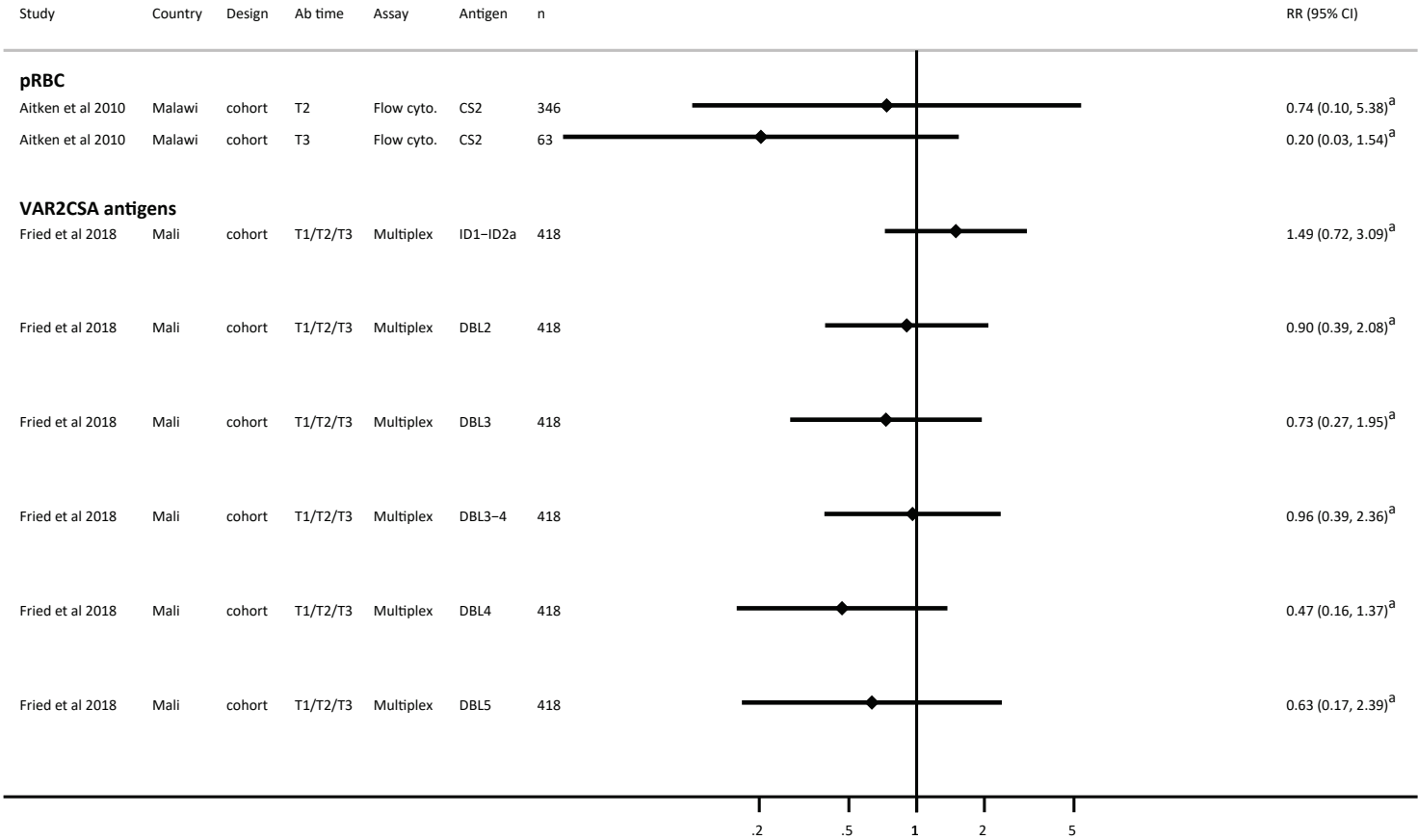


Supplementary Figure 8. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and low birthweight in primigravidae. A. Estimates represent the odds of low birthweight birth in Ab responders compared with Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of low birthweight birth in Ab responders compared with Ab non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). ^aData supplied by original authors and estimate calculated by current authors. CC, case-control; CS, cross-sectional; n, number of participants included in estimate; OR, odds ratio; pRBC, parasitized red blood cells; RCT, randomized controlled trial; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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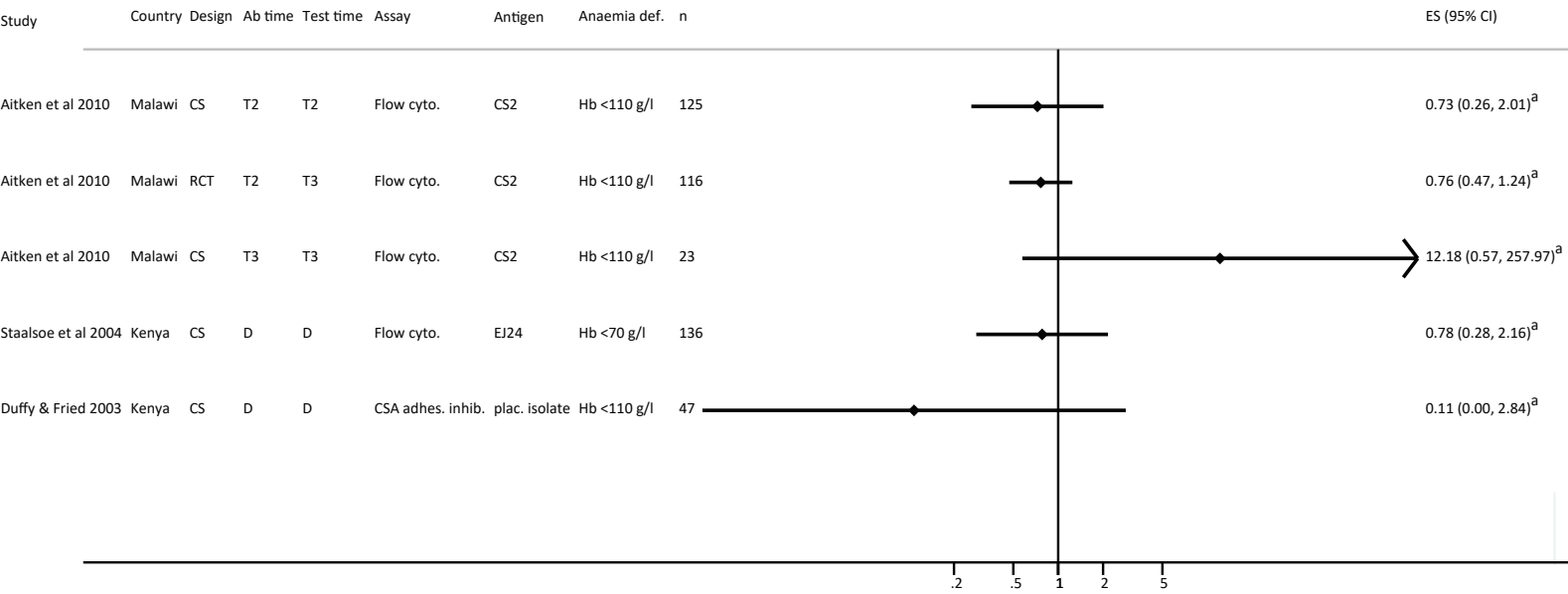


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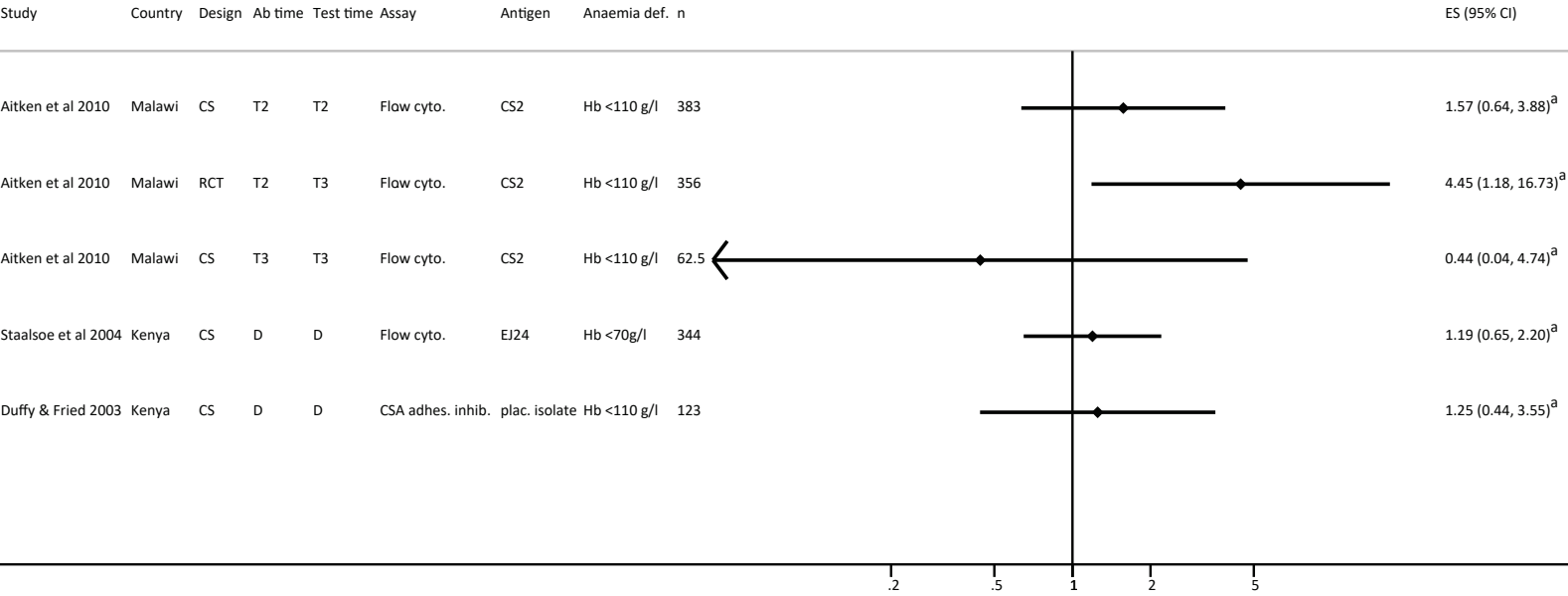


Supplementary Figure 9. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and low birthweight in secundigravidae and multigravidae. A. Estimates represent the odds of low birthweight birth in Ab responders compared with Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of low birthweight birth in Ab responders compared with Ab non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). ^aData supplied by original authors and estimate calculated by current authors. CC, case-control; CS, cross-sectional; ES, estimate; n, number of participants included in estimate; pRBC, parasitized red blood cells; RCT, randomized controlled trial; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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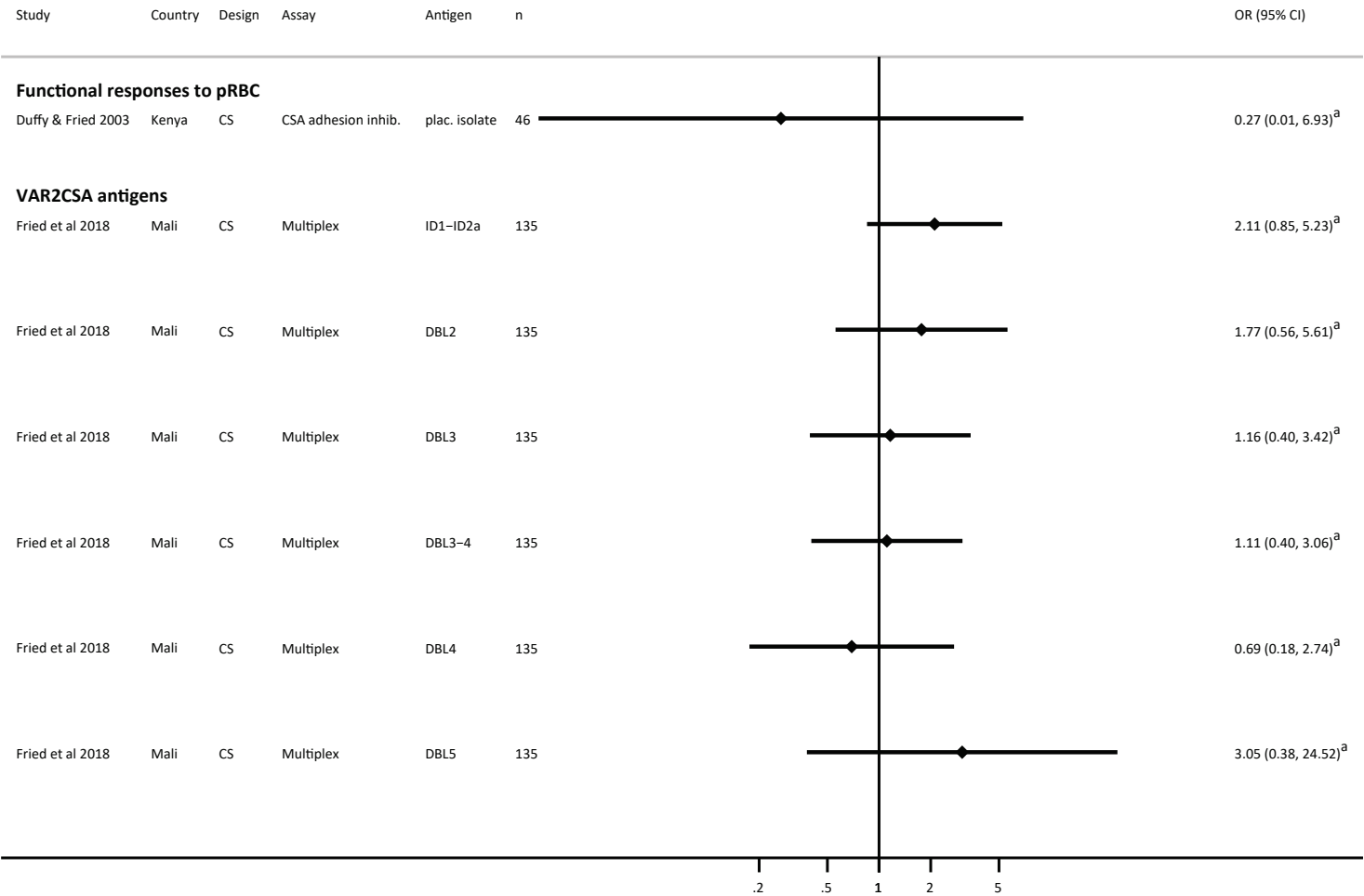


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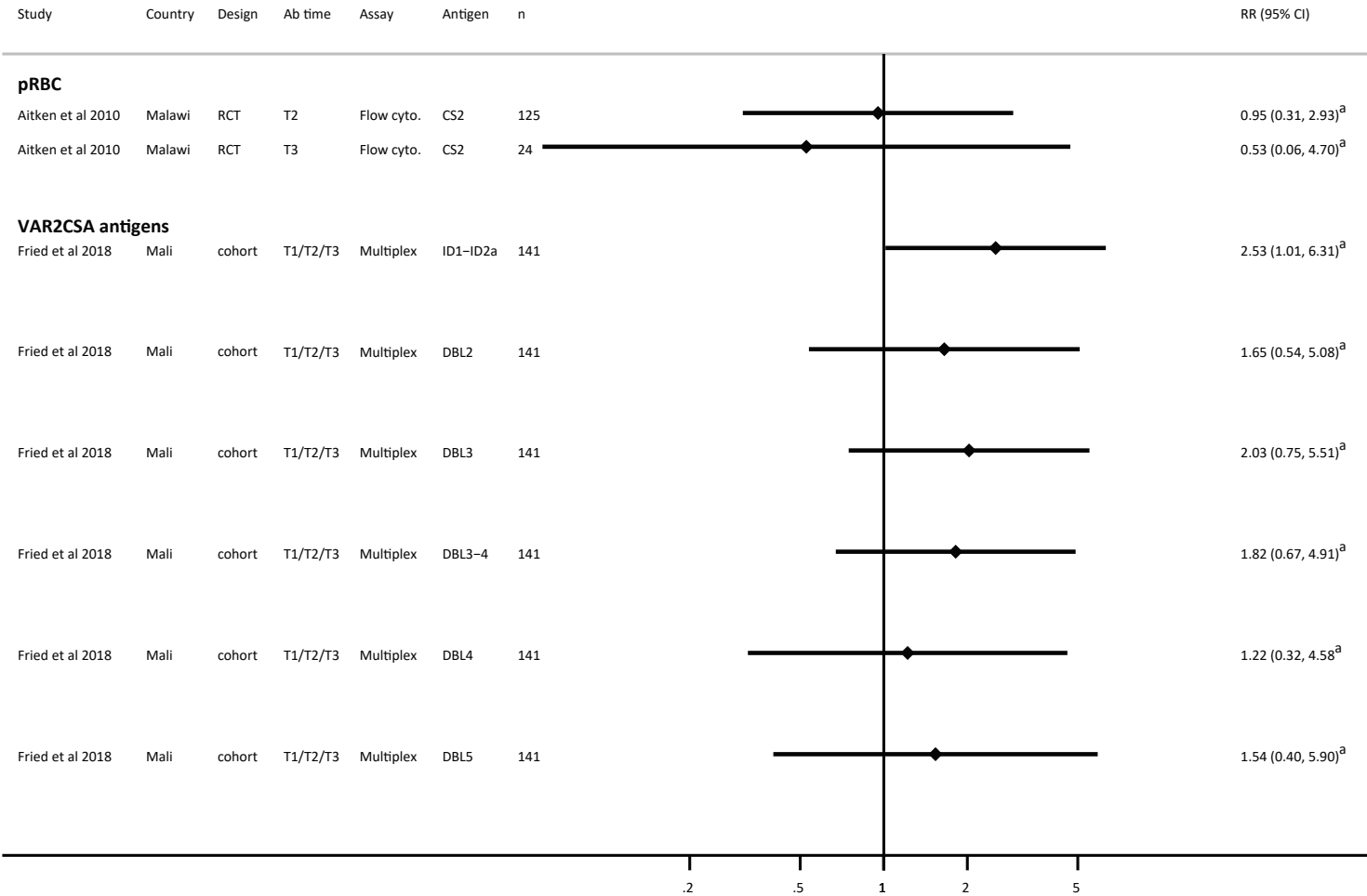


Supplementary Figure 10. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and anaemia in primigravidae (A) and secundigravidae and multigravidae (B). Estimates represent the odds (CS and CC studies) or risk (RCT) of anaemia or severe anaemia, as defined in individual publications, in Ab responders compared to Ab non-responders. Timing of antibody determination (Ab time) and anaemia determination (Test time) are as indicated. ^aData supplied by original authors and estimate calculated by current authors; ^bEstimate calculated by current authors from data in original publication. CS, cross-sectional; CSA adhes. inhib., CSA adhesion inhibition assay; CC, case-control; ES, estimate; Flow cyto., flow cytometry; Hb, haemoglobin; n, number of participants included in estimate; plac. isolate, placental isolate; PCV, packed cell volume; RCT, randomized controlled trial; T2, second trimester; T3, third trimester.

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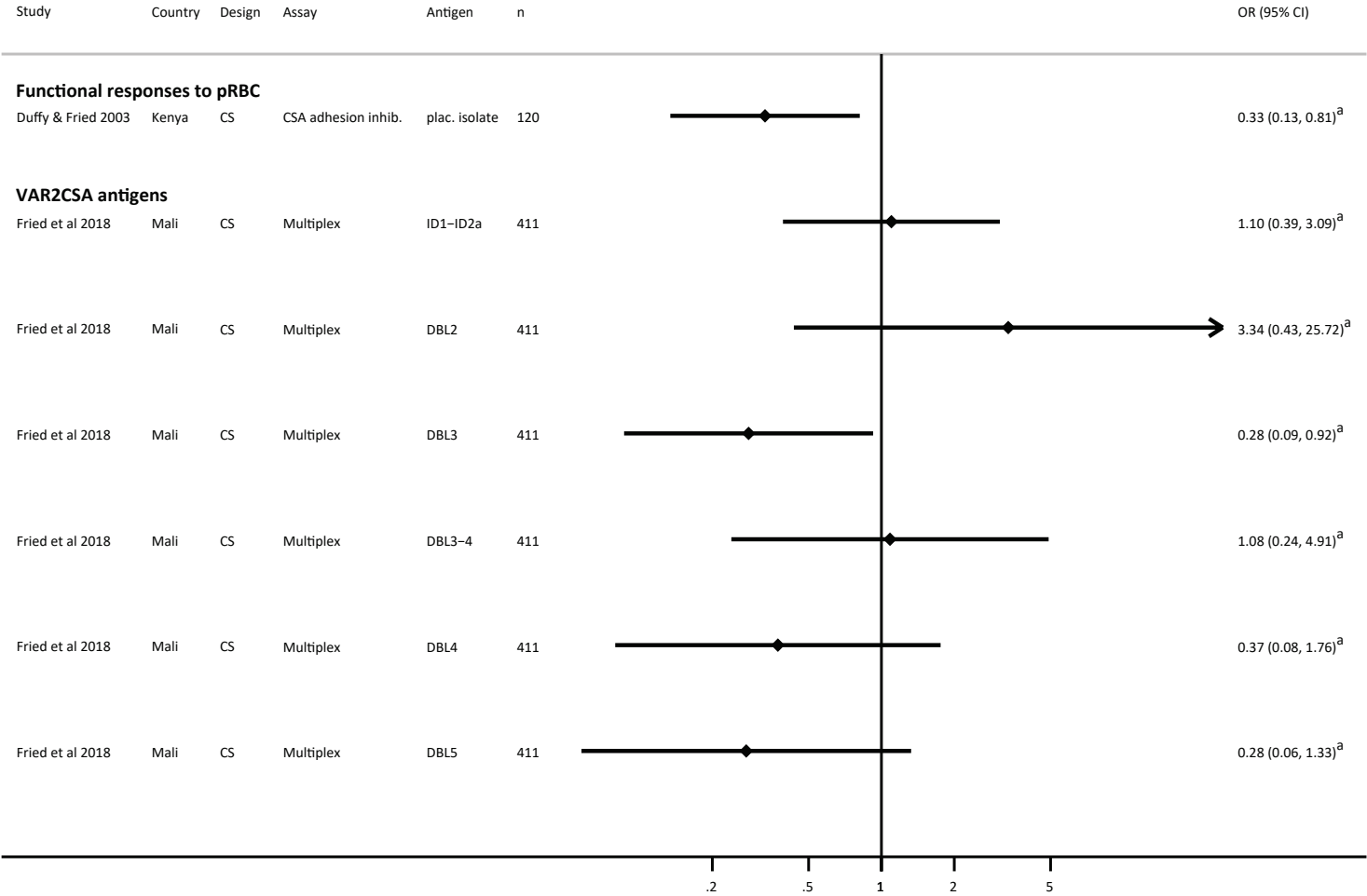


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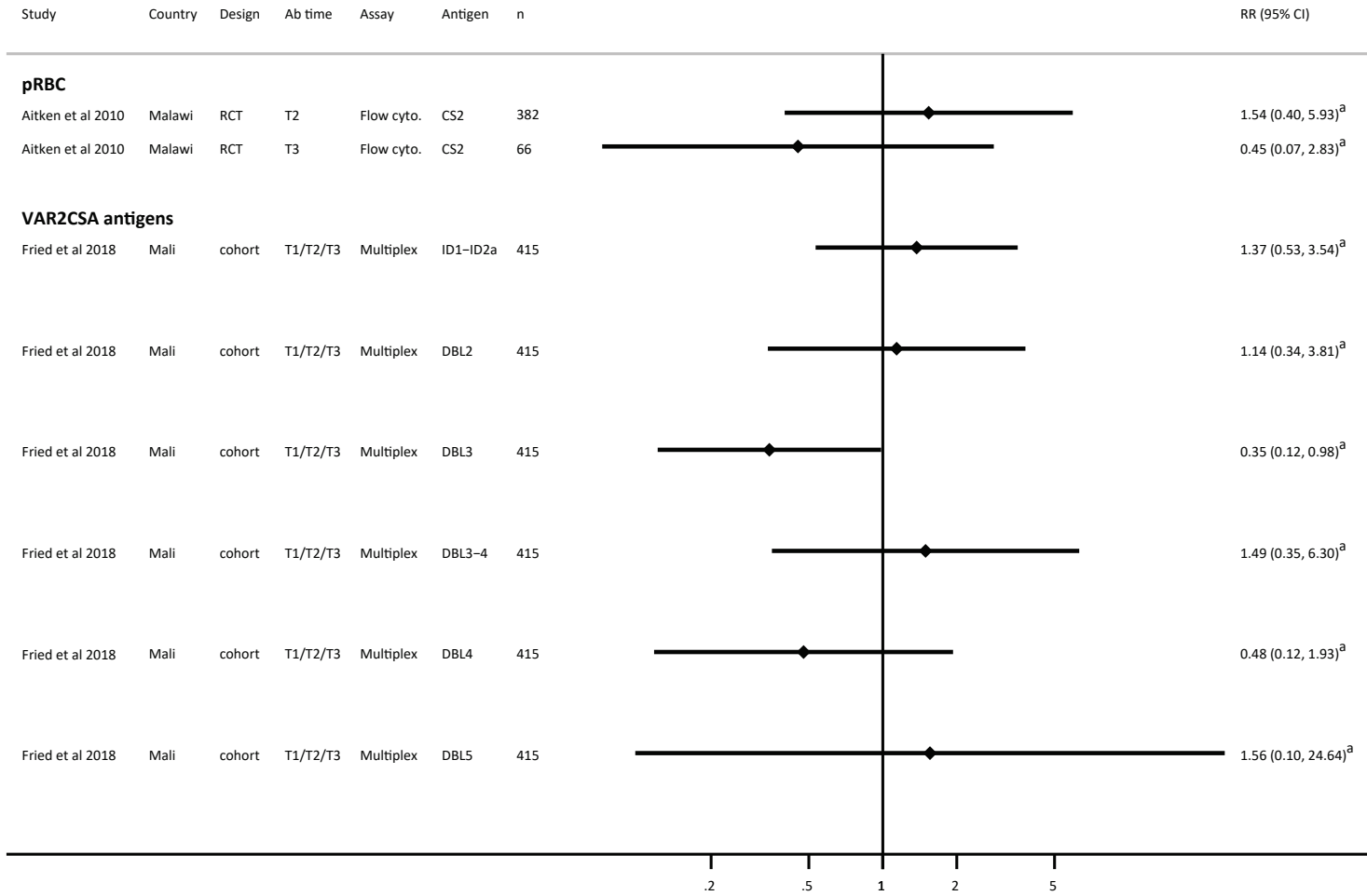


Supplementary Figure 11. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and preterm birth in primigravidae. A. Estimates represent the odds of preterm birth in Ab responders compared to Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of preterm birth in Ab responders compared to non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). Meta-analysis was not performed as antigens differed between studies ^aData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; CSA adhesion inhib., CSA adhesion inhibition assay; Flow cyto., flow cytometry; n, number of participants included in estimate; OR, odds ratio; plac. isolate, placental isolate; pRBC, parasitized red blood cells; RCT, randomized controlled trial; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.

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Supplementary Figure 12. Forest plot of the association between antibodies to pregnancy-associated *P. falciparum* antigens and preterm birth in secundigravidae and multigravidae. A.

Estimates represent the odds of preterm birth in Ab responders compared to Ab non-responders, where antibodies were measured at delivery (cross-sectional studies). B. Estimates represent the risk of preterm birth in Ab responders compared to non-responders, where antibodies were measured at time-points prior to delivery, as indicated (prospective studies). Meta-analysis was not performed as antigens differed between studies ^aData supplied by original authors and estimate calculated by current authors. CC, case control; CS, cross-sectional; CSA adhesion inhib., CSA adhesion inhibition assay; Flow cyto., flow cytometry; n, number of participants included in estimate; OR, odds ratio; plac. isolate, placental isolate; pRBC, parasitized red blood cells; RCT, randomized controlled trial; RR, risk ratio; T1, first trimester; T2, second trimester; T3, third trimester.