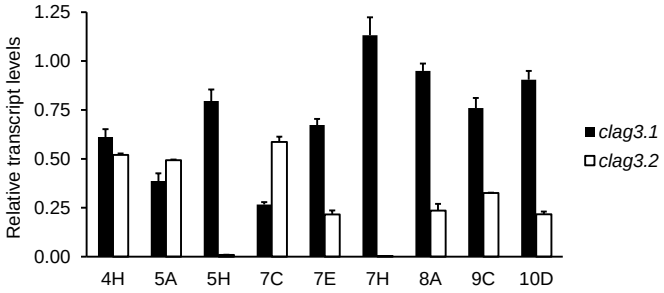

Supplemental Fig. S3. Expression of *clag3.1* and *clag3.2* in subclones of isolate V033 (obtained from a volunteer infected with parasites of the NF54 line). Subclones were obtained by limiting dilution. Two out of nine subclones (5H and 7H) expressed *clag3.1* almost exclusively, whereas all other subclones likely originated from *clag3.2*-expressing single parasites. In the latter subclones, during the 3-4 weeks in culture elapsed between limiting dilution and transcriptional analysis parasites that spontaneously switched to *clag3.1* expression were progressively selected. This interpretation is based on the observation that in the NF54 genetic background parasites that express *clag3.1* are selected under culture conditions (Fig. 4C). The similar transcript levels of *clag3.1* and *clag3.2* observed in parasites from volunteer V033 (Fig. 4B) are consistent with the number of *clag3.1*-expressing subclones obtained (two out of nine) because *clag3.1* has a stronger promoter than *clag3.2* [1, 2], implying that similar transcript levels of *clag3.1* and *clag3.2* reflect a higher proportion of *clag3.2*-expressing parasites. Altogether, the transcriptional analysis of V033 subclones revealing two clearly distinct types of subclones suggests that the V033 isolate predominantly consists of a mixture of parasites expressing *clag3.1* and parasites expressing *clag3.2*, rather than a homogeneous population of parasites expressing the two genes simultaneously, but mutual exclusion is directly demonstrated only for parasites expressing *clag3.1*, and inferred for parasites expressing *clag3.2*. Transcript levels are normalized against *rhoph2*. Error bars are SD.

References.

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