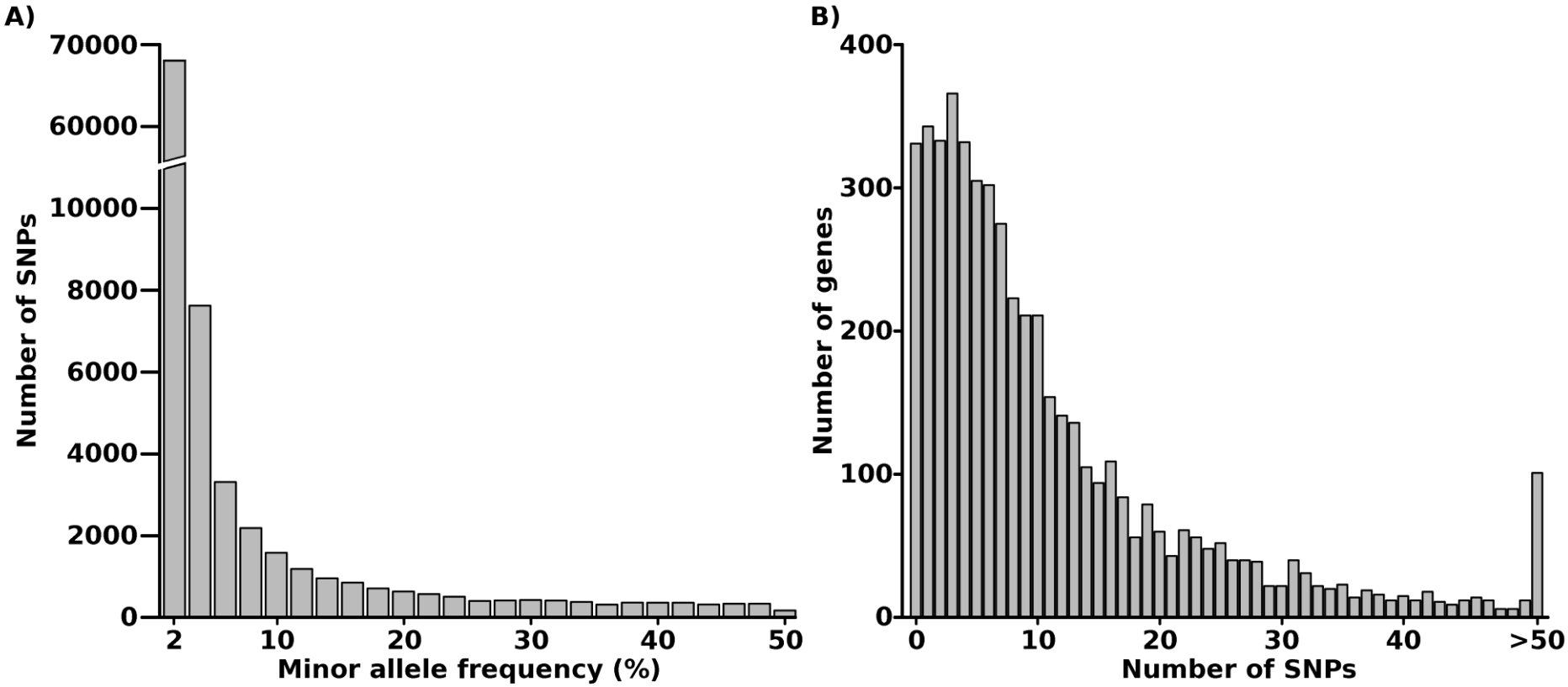
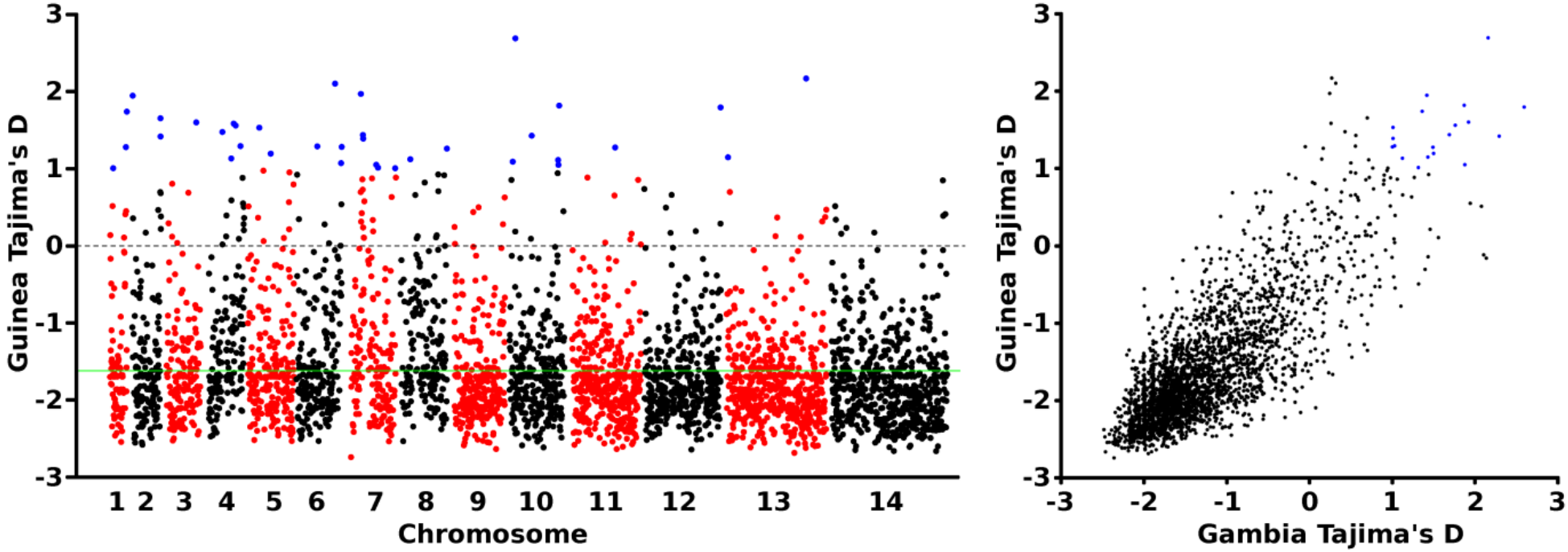
**Supplementary Analysis**

A subset of *P. falciparum* infections that each contained predominantly a single genotype were identified in both the Guinea and The Gambia populations using the $F_{ws}$ metric, with scores of > 0.95 indicating that a single parasite genotype accounted for the majority of observable reads within the isolate (Figure 2, Supplementary Table S2). The predominantly single genotype populations consisted of 50 Guinean and 33 Gambian isolates and 106009 biallelic SNPs. In order to ensure mixed genotype infections had not had a negative impact on the accuracy of allele calls the primary analysis was repeated in full using only those isolates identified as being predominantly single genotype, the results of which are presented below and which show close general agreement with the analysis of the complete 152 isolate dataset presented in the main paper.

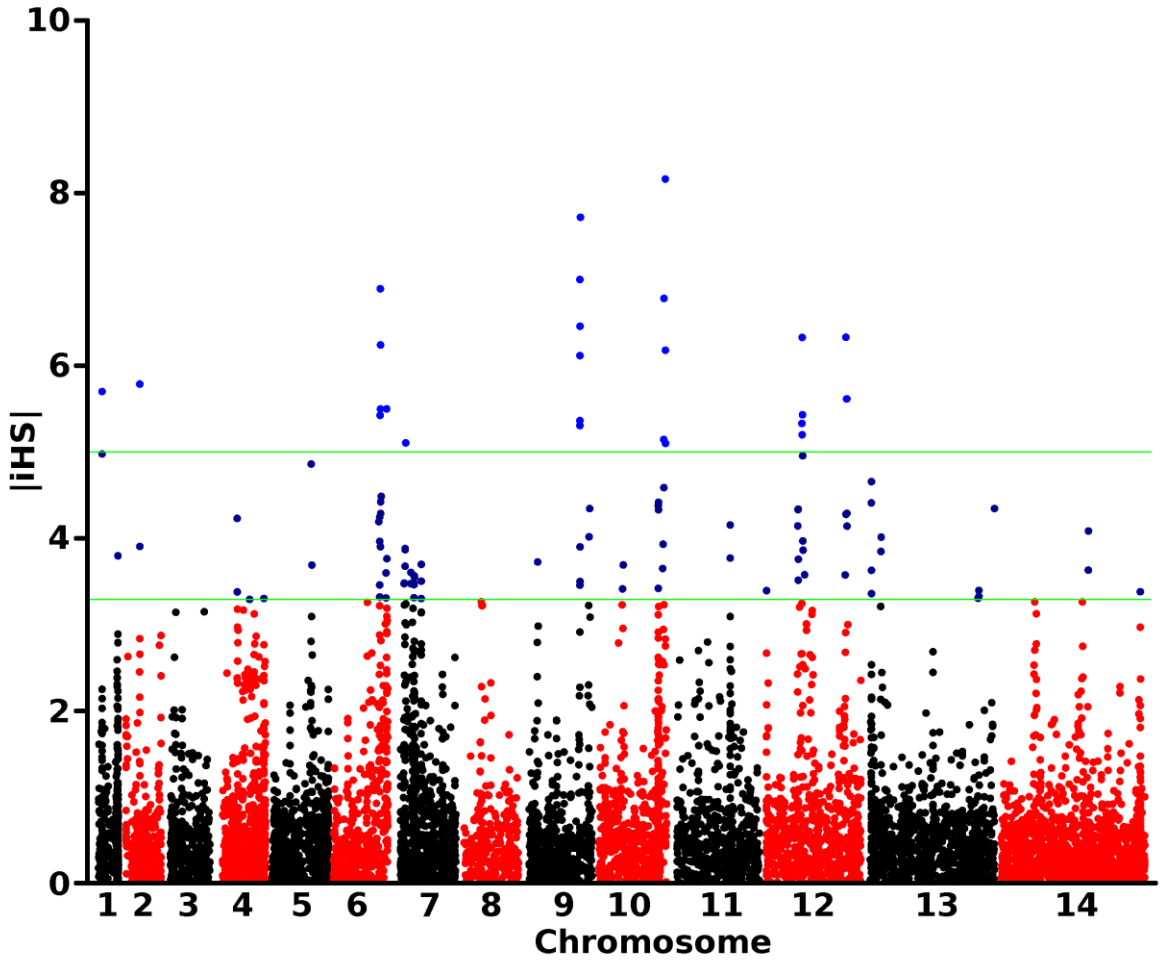
**Supplementary Analysis Figure I. A.** Frequency distribution of the minor alleles for each of the SNPs scored in a population sample of 50 predominantly single genotype *P. falciparum* isolates from N'Zerekore in Guinea. **B.** Distribution of numbers of genes (N = 5188 analysed in total) with each given number of SNPs in the N'Zerekore population sample.



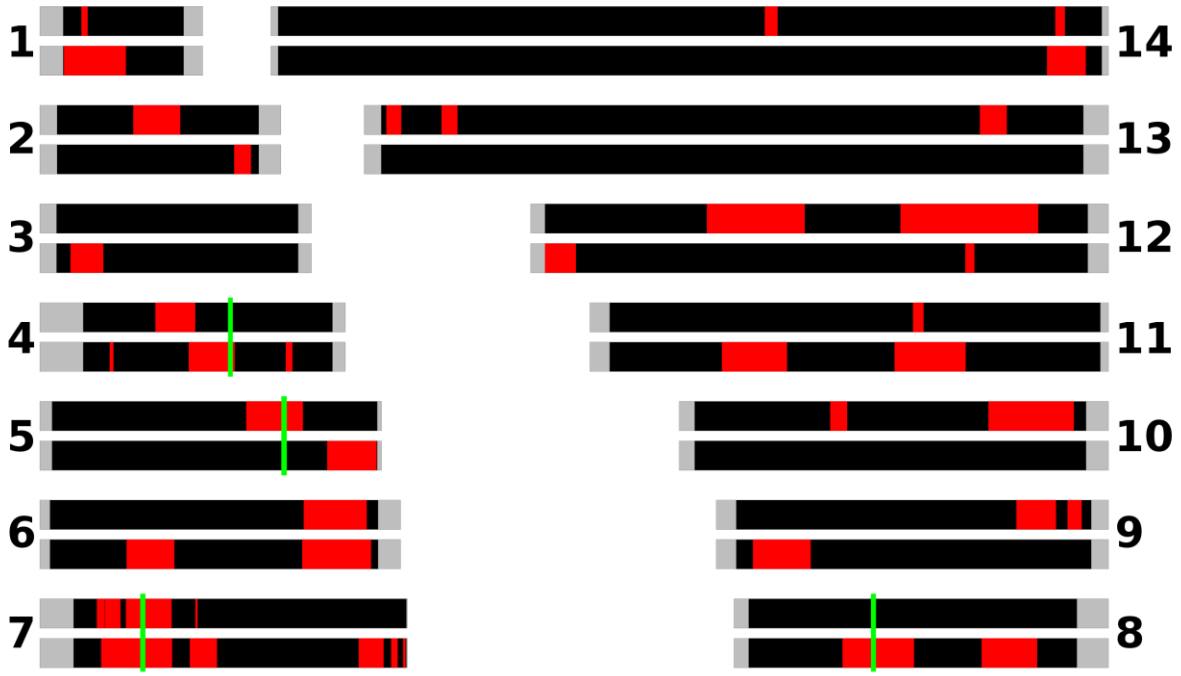
**Supplementary Analysis Figure II**. Genome wide distribution of Tajima's D values summarising the allele frequency spectra for *P. falciparum* genes with 3 or more SNPs. **A.** Tajima's D values for each of 3696 *P. falciparum* genes with 3 or more SNPs in Guinea (N'Zerekore predominantly single genotype population sample of 50 isolate sequences). Individual chromosomes are identified by the alternate black and red colouring, with genes plotted as individual points based on their position within each chromosome. Detailed data for each of the genes are given in Supplementary Analysis Table IV. **B.** Correlation between Tajima's D scores for the Guinea (N'Zerekore) population and a previously sampled population from The Gambia (Greater Banjul area), analysing 2955 genes that had 3 or more SNPs in each of the populations. Genes with a Tajima's D value of > 1 in both populations are highlighted in blue (and identified in Table 1).



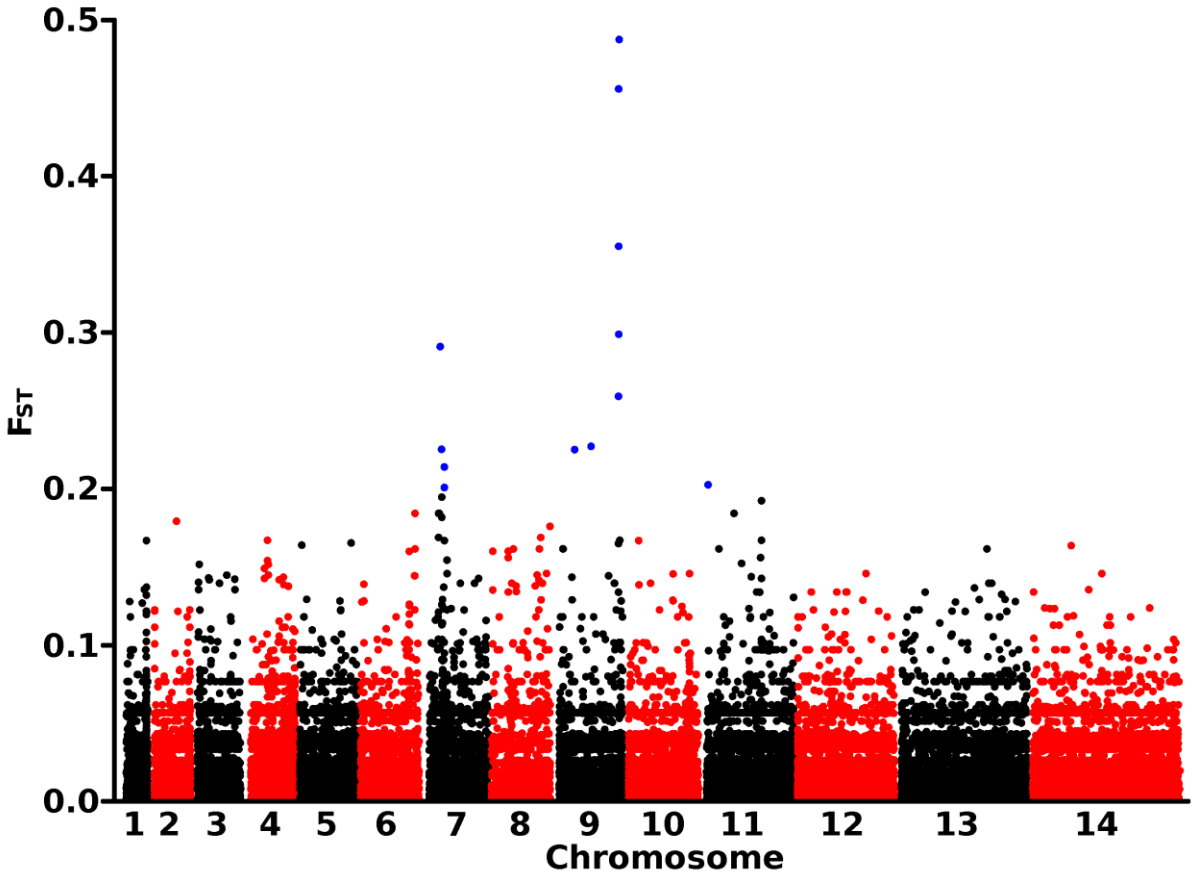
**Supplementary Analysis Figure III**. Genome wide scan of standardised integrated haplotype scores (|iHS|) for *P. falciparum* SNPs with minor allele frequency of at least 5% in N'Zerekore (Guinea, sequence analysis of 50 predominantly single genotype clinical isolates). Individual chromosomes are identified by alternate black and red colouring of their SNPs, with high scoring SNPs highlighted (|iHS| > 3.29 [top 1% of expected distribution] in dark blue and > 5 in light blue) indicating loci most likely to have been under recent positive directional selection.



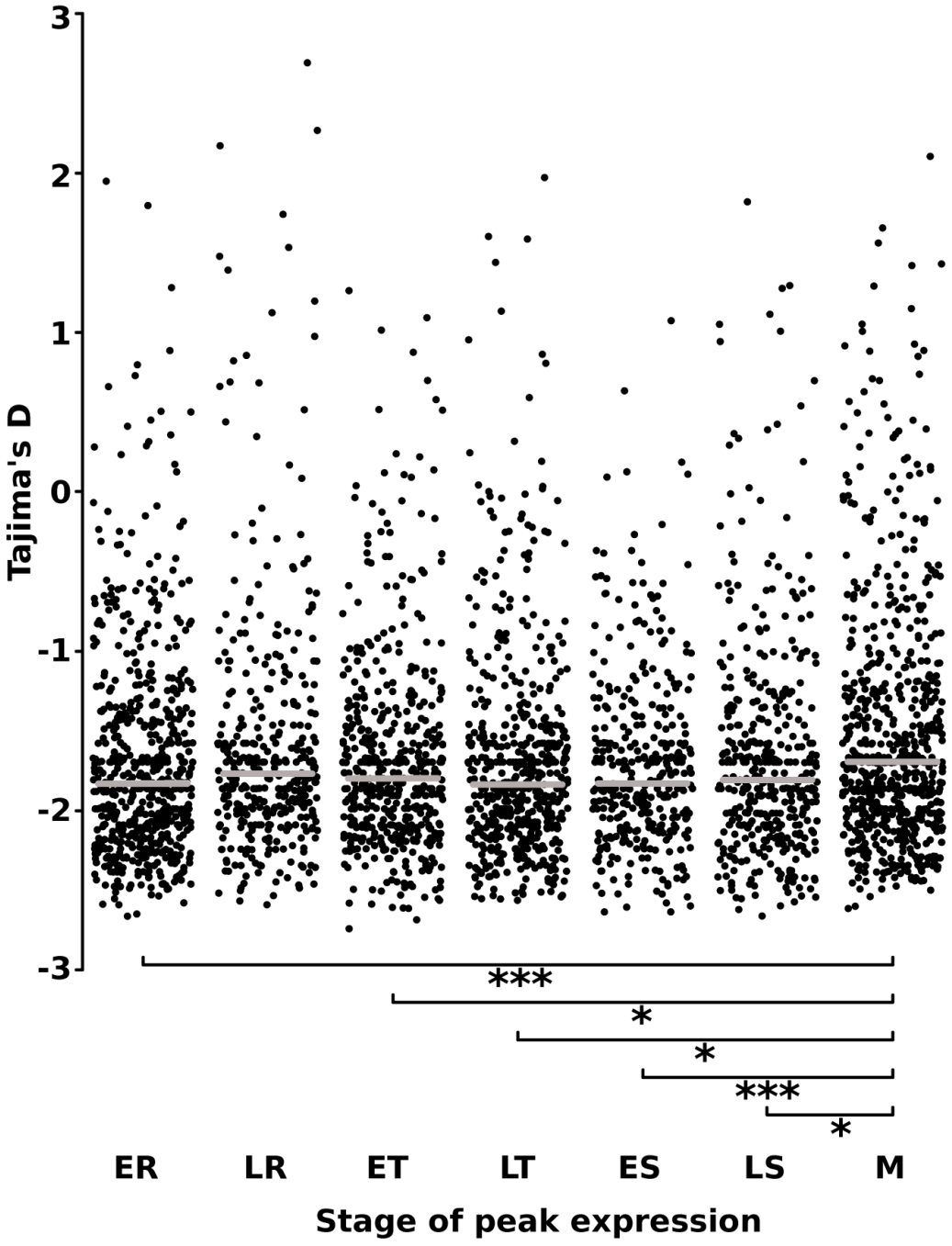
**Supplementary Analysis Figure IV.** Regions of the 14 *P. falciparum* chromosomes showing signatures consistent with recent positive directional selection in the predominantly single genotype Guinea population sample (N'Zerekore) compared with the Gambian population sampled previously (Nwakanma et al. 2013). For each chromosome the top bar represents the Guinea population, the bottom bar the Gambian population. Red shading indicates the regions containing 2 or more SNPs with elevated |iHS| values in either population; grey shading indicates the sub-telomeric regions that were not analysed; green bars indicate the positions of antimalarial drug resistance genes *dhfr*, *mr1*, *crt* and *dhps* on chromosomes 4, 5, 7 and 8 respectively.



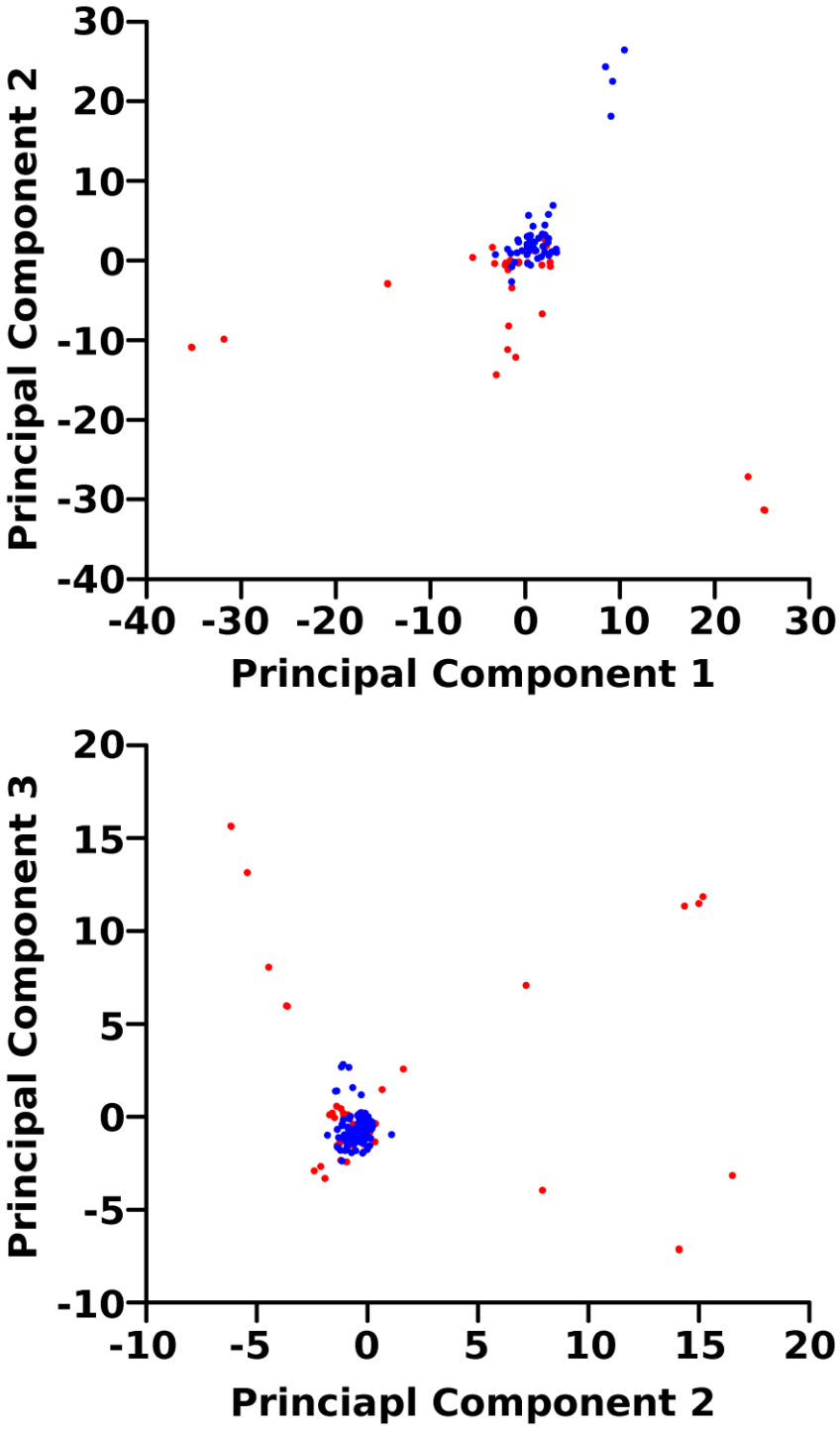
**Supplementary Analysis Figure V**. Genome wide $F_{ST}$ between the 2011 Guinean and the 2008 Gambian predominantly single genotype populations. $F_{ST}$ scores were calculated for 106009 biallelic SNPs across the genome, with each chromosome identified by the alternating black / red colouring and SNPs with $F_{ST} > 0.2$ being shown in blue (Supplementary Analysis Table III).



**Supplementary Analysis Figure VI.** Tajima's D value distribution is highest for genes predicted to have peak expression at the merozoite stage. Expression data for 3523 genes from microarray studies (Le Roch et al. 2003) retrieved from PlasmoDB (Aurrecoechea et al. 2009) were used to group genes by stage of peak expression. Points show the Tajima's D score for each gene in the Guinean population (grey bars show the medians for all genes with the same stage of peak expression) with predicted peak expression at each stage in the erythrocytic life cycle (ER, early ring; LR, late ring; ET, early trophozoite; LT, late trophozoite; ES, early schizont; LS, late schizont; M, merozoite). Asterisks indicate p values given by Mann-Whitney tests for the merozoite stage versus each other stage individually (* $p<0.05$, *** $p<0.0001$).



**Supplementary Analysis Figure VII.** Principal component analysis of 83 predominantly single genotype isolates from Guinea and Gambia utilising 112089 SNPs. A. First component and second component (3.5% and 3.4% of total variation respectively). B. Second and third component (3.4% of variation).



**Supplementary Analysis Table I**. 19 genes with Tajima's D scores of > 1 in both the Guinean and Gambian predominantly single genotype populations. Tajima's D scores were calculated for all genes with 3 or more SNPs following masking or repeat regions and exclusion of SNPs within introns.

| Gene ID | Old Gene ID | Number of SNPs (Guinea) | Tajima's D (Guinea) | Number of SNPs (Gambia) | Tajima's D (Gambia) | Product Description |
|---|---|---|---|---|---|---|
| PF3D7_0113800 | PFA0665w | 223 | 1.28 | 207 | 1.00 | DBL containing protein |
| PF3D7_0114500 | PFA0700c | 13 | 1.74 | 14 | 1.36 | Plasmodium exported protein (hyp10) |
| PF3D7_0201600 | PFB0080c | 23 | 1.95 | 21 | 1.42 | Plasmodium exported protein (PHISTb) |
| PF3D7_0221000 | PFB0950w | 21 | 1.42 | 17 | 2.29 | Plasmodium exported protein |
| PF3D7_0321200 | PFC0935c | 17 | 1.60 | 14 | 1.92 | N-acetylglucosamine-1-phosphate transferase, putative |
| PF3D7_0417800 | PFD0865c | 9 | 1.13 | 8 | 1.12 | Cdc2-related protein kinase 1 (CRK1) |
| PF3D7_0420200 | PFD0980w | 15 | 1.56 | 13 | 1.76 | Holo-(acyl-carrier protein) synthase |
| PF3D7_0422800 | PFD1045w | 13 | 1.29 | 13 | 1.03 | Serpentine receptor, putative (SR12) |
| PF3D7_0508800 | PFE0435c | 4 | 1.53 | 3 | 1.01 | Single-stranded DNA-binding protein (SSB) |
| PF3D7_0516300 | PFE0815w | 14 | 1.20 | 13 | 1.50 | tRNA pseudouridine synthase |
| PF3D7_0710200 | PF07_0042 | 119 | 1.44 | 108 | 1.69 | Conserved Plasmodium protein |
| PF3D7_0710400 | MAL7P1.32 | 8 | 1.39 | 8 | 1.01 | Nucleotide excision repair protein |
| PF3D7_0720400 | PF07_0085 | 11 | 1.01 | 11 | 1.32 | Ferrodoxin reductase-like protein |
| PF3D7_1004800 | PF10_0051 | 16 | 2.69 | 17 | 2.16 | ADP/ATP carrier protein |
| PF3D7_1035700 | PF10_0348 | 25 | 1.05 | 21 | 1.88 | Duffy binding-like merozoite surface protein (DBLMSP) |
| PF3D7_1036300 | PF10_0355 | 83 | 1.82 | 85 | 1.87 | Merozoite surface protein (DBLMSP2) |
| PF3D7_1133400 | PF11_0344 | 68 | 1.28 | 62 | 1.49 | Apical membrane antigen 1 (AMA1) |
| PF3D7_1253100 | PFL2555w | 11 | 1.80 | 9 | 2.59 | Plasmodium exported protein (PHISTa) |
| PF3D7_1301800 | PF13_0075 | 138 | 1.15 | 119 | 1.43 | Surface-associated interspersed protein 13.1 (SURFIN 13.1) |

**Supplementary Analysis Table II.** Windows of directional selection for all *P. falciparum* genomic regions with at least 2 SNPs with |iHS| scores of > 3.29 (top 1% of expected distribution) for the Guinea predominantly single genotype population sample.

| Chromosome | Window start (kb along chromosome) | Window end (kb along chromosome) | Region size (kb) | Number of SNPs | Genes within region |
|---|---|---|---|---|---|
| 1 | 163 | 188 | 25 | 2 | PF3D7_0103600 - PF3D7_0104200 |
| 2 | 367 | 552 | 184 | 2 | PF3D7_0208900 - PF3D7_0213600 |
| 4 | 454 | 611 | 158 | 2 | PF3D7_0409600 - PF3D7_0413300 |
| **5** | **811** | **1034** | **222** | **2** | **PF3D7_0519600 - PF3D7_0524900** |
| 6 | 1037 | 1285 | 249 | 22 | PF3D7_0625600 - PF3D7_0630600 |
| 7 | 224 | 253 | 29 | 3 | PF3D7_0704600 - PF3D7_0705100 |
| 7 | 254 | 317 | 63 | 4 | PF3D7_0705100 - PF3D7_0706500 |
| **7** | **338** | **518** | **180** | **9** | **PF3D7_0707300 - PF3D7_0711700** |
| 7 | 611 | 629 | 9 | 4 | PF3D7_0713400 - PF3D7_0713900 |
| 9 | 1180 | 1336 | 156 | 27 | PF3D7_0929400 - PF3D7_0933800 |
| 9 | 1381 | 1437 | 56 | 2 | PF3D7_0935500 - PF3D7_0936300 |
| 10 | 594 | 661 | 67 | 2 | PF3D7_1014700 - PF3D7_1016400 |
| 10 | 1216 | 1552 | 336 | 12 | PF3D7_1029900 - PF3D7_1038600 |
| 11 | 1270 | 1312 | 41 | 2 | PF3D7_1132800 - PF3D7_1133800 |
| 12 | 693 | 1078 | 385 | 13 | PF3D7_1217500 - PF3D7_1226600 |
| 12 | 1454 | 1996 | 542 | 7 | PF3D7_1234800 - PF3D7_1248700 |
| 13 | 87 | 146 | 59 | 4 | PF3D7_1301600 - PF3D7_1302700 |
| 13 | 304 | 367 | 63 | 2 | PF3D7_1306500 - PF3D7_1308200 |
| 13 | 2420 | 2526 | 106 | 3 | PF3D7_1360500 - PF3D7_1362800 |
| 14 | 1941 | 1992 | 51 | 2 | PF3D7_1447500 - PF3D7_1448500 |

Bold, windows which overlap *mdr1* and *crt* on chromosomes 5 and 7 respectively.

**Supplementary Analysis Table III**. List of the most highly differentiated SNP allele frequencies between the Guinean and Gambian predominantly single genotype populations. $F_{ST}$ scores were calculated for 106009 biallelic SNPs genome-wide (with a mean $F_{ST}$ = 0.015).

| Chromosome | SNP Position | Gene | Reference allele frequency (Guinea) | Reference allele frequency (Gambia) | $F_{ST}$ | Coding effect | Amino Acid Change |
|---|---|---|---|---|---|---|---|
| 7 | 375792 | PF3D7_0708200 | 0.88 | 0.36 | 0.29 | Synonymous | - |
| 7 | 405600 | PF3D7_0709000 | 0.78 | 0.30 | 0.23 | Non-synonymous | I -> T |
| 7 | 466461 | PF3D7_0710200 | 0.30 | 0.76 | 0.20 | Non-synonymous | N -> Y |
| 7 | 466458 | PF3D7_0710200 | 0.38 | 0.89 | 0.21 | Non-synonymous | D -> E |
| 7 | 466465 | PF3D7_0710200 | 0.38 | 0.89 | 0.21 | Non-synonymous | N -> Y |
| 7 | 466466 | PF3D7_0710200 | 0.38 | 0.89 | 0.21 | Non-synonymous | N -> I |
| 7 | 466482 | PF3D7_0710200 | 0.38 | 0.89 | 0.21 | Synonymous | - |
| 9 | 413988 | intergenic | 0.88 | 0.44 | 0.23 | - | - |
| 9 | 775403 | intergenic | 1.00 | 0.68 | 0.23 | - | - |
| 9 | 1378602 | PF3D7_0935400 | 0.60 | 0.09 | 0.26 | Non-synonymous | P -> H |
| 9 | 1382170 | intergenic | 0.76 | 0.15 | 0.36 | - | - |
| 9 | 1383344 | intergenic | 0.72 | 0.03 | 0.46 | - | - |
| 9 | 1384752 | intergenic | 0.74 | 0.18 | 0.30 | - | - |
| 9 | 1393934 | intergenic | 0.88 | 0.18 | 0.49 | - | - |
| 11 | 119497 | PF3D7_1102500 | 0.96 | 0.61 | 0.20 | Non-synonymous | S -> F |

**Supplementary Table IV**. Tajima's D values genome-wide for all genes with 3 or more SNPs in the predominantly single genotype Guinean or Gambian populations. (Large EXCEL file given separately as part of Supplementary Table S3)

**Supplementary Analysis Table V-A.** Gene ontology terms significantly enriched amongst genes with a Tajima's D value of > 1.

| GO ID | Term | Annotated | Significant | Expected | p-value |
|---|---|---|---|---|---|
| **Molecular function** | | | | | |
| **Guinea** | | | | | |
| GO:0004872 | receptor activity | 24 | 4 | 0.21 | $1.0 \times 10^{-6}$ |
| GO:0016780 | phosphotransferase activity for other substituted phosphate groups | 5 | 2 | 0.05 | $8.9 \times 10^{-4}$ |
| **Gambia** | | | | | |
| GO:0004872 | receptor activity | 23 | 4 | 0.30 | $6.3 \times 10^{-5}$ |
| GO:0016780 | phosphotransferase activity for other substituted phosphate groups | 5 | 2 | 0.06 | $1.6 \times 10^{-3}$ |
| **Biological process** | | | | | |
| **Guinea** | | | | | |
| GO:0009405 | pathogenesis | 17 | 5 | 0.21 | $1.0 \times 10^{-6}$ |
| GO:0030260 | entry into host cell | 12 | 3 | 0.15 | $3.4 \times 10^{-4}$ |
| **Gambia** | | | | | |
| GO:0009405 | pathogenesis | 17 | 5 | 0.25 | $2.2 \times 10^{-6}$ |
| **Cellular compartment** | | | | | |
| **Guinea** | | | | | |
| GO:0016021 | integral to membrane | 306 | 9 | 3.35 | $3.6 \times 10^{-3}$ |

All terms that were highly significantly enriched ($p<0.01$) are shown for both predominantly single genotype populations. The total number of genes used in the analysis annotated with each term is shown along with the number of these genes that were significant (Tajima's D scores > 1). The number of genes expected to be significant given a random distribution is also shown. P-values calculated by Fisher's exact test and adjusted to account for the GO graph structure (Alexa et al 2006).

**Supplementary Table V-B.** Genes contributing to significantly enriched GO terms.

| GO ID | Term | Gene ID | Product | Tajima's D Guinea | Tajima's D Gambia |
|---|---|---|---|---|---|
| GO:0004872 | receptor activity | PF3D7_0113800 | DBL containing protein, unknown function | 1.28 | 1.00 |
| | | PF3D7_0424300 | erythrocyte binding antigen-165, pseudogene (EBA165) | -0.40 | 1.02 |
| | | PF3D7_0731500 | erythrocyte binding antigen-175 (EBA175) | 1.01 | 0.95 |
| | | PF3D7_1035700 | duffy binding-like merozoite surface protein (DBLMSP) | 1.05 | 1.88 |
| | | PF3D7_1036300 | merozoite surface protein (DBLMSP2) | 1.82 | 1.87 |
| GO:0016780 | phosphotransferase activity for other substituted phosphate groups | PF3D7_0321200 | N-acetylglucosamine-1-phosphate transferase, putative | 1.60 | 1.92 |
| | | PF3D7_0420200 | holo-(acyl-carrier protein) synthase, putative | 1.56 | 1.76 |
| GO:0009405 | pathogenesis | PF3D7_0113800 | DBL containing protein, unknown function | 1.28 | 1.00 |
| | | PF3D7_0424300 | erythrocyte binding antigen-165, pseudogene (EBA165) | -0.40 | 1.02 |
| | | PF3D7_0731500 | erythrocyte binding antigen-175 (EBA175) | 1.01 | 0.95 |
| | | PF3D7_1035700 | duffy binding-like merozoite surface protein (DBLMSP) | 1.05 | 1.88 |
| | | PF3D7_1036300 | merozoite surface protein (DBLMSP2) | 1.82 | 1.87 |
| | | PF3D7_1133400 | apical membrane antigen 1 (AMA1) | 1.28 | 1.49 |
| GO:0030260 | entry into host cell | PF3D7_0731500 | erythrocyte binding antigen-175 (EBA175) | 1.01 | 0.95 |
| | | PF3D7_1035400 | merozoite surface protein 3 (MSP3) | 1.11 | 0.72 |
| | | PF3D7_1133400 | apical membrane antigen 1 (AMA1) | 1.28 | 1.49 |
| GO:0046812 | integral to membrane | PF3D7_0104100 | conserved Plasmodium membrane protein, unknown function | 1.01 | 0.76 |
| | | PF3D7_0113800 | DBL containing protein, unknown function | 1.28 | 1.00 |

| | | | |
|---|---|---|---|
| PF3D7_0114500 | Plasmodium exported protein (hyp10), unknown function | 1.74 | 1.36 |
| PF3D7_0321200 | N-acetylglucosamine-1-phosphate transferase, putative | 1.60 | 1.92 |
| PF3D7_0501300 | skeleton-binding protein 1 (SBP1) | 1.05 | 1.88 |
| PF3D7_0731500 | erythrocyte binding antigen-175 (EBA175) | 1.01 | 0.95 |
| PF3D7_1035700 | duffy binding-like merozoite surface protein (DBLMSP) | 1.05 | 1.88 |
| PF3D7_1036300 | merozoite surface protein (DBLMSP2) | 1.82 | 1.87 |
| PF3D7_1133400 | apical membrane antigen 1 (AMA1) | 1.28 | 1.49 |

For each GO term that is significantly enriched the associated genes that have Tajima's D scores above one in either population are shown along with their products and Tajima's D score in both the Guinean and Gambian population.

**Supplementary Table VI.** Linkage disequilibrium between the five high $F_{ST}$ SNPs surrounding *gdv1* on *P. falciparum* chromosome 9. SNPs with significant linkage disequilibrium are highlighted in bold for each population sample.

| | Guinea | | | Gambia | | |
|---|---|---|---|---|---|---|
| **SNPs being compared** | D' | $R^2$ | *p* value | D' | $R^2$ | *p* value |
| 1378602 / 1382170 | 0.306 | 0.044 | 0.192 | 0.214 | 0.026 | 0.401 |
| 1378602 / 1383344 | **0.524** | **0.160** | **0.006** | 0.468 | 0.219 | 0.059 |
| 1378602 / 1384752 | **0.615** | **0.200** | **0.002** | 0.185 | 0.015 | 0.475 |
| 1378602 / 1393934 | **1.000** | **0.205** | **0.002** | 0.185 | 0.015 | 0.466 |
| 1382170 / 1383344 | **0.884** | **0.635** | **< 0.001** | 1.000 | 0.174 | 0.159 |
| 1382170 / 1384752 | **0.662** | **0.394** | **< 0.001** | 0.022 | 0.000 | 0.546 |
| 1382170 / 1393934 | **1.000** | **0.432** | **< 0.001** | **0.756** | **0.459** | **0.002** |
| 1383344 / 1384752 | **0.679** | **0.417** | **< 0.001** | 0.389 | 0.044 | 0.175 |
| 1383344 / 1393934 | **1.000** | **0.351** | **< 0.001** | 1.000 | 0.140 | 0.197 |
| 1384752 / 1393934 | **1.000** | **0.388** | **< 0.001** | -0.019 | 0.000 | 1.000 |