RESEARCH ARTICLE

Figures and figure supplements

Local emergence in Amazonia of *Plasmodium falciparum k13* C580Y mutants associated with *in vitro* artemisinin resistance

Luana C Mathieu *et al*

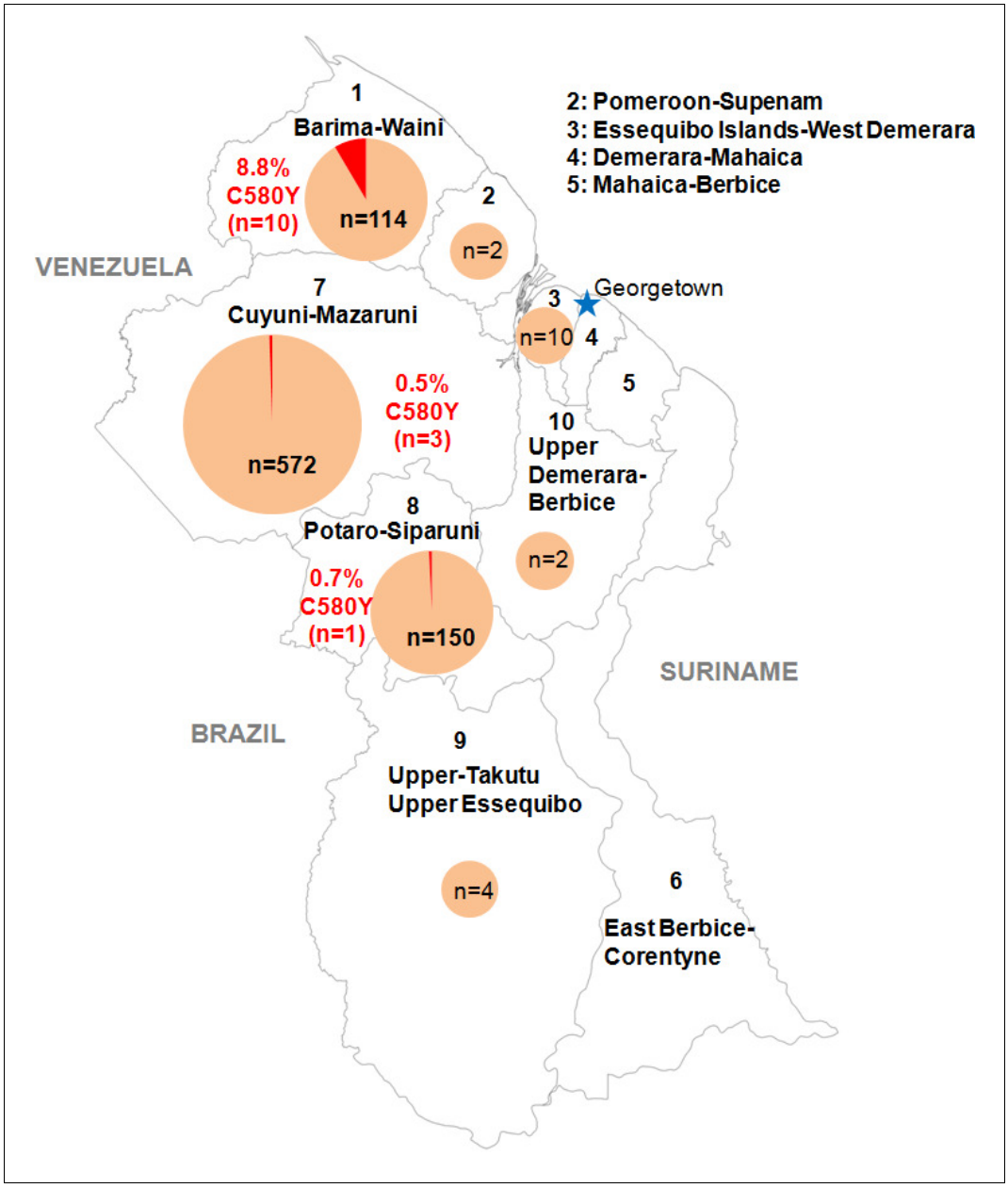

Figure 1. Distribution of the *pfk13* C580Y mutant parasites among Guyana regions. Pie charts represent the total number of isolates analyzed per region. Mutants are represented in red.

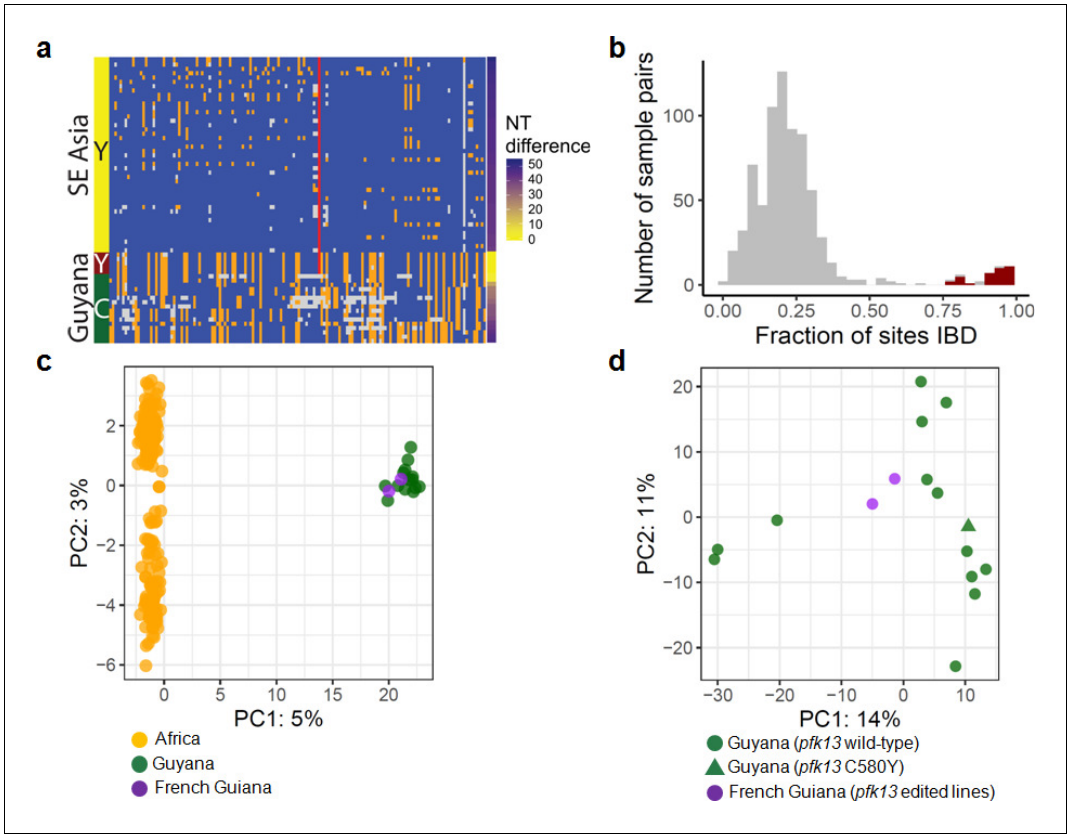

Figure 2. Whole-genome sequence analysis of *pfk13* C580Y mutant parasites in Guyana. (**a**) Comparison of the haplotypic background of *pfk13* C580Y mutant parasites from Guyana, 2016, and Southeast Asia, 2010–2012. Across Pf3k samples from Cambodia, Thailand, and Vietnam, 45 unique C580Y-coding haplotypic backgrounds were identified and compared to haplotypes from Guyana. Columns represent 149 sites containing non-singleton single nucleotide polymorphisms (SNPs) found within a 150 kb segment surrounding the *pfk13* C580Y-coding allele. At a given site, the more common allele is marked blue, the less common allele is orange, and missing calls are grey. The Y-coding variant for codon 580 of *pfk13* is represented by the red blocks; wild-type is blue. Only the five *pfk13* C580Y mutant samples with fewer than 15% missing calls are depicted here. (**b**) Analysis of relatedness at the whole-genome level among Guyana clones. Pairwise identity-by-descent (IBD) was estimated for all pairs of Guyana samples with high quality whole-genome sequence data (<70% missing calls). Pairwise comparisons between samples exhibiting the *pfk13* C580Y allele are indicated in red, and show uniformly high levels of relatedness, suggesting a single clonal lineage harboring the resistance mutation. (**c, d**) Principal components analysis of parasites from Guyana or other geographic regions using SNP calls from whole-genome sequence data. (**c**) The parasites from Guyana and French Guiana form a single cluster when compared with parasites from Africa. (**d**) The two edited parasite lines from French Guiana are highly similar to the sequenced parasite samples from Guyana including a *pfk13* mutant.

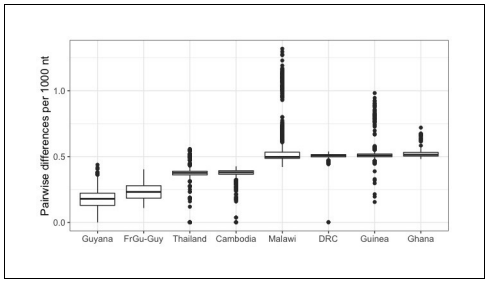

Figure 2—figure supplement 1. Number of single nucleotide differences between pairs of parasites within different geographic locations. The median number of nucleotide differences between the two edited French Guianan lines and Guyanese parasites (FrGu-Guy; 0.23 per thousand nucleotides) is lower than the median nucleotide differences between parasite pairs drawn from within any of the other analyzed populations. To account for potential differences in sequencing depth and quality across populations, calculations were made using a set of high quality SNP calls (GATK quality score >20;<80% missing calls for the given population). FrGu: French Guiana, Guy: Guyana, DRC: Democratic Republic of Congo.

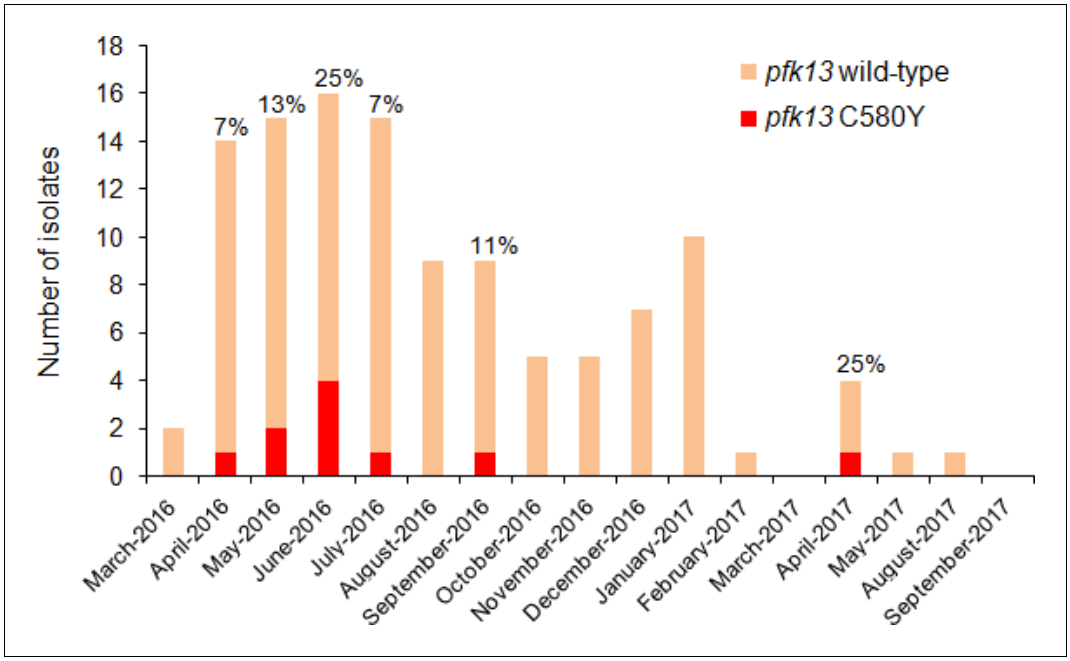

Figure 3. Temporal distribution of *pfk13* C580Y mutants in Region 1 of Guyana per month of collection, 2016–2017. The percentage of *pfk13* C580Y mutants for each month of identification is represented above each bar.

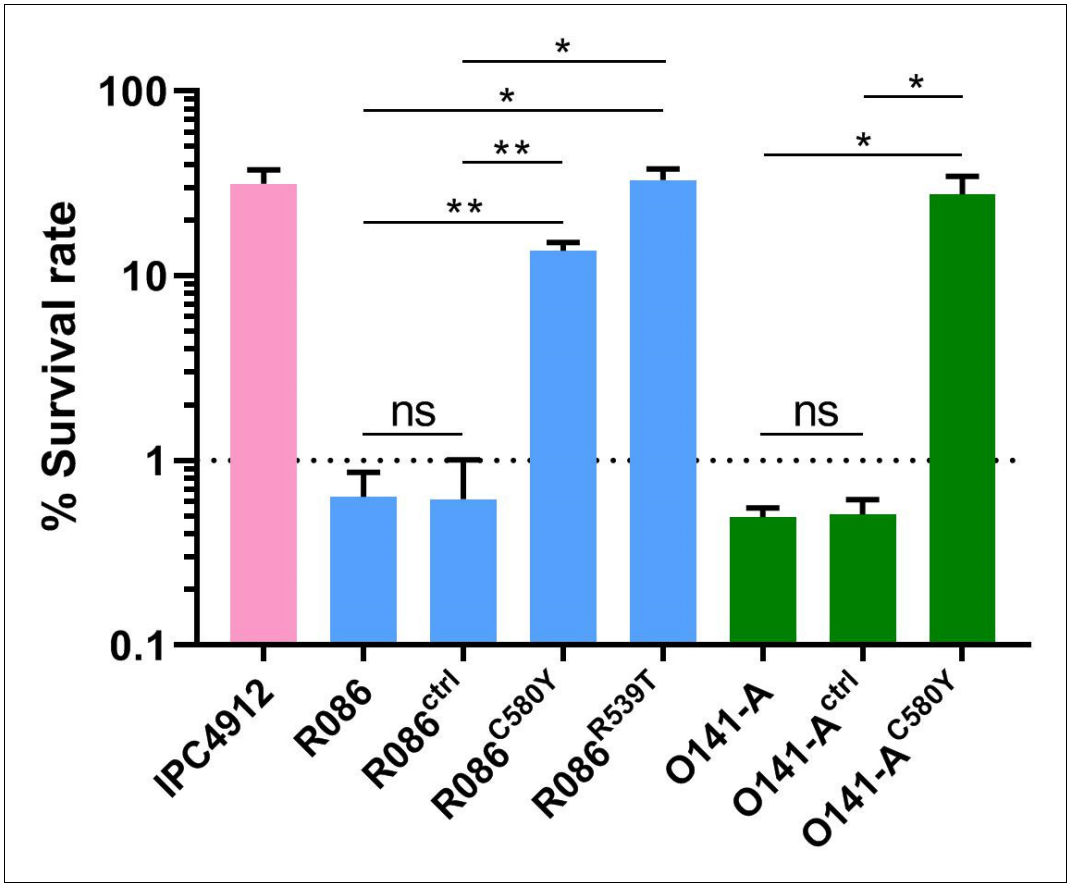

Figure 4. Ring-stage Survival Assays in parasites from French Guiana. Data show survival rates of ring-stage parasites (0–3 hr post invasion of human erythrocytes) after a 6 hr pulse of 700 nM DHA, as measured by microscopy 66 hr later. Data illustrate mean ± SEM percent survival from three independent repeats compared with dimethyl sulfoxide (DMSO)-treated parasites as a control for two isolates from French Guiana (O141-A, R086). Parents harbored wild-type *pfk13* allele, and for zinc-finger nuclease edited isogenic parasites, control (ctrl) isolates harbored wild-type *pfk13* allele with silent mutations or *pfk13* mutations (C580Y or R539T). IPC4912, a Cambodian reference strain harboring the I543T *pfk13* mutation was used as a control. A parasite line is considered resistant when the survival rate is greater than 1%. Student's t-test was used to assess significant differences between survival rates of parental and *pfk13*-edited parasites. *p<0.05; **p<0.01; ns: not significant.

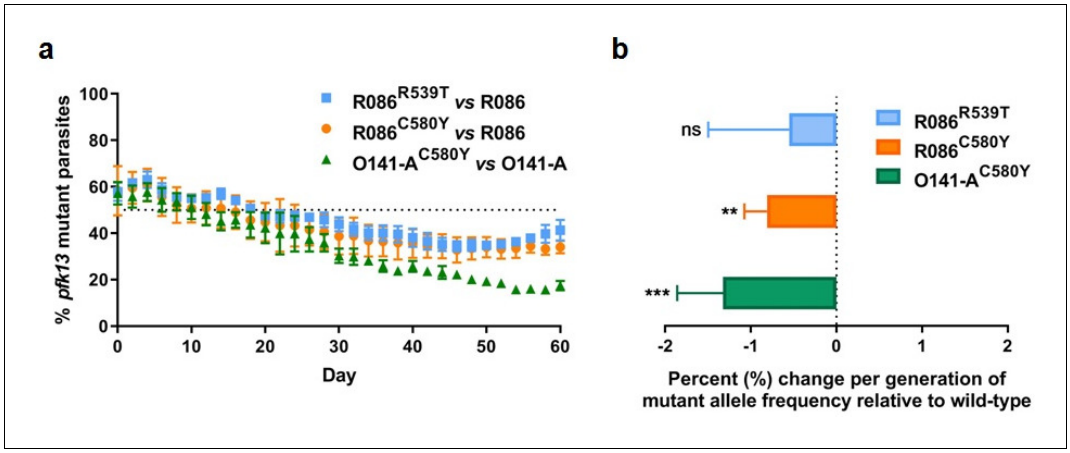

Figure 5. Competition growth assays of *pfk13* mutant and wild-type parasites. (**a**) Frequency of wild-type and mutant parasites in co-culture, as measured by TaqMan allelic discrimination qPCR. Data show the percentage of *pfk13* mutant parasites in the culture over 60 days with sampling every two days. Error bars represent the SEM of *pfk13* mutant allele frequency between the two biological replicates (including two technical replicates for qPCR). A percentage below 50% indicates the mutant was less fit than the isogenic *pfk13* wild-type line. (**b**) Percentage change per generation of *pfk13* mutant allele frequency relative to wild-type. Data show that *pfk13* mutations confer an *in vitro* fitness cost in both parasite lines. Differences in growth rates were calculated as the percent change in *pfk13* mutant allele frequency averaged over 30 generations. Error bars represent the SEM of percentage growth change between the two biological sampling experiments calculated for every generation in each co-culture. Significance was calculated using the Wilcoxon signed-rank test in every generation across the two biological replicate experiments. **p<0.01, ***p<0.001; ns: not significant.

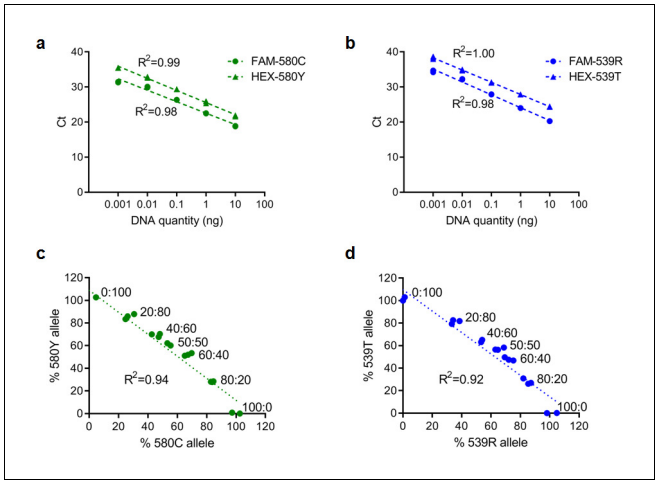

Figure 5—figure supplement 1. Representative standard curves for qPCR reactions targeting *pfk13* C580/C580Y or *pfk13* R539/R539T allele. (**a, b**) Standard curves showing good amplification efficiency (between 88% and 95%) and high sensitivity using 10-fold serially diluted genomic DNA obtained from wild-type *pfk13* C580 and R539 or mutant *pfk13* C580Y or R539T parasites. (**c, d**) Scatter plots for percent wild-type and mutant alleles in multiplexed qPCR assays using pre-defined mixtures of plasmids. We used mixtures comprising of *pfk13* C580 and C580Y or R539 and R539T expressing plasmids in fixed molar ratios of wild-type: mutant alleles (0:100, 20:80, 40:60, 50:50, 60:40, 80:20, 100:0) to validate the specificity of using TaqMan qPCR assays to determine the *pfk13* allele frequency.

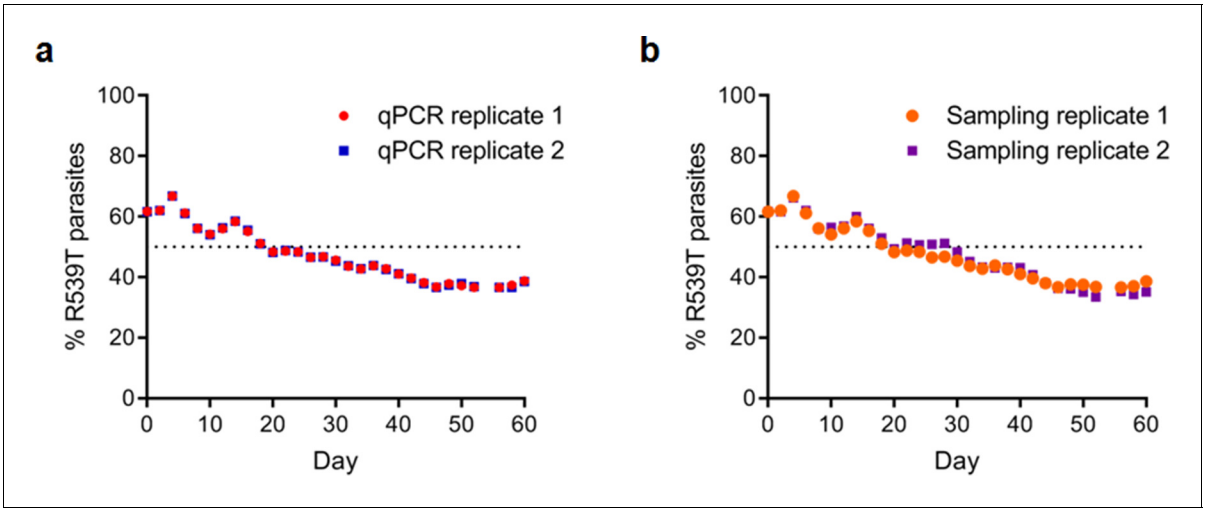

Figure 5—figure supplement 2. Reproducibility of TaqMan allelic discrimination qPCR performed on R086^{R539T} and R086 parasites. (**a**) Scatter plots show the percentage of *pfk13* R539T parasites in two separate qPCR technical replicate runs which correlate perfectly. (**b**) Scatter plots show the percentage of *pfk13* R539T parasites in two independent sampling replicates over 60 days in culture, which showed consistent trends.