

Supplementary Material

Journal: Applied Microbiology and Biotechnology

European Farmhouse Brewing Yeasts Form a Distinct Genetic Group

Richard Preiss¹, Eugene Fletcher^{1,a}, Lars Marius Garshol², Barret Foster³, Emine Ozsahin³, Mark Lubberts³, George van der Merwe³, Kristoffer Krogerus^{4*}

¹ Escarpment Laboratories, Guelph, ON, Canada

² Independent researcher, Rælingen, Norway

³ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

⁴ VTT Technical Research Centre of Finland, Tekniikantie 21, 02150, Espoo, Finland

^a Current affiliation: Carleton University, Canada

* Address correspondence to Kristoffer Krogerus, kristoffer.krogerus@vtt.fi

Supplementary Figure and Table legends

Supplementary Figure S1. Visualisation of phased haplotigs detected by nPhase in the 17 phased strains.

Supplementary Figure S2. Median coverage in 10 kbp windows across the genome of the 105 *S. cerevisiae* strains included in the genomic analysis.

Supplementary Figure S3. Allele frequency distributions across the genome of the 105 *S. cerevisiae* strains included in the genomic analysis.

Supplementary Figure S4. A neighbour-joining tree generated based on MinHash distances in the (A) assemblies of 1,011 yeast genomes (Peter et al. 2018), and (B) 142 assemblies of ScRAP (O'Donnell et al. 2023), together with long-read assemblies of 9 landrace and 6 control strains generated here. The branches containing the 'Mixed origin', 'European farmhouse' and 'Beer 1'/Ale beer' populations are highlighted.

Supplementary Figure S5. (A) Maximum likelihood phylogenetic tree based on SNPs at 310688 sites in 1,011 yeast genome strains (Peter et al. 2018), the 35 landrace and five brewing control strains sequenced here, and the five sequenced NCYC 'kveik' strains. (B) A neighbour-joining tree based on SNPs at 1652813 biallelic sites in 1,011 yeast genome strains (Peter et al. 2018), the 35 landrace and five brewing control strains sequenced here, and the five sequenced NCYC 'kveik' strains. The branches containing the 'Mixed origin', 'European farmhouse' and 'Beer 1'/Ale beer' populations are highlighted.

Supplementary Figure S6. Population structure of a merged set of 35 *S. cerevisiae* landrace brewing strains and the 1,011 yeast genomes strains estimated with ADMIXTURE based on SNPs at 31766 sites. Each strain along the x-axis is represented by a vertical bar partitioned into colors based on estimated membership fractions to the resolved populations for $K = 8$ to 20 assumed ancestral populations.

Supplementary Figure S7. Heatmaps visualising D , f_4 -ratio and f -branch statistics between populations as calculated with DSuite. (A) The f -branch statistics between population tree tips and nodes. A warmer colour represents a higher estimated f -branch value, which indicates the proportion of the genome affected by admixture. Grey shading indicates tests that could not be made. The maximum (B) D and (C) f_4 -ratio statistics between pairs of populations. A red color indicates a higher value, while a blue color a lower value.

Supplementary Figure S8. The network graph topology with highest posterior probability as derived by AdmixtureBayes from the merged data set of 1,011 yeast genome strains (Peter et al. 2018), the 35 landrace and five brewing control strains sequenced here, and the five sequenced NCYC ‘kveik’ strains. The data set converged to and was best described by 20 admixture events. Red squares indicate admixture events, while blue ovals indicate populations.

Supplementary Figure S9. Change in specific gravity over time in the wort fermentations with landrace and control brewing strains. Error bars show standard deviation of three replicates.

Supplementary Figure S10. Concentration of isoamyl, isobutyl and phenylethyl acetate in the beers fermented with landrace and control brewing strains. Error bars show standard deviation of three replicates. Different letters above bars indicate significant differences by one-way ANOVA ($p < 0.05$).

Supplementary Figure S11. Relative total ester formation by group among the landrace and control brewing strains during wort fermentations. Different letters above bars indicate significant differences by one-way ANOVA ($p < 0.05$).

Supplementary Figure S12. Average copy number of genes related to maltose and maltotriose transport and metabolism among the landrace strains sequenced here and the 1,011 yeast genome strains (Peter et al. 2018) grouped by population.

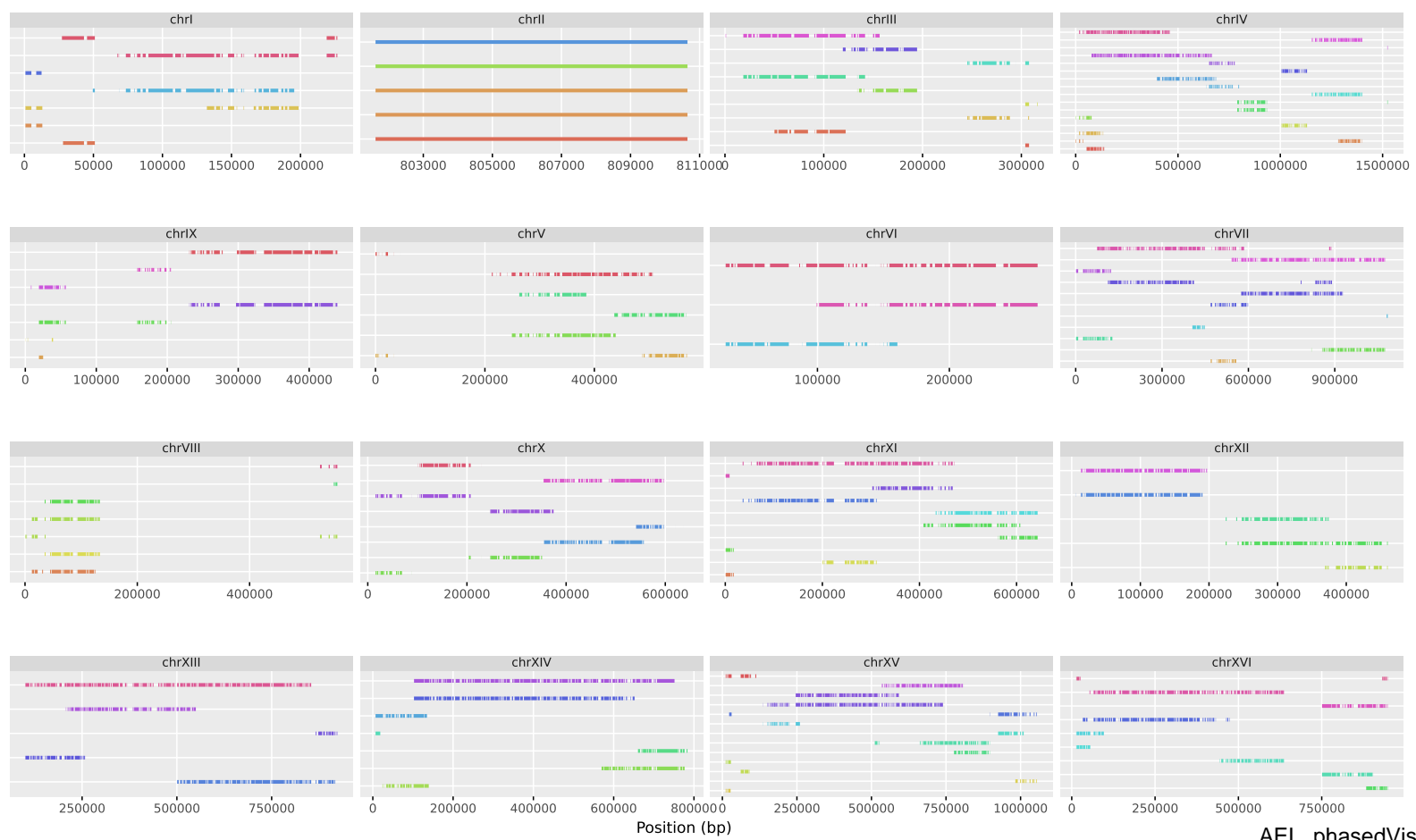
Supplementary Figure S13. Copy number of *RTMI* among the 1,011 yeast genomes strains (Peter et al. 2018) grouped by population.

Supplementary Figure S14. The *RTMI* cluster in the long-read assemblies of the landrace and control brewing strains. *RTMI* is highlighted in red, while *SUC2* is highlighted in blue.

Supplementary Table S1. A list of strains included in the genomic analysis, the number of homozygous and heterozygous SNPs detected, and their accession numbers.

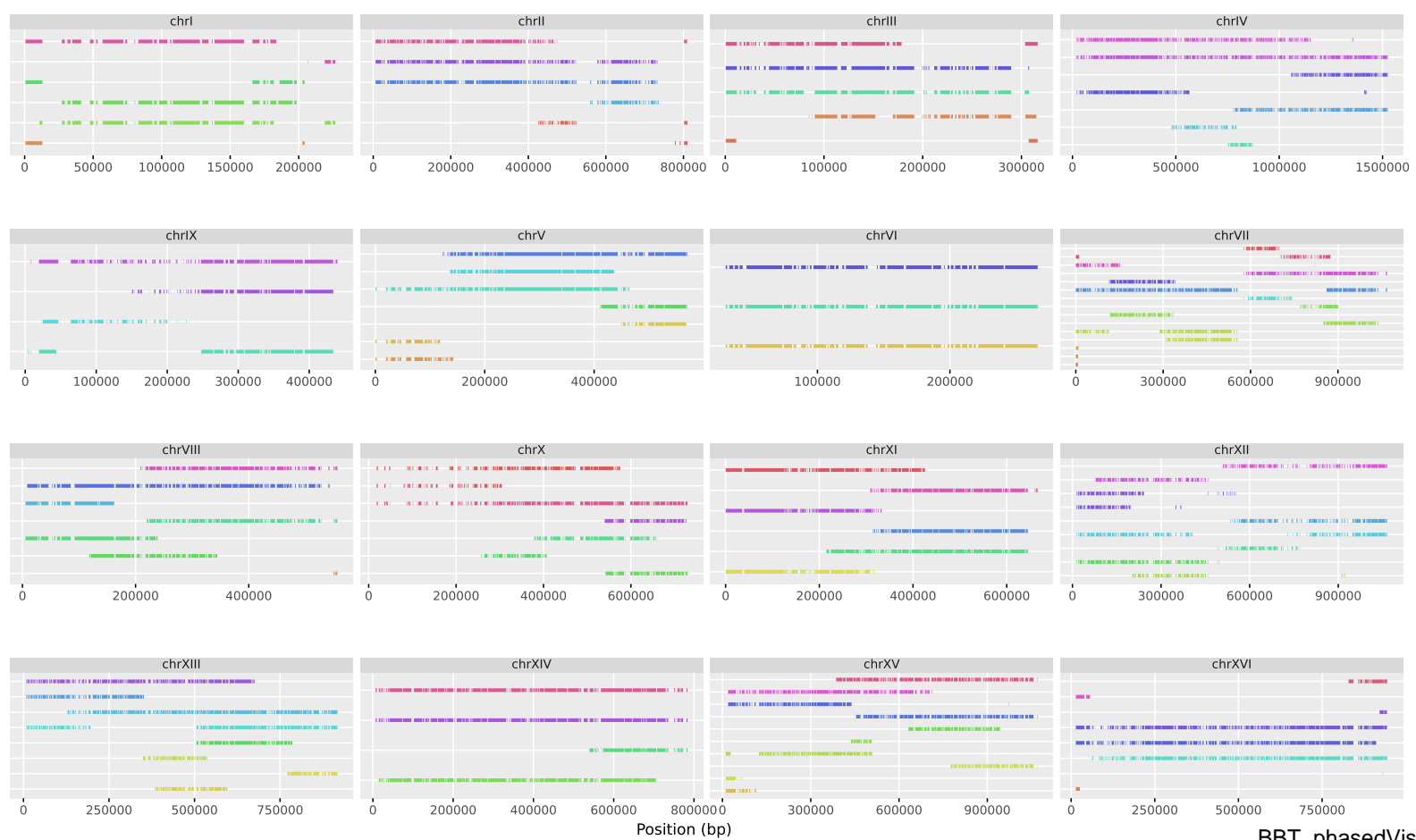
Supplementary Table S2. Assembly statistics for the 15 long-read assemblies generated here.

Supplementary Figure S1



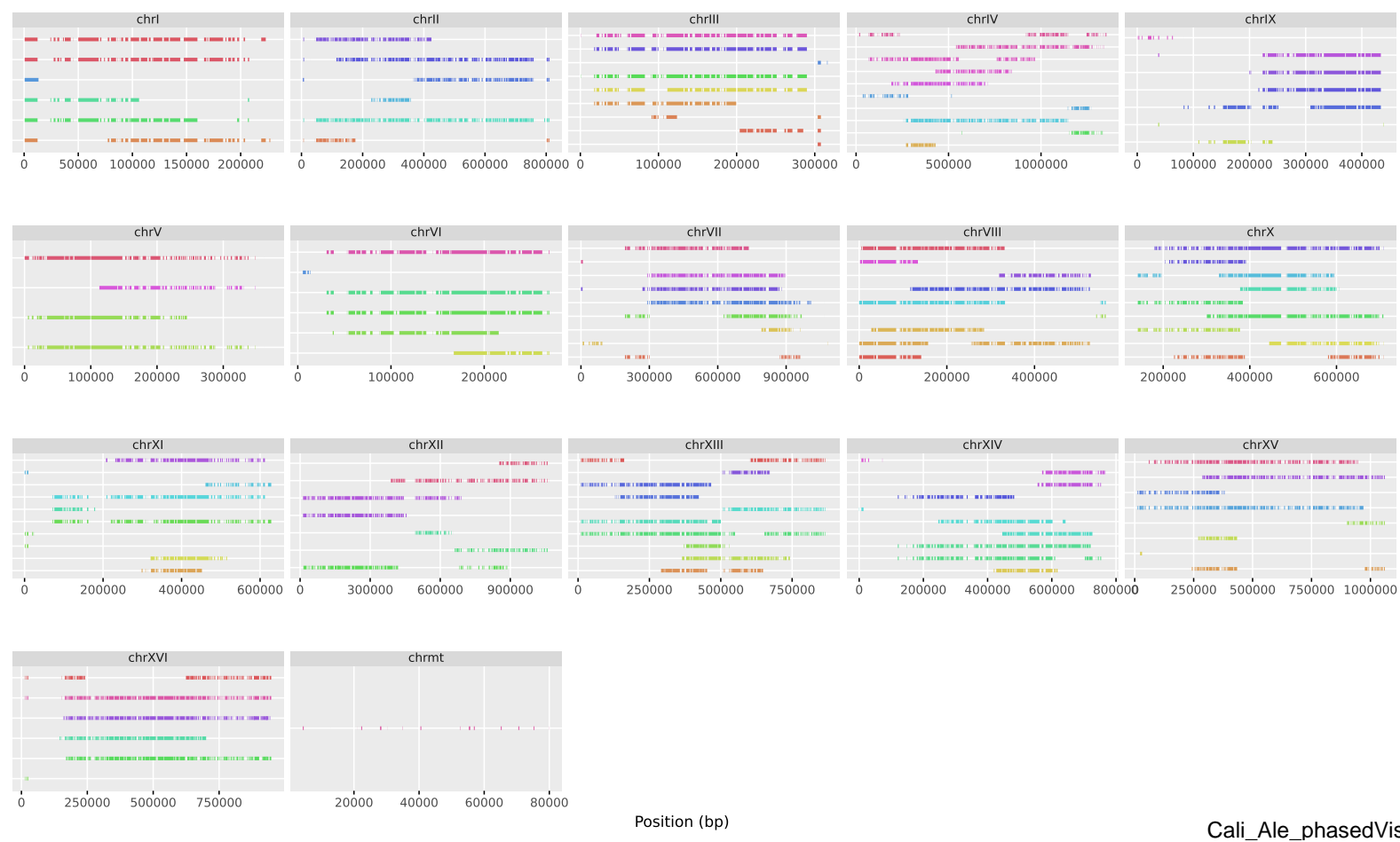
AEL_phasedVis

Supplementary Figure S1

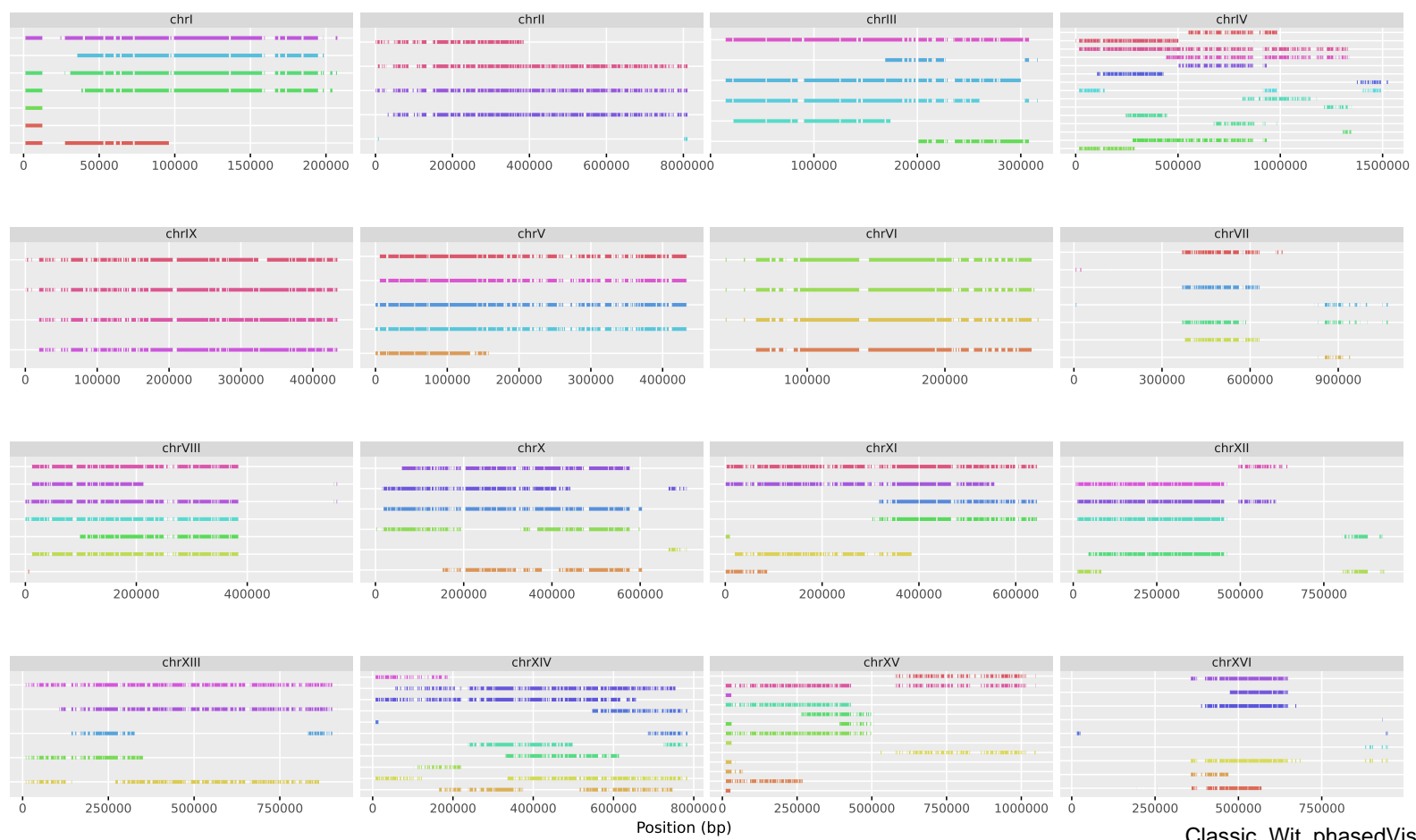


BBT_phasedVis

Supplementary Figure S1

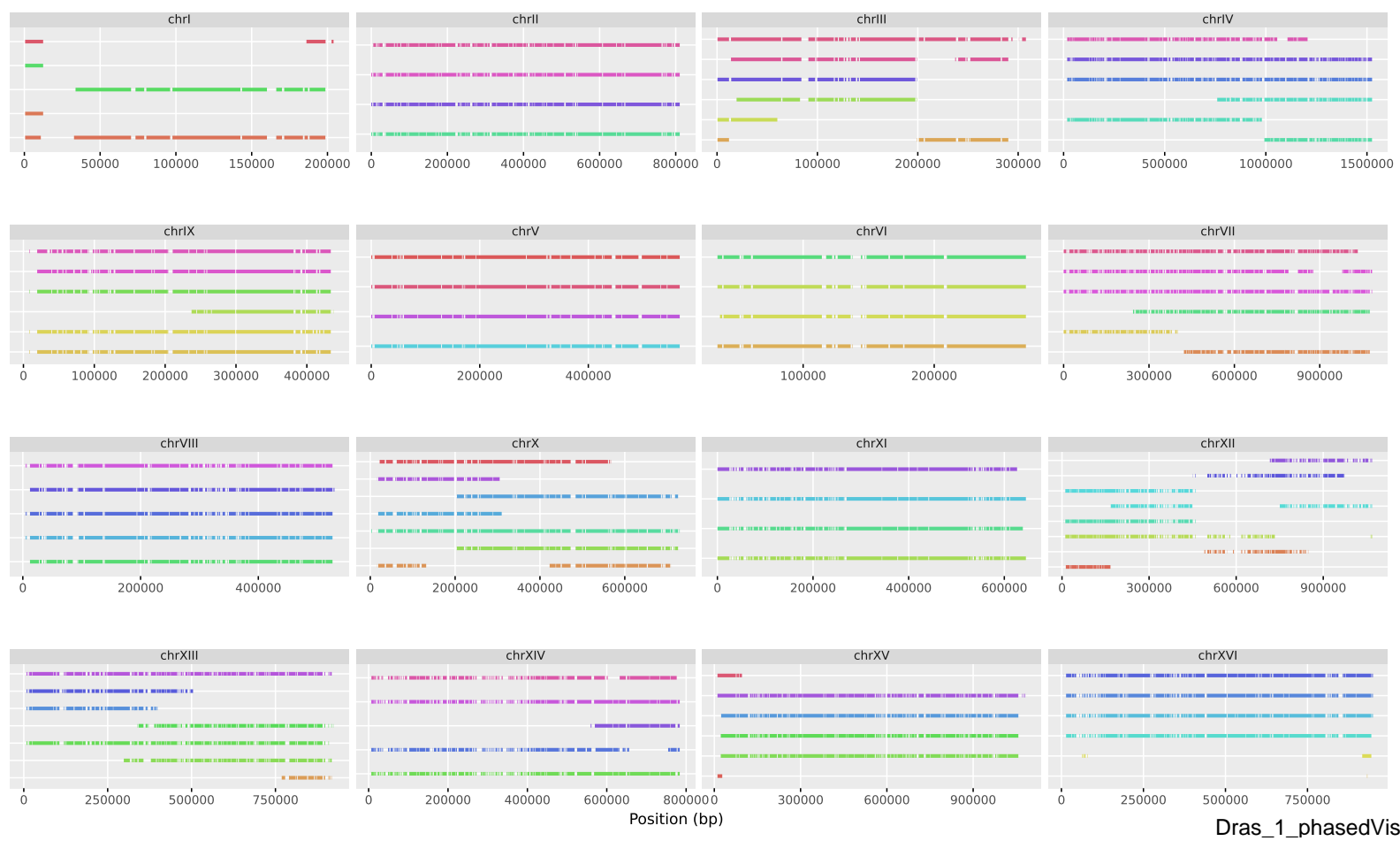


Supplementary Figure S1

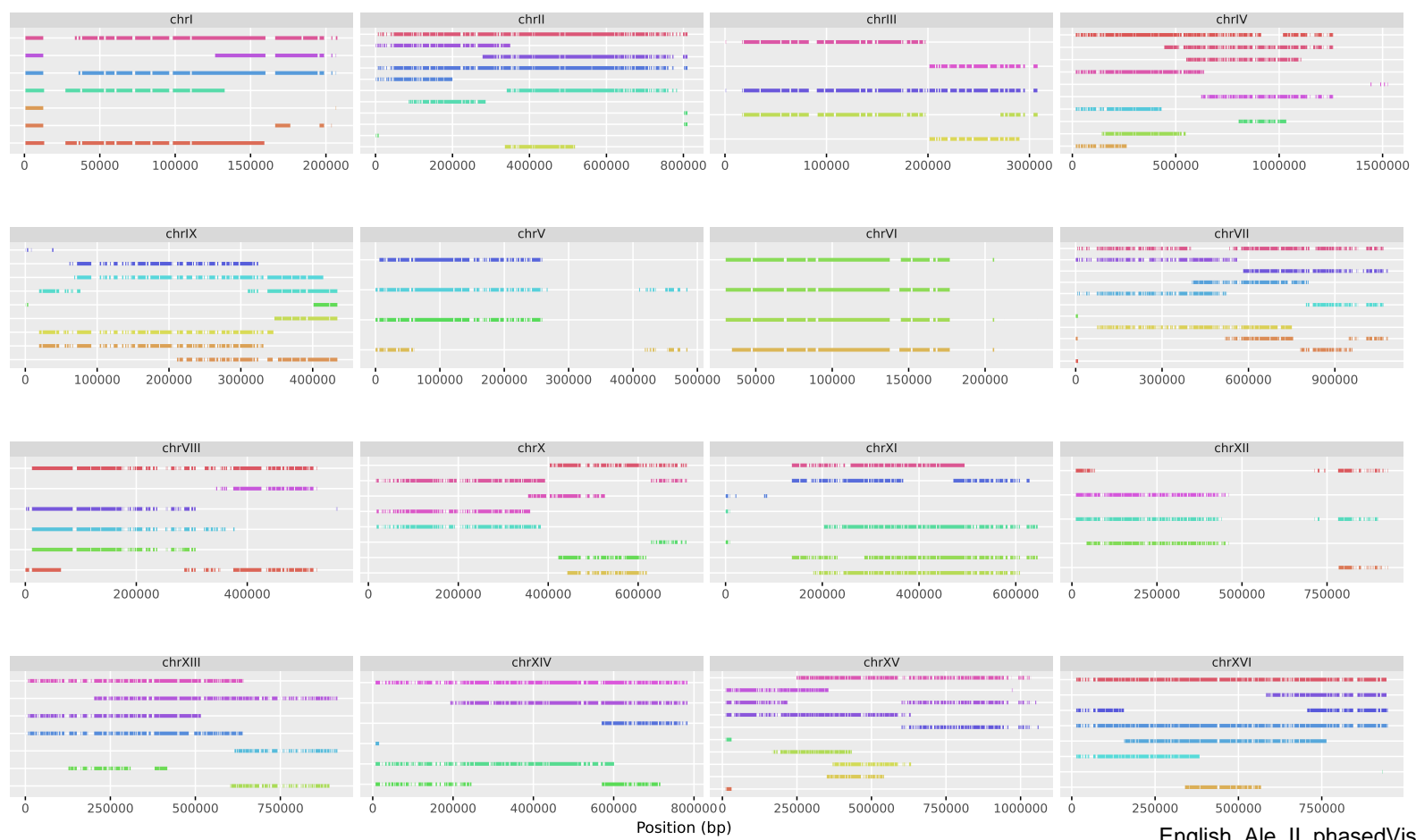


Classic_Wit_phasedVis

Supplementary Figure S1

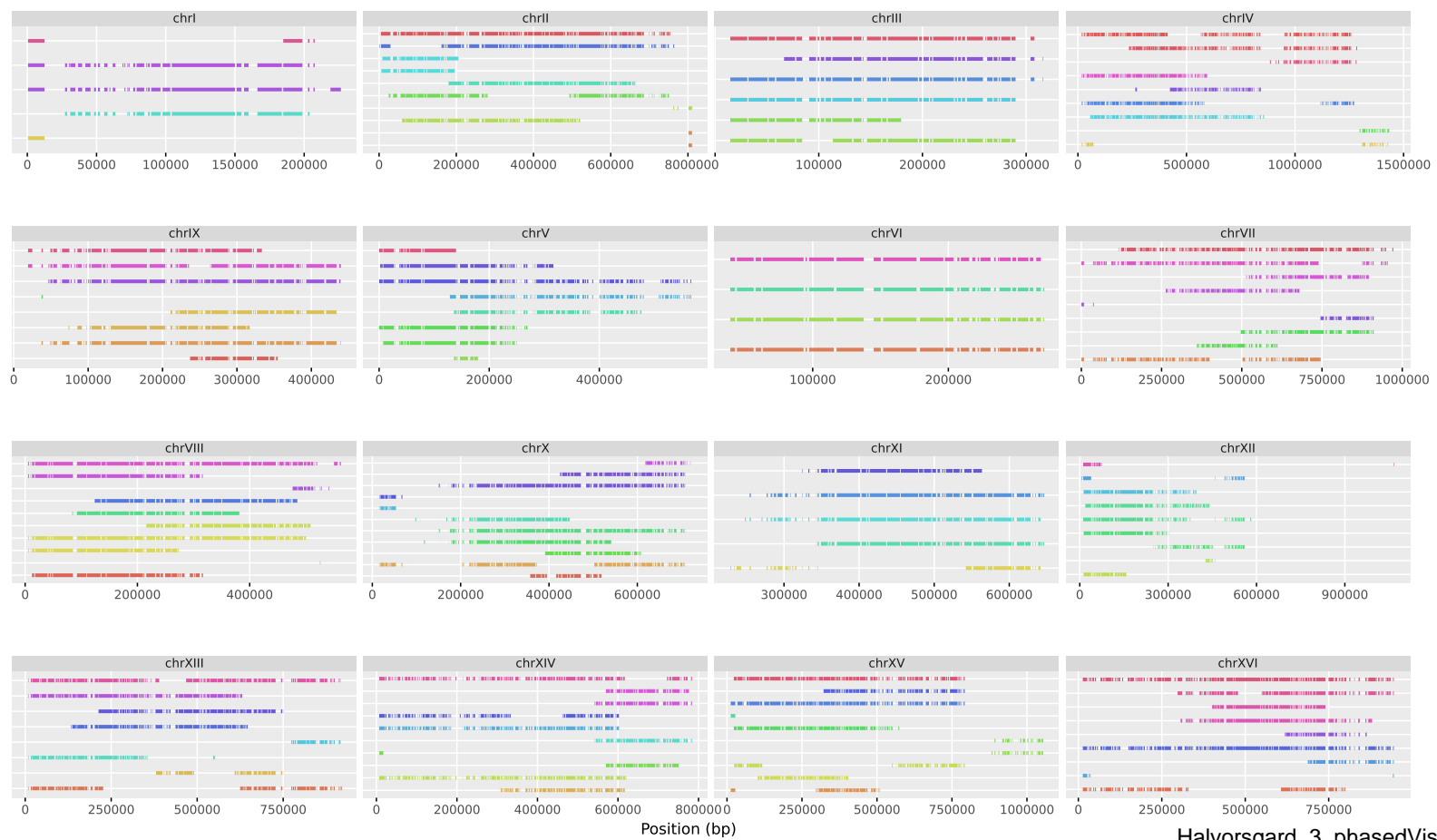


Supplementary Figure S1

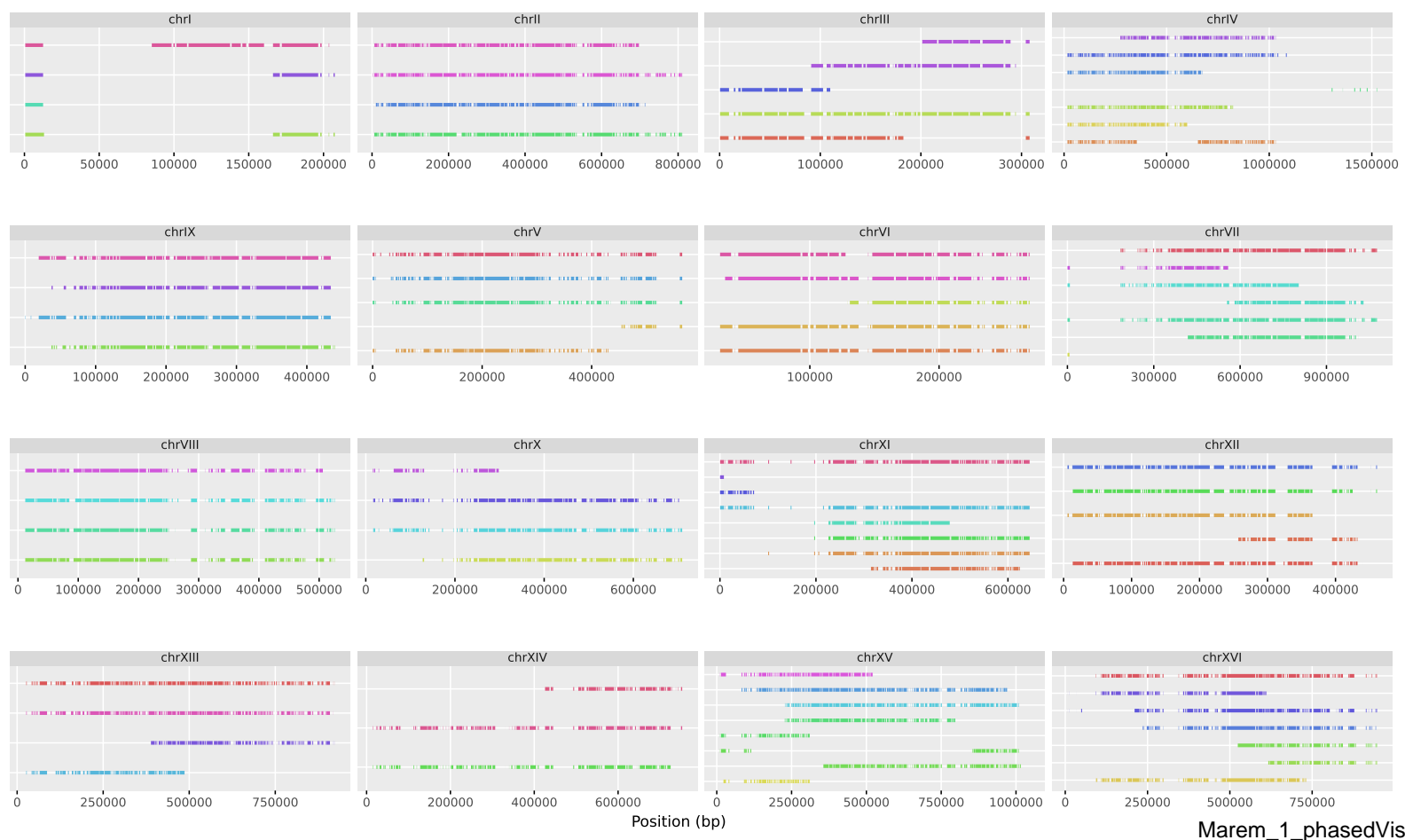


English_Ale_II_phasedVis

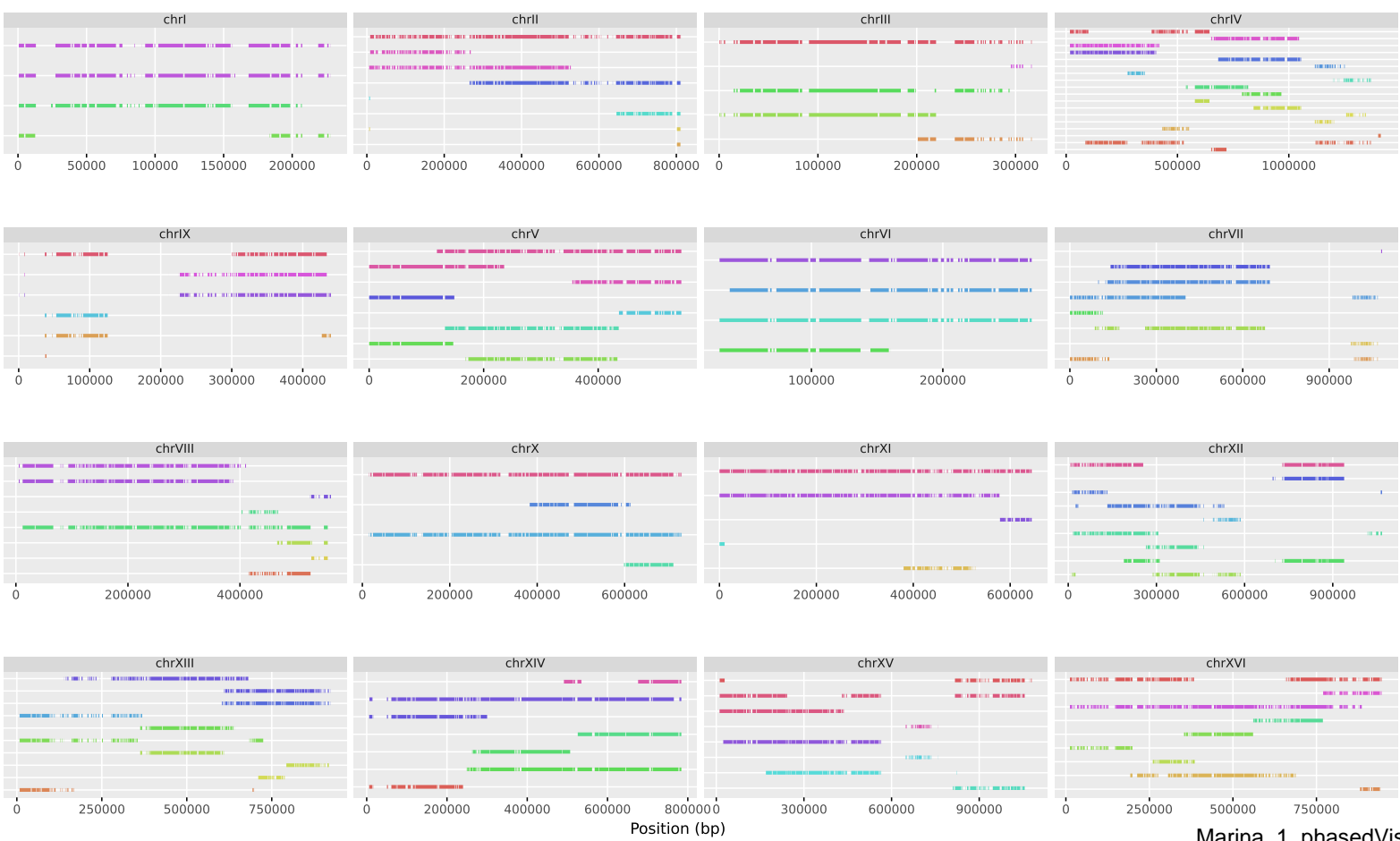
Supplementary Figure S1



Supplementary Figure S1

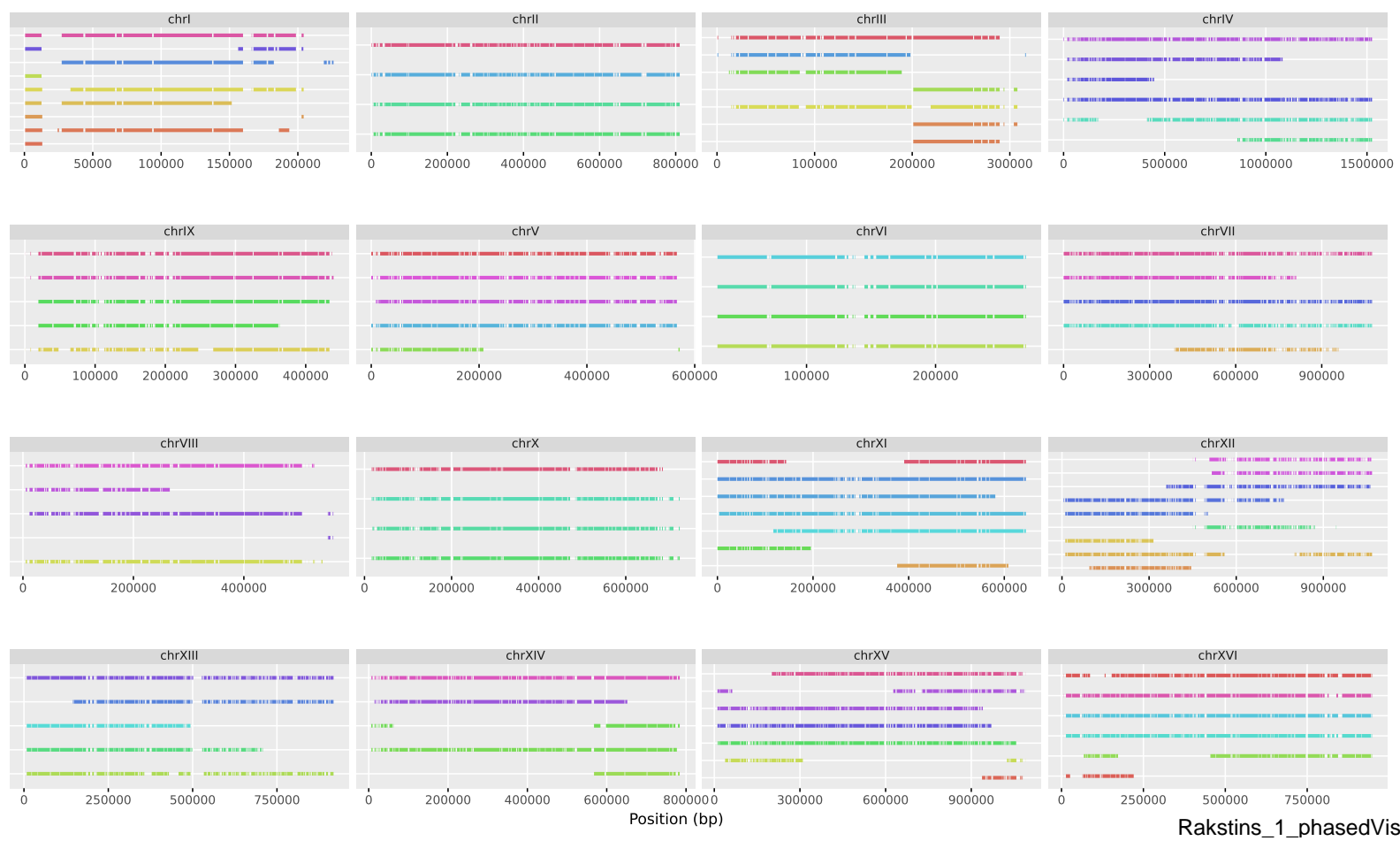


Supplementary Figure S1

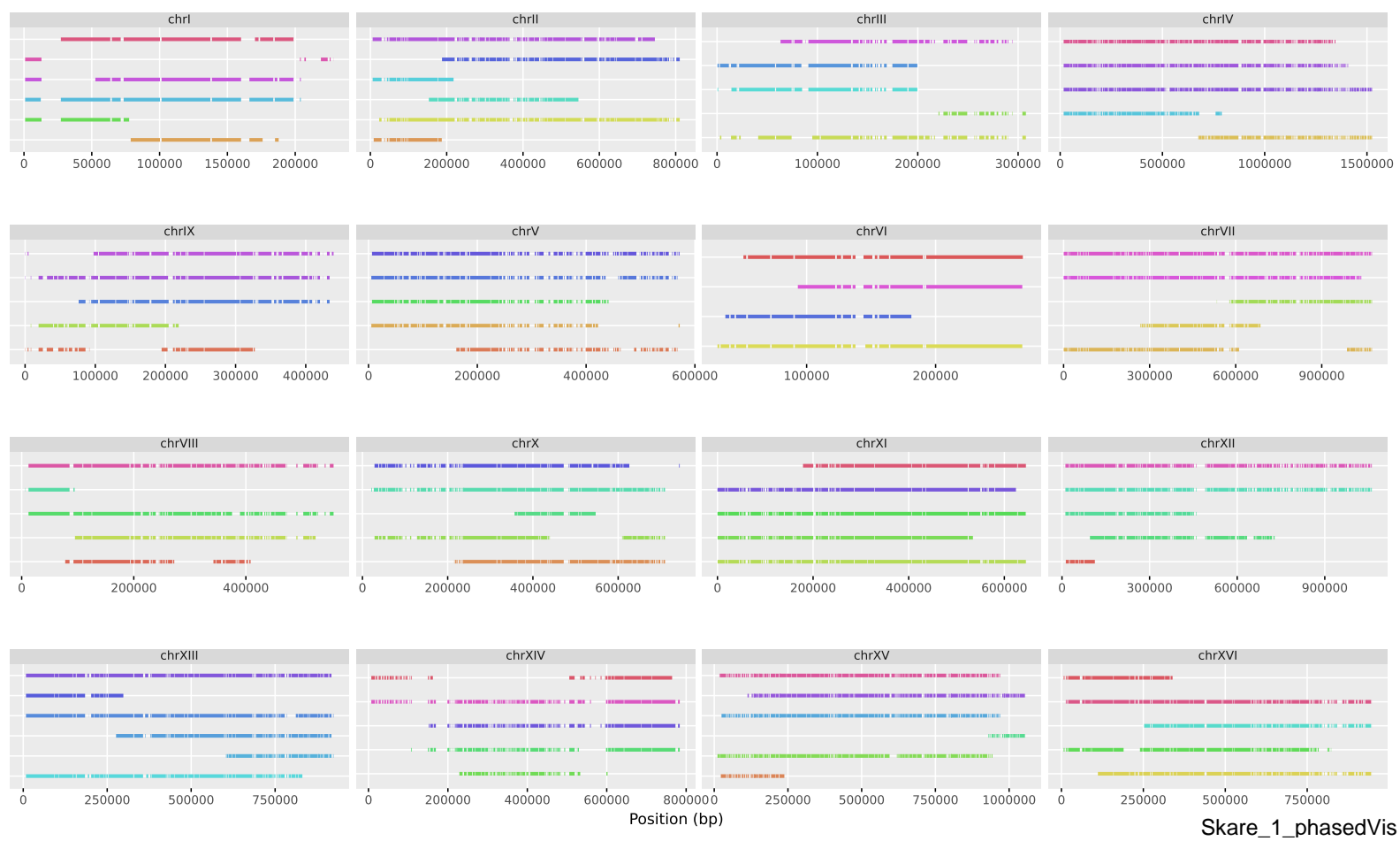


Marina_1_phasedVis

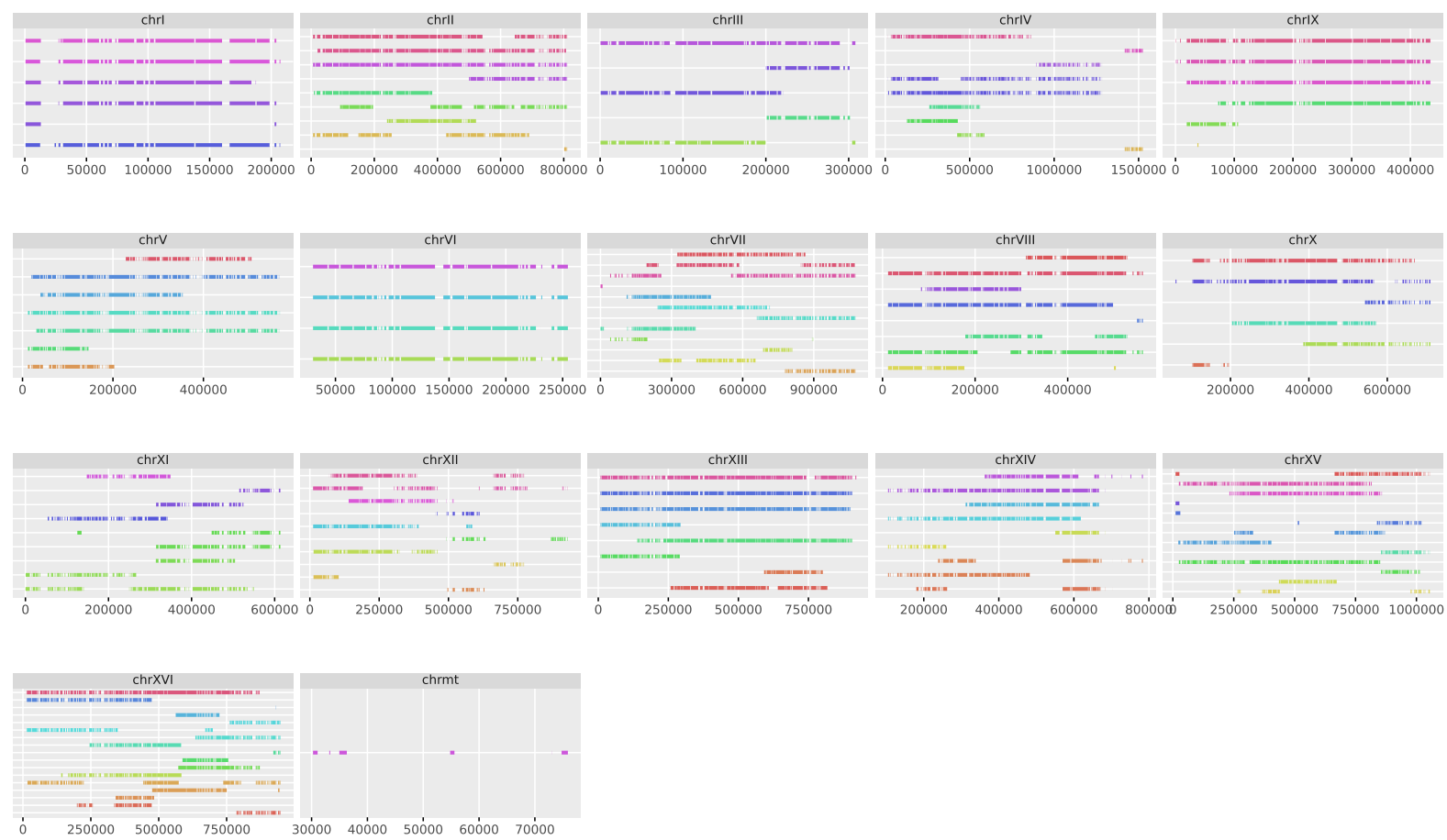
Supplementary Figure S1



Supplementary Figure S1



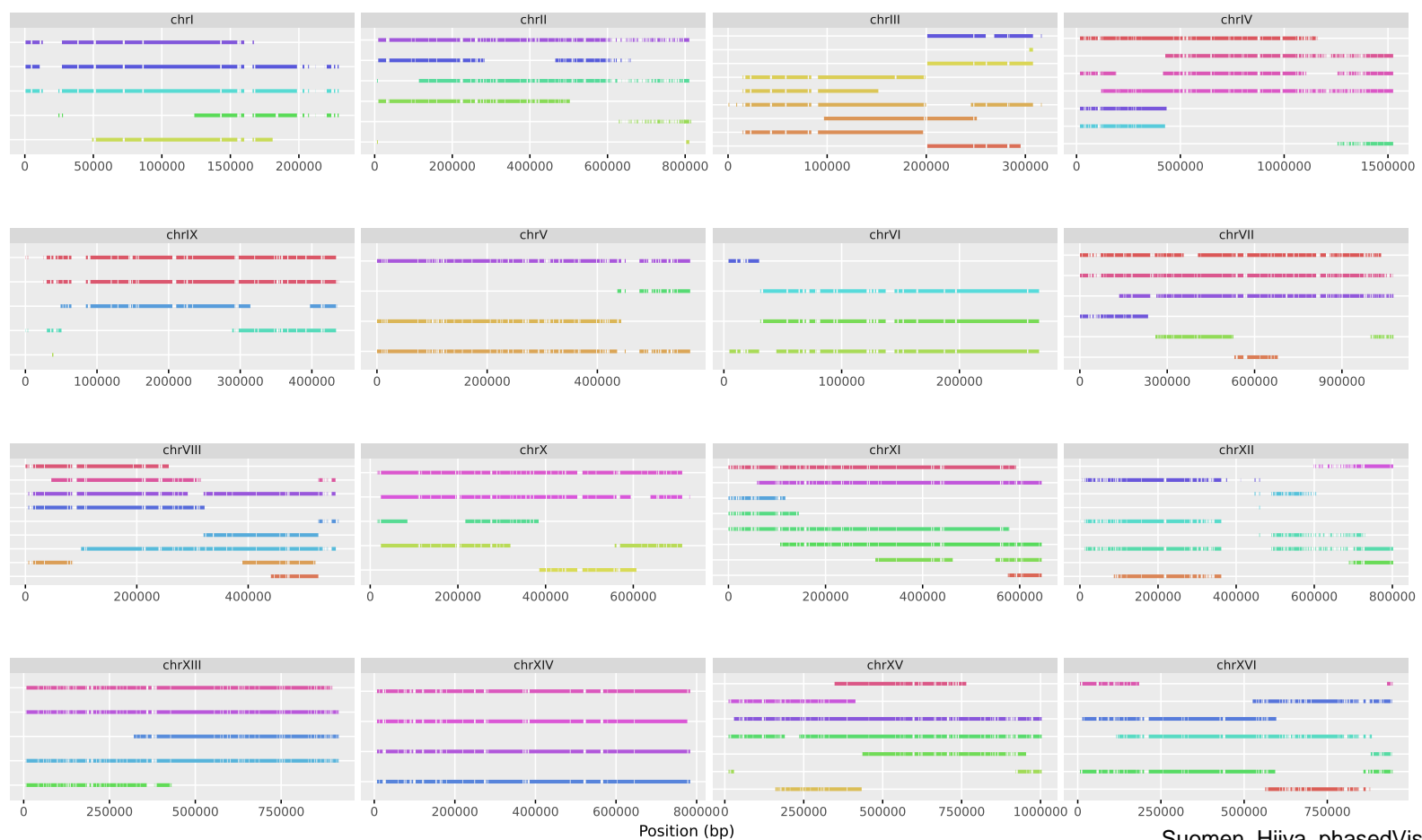
Supplementary Figure S1



Position (bp)

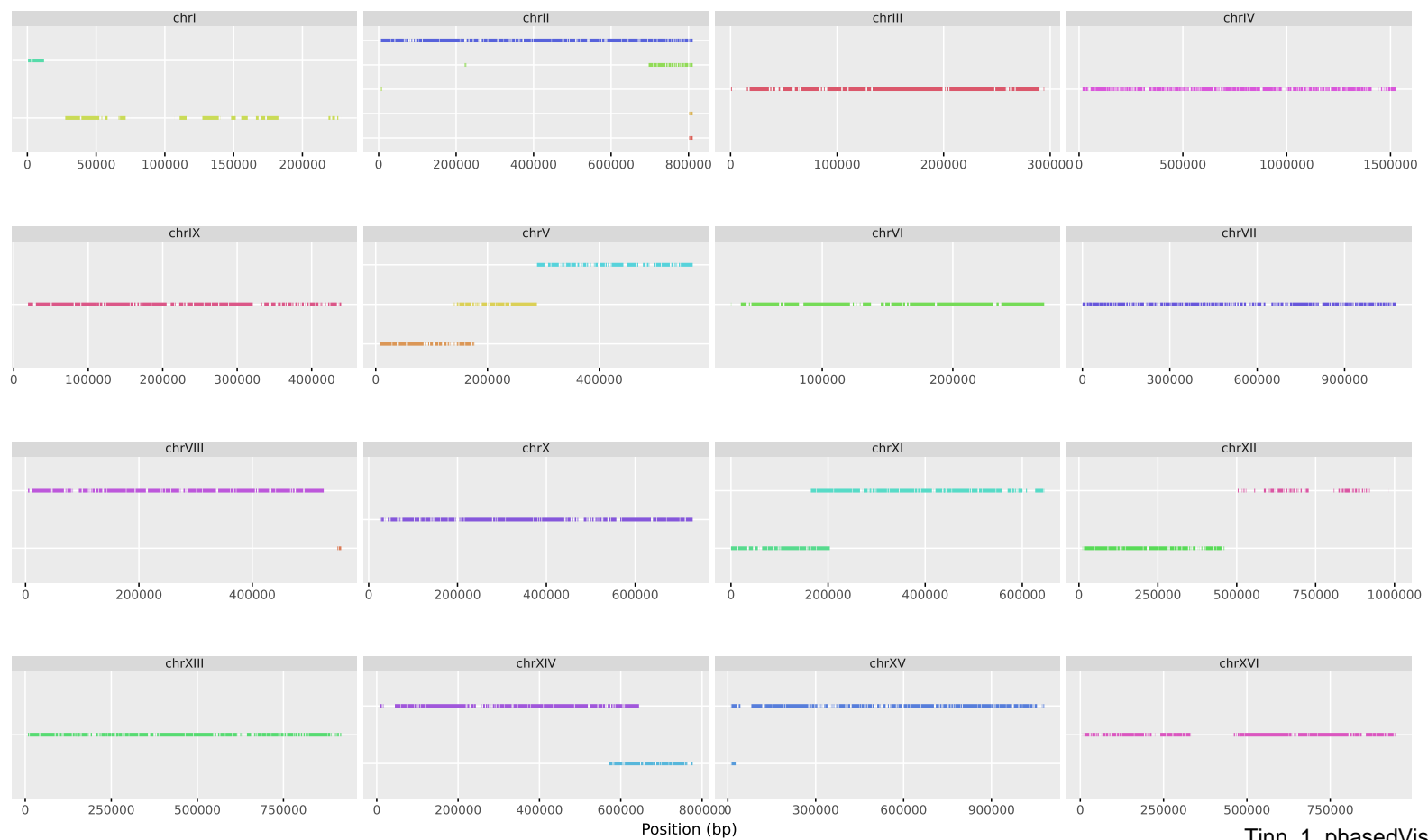
Stordal_Ebbegarden_1_phasedVis

Supplementary Figure S1



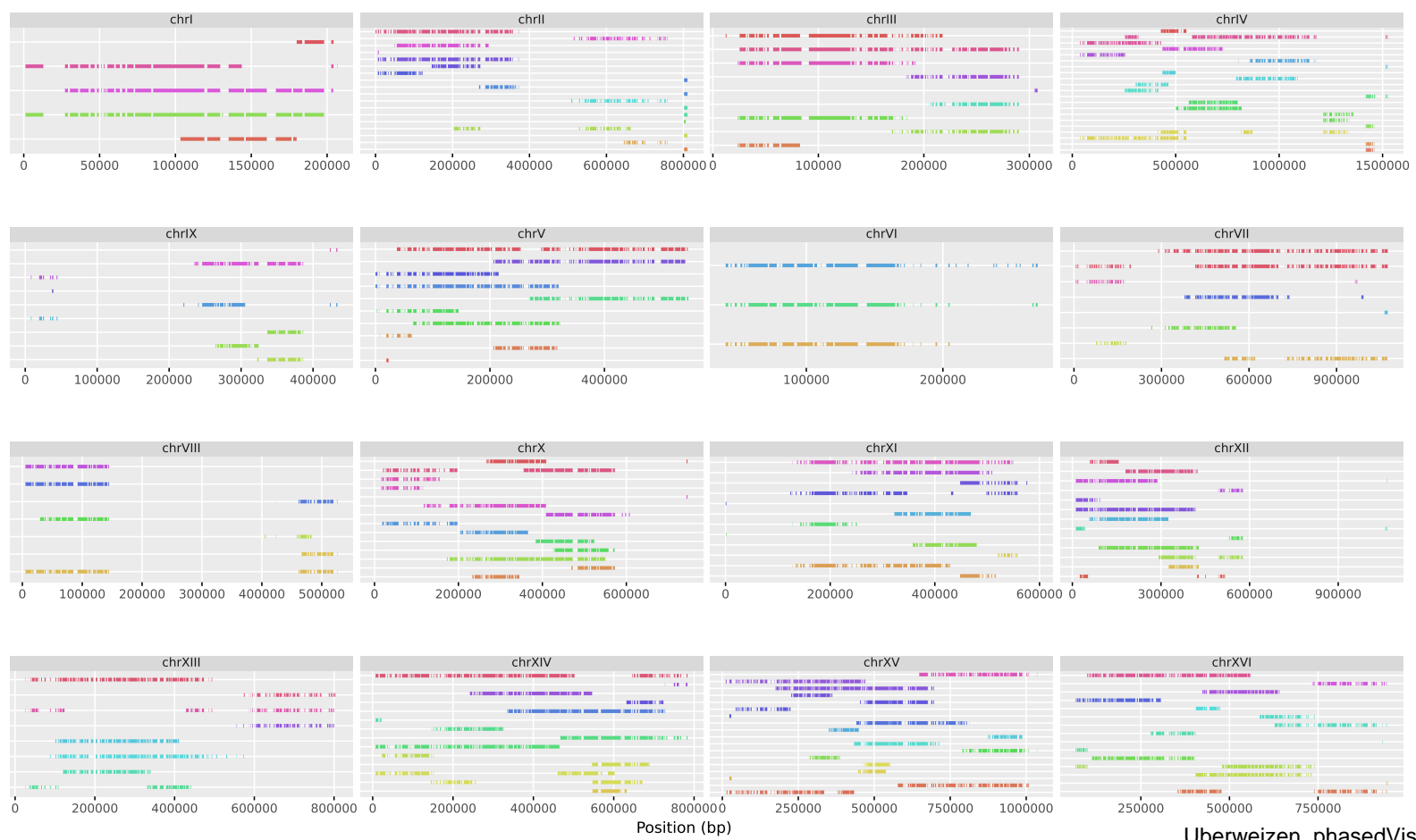
Suomen_Hiiva_phasedVis

Supplementary Figure S1



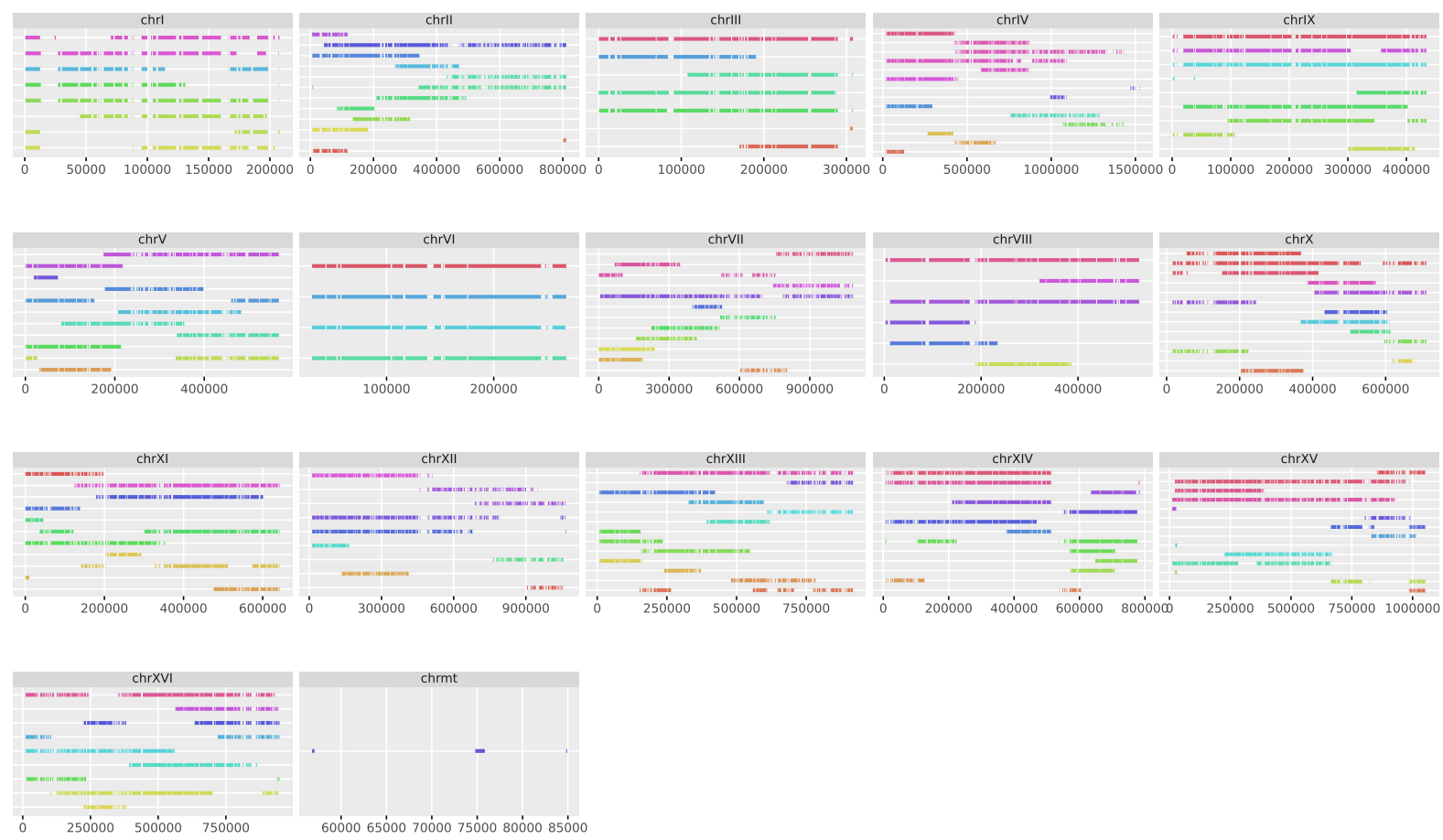
Tinn_1_phasedVis

Supplementary Figure S1



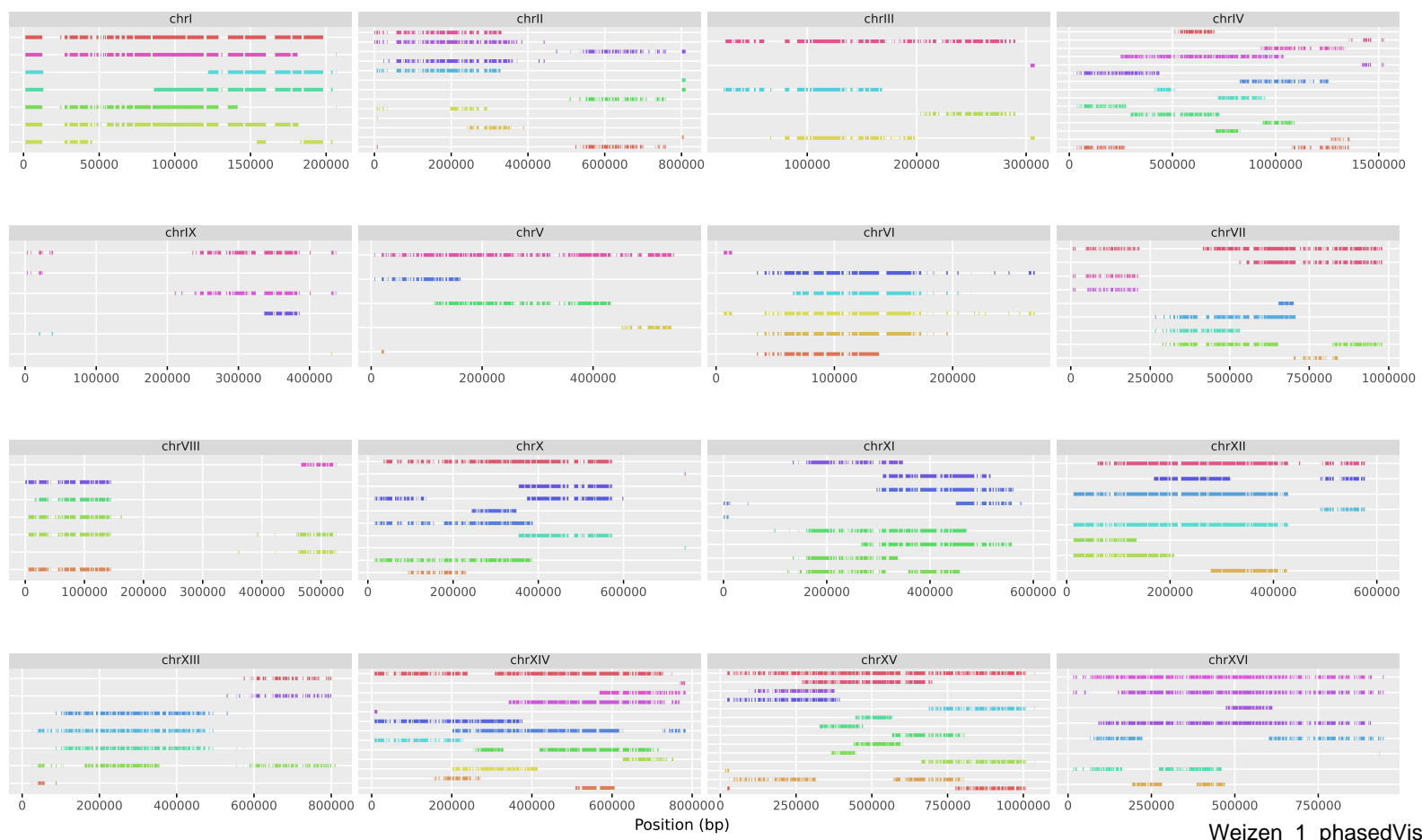
Überweizen_phasedVis

Supplementary Figure S1

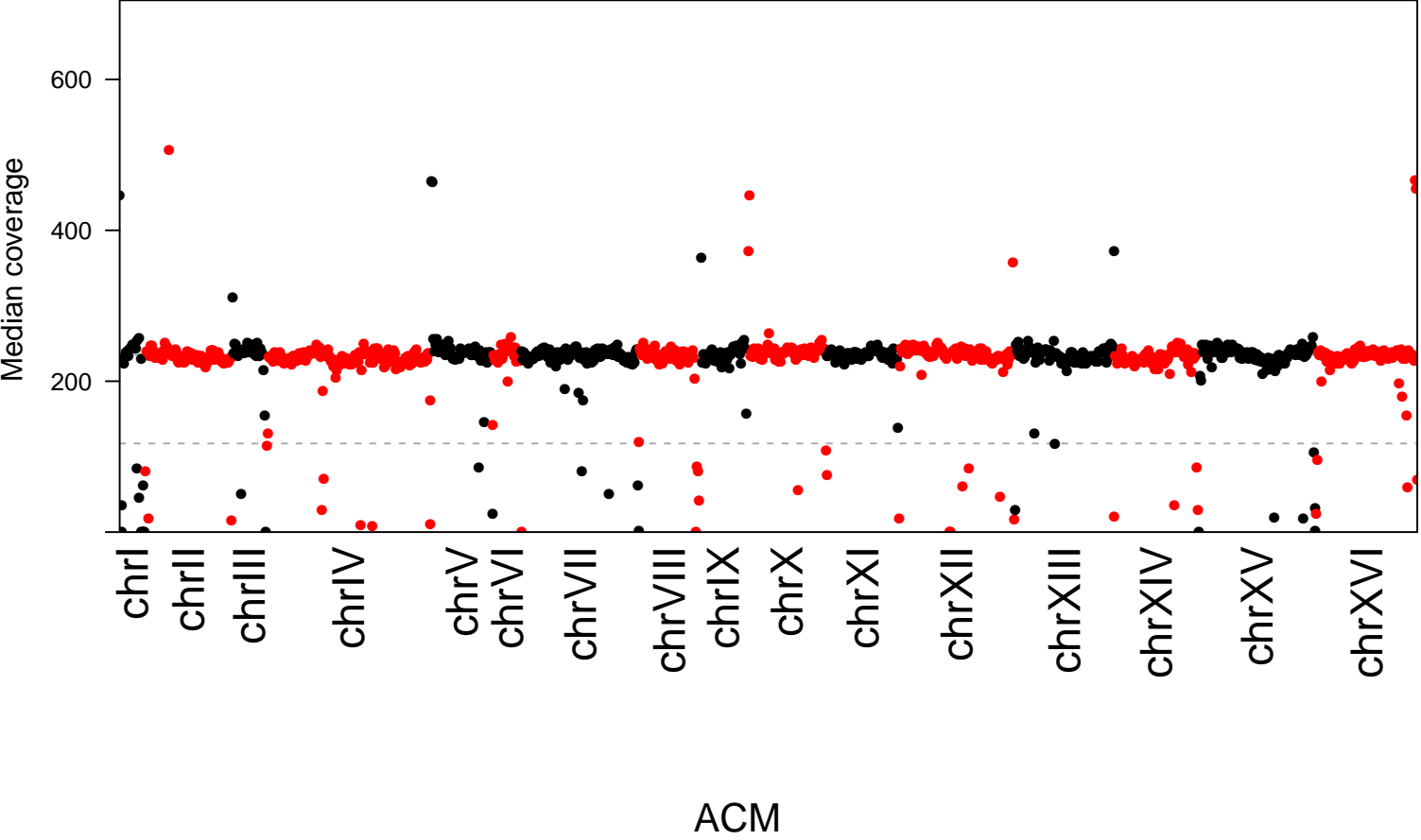


Voss_1_phasedVis

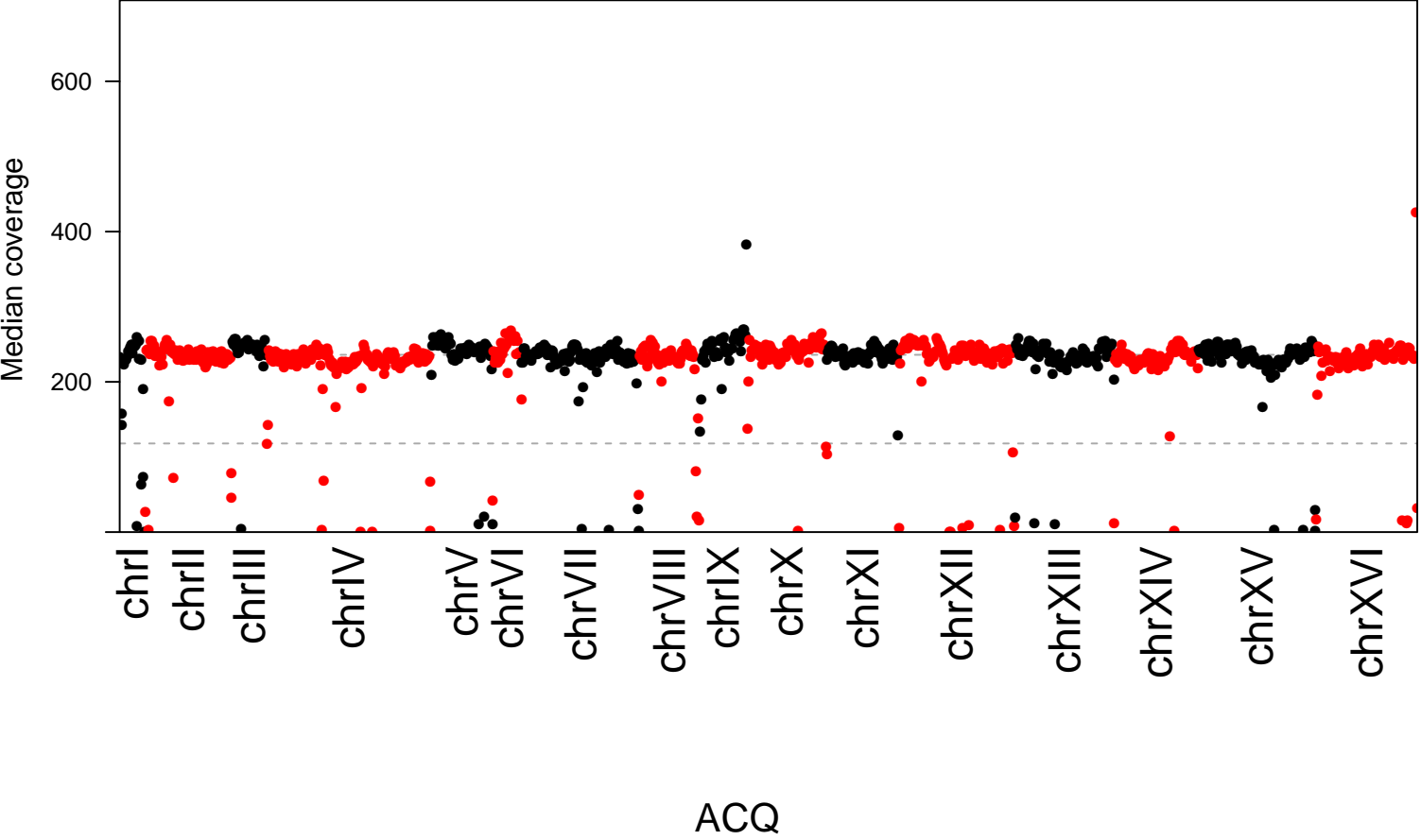
Supplementary Figure S1



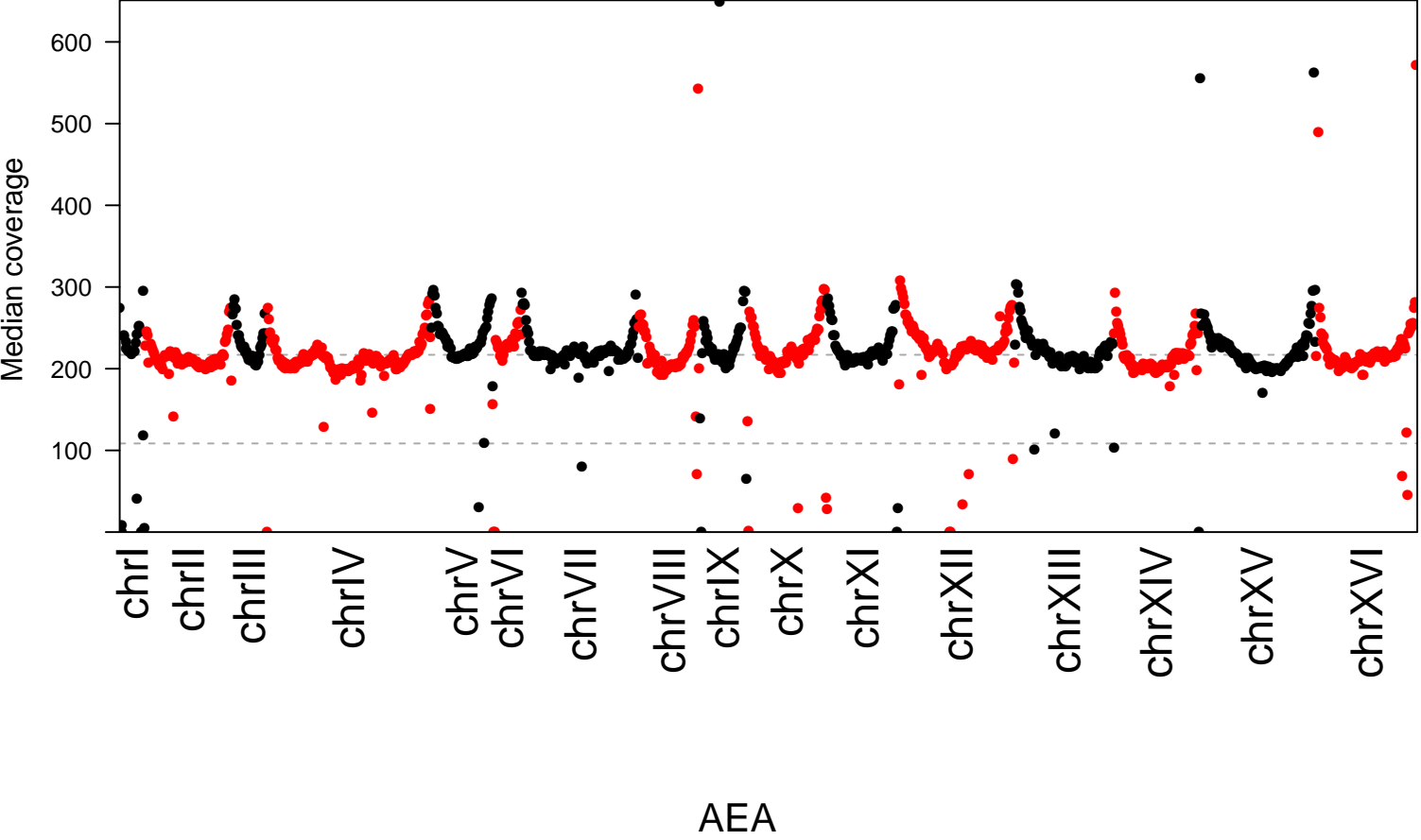
Supplementary Figure S2



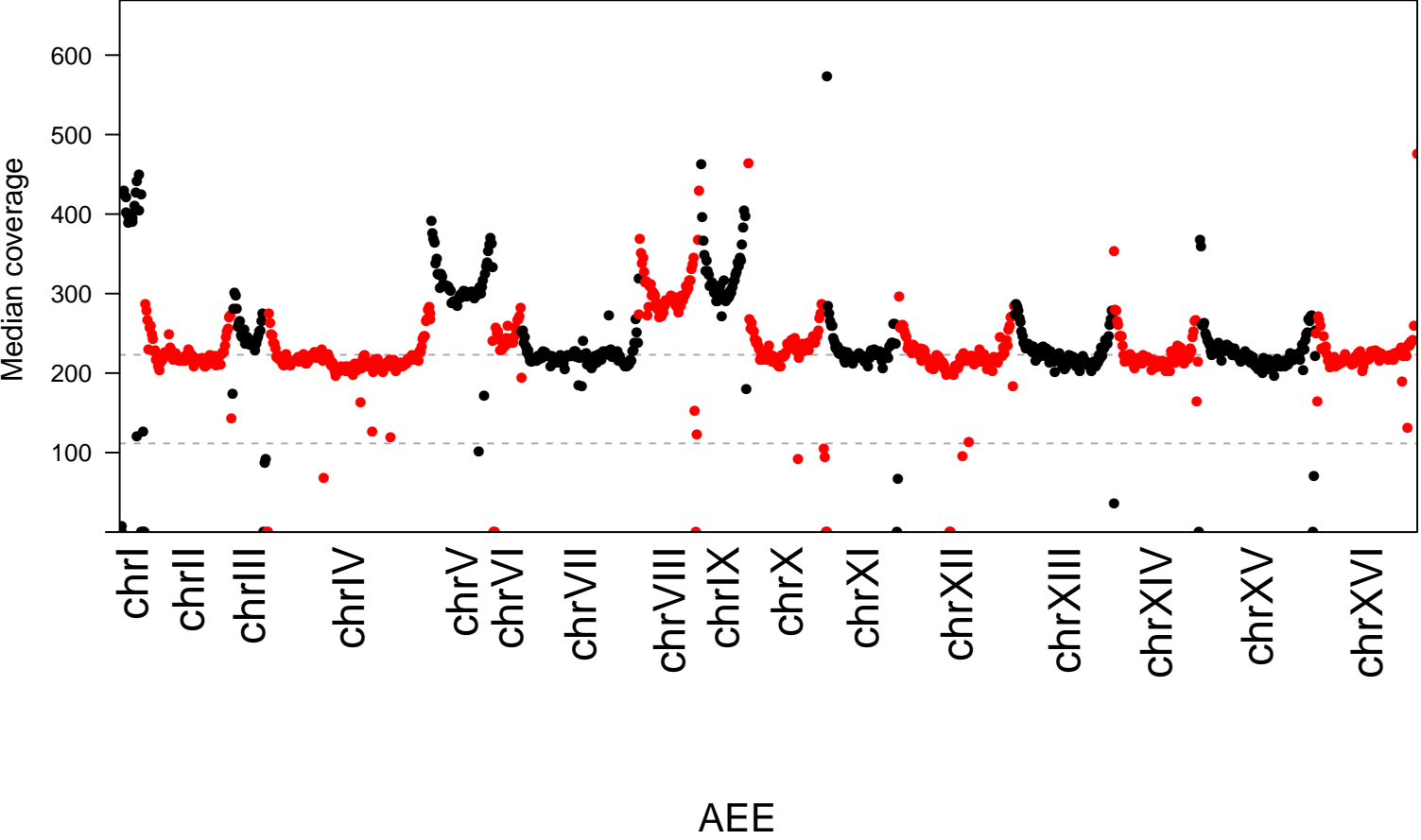
Supplementary Figure S2



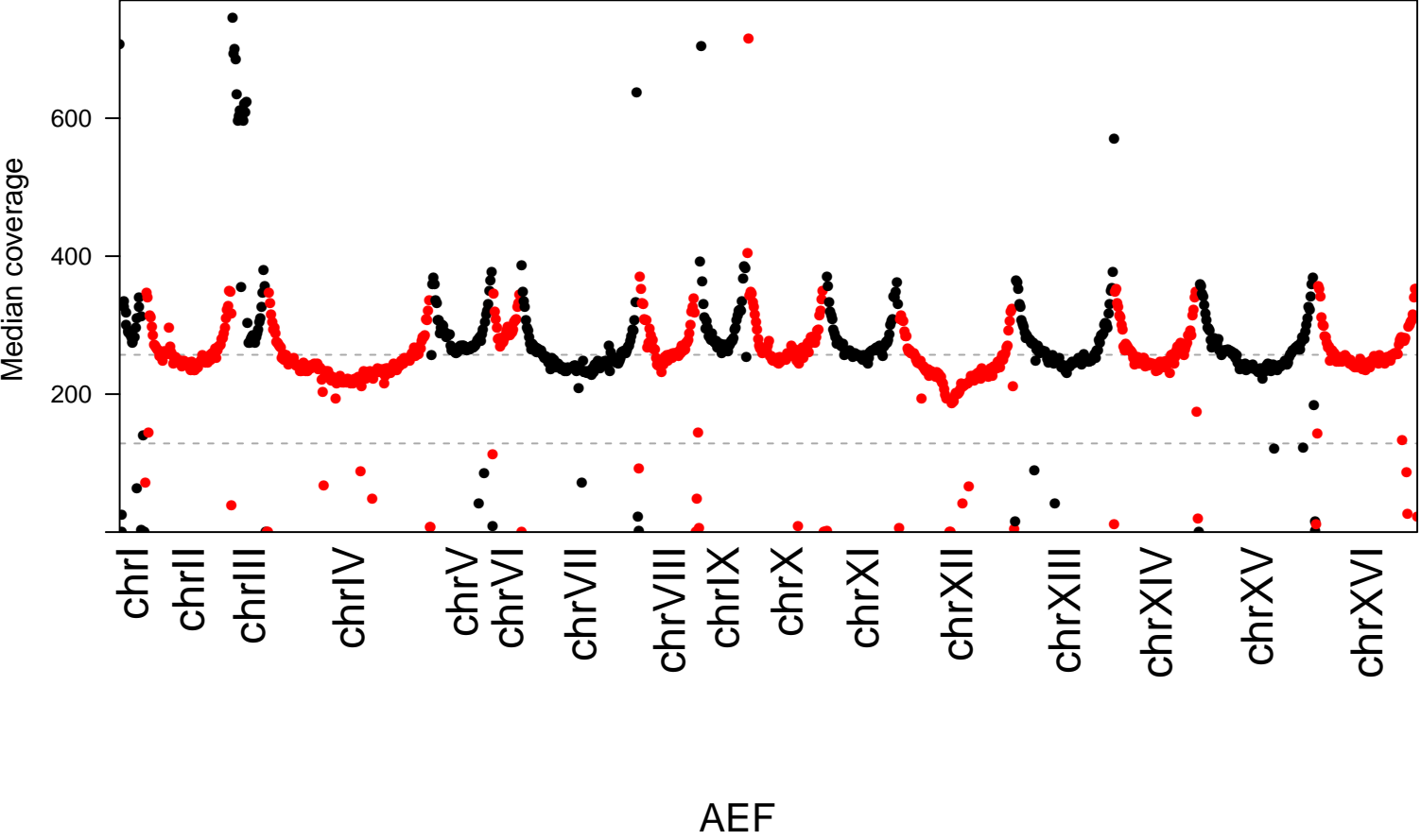
Supplementary Figure S2



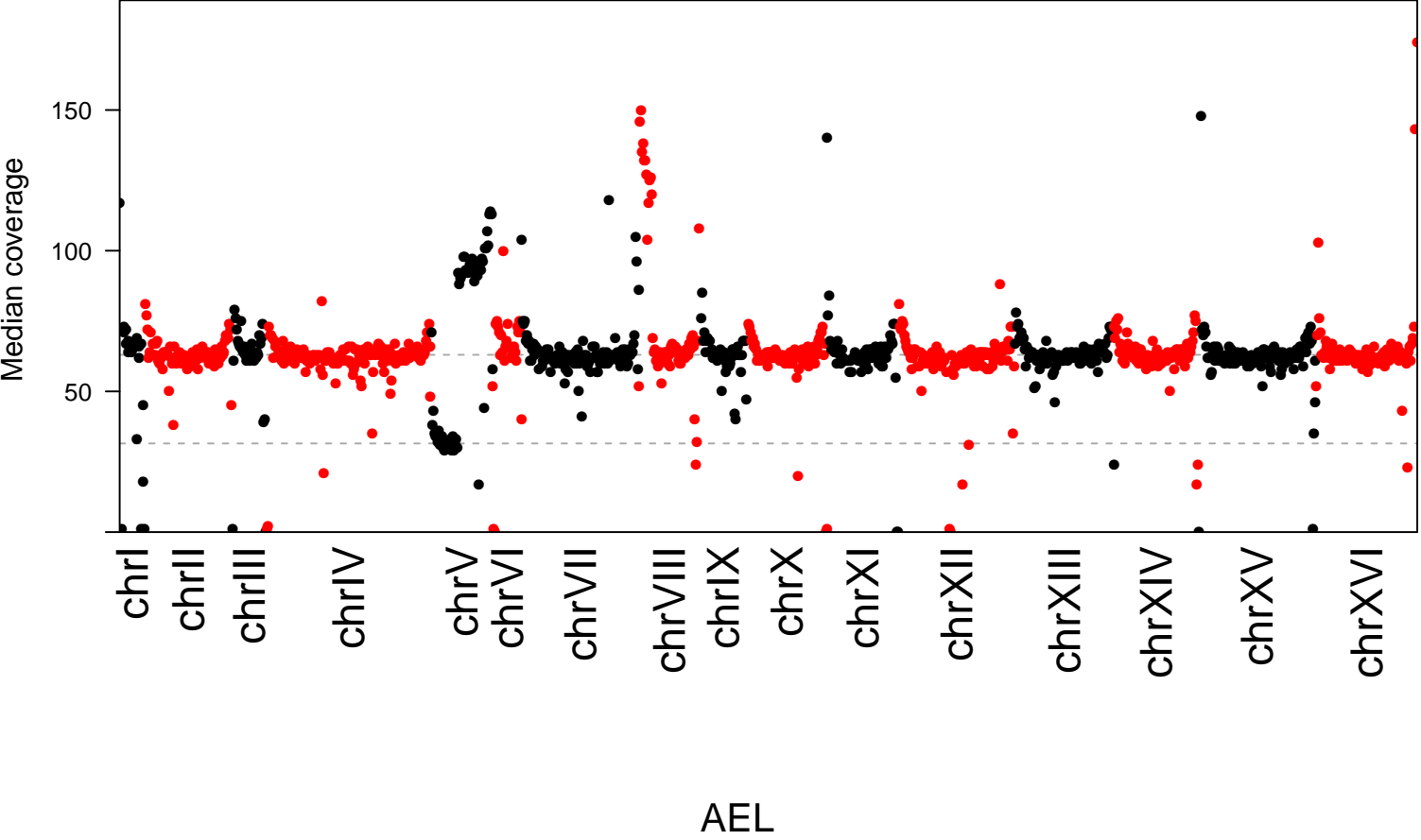
Supplementary Figure S2



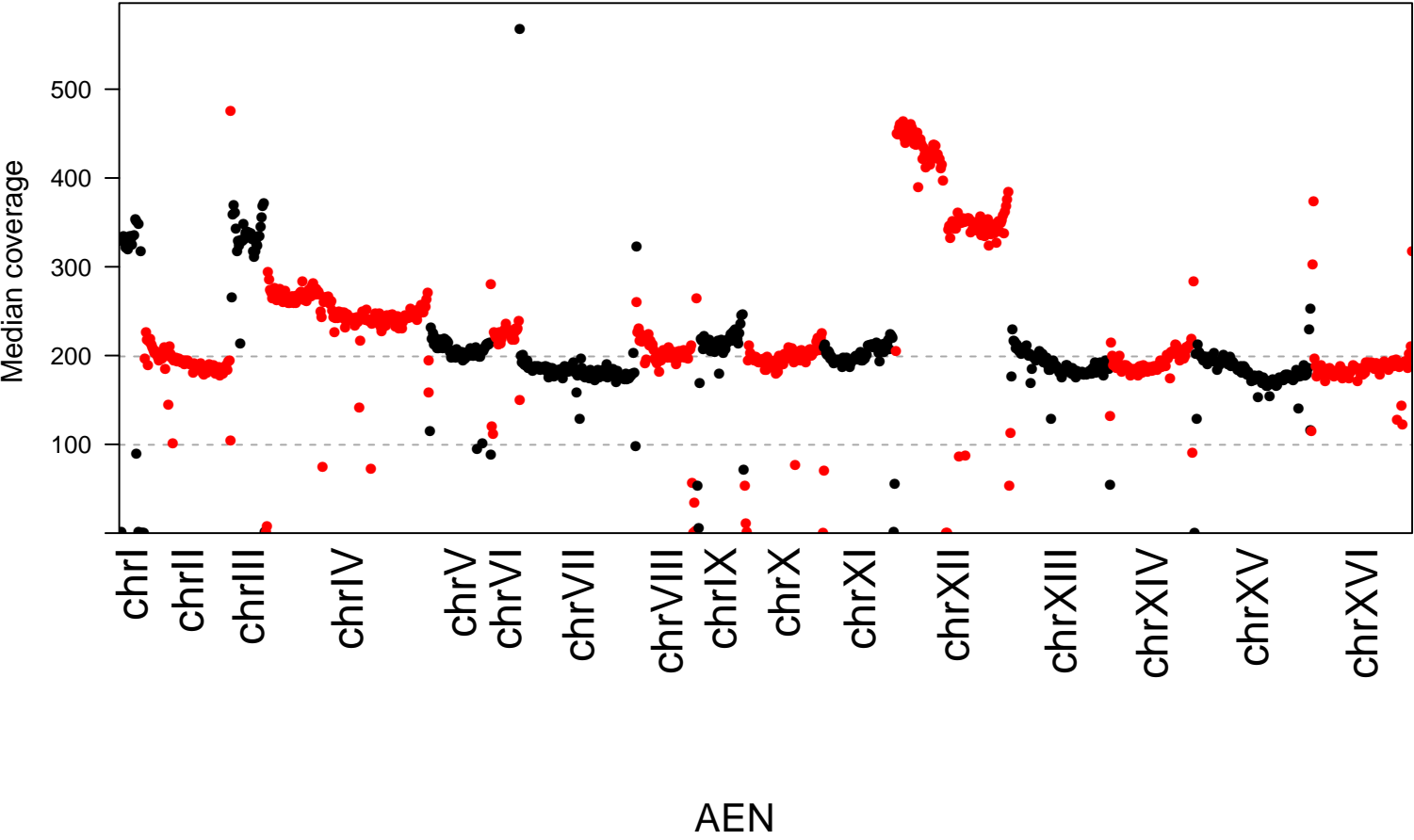
Supplementary Figure S2



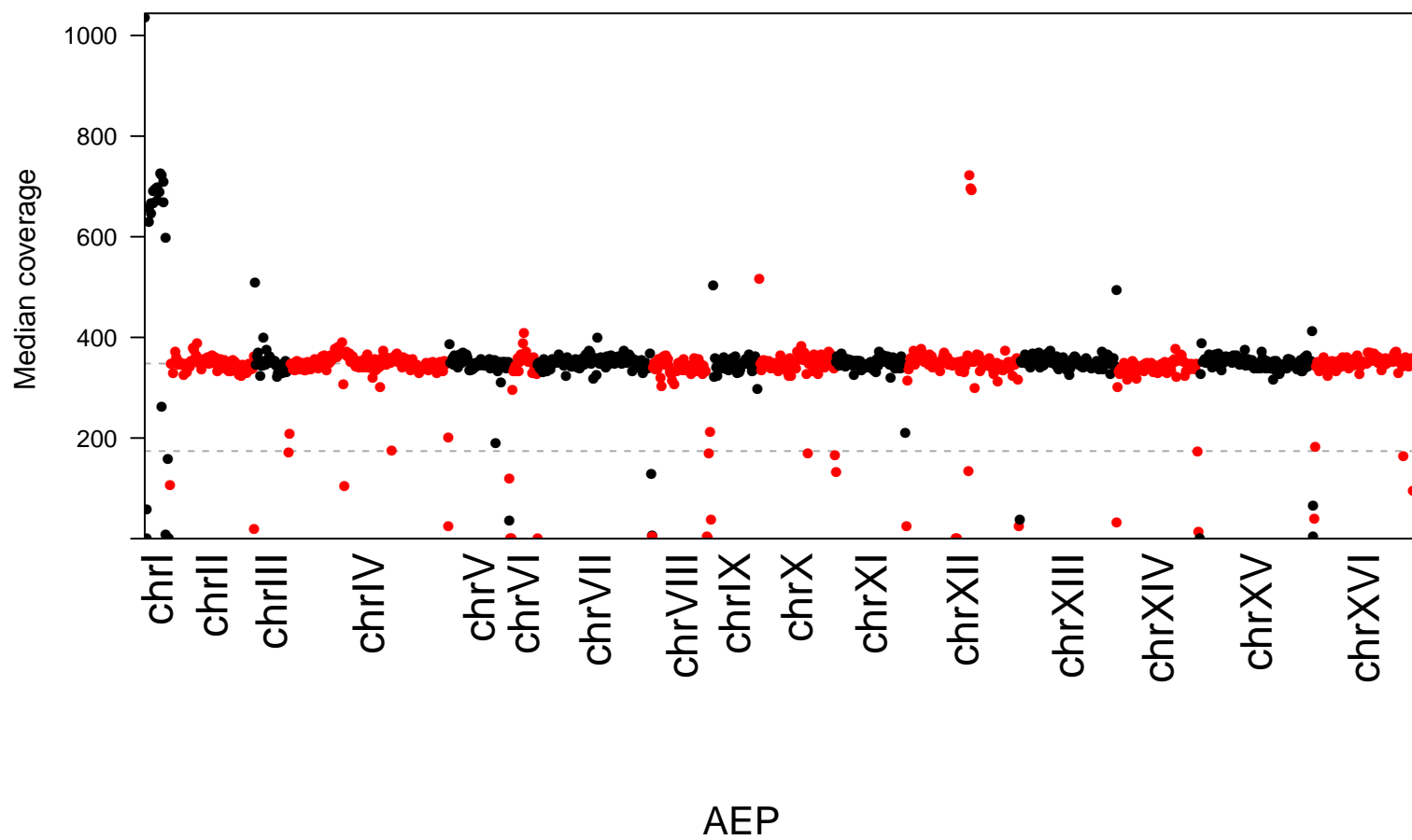
Supplementary Figure S2



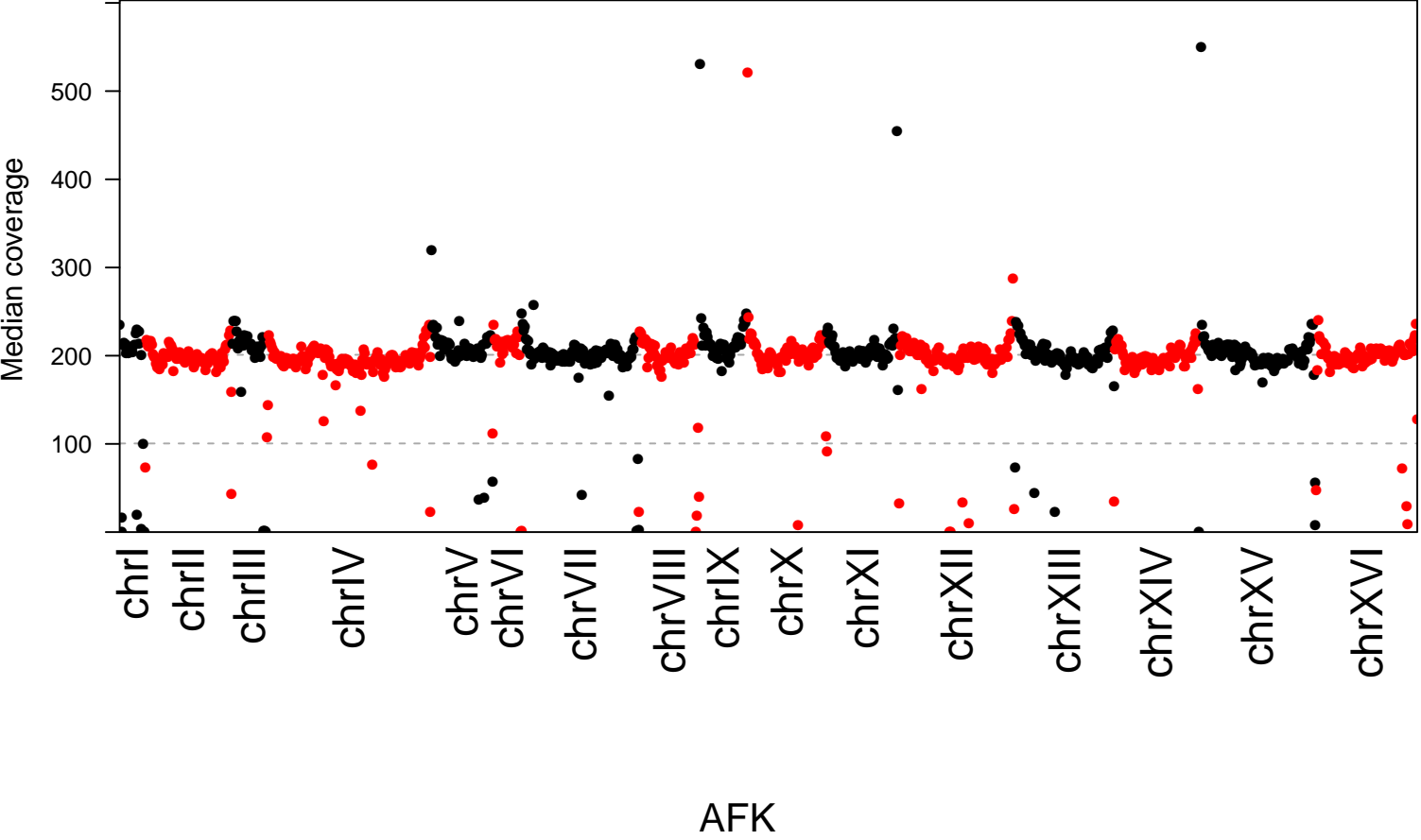
Supplementary Figure S2



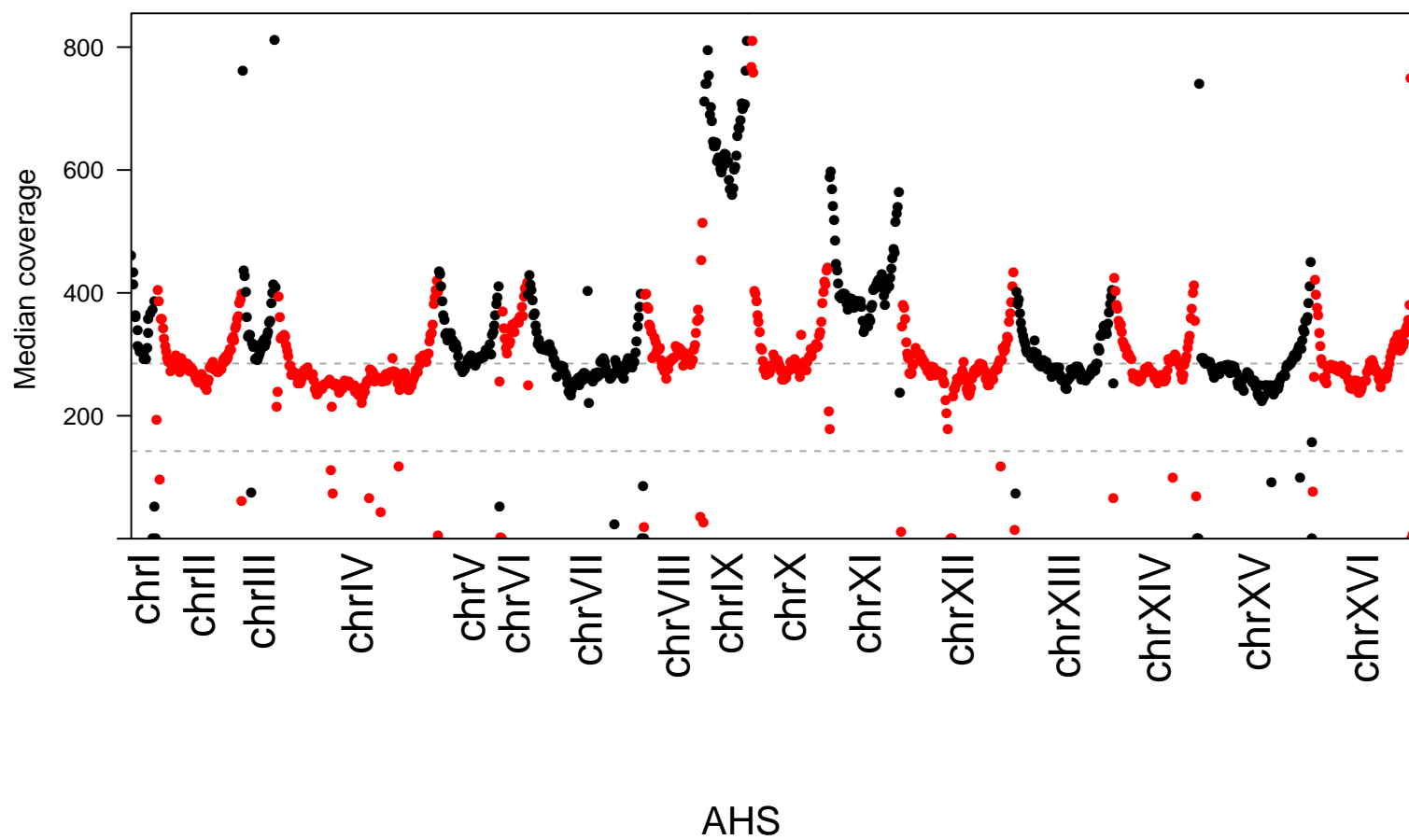
Supplementary Figure S2



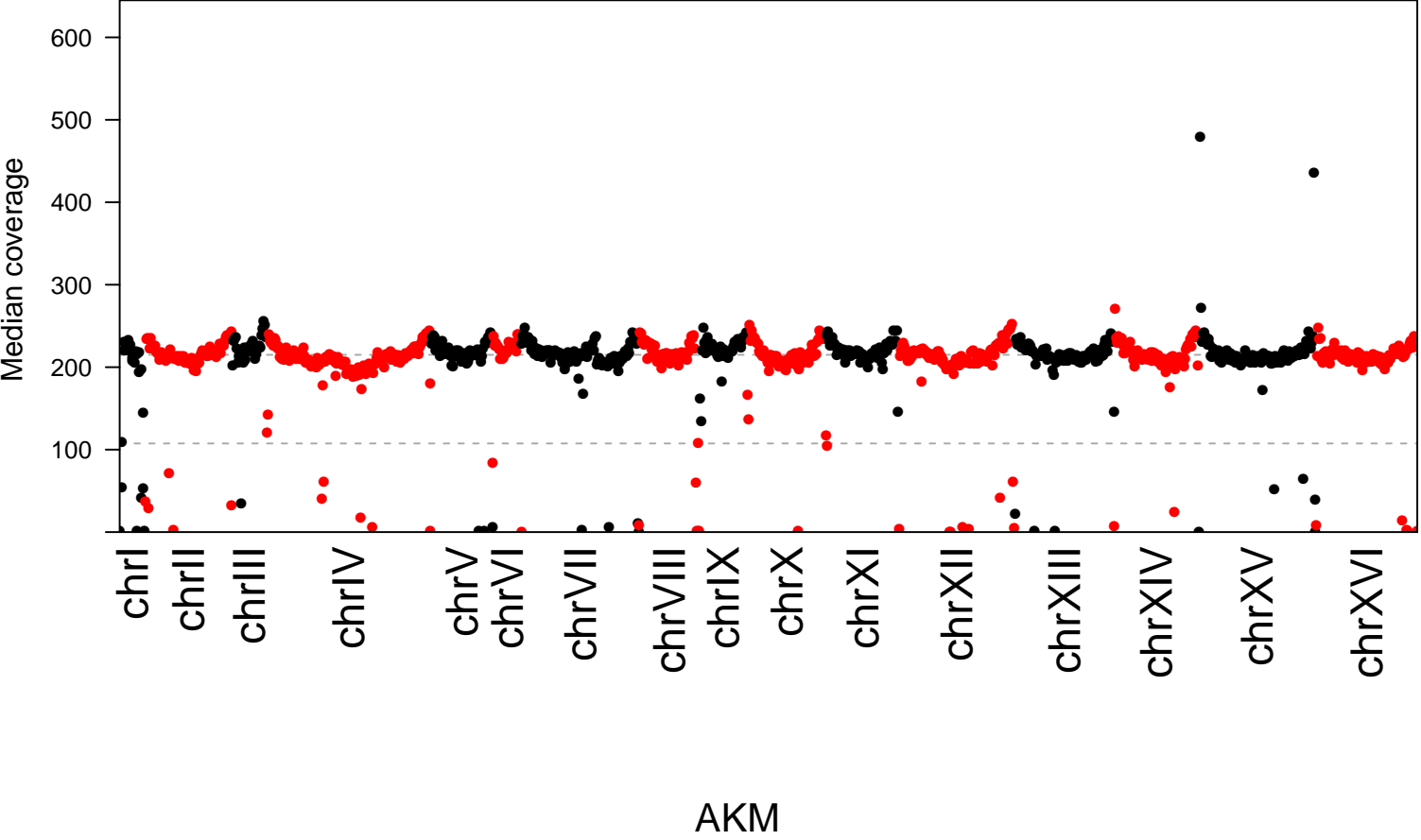
Supplementary Figure S2



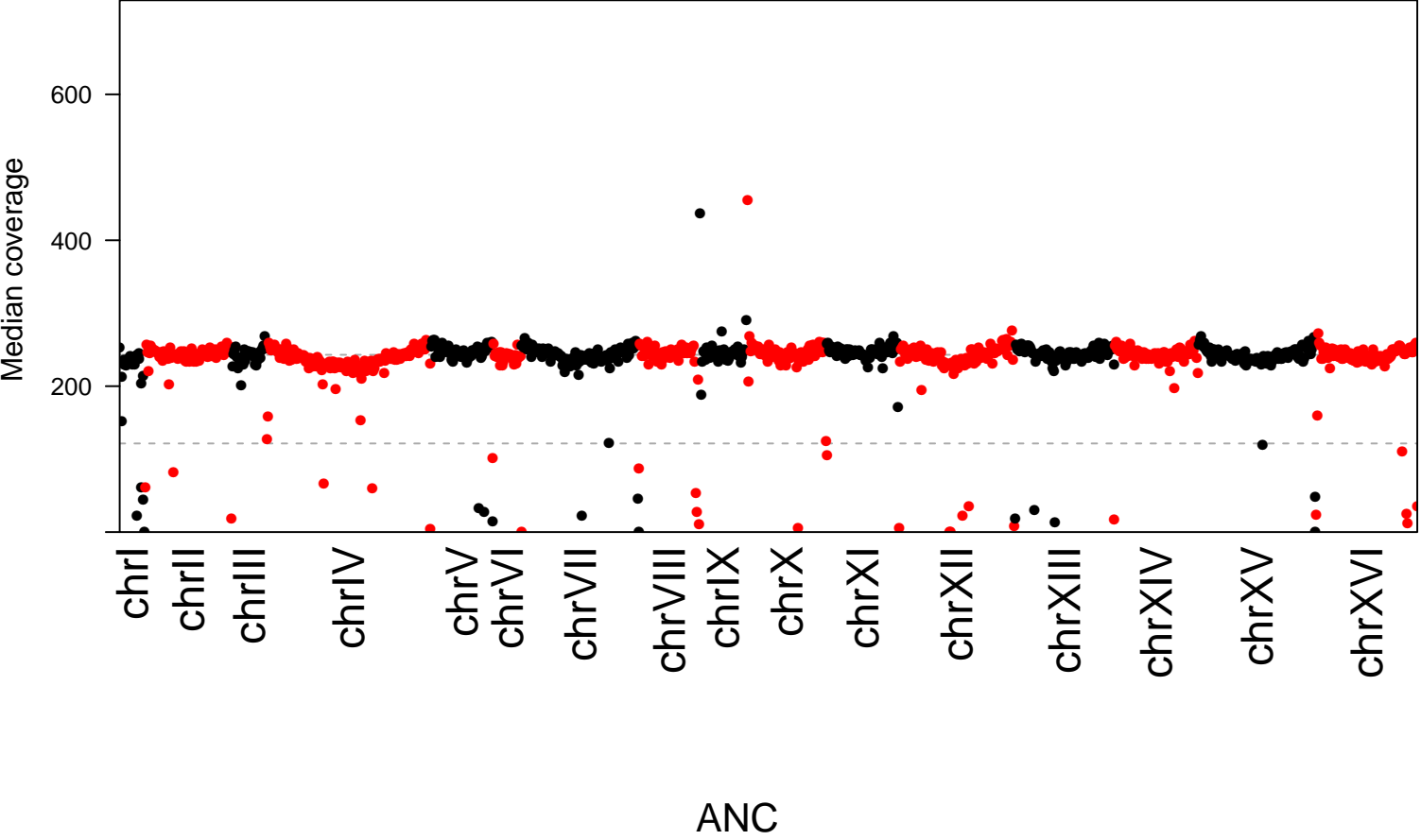
Supplementary Figure S2



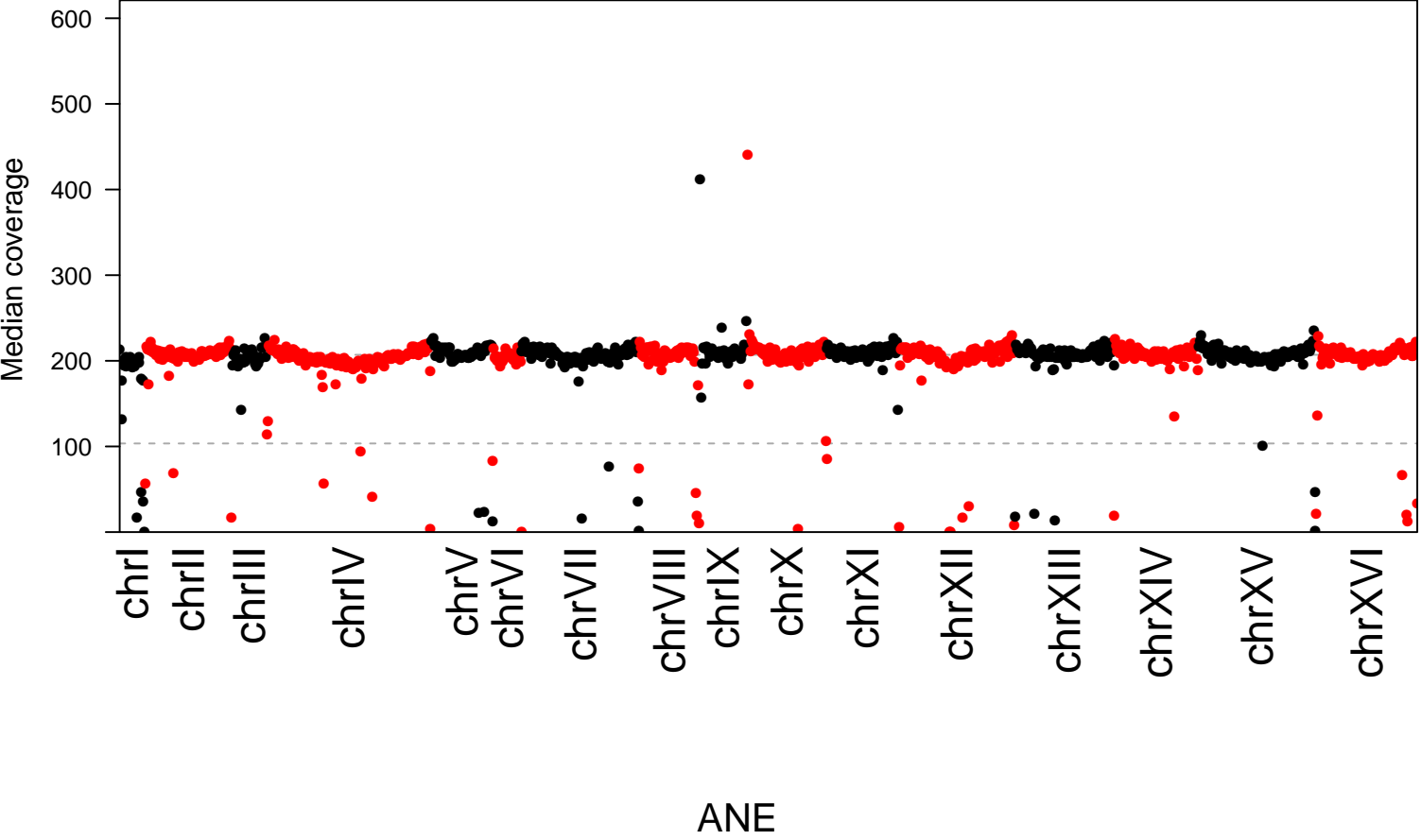
Supplementary Figure S2



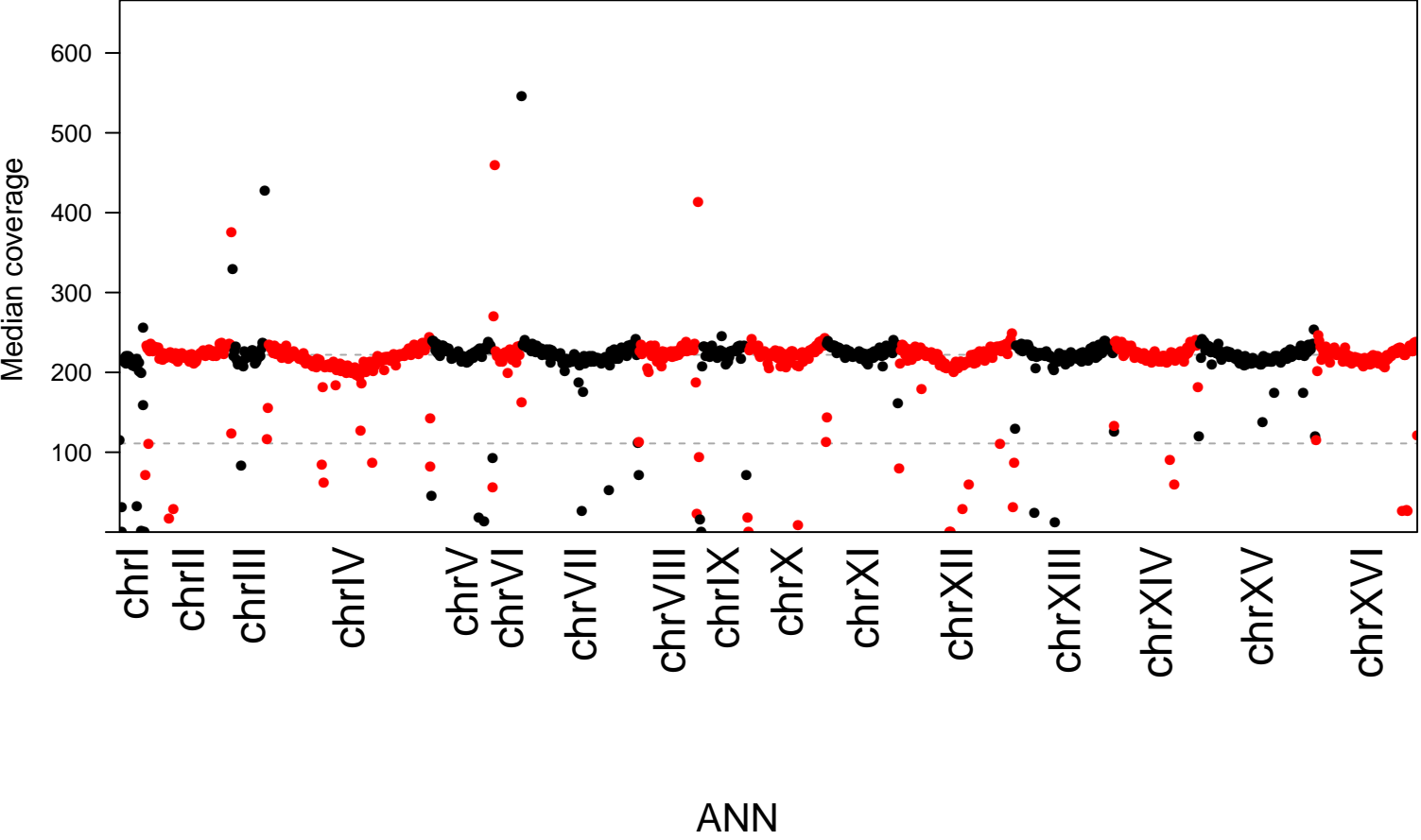
Supplementary Figure S2



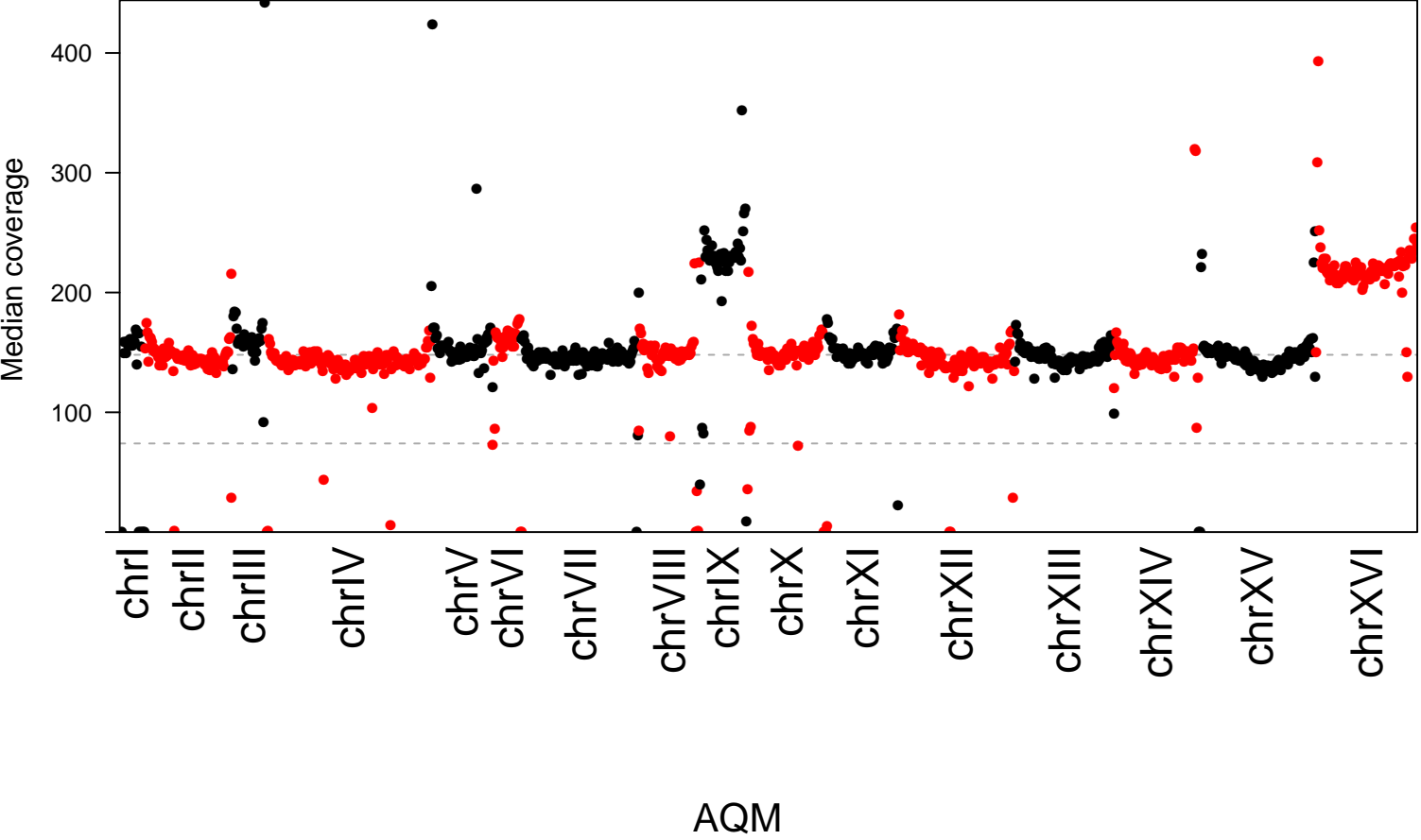
Supplementary Figure S2



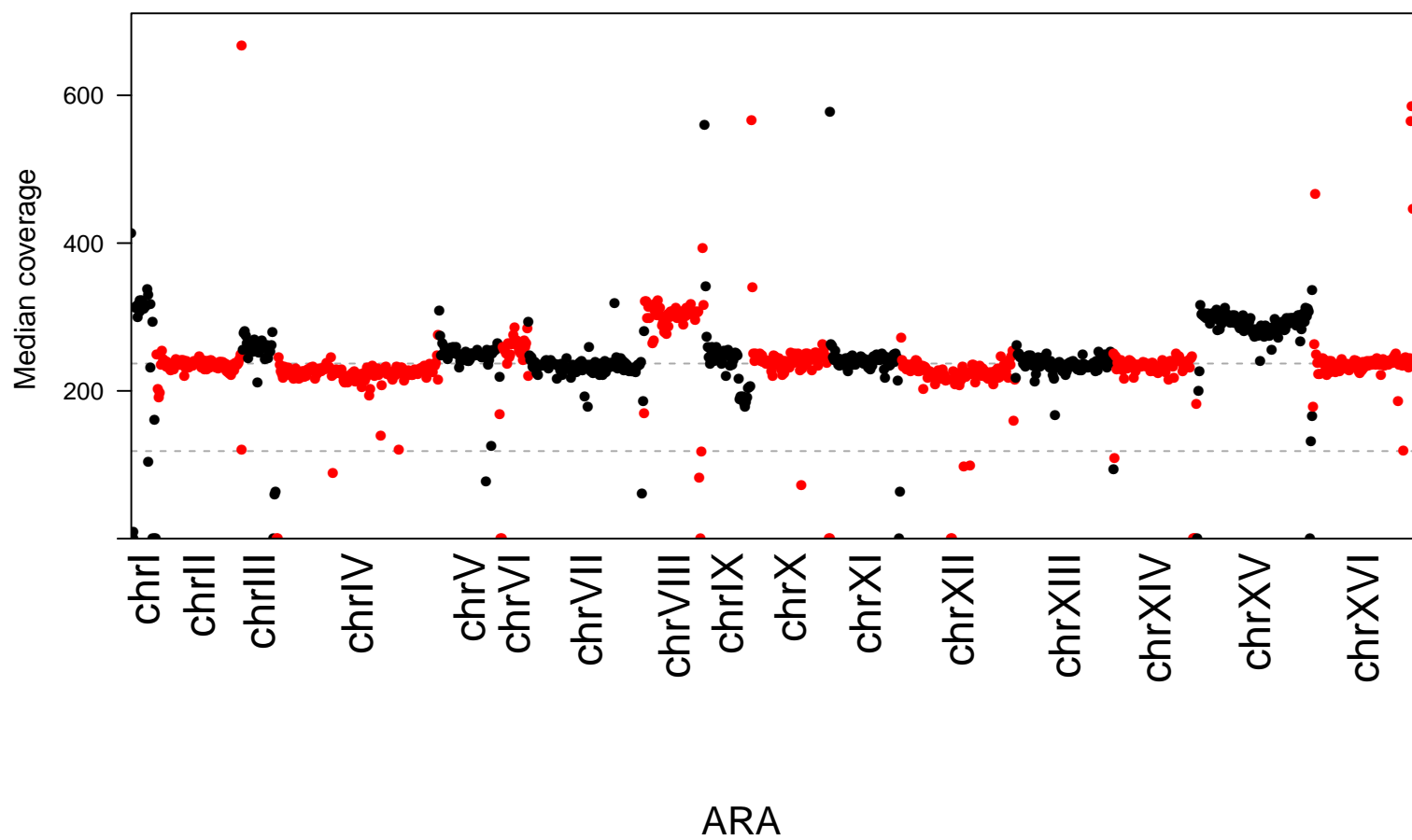
Supplementary Figure S2



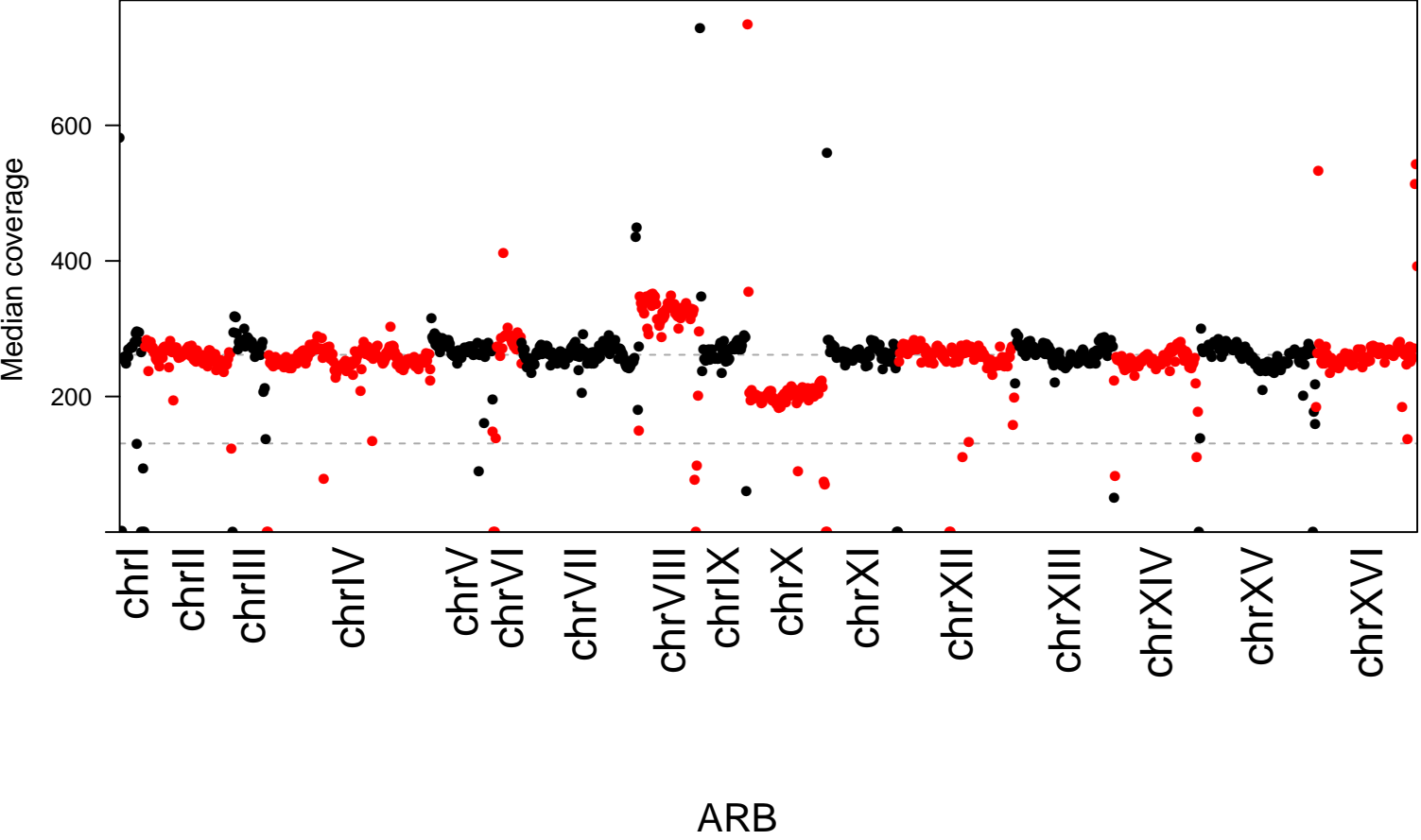
Supplementary Figure S2



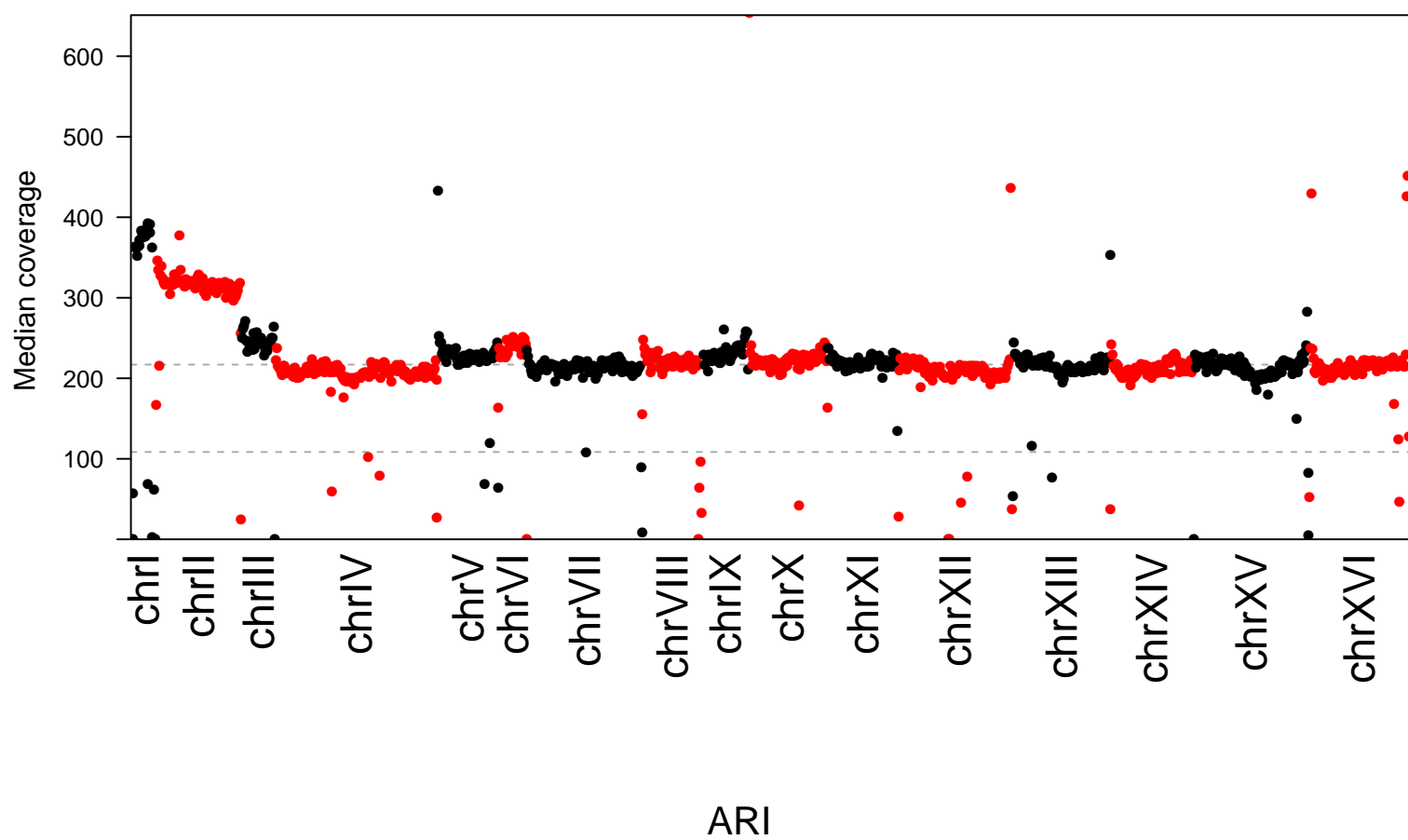
Supplementary Figure S2



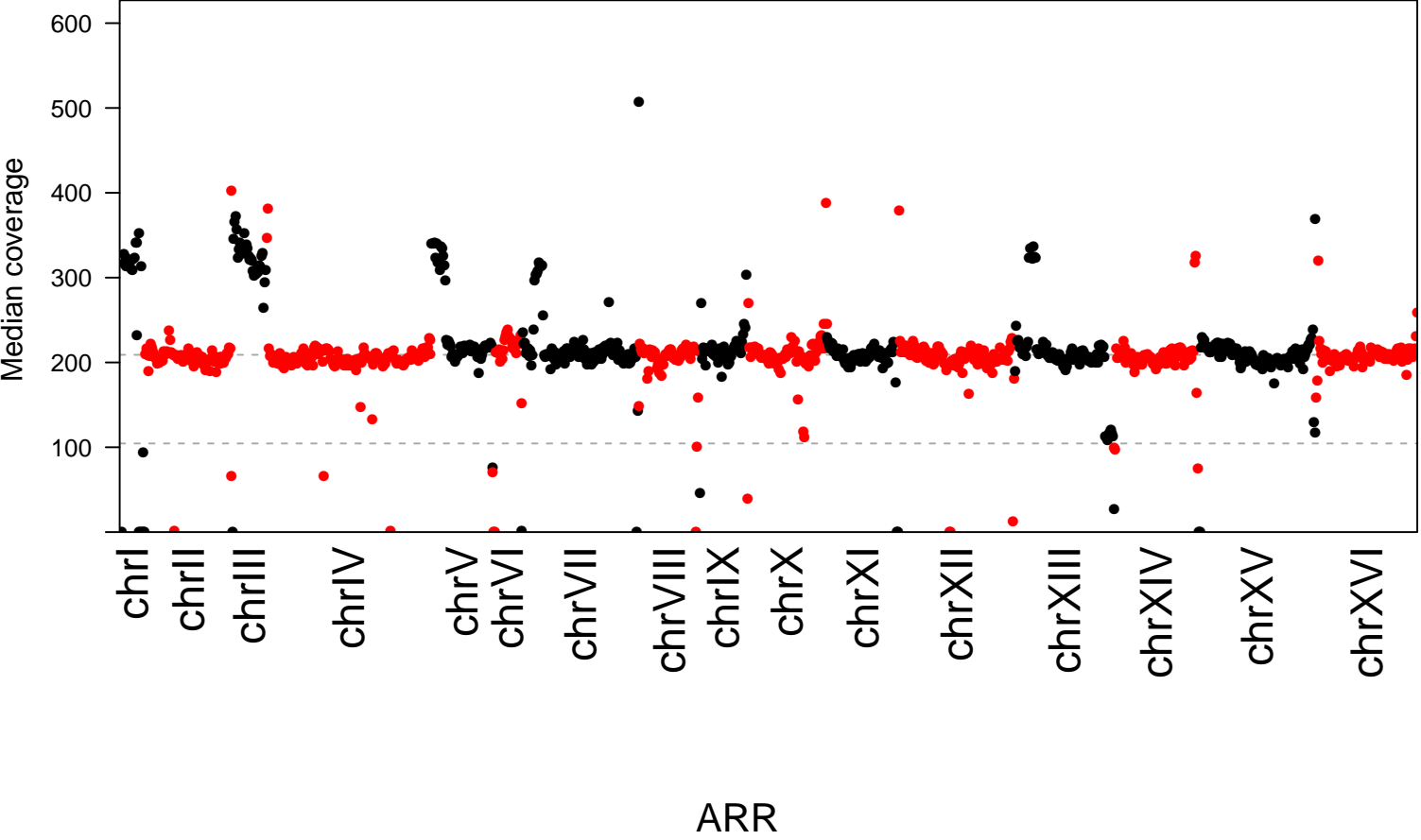
Supplementary Figure S2



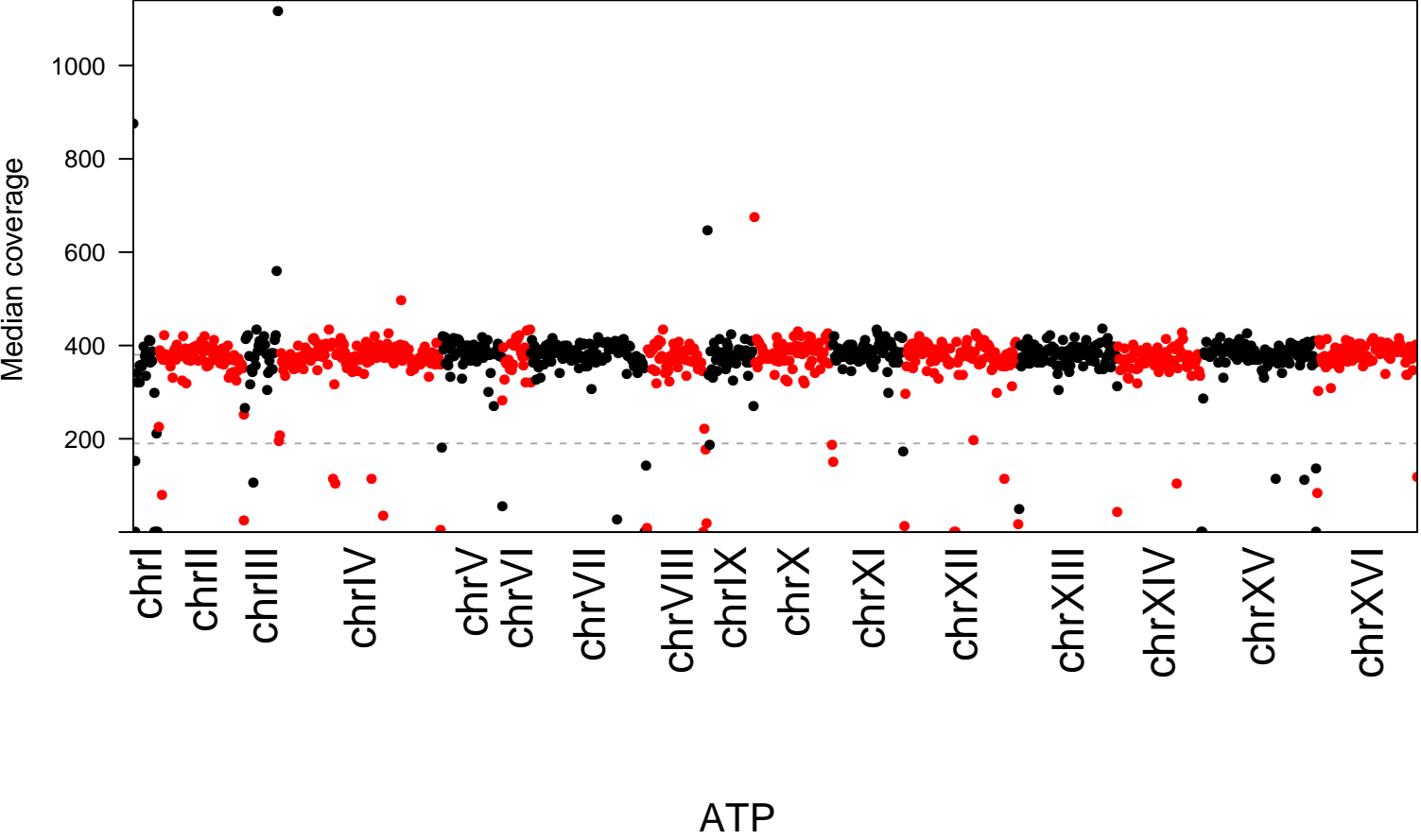
Supplementary Figure S2



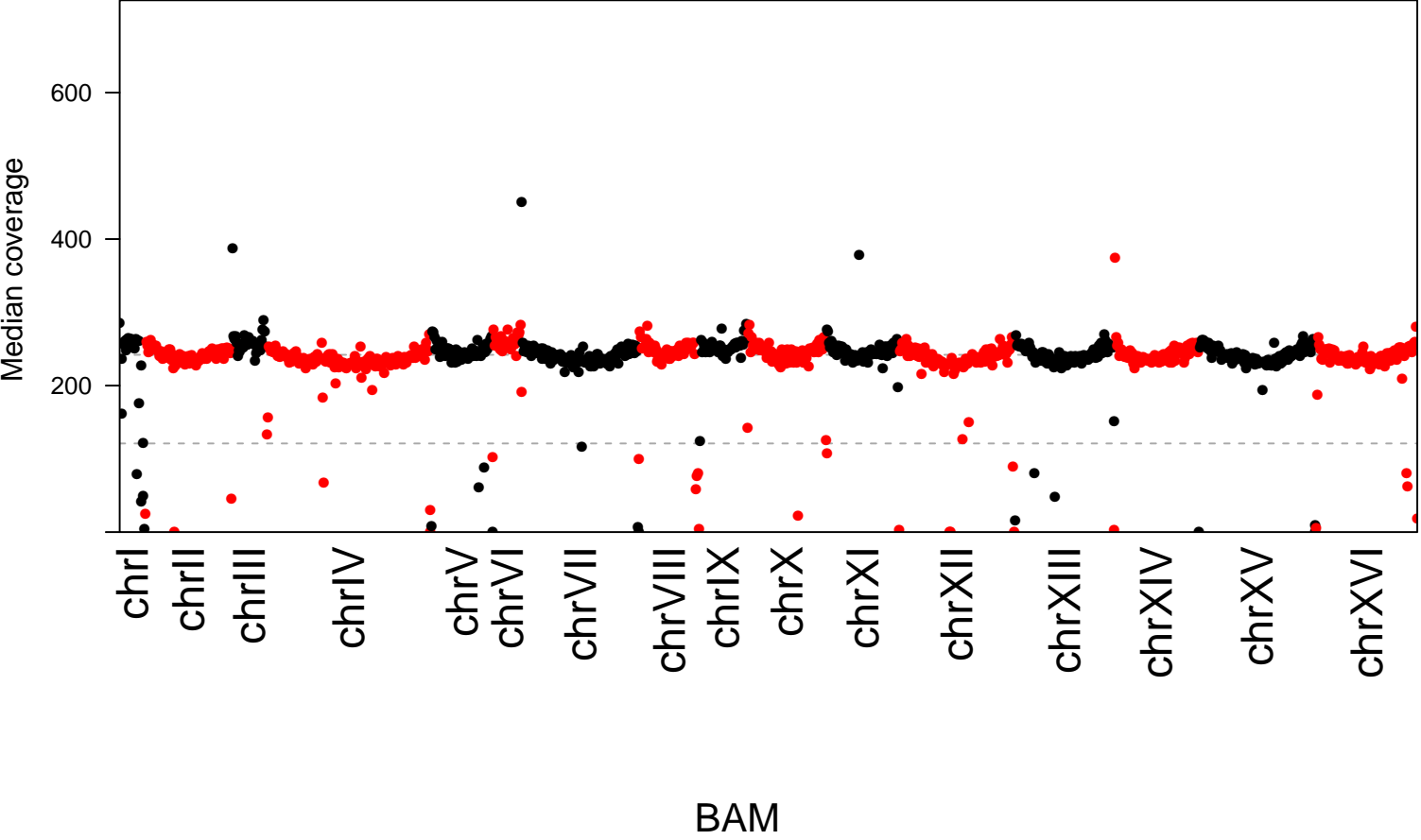
Supplementary Figure S2



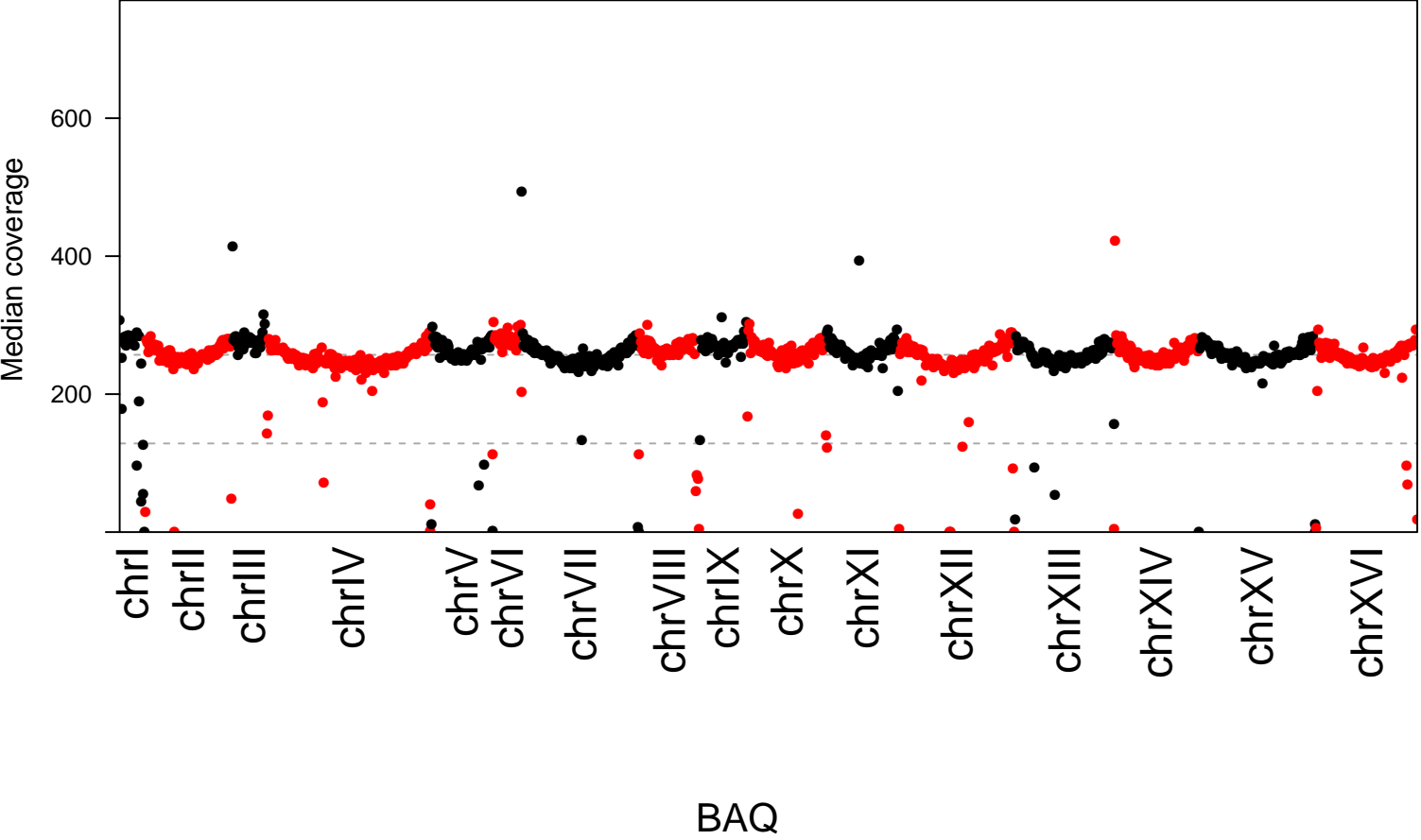
Supplementary Figure S2



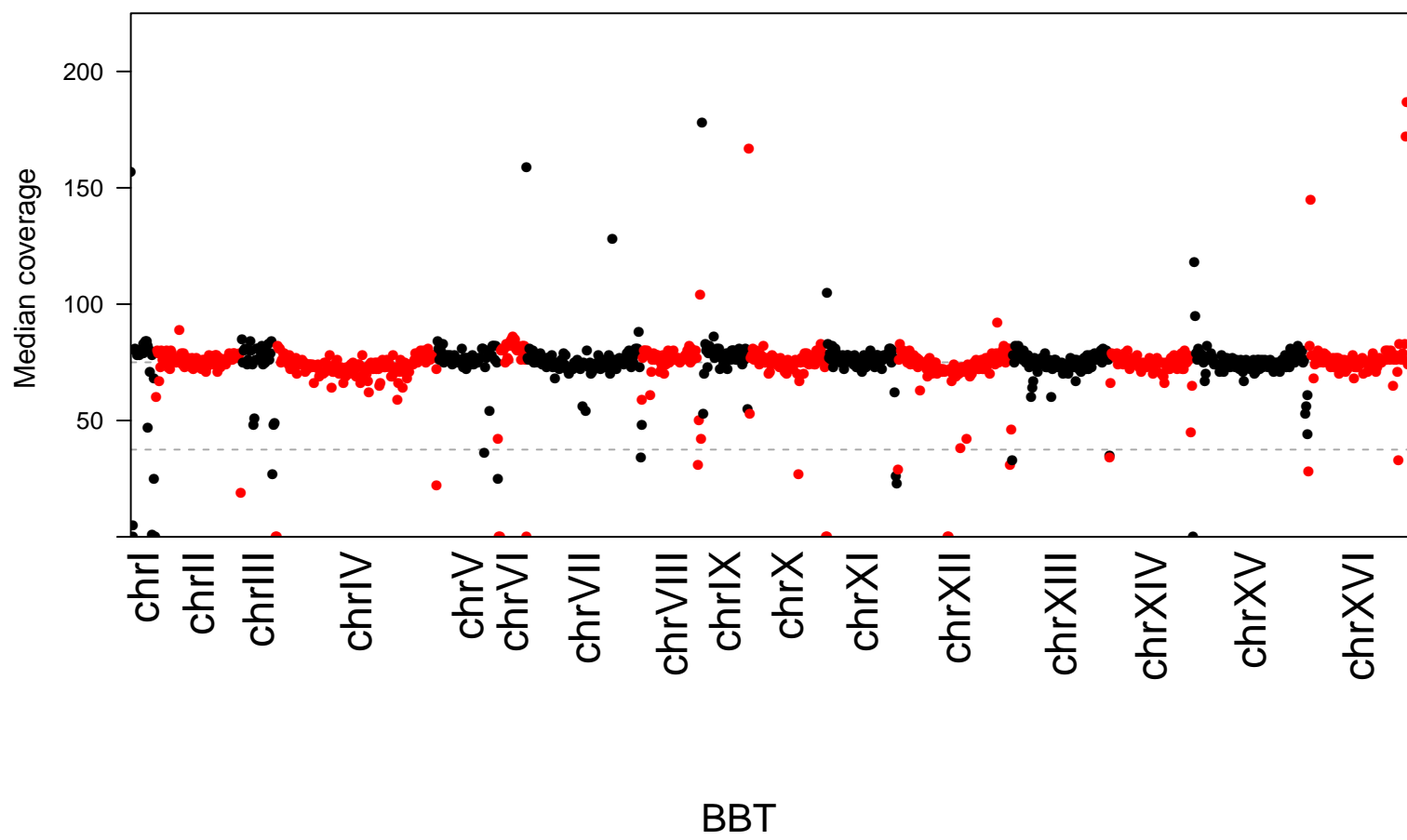
Supplementary Figure S2



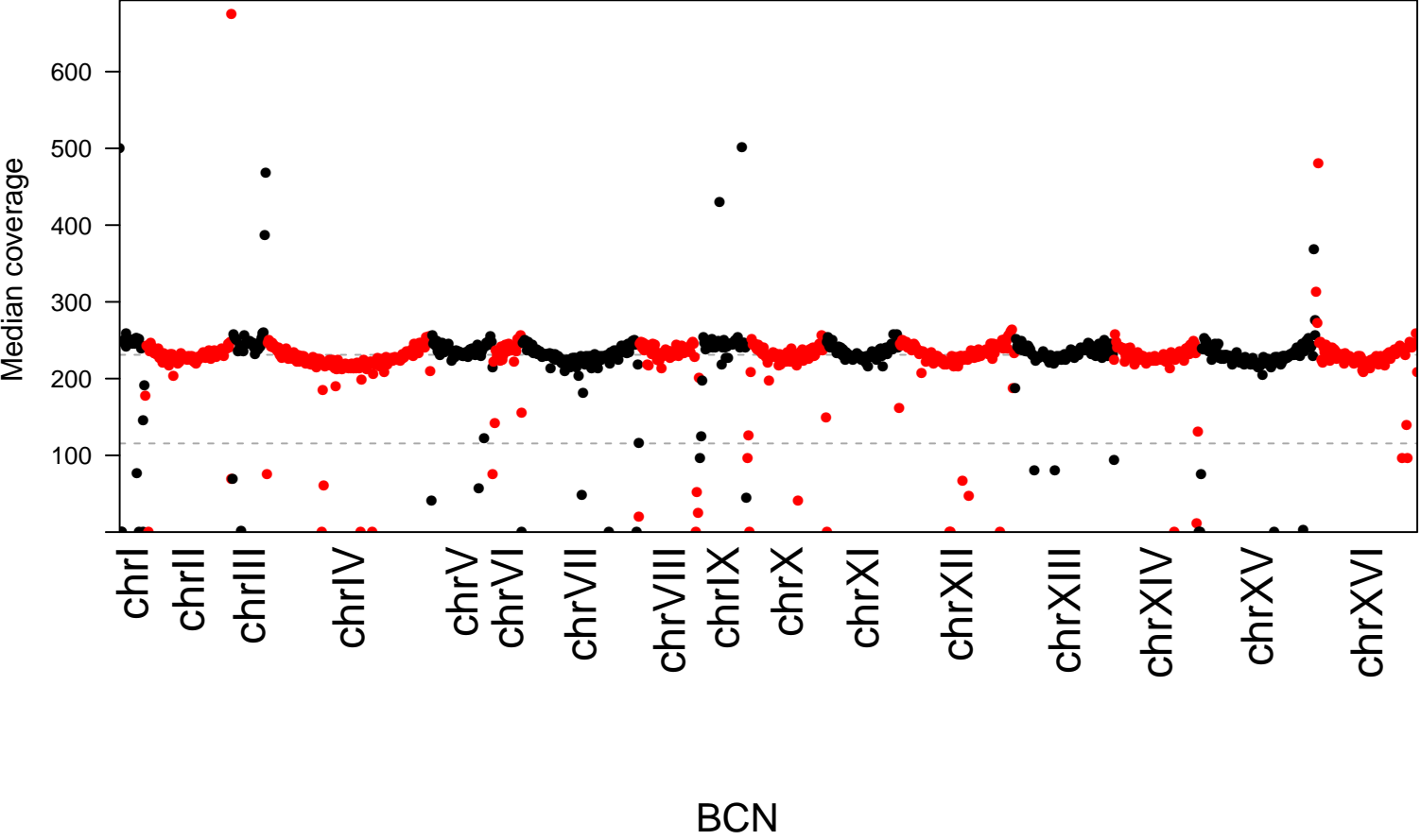
Supplementary Figure S2



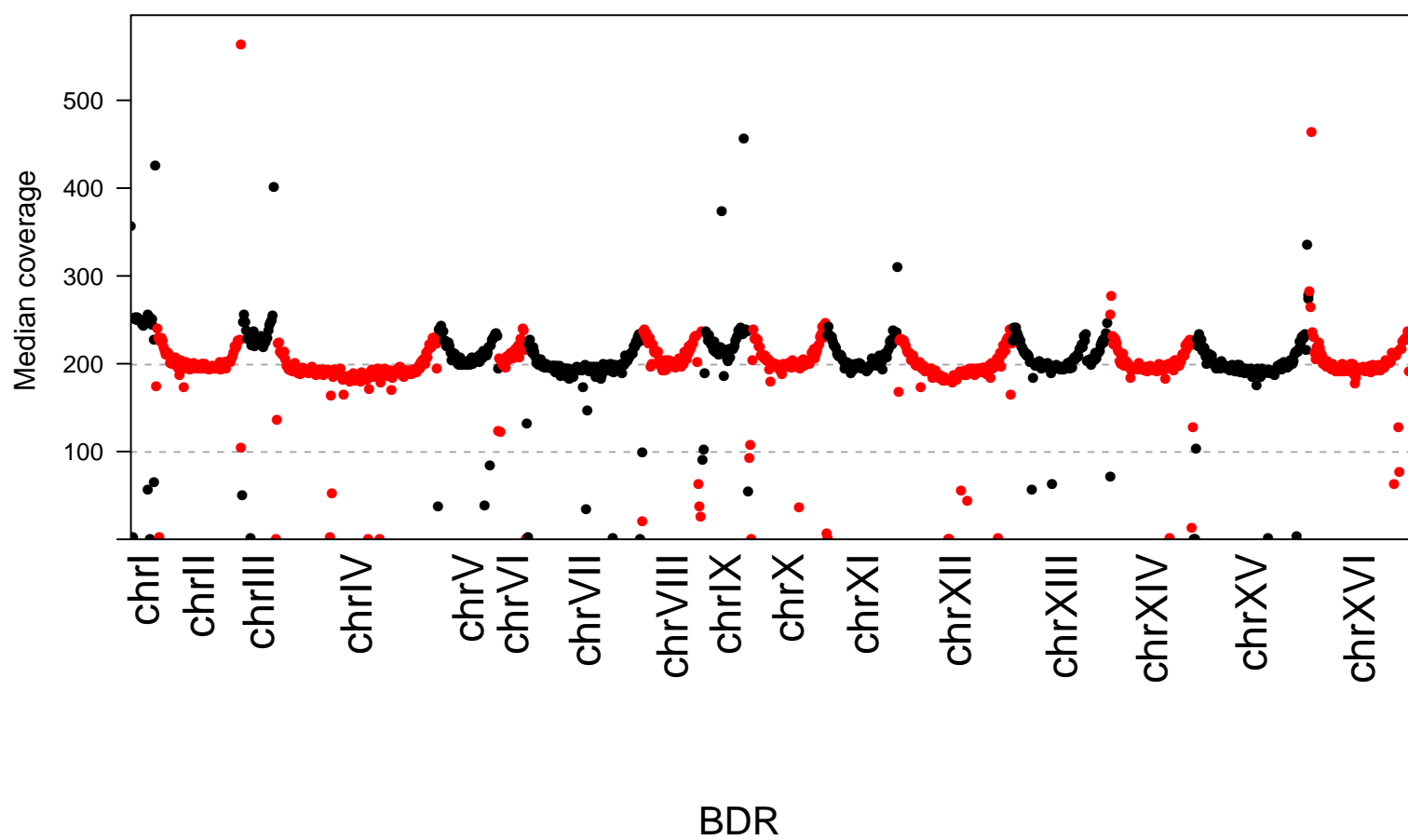
Supplementary Figure S2



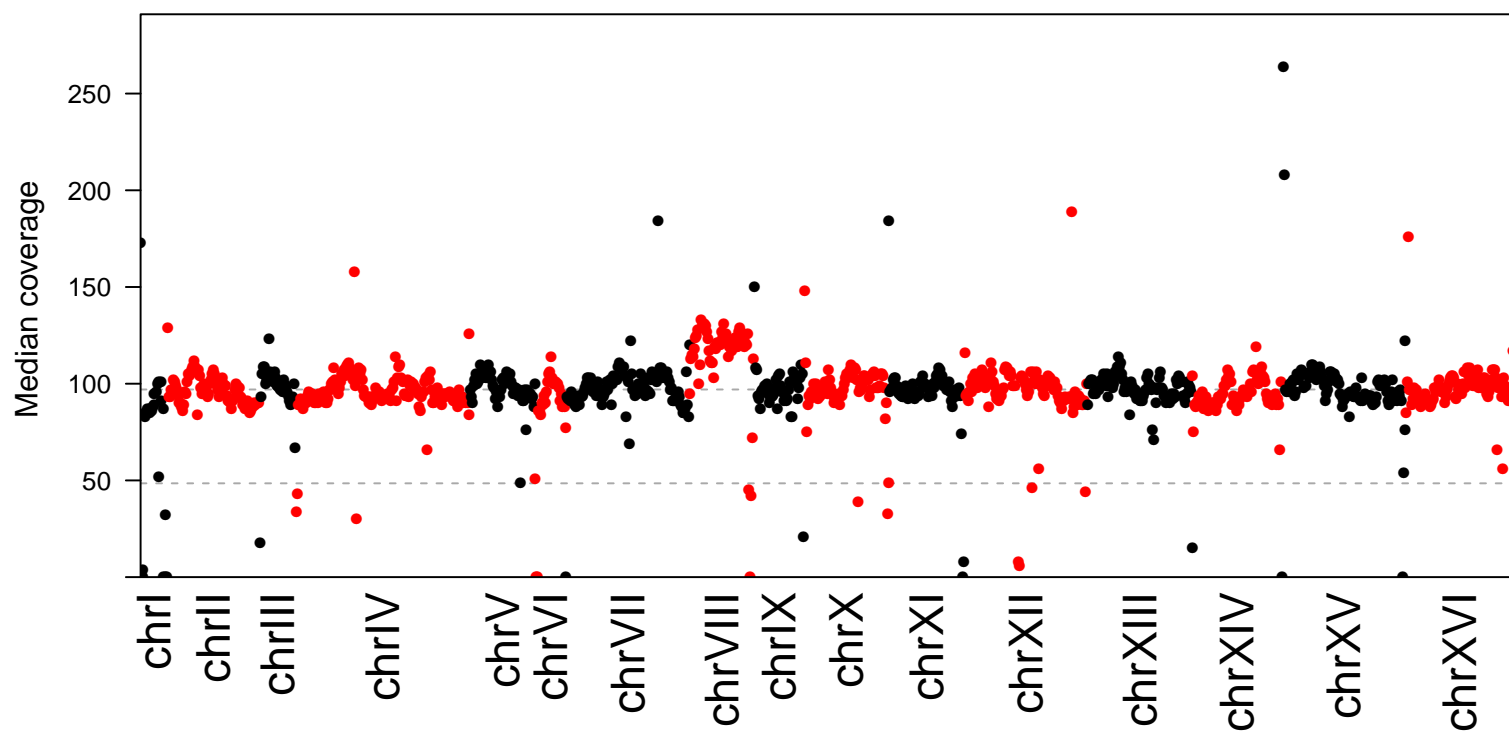
Supplementary Figure S2



Supplementary Figure S2

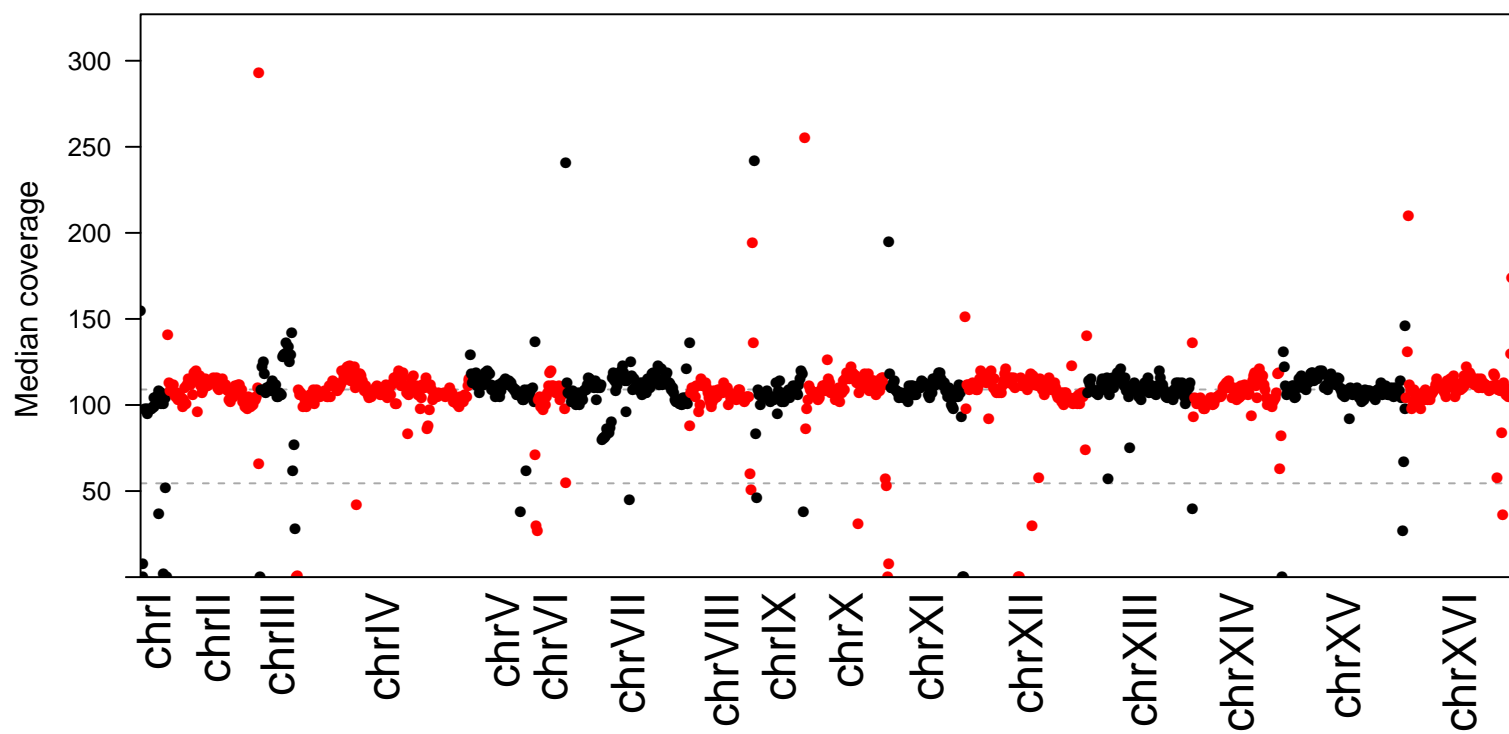


Supplementary Figure S2



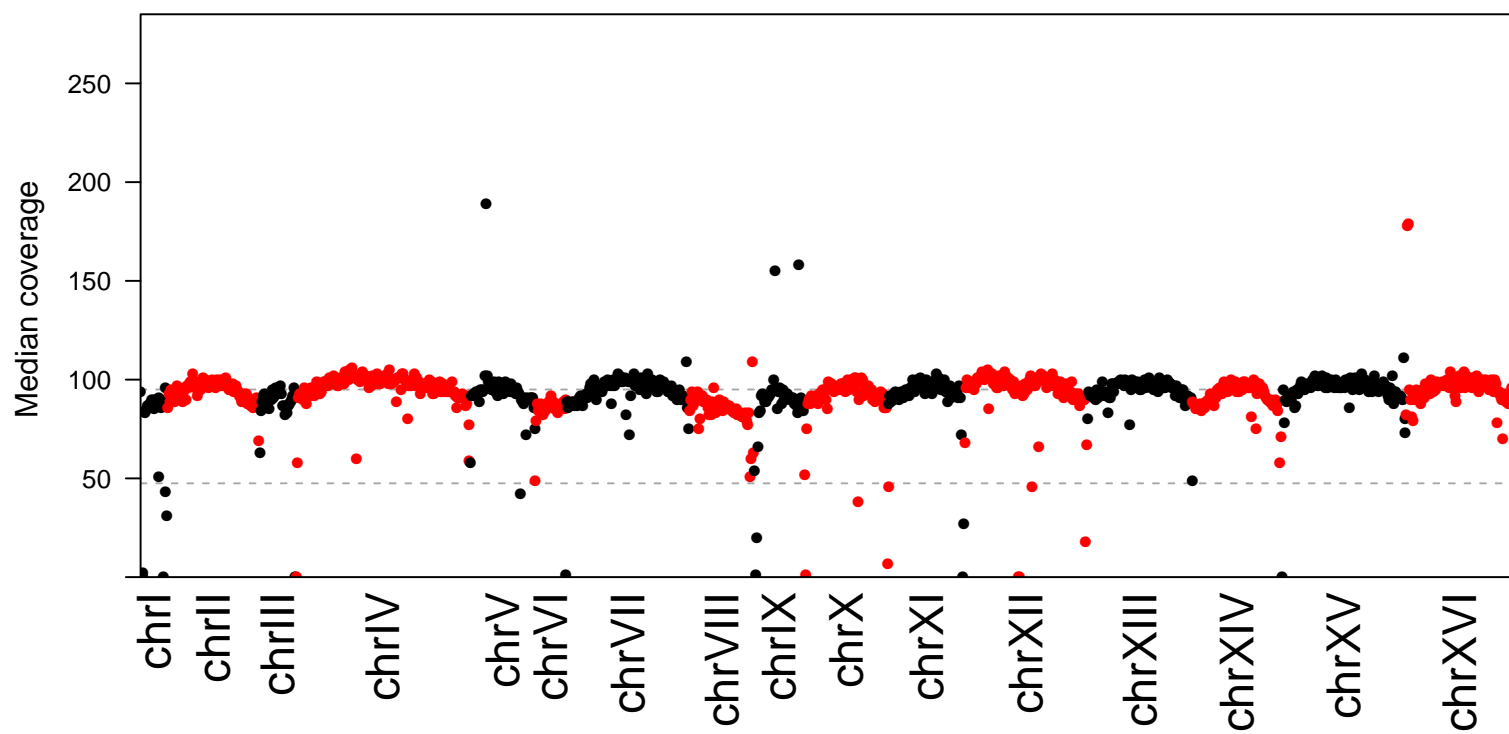
beer022

Supplementary Figure S2



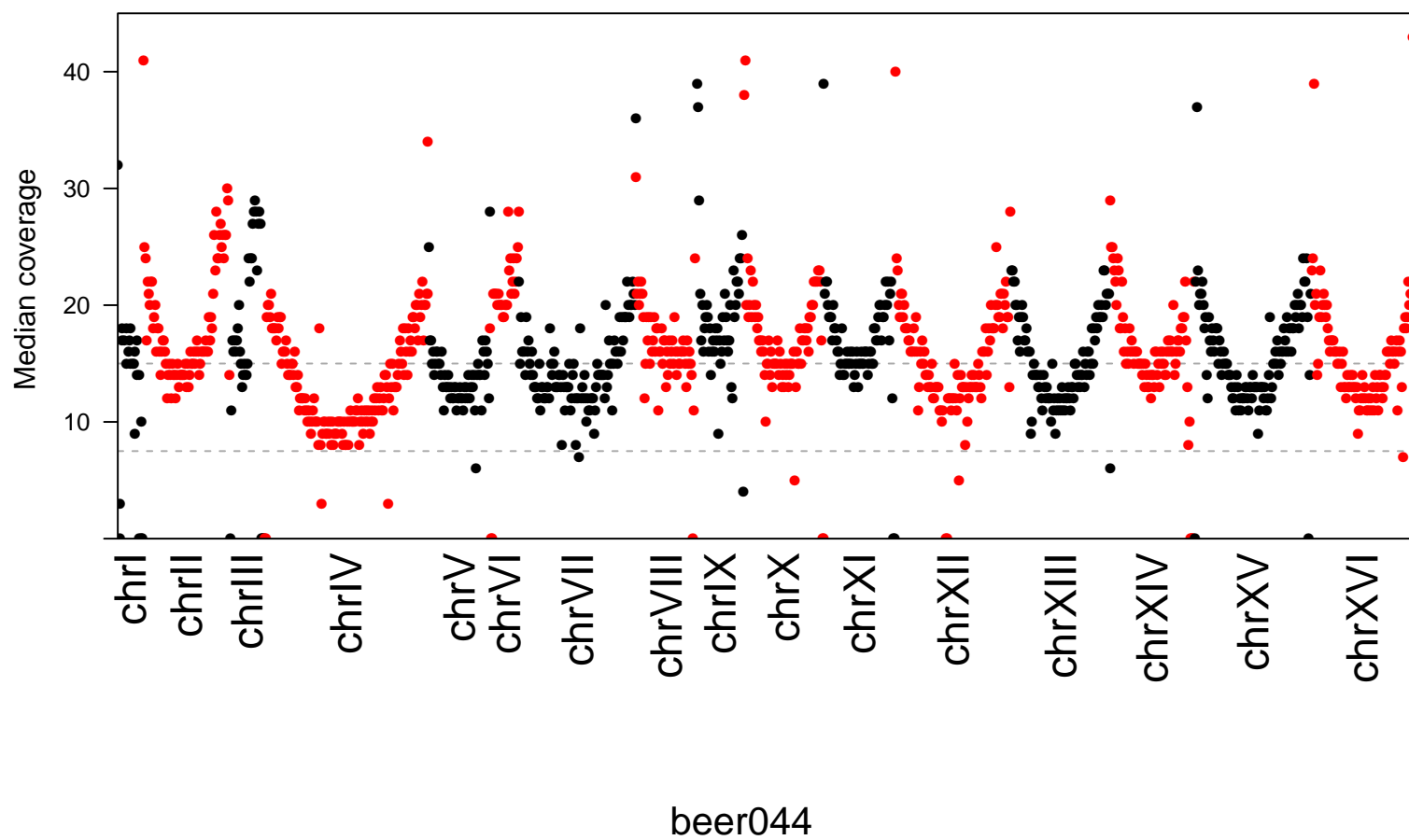
beer028

Supplementary Figure S2

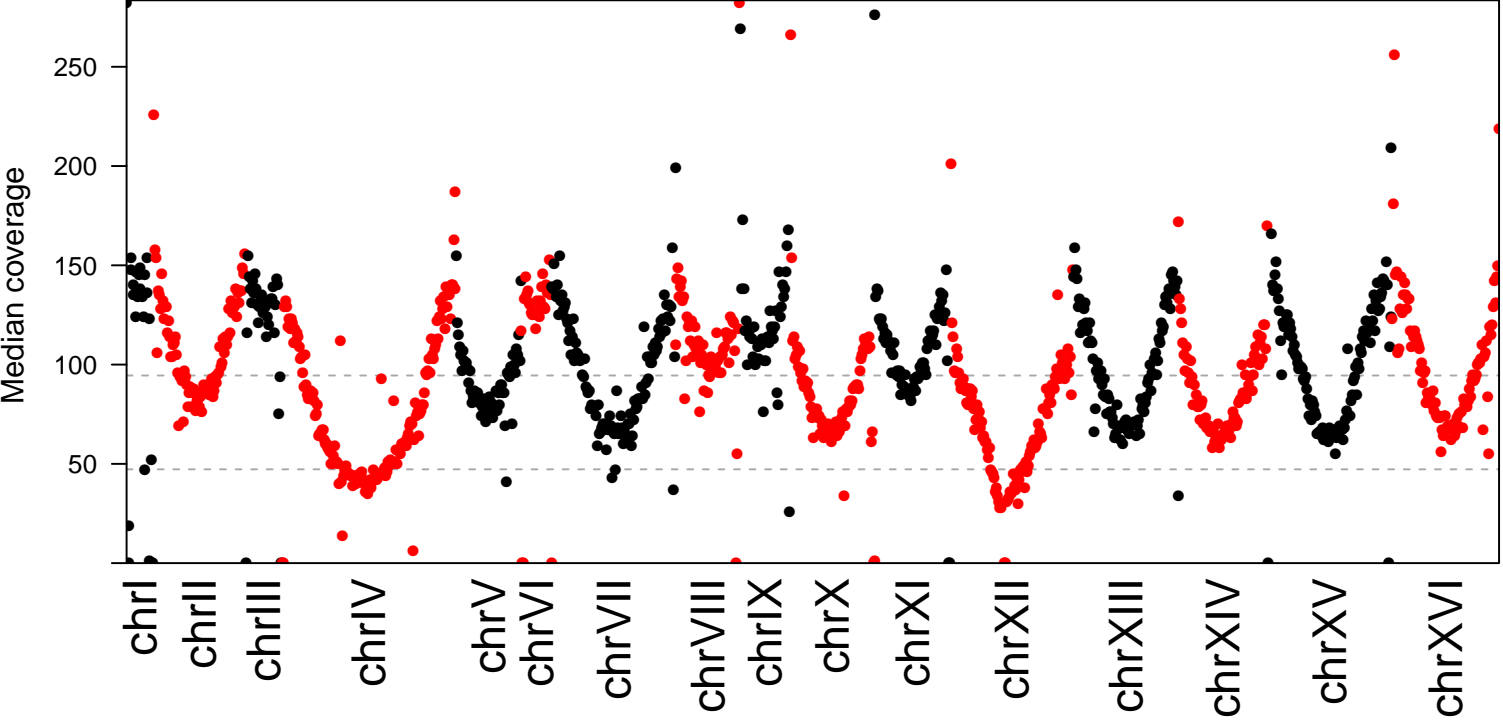


beer034

Supplementary Figure S2

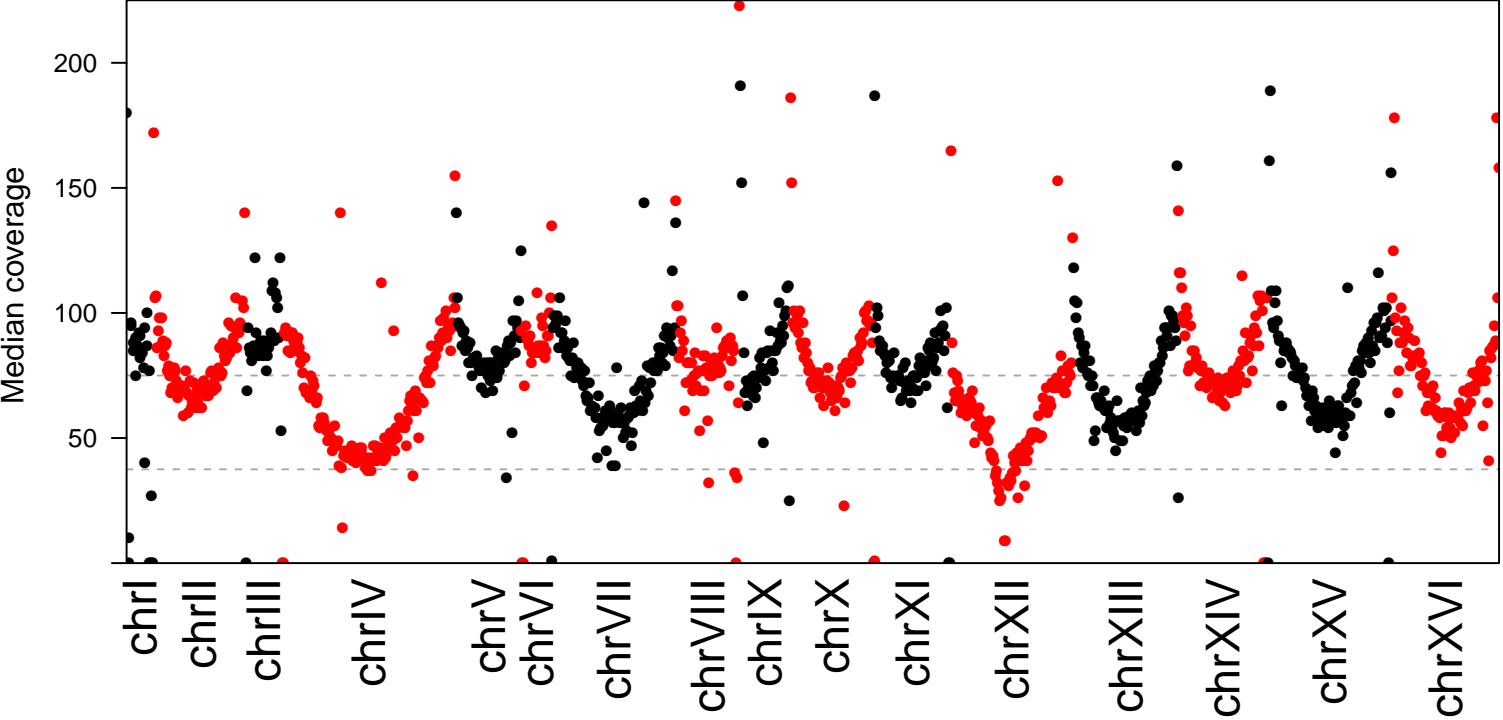


Supplementary Figure S2



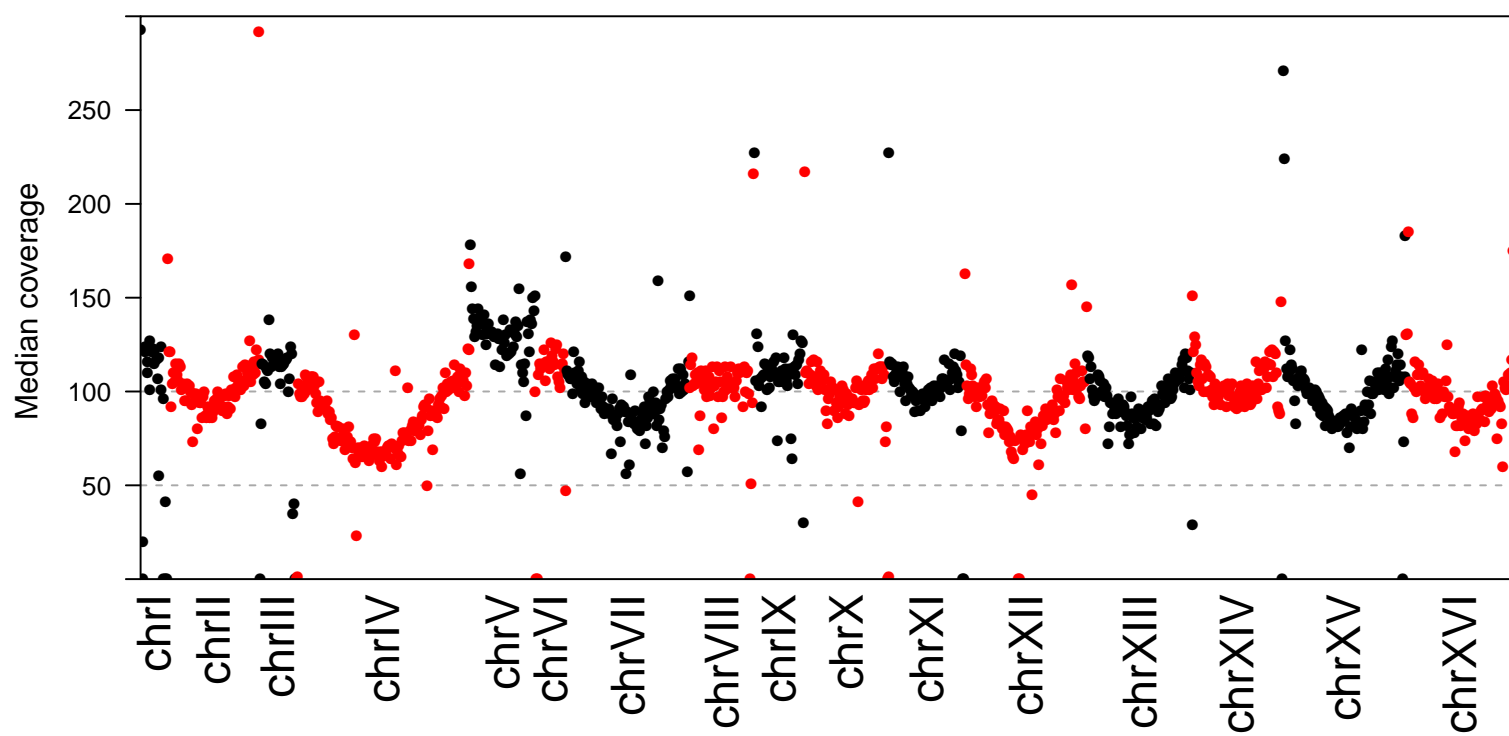
beer045

Supplementary Figure S2



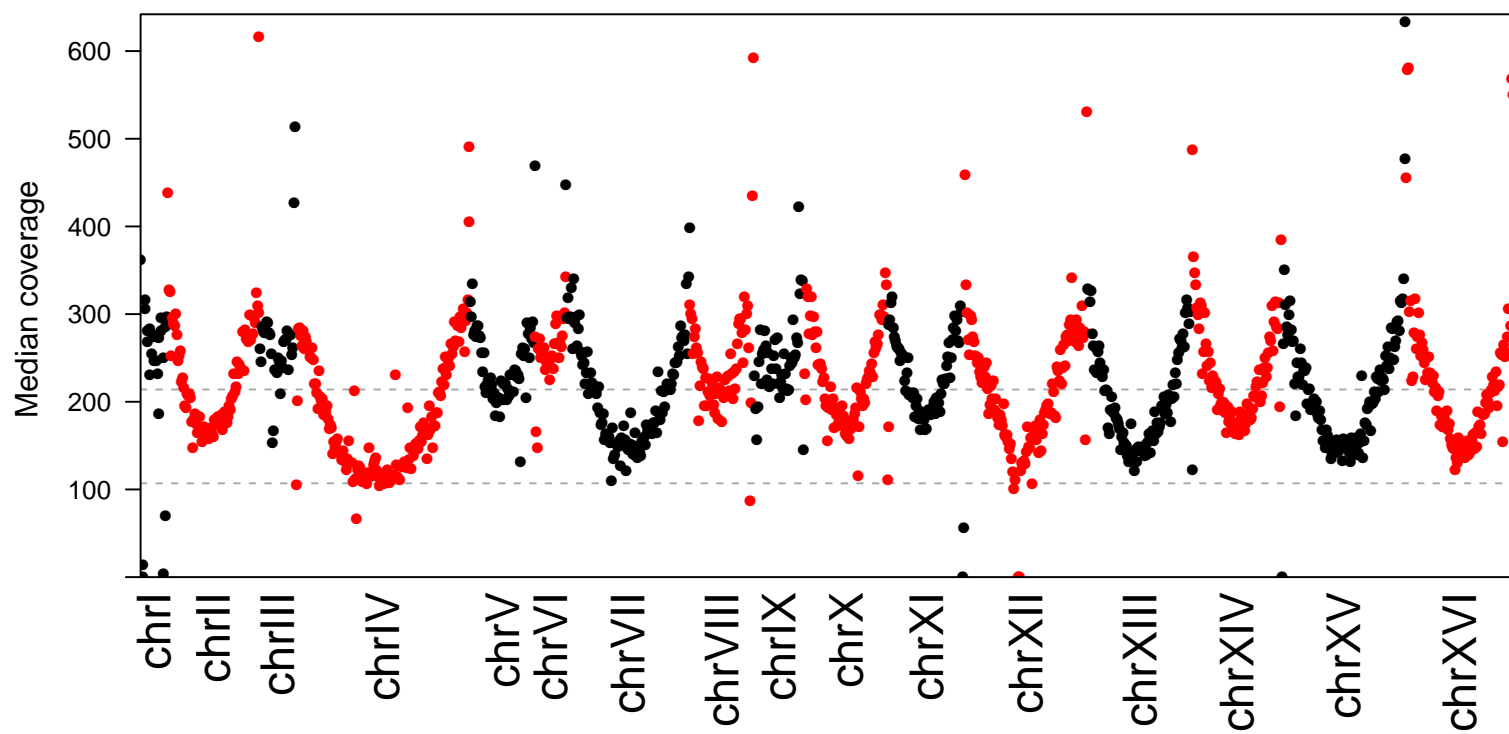
beer046

Supplementary Figure S2



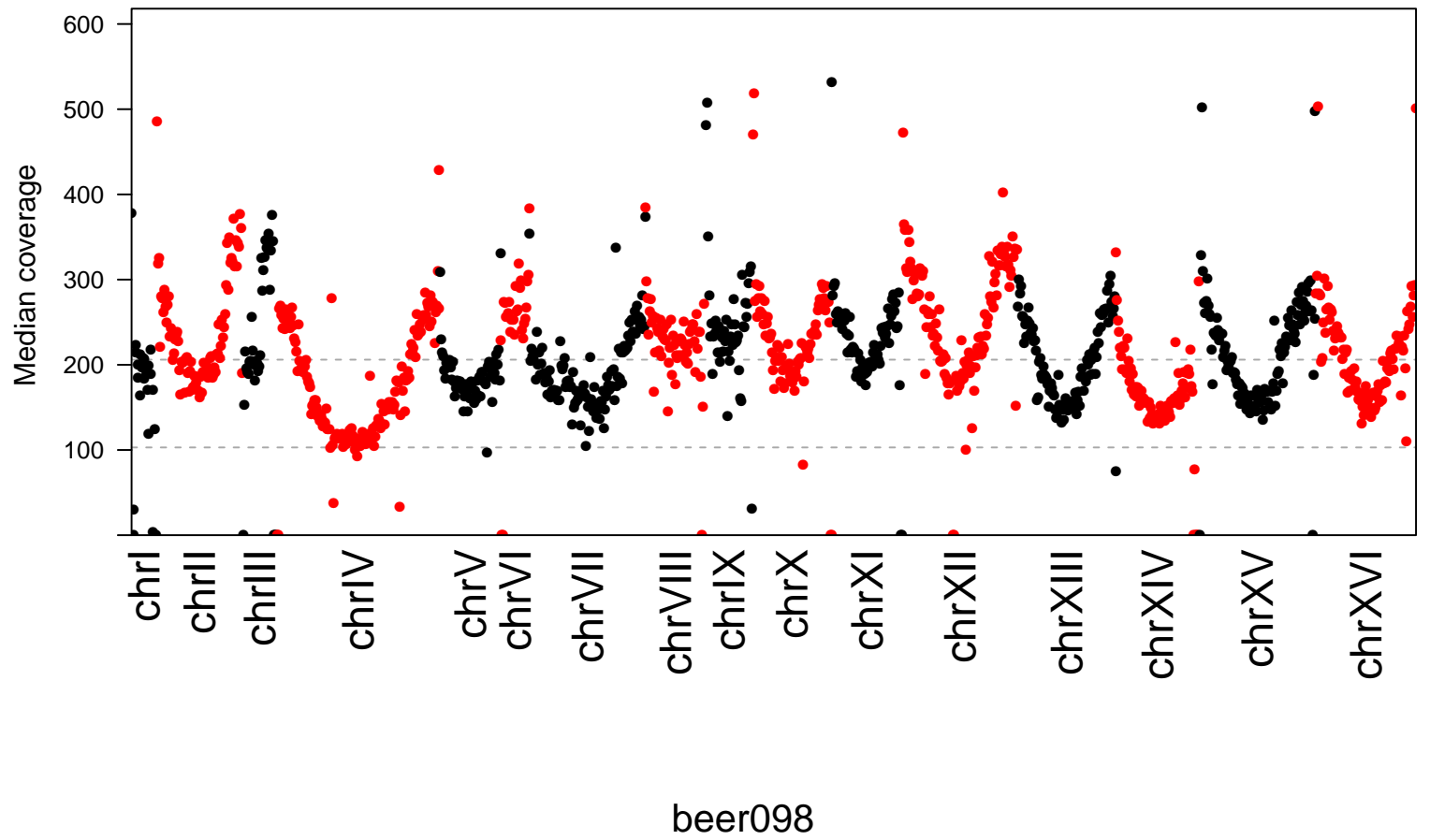
beer079

Supplementary Figure S2

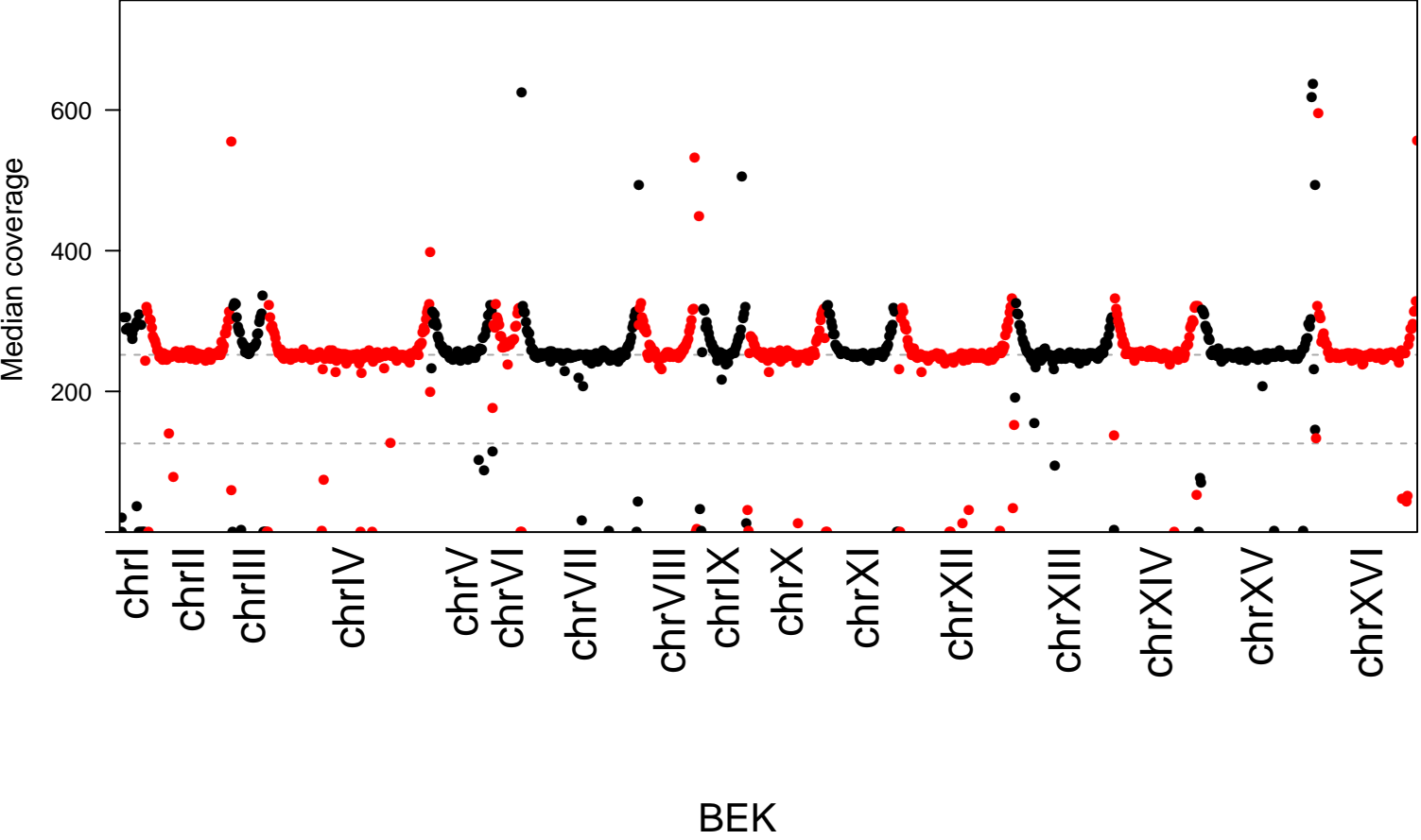


beer085

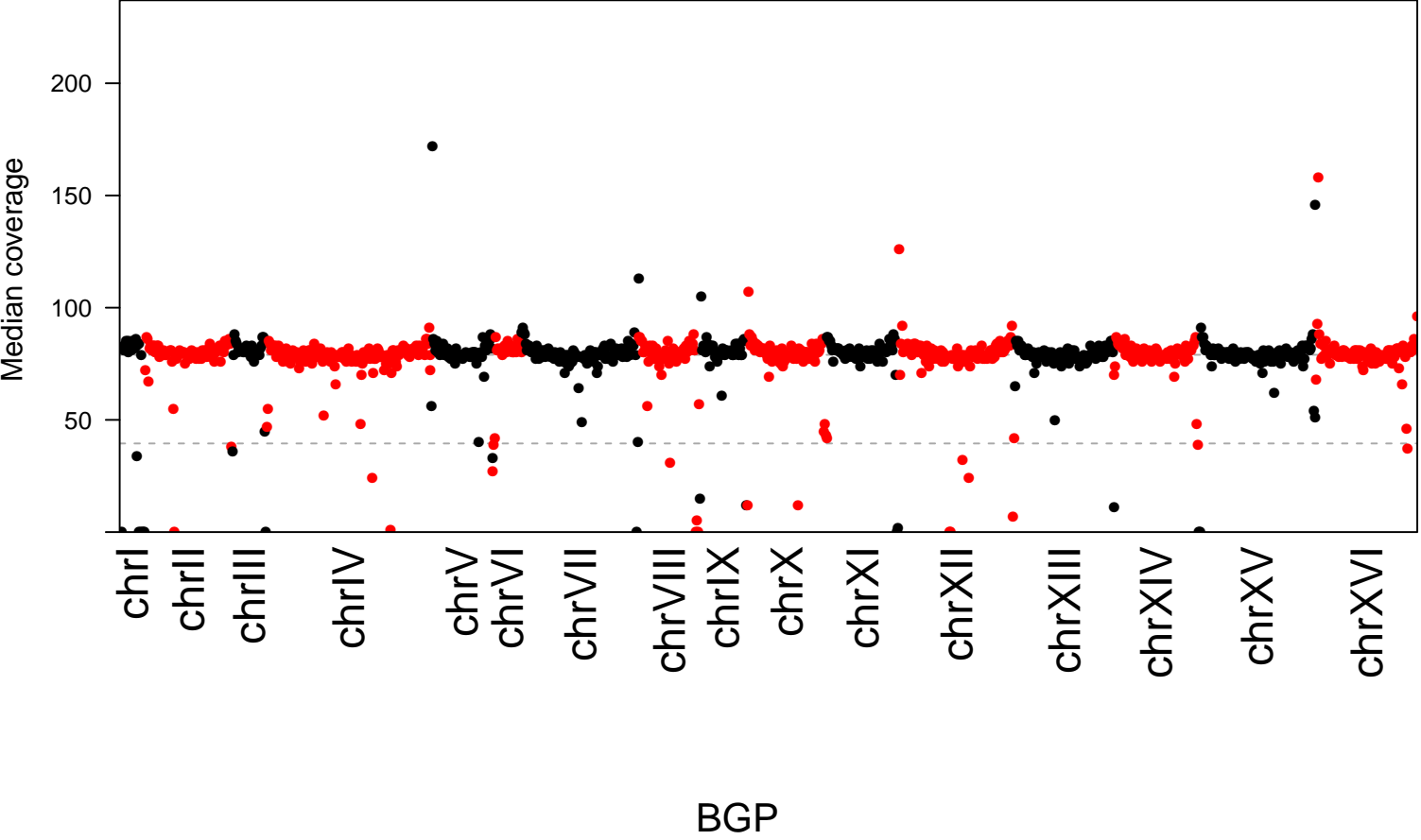
Supplementary Figure S2



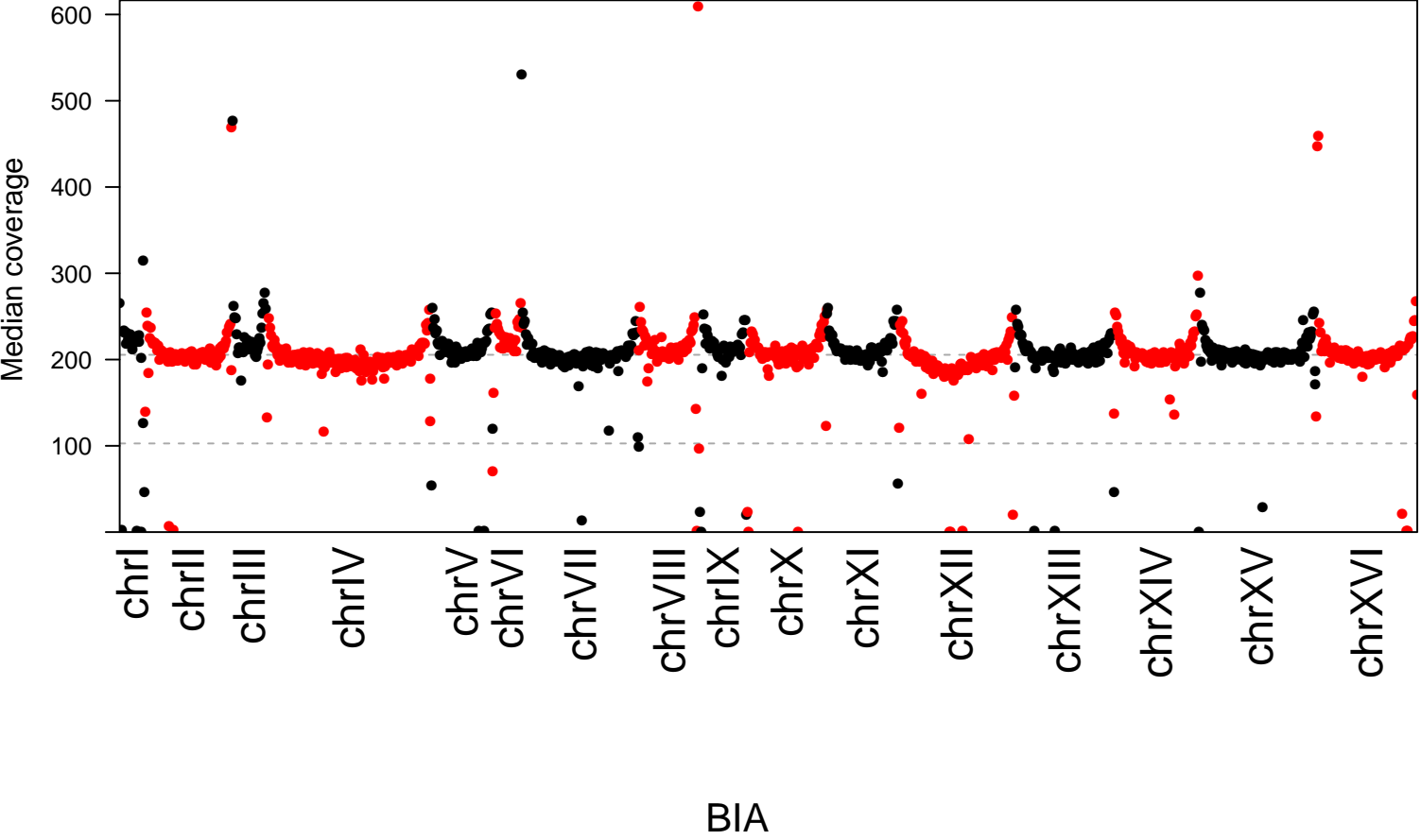
Supplementary Figure S2



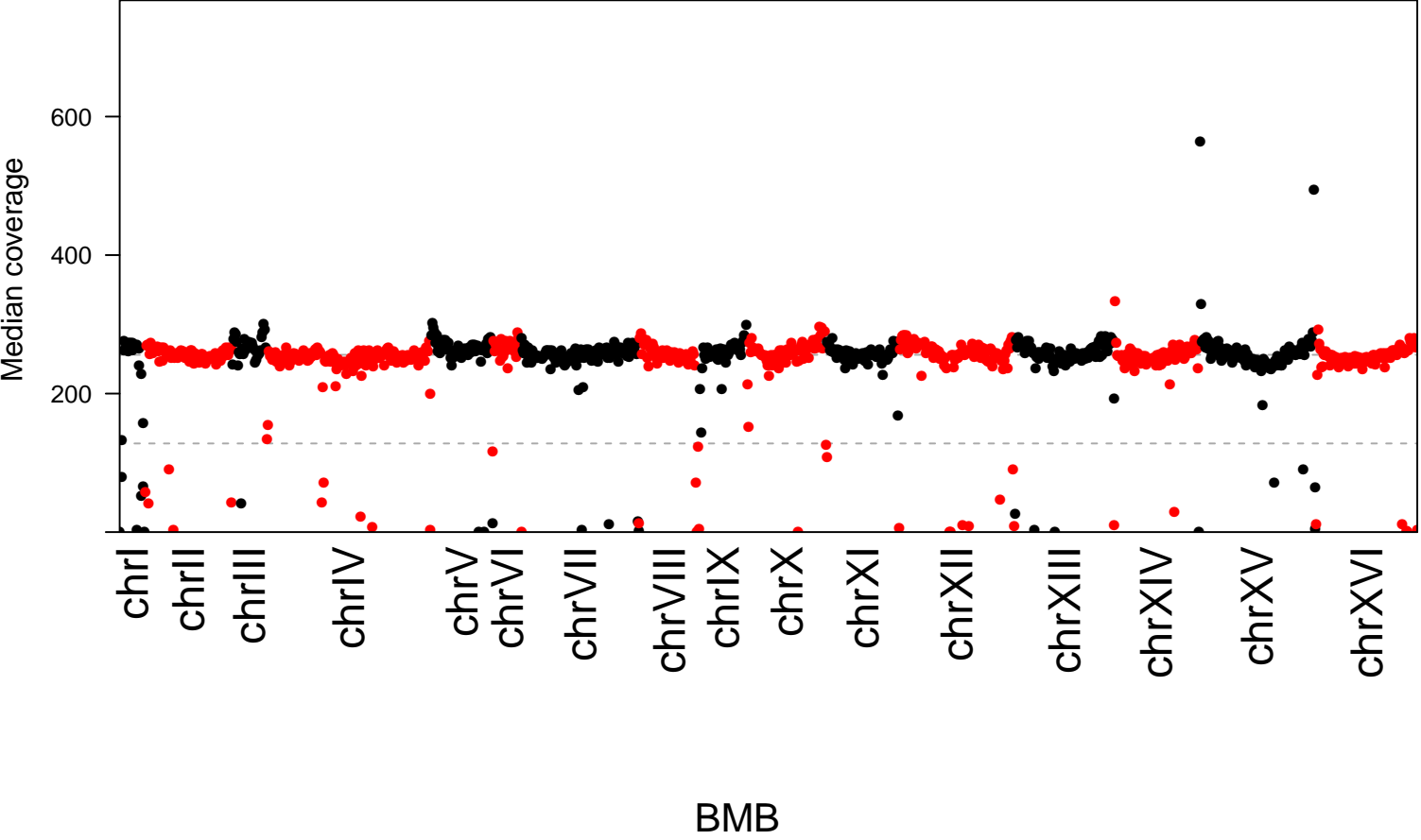
Supplementary Figure S2



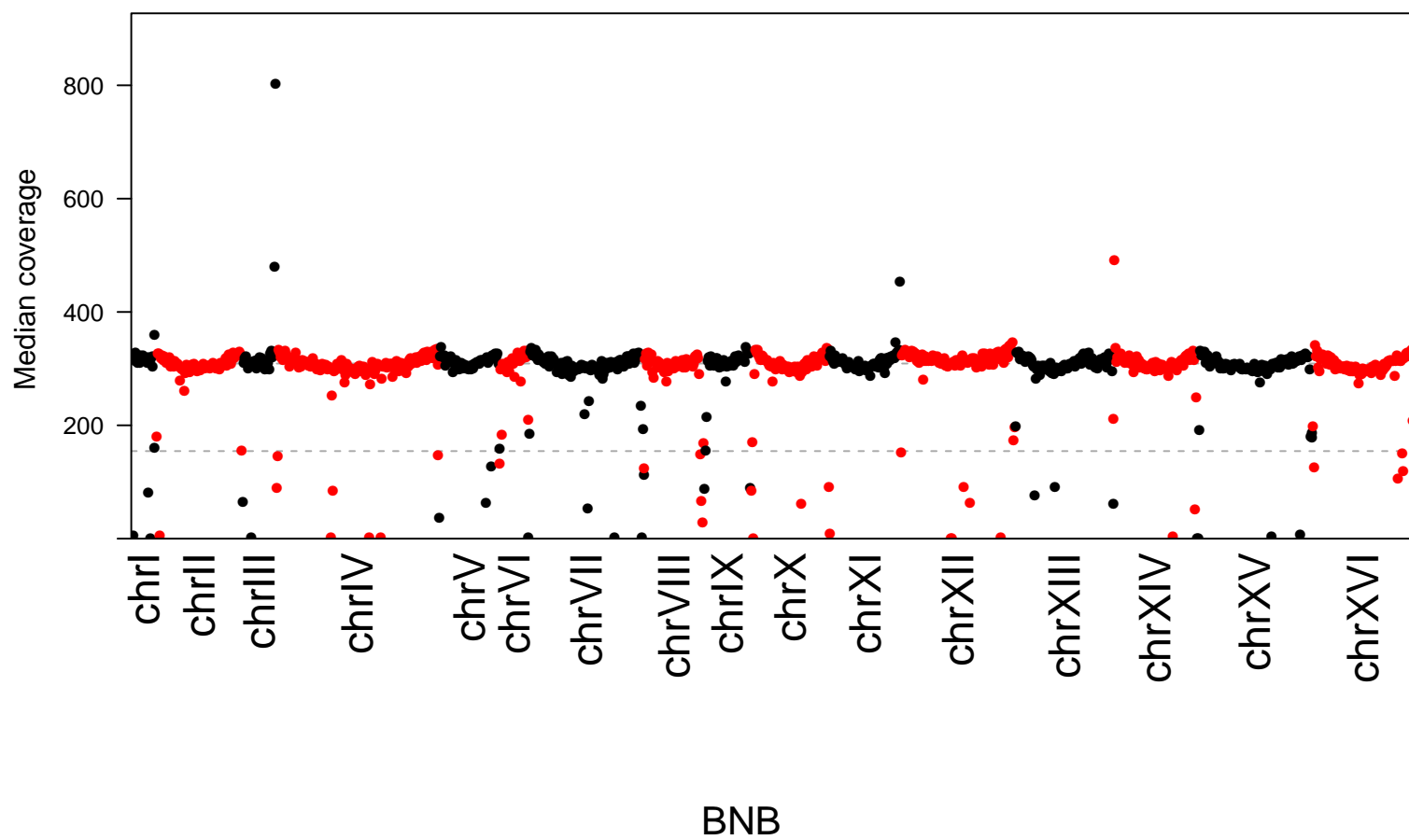
Supplementary Figure S2



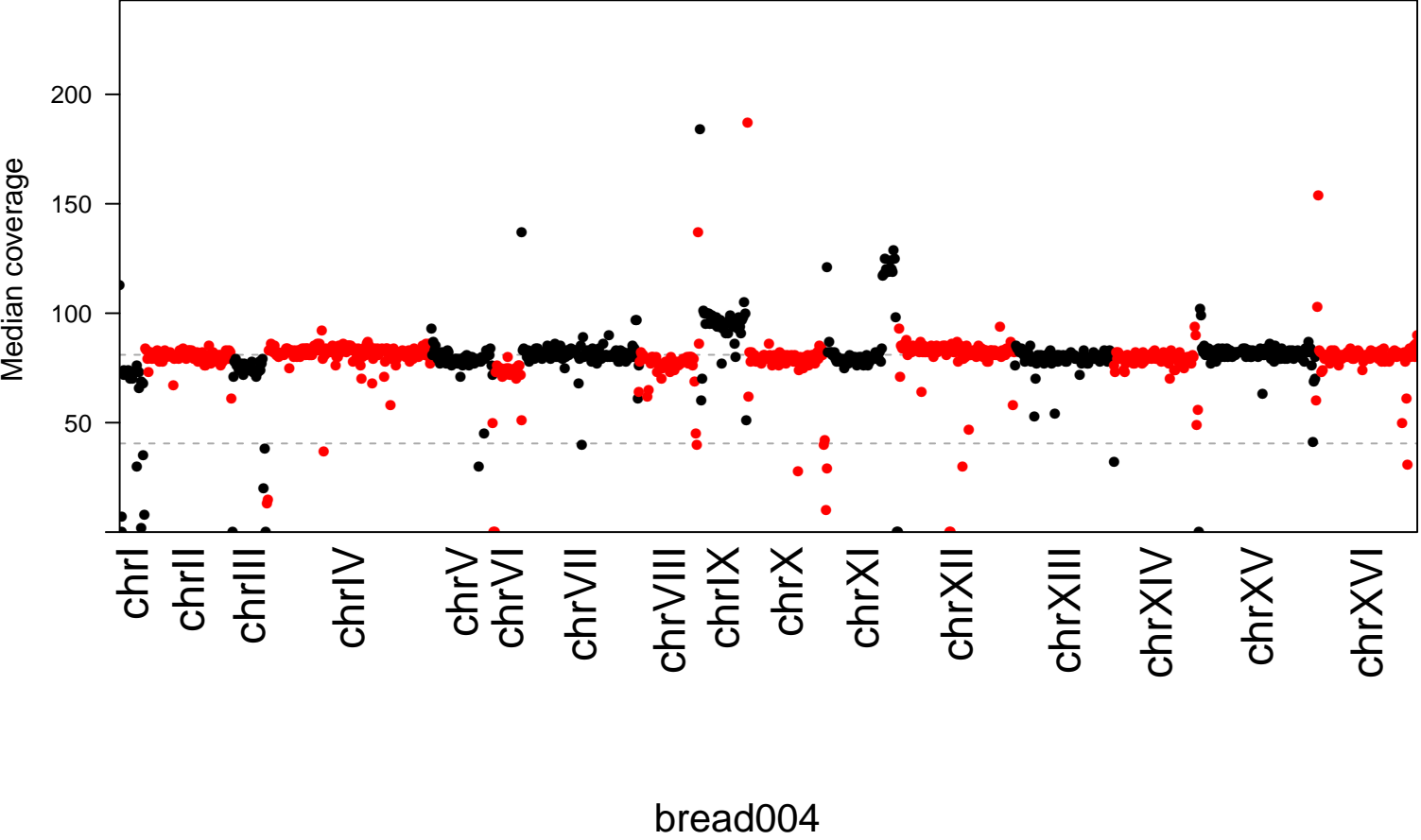
Supplementary Figure S2



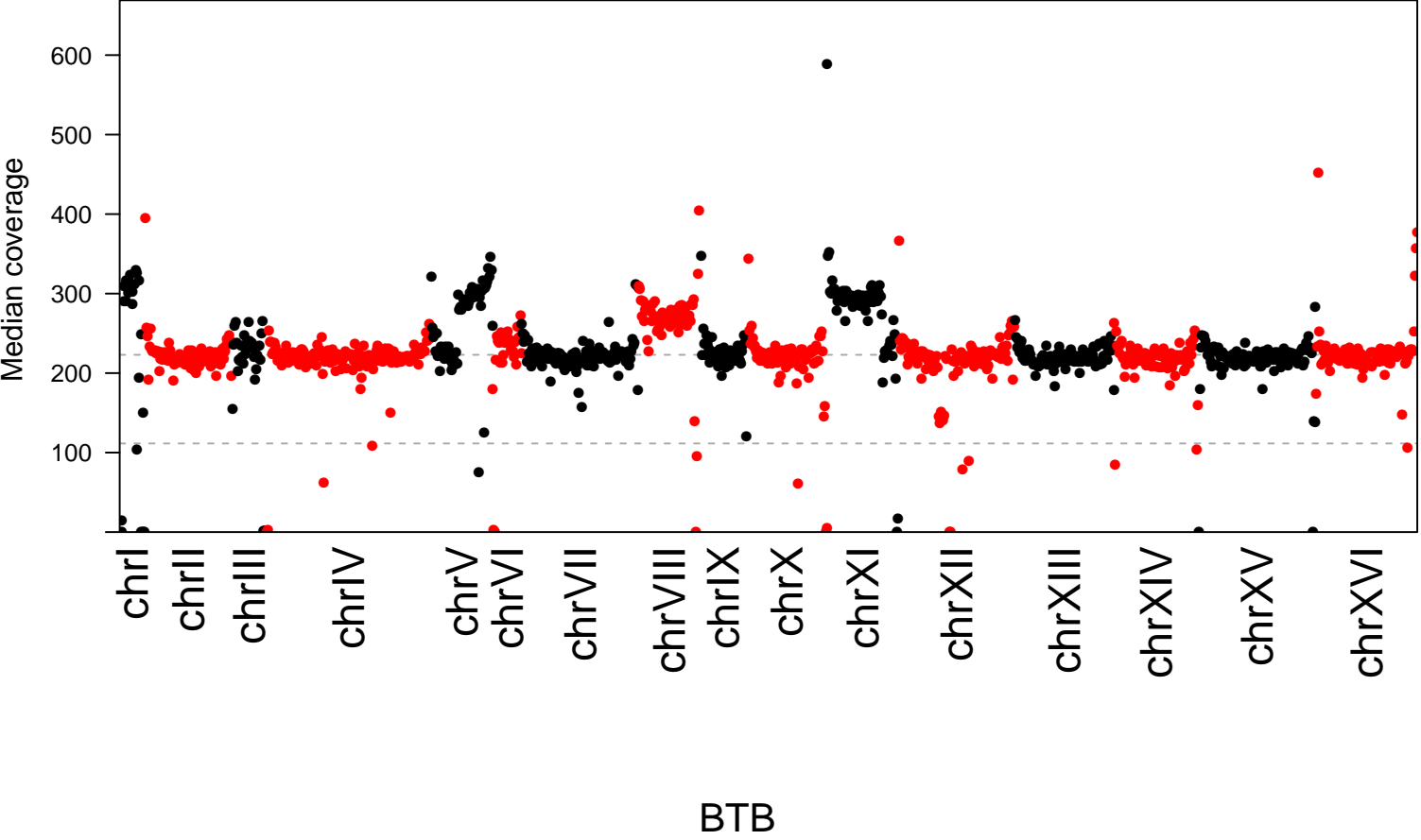
Supplementary Figure S2



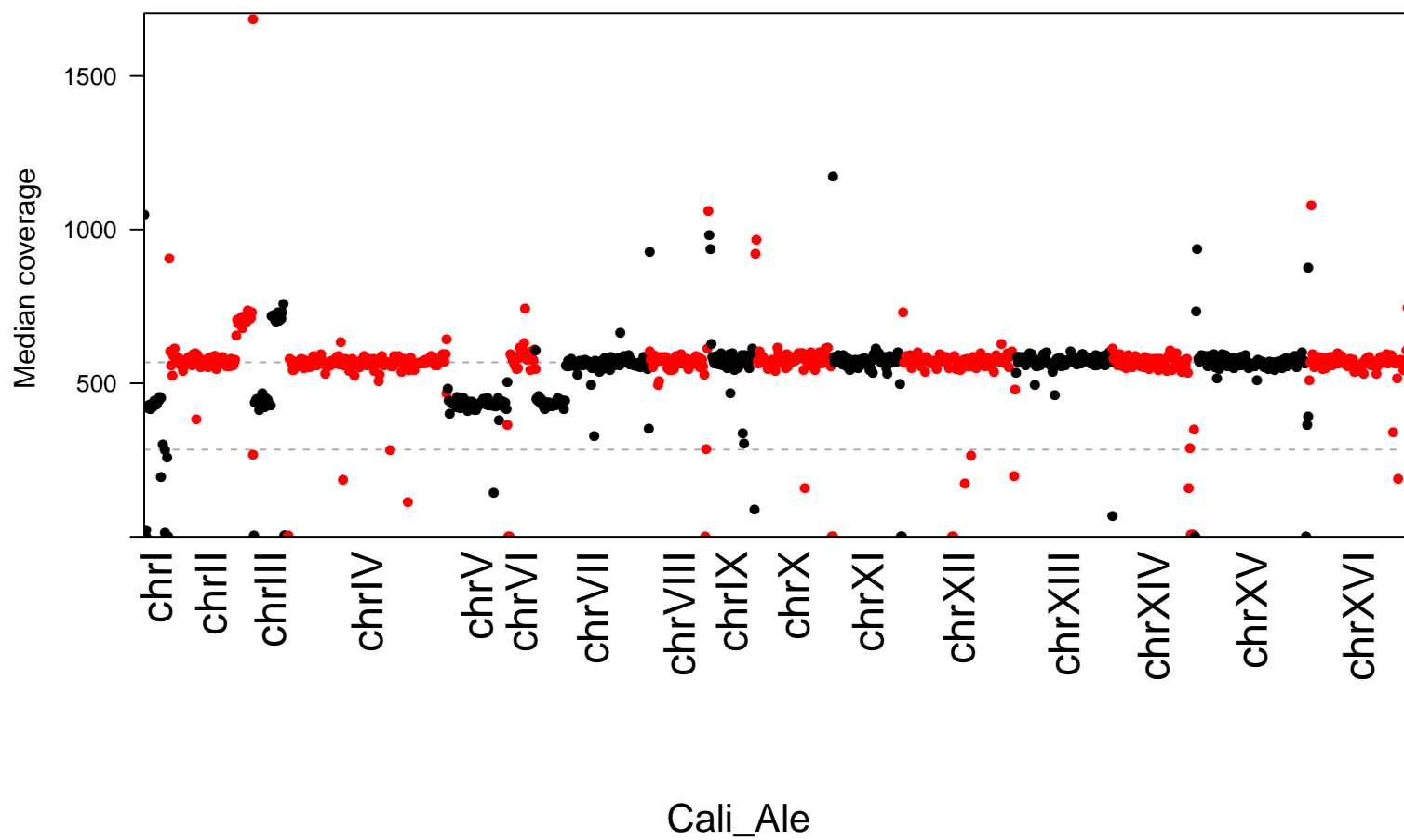
Supplementary Figure S2



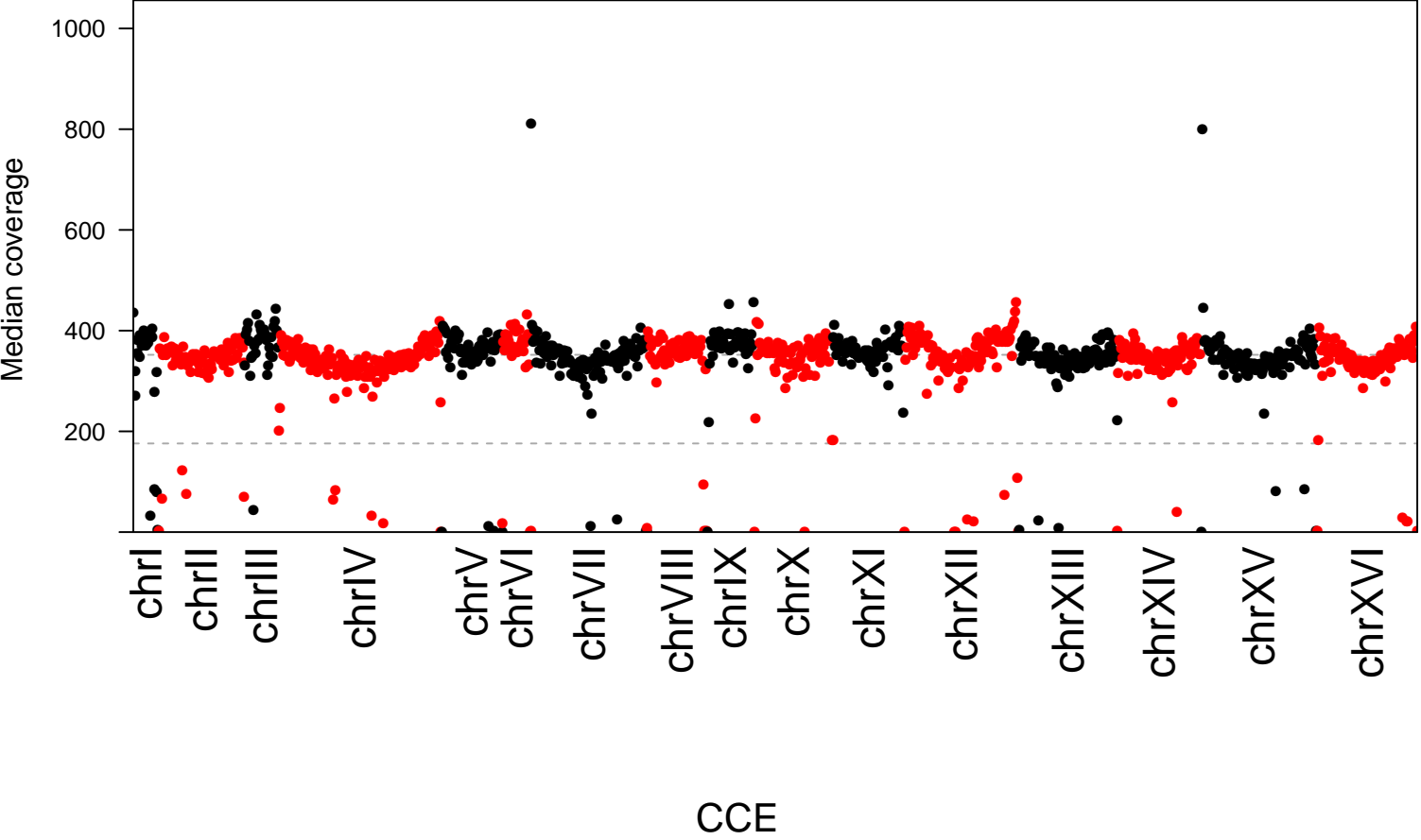
Supplementary Figure S2



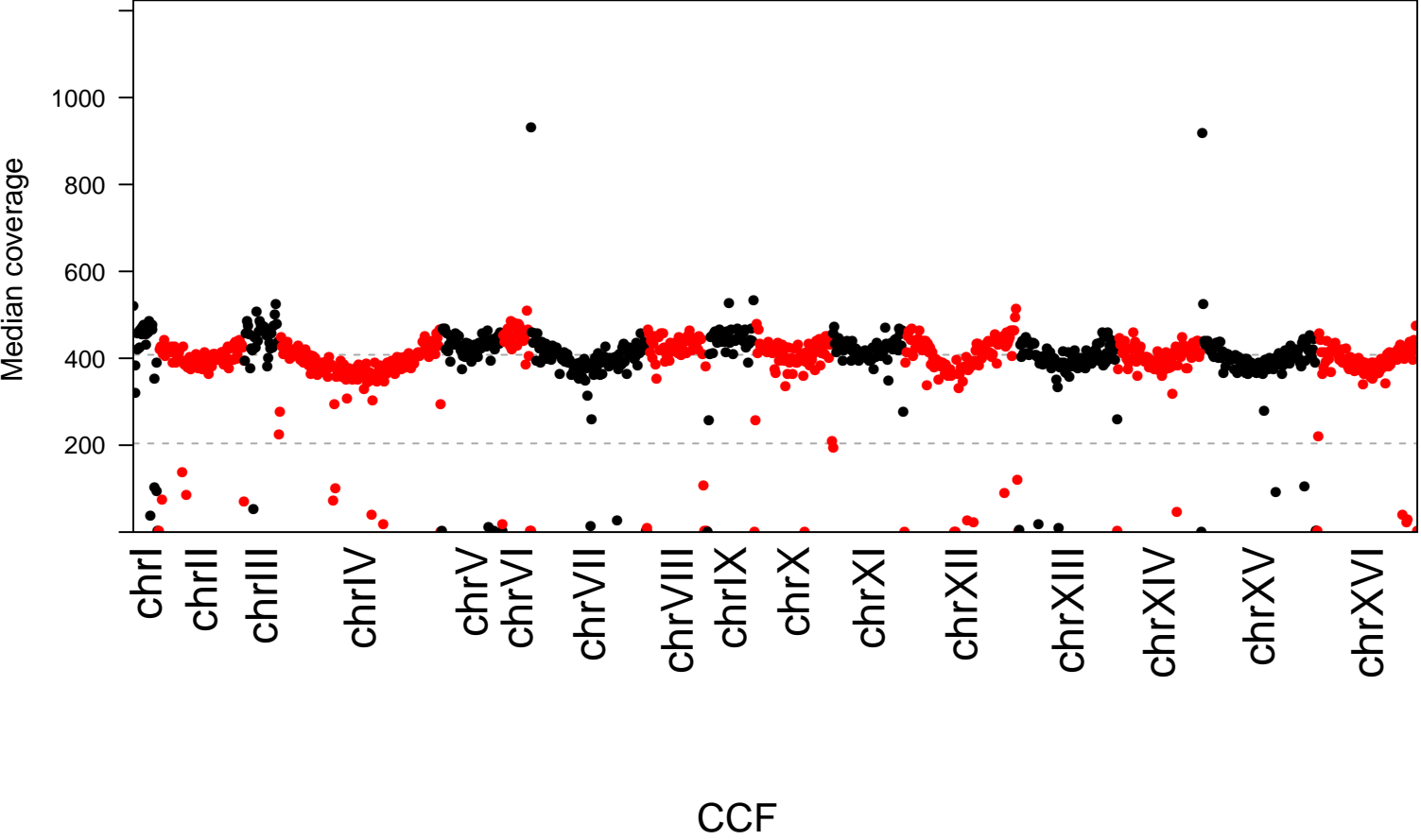
Supplementary Figure S2



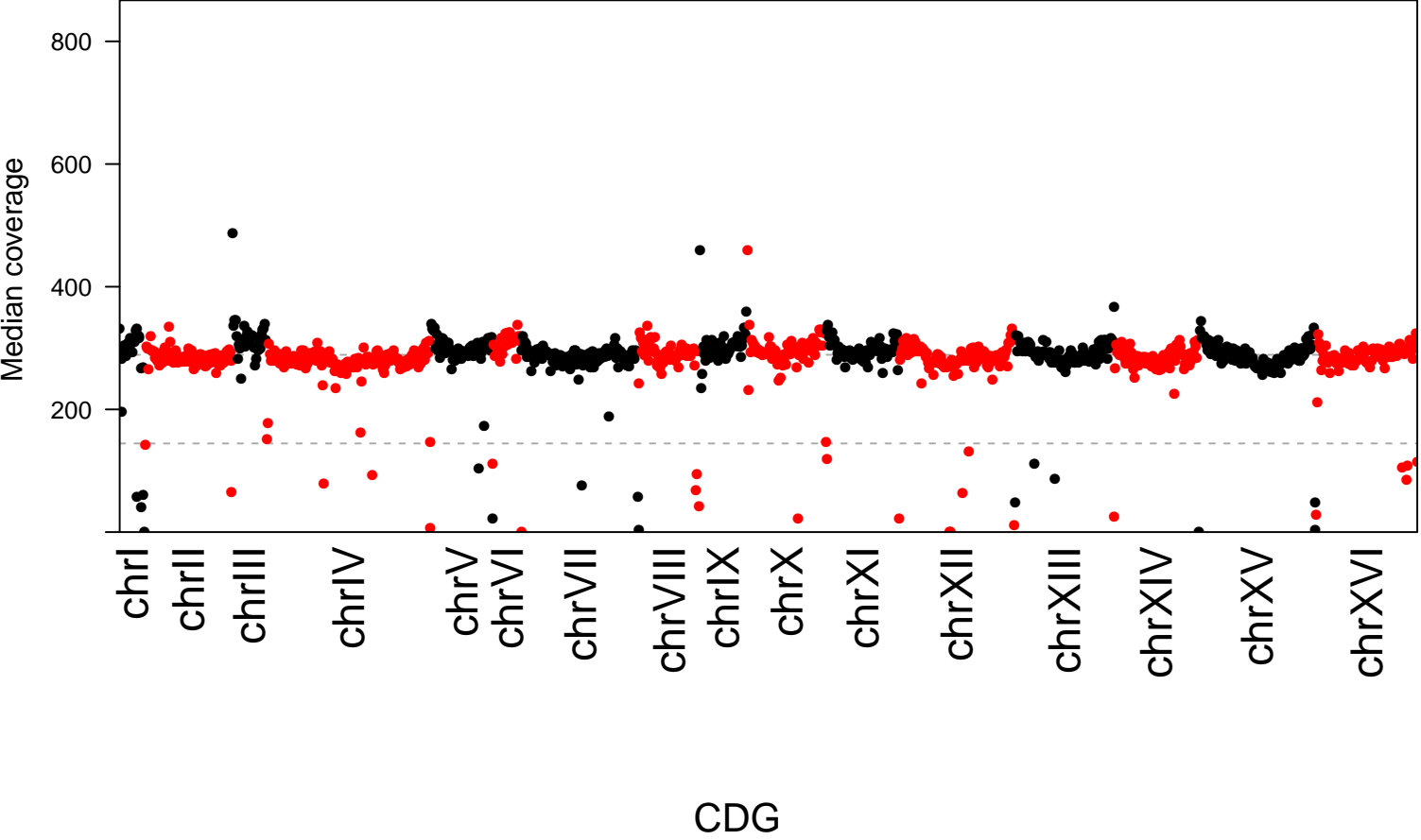
Supplementary Figure S2



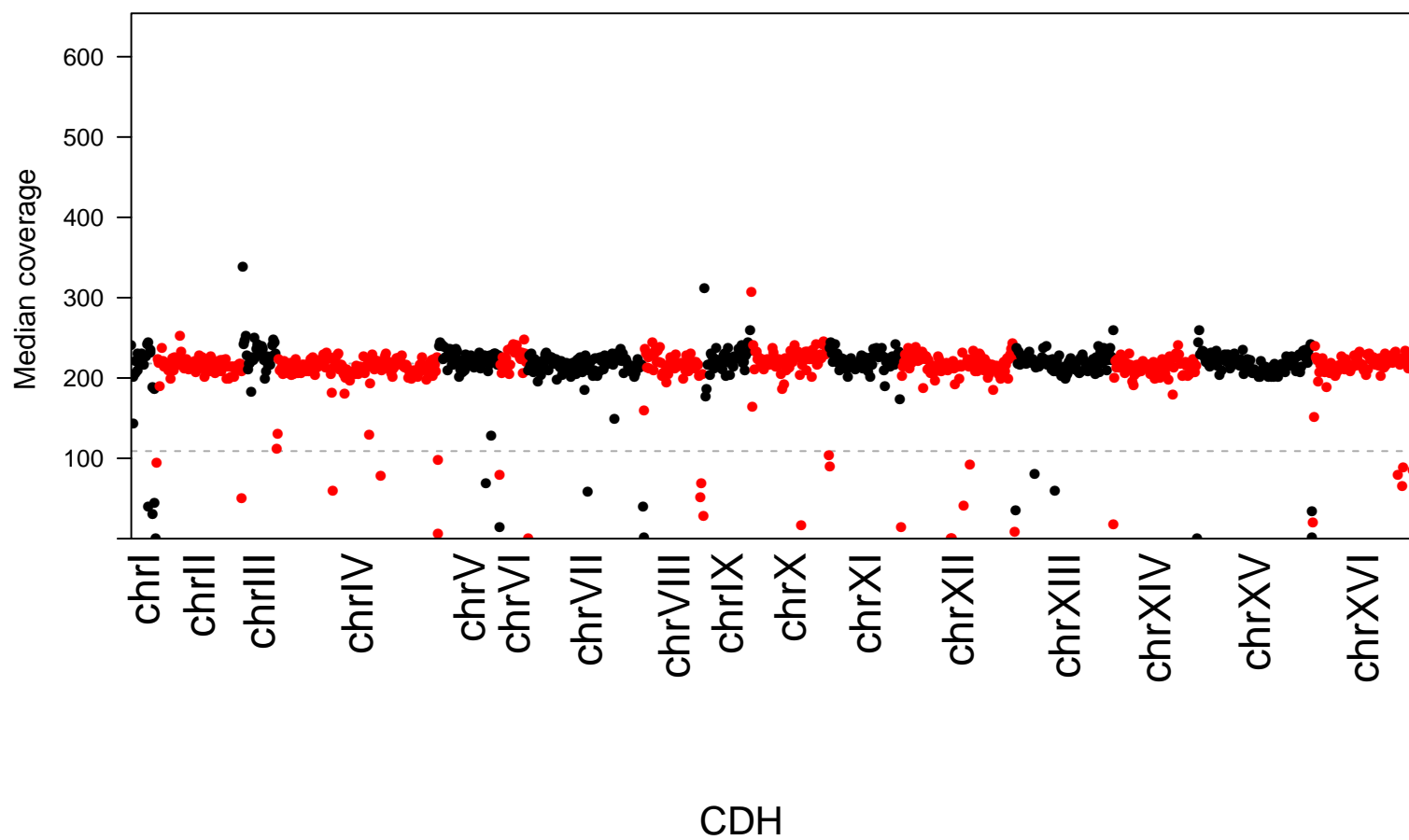
Supplementary Figure S2



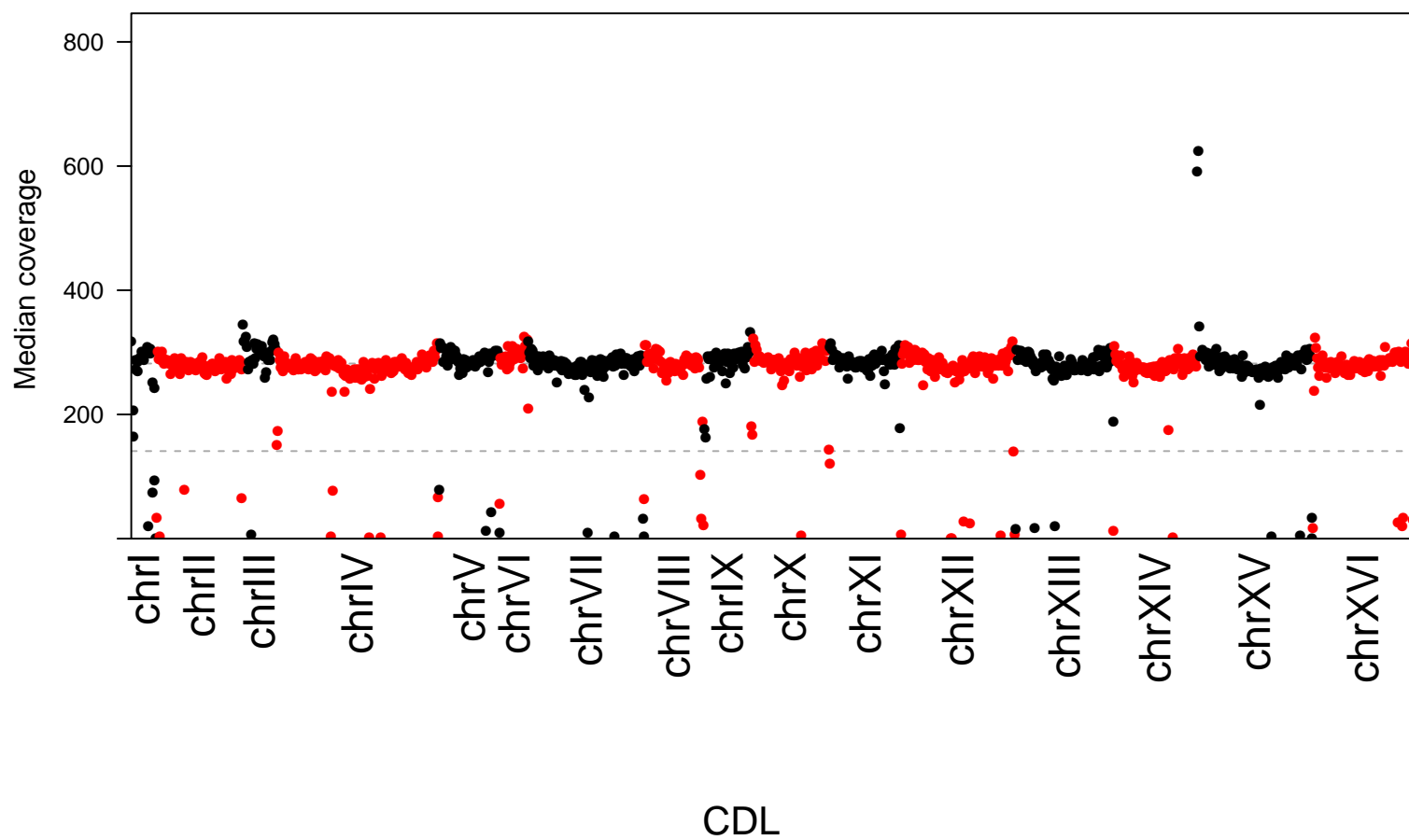
Supplementary Figure S2



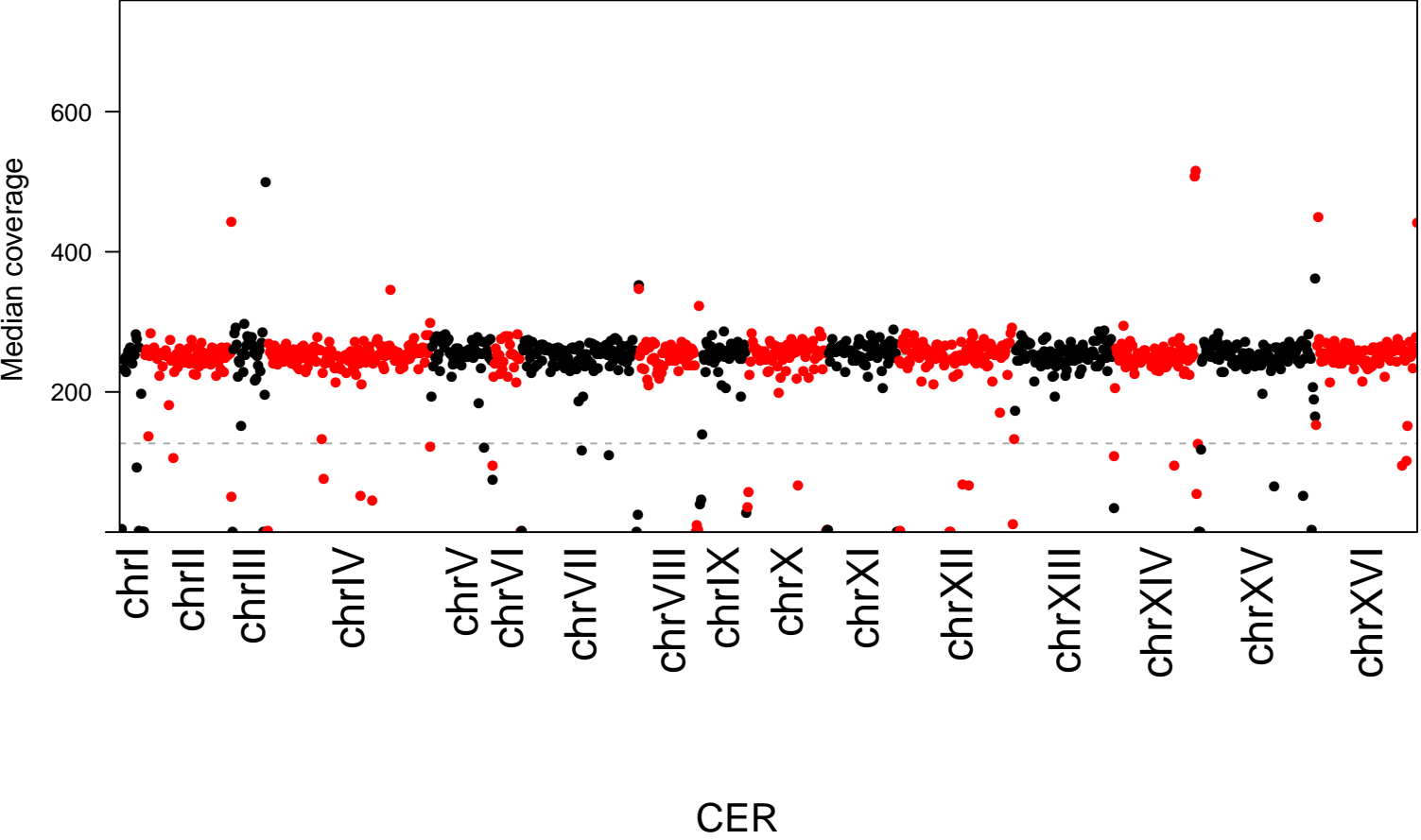
Supplementary Figure S2



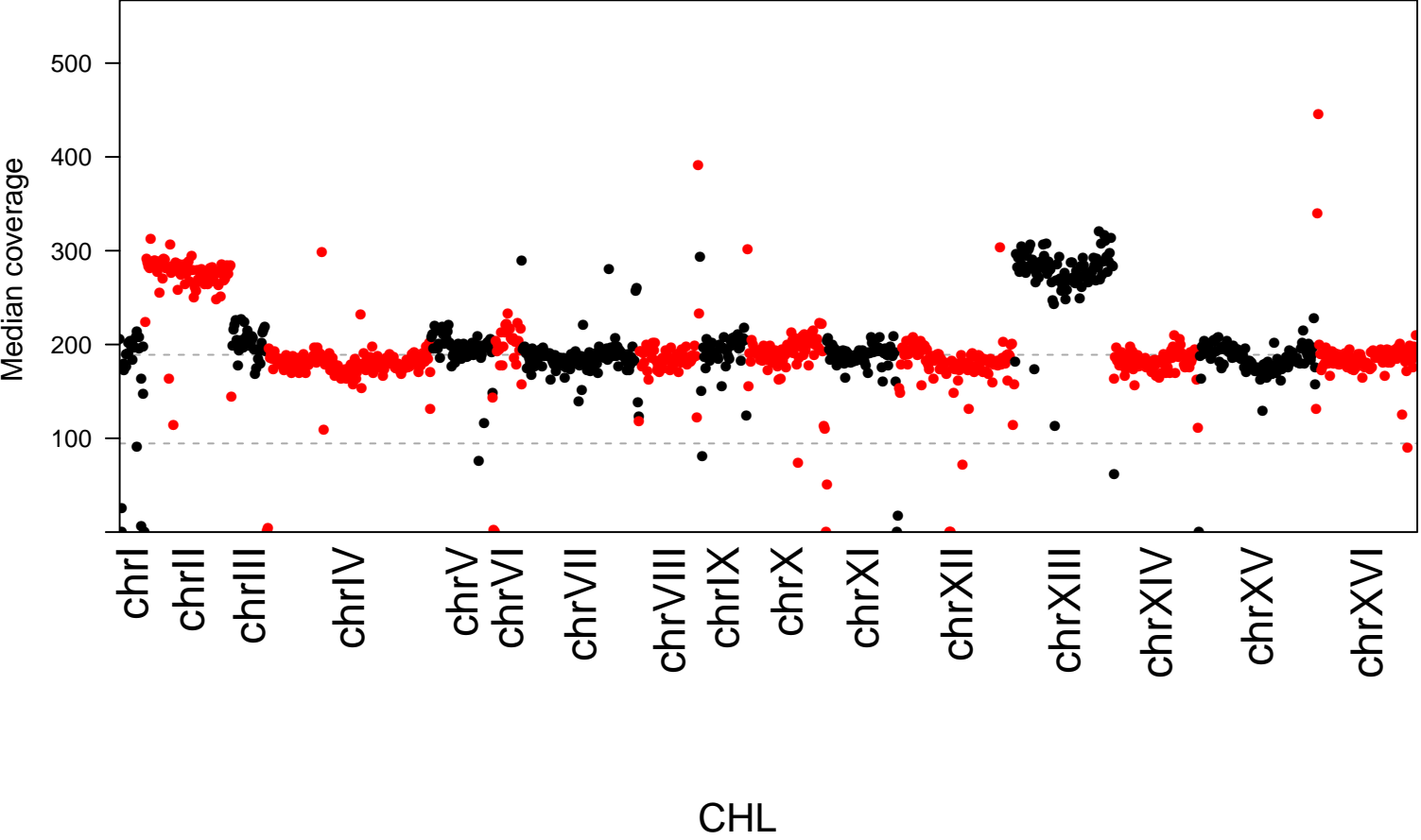
Supplementary Figure S2



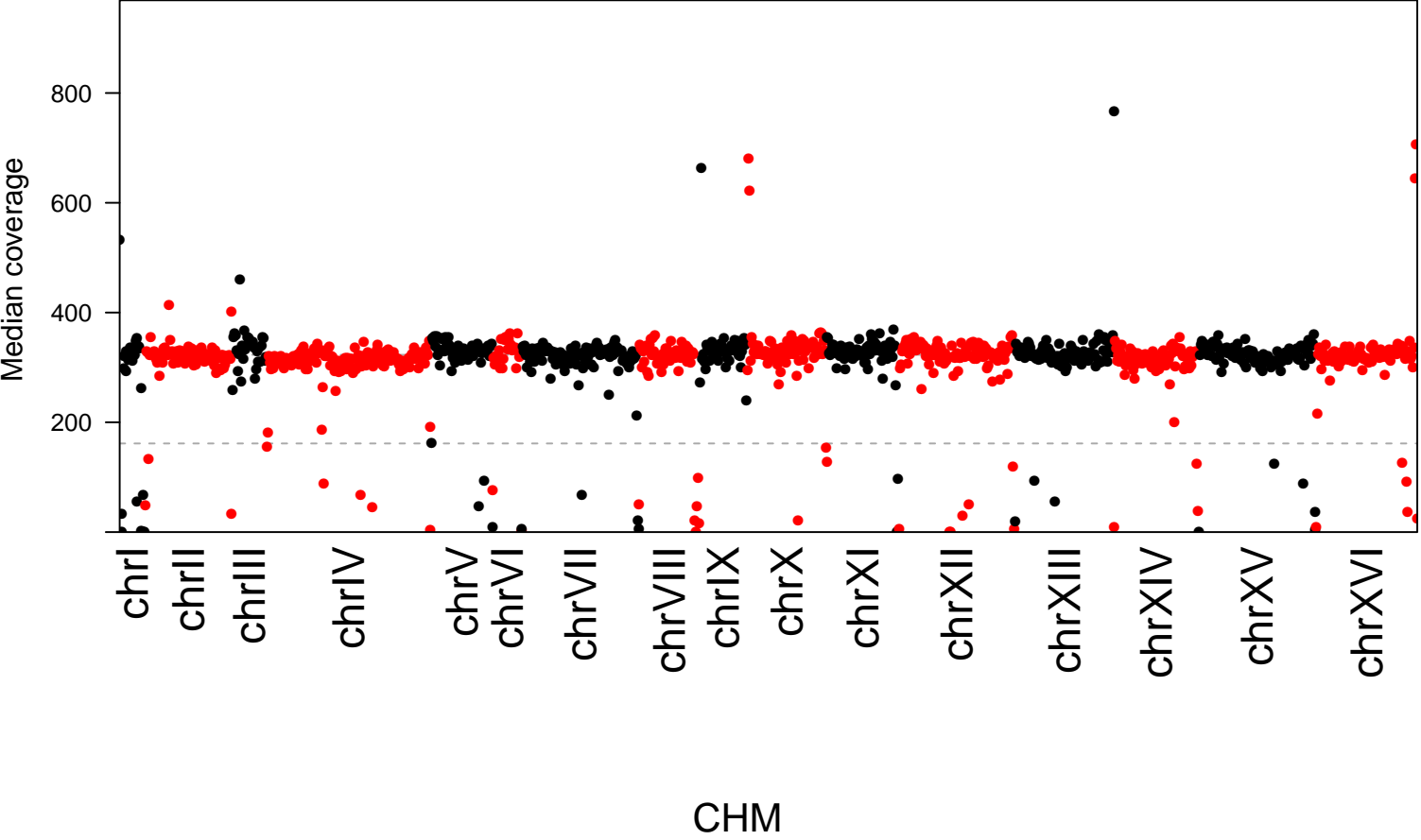
Supplementary Figure S2



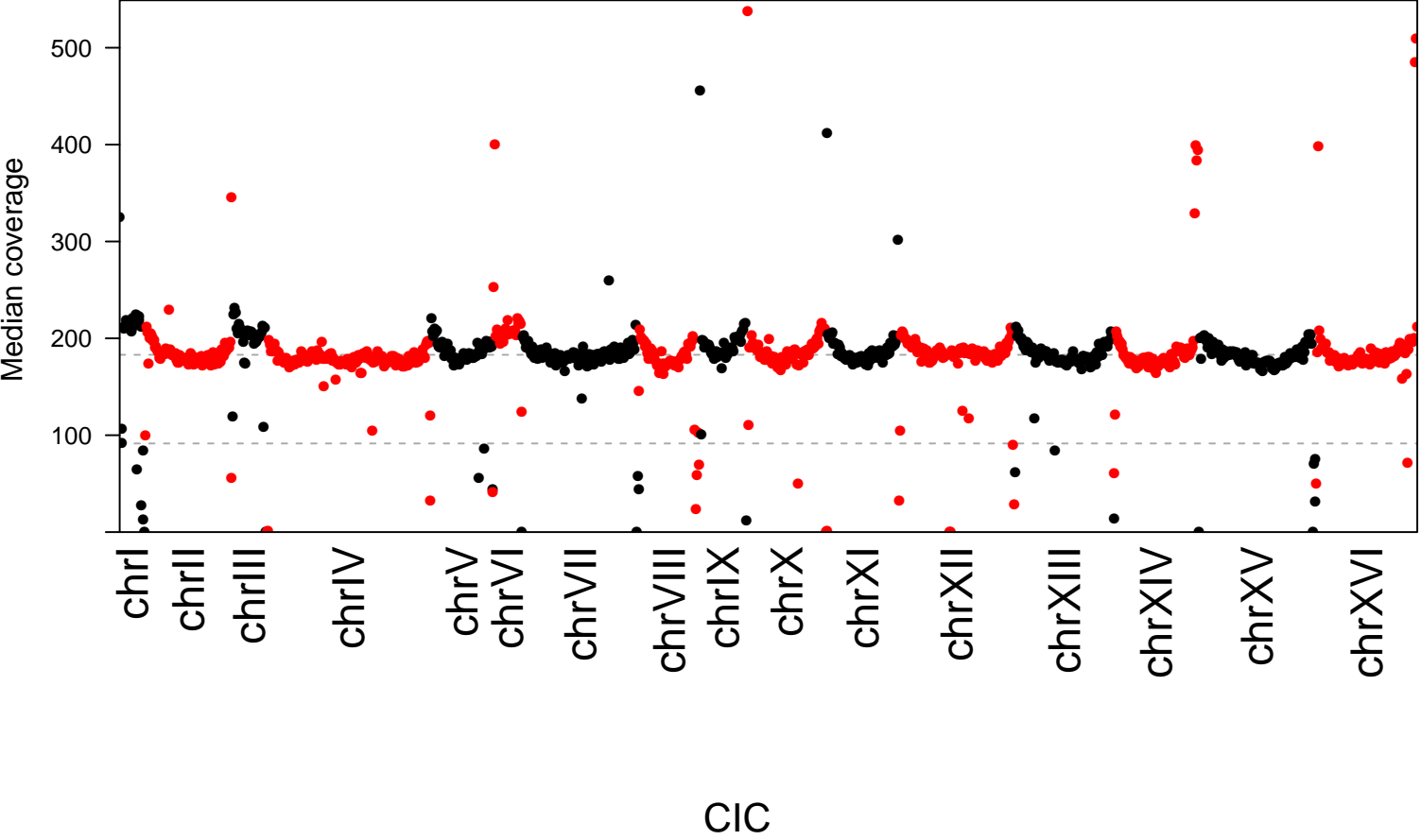
Supplementary Figure S2



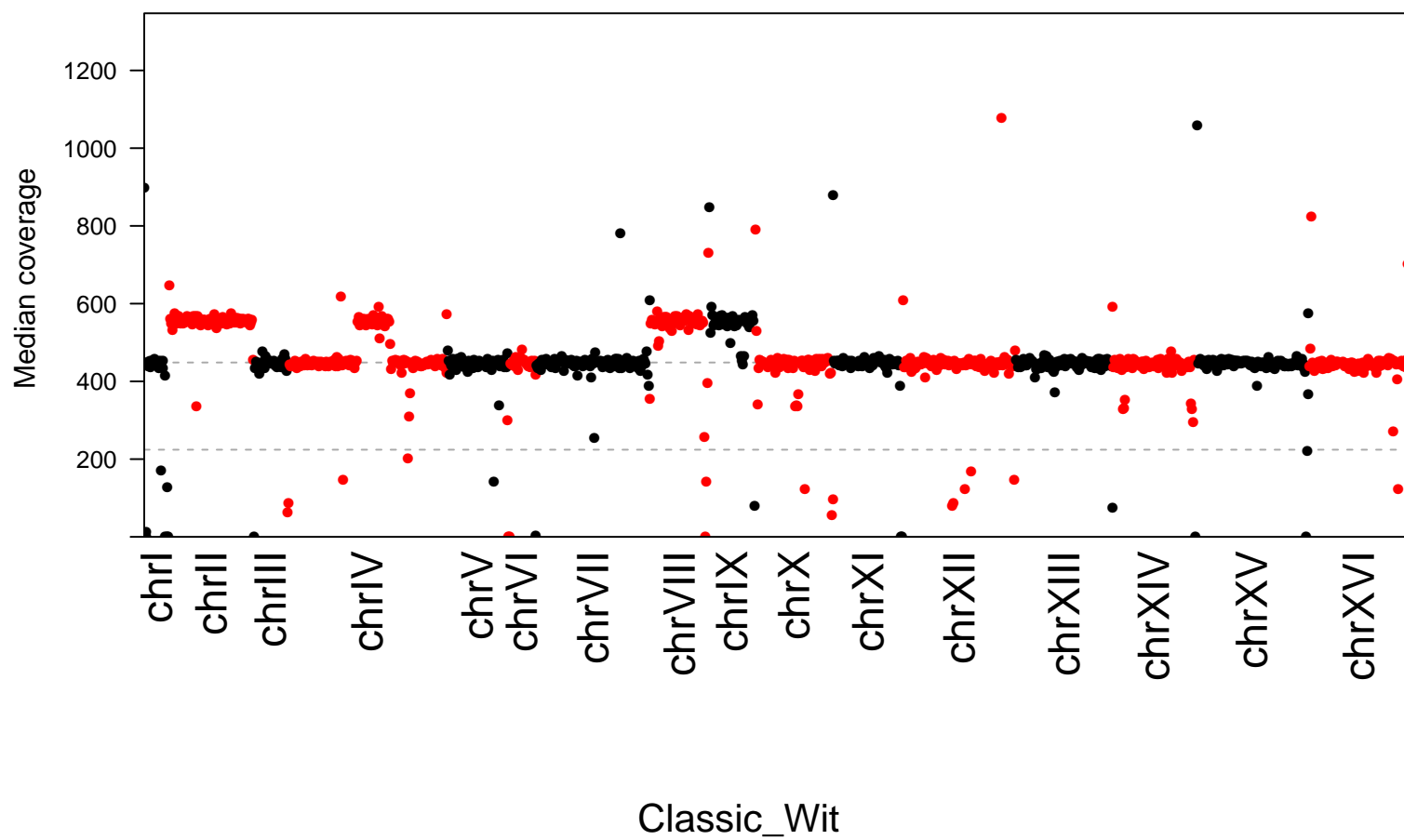
Supplementary Figure S2



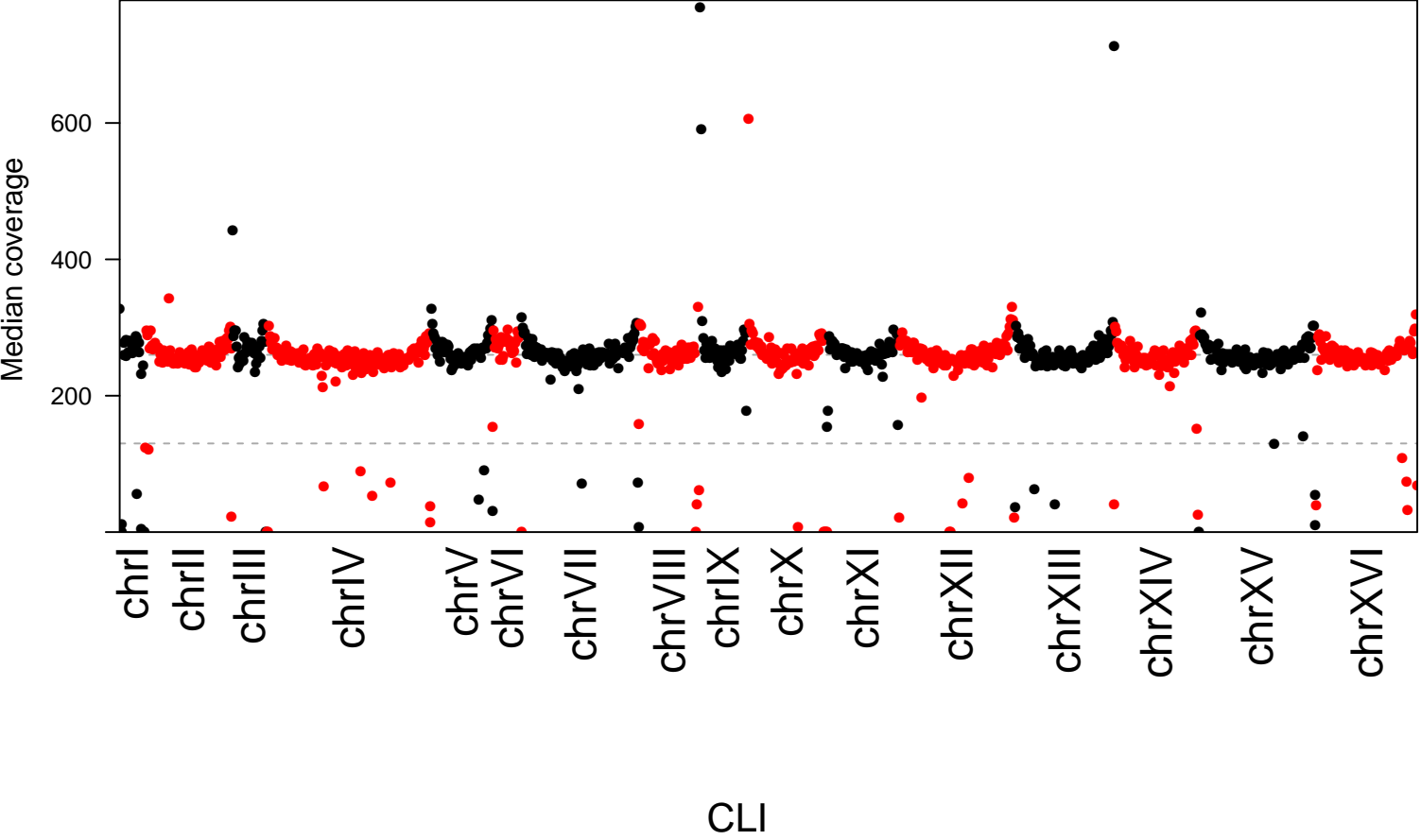
Supplementary Figure S2



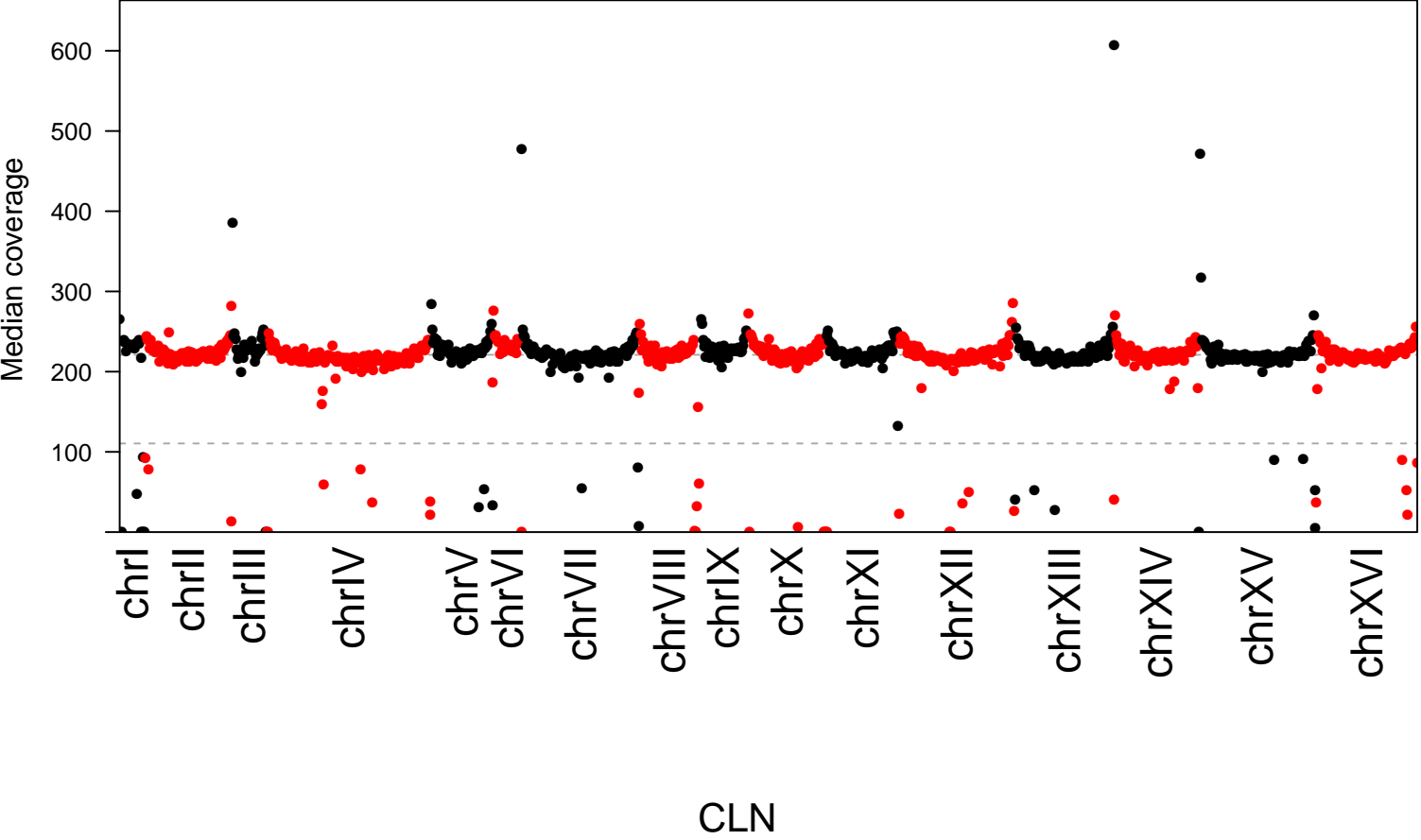
Supplementary Figure S2



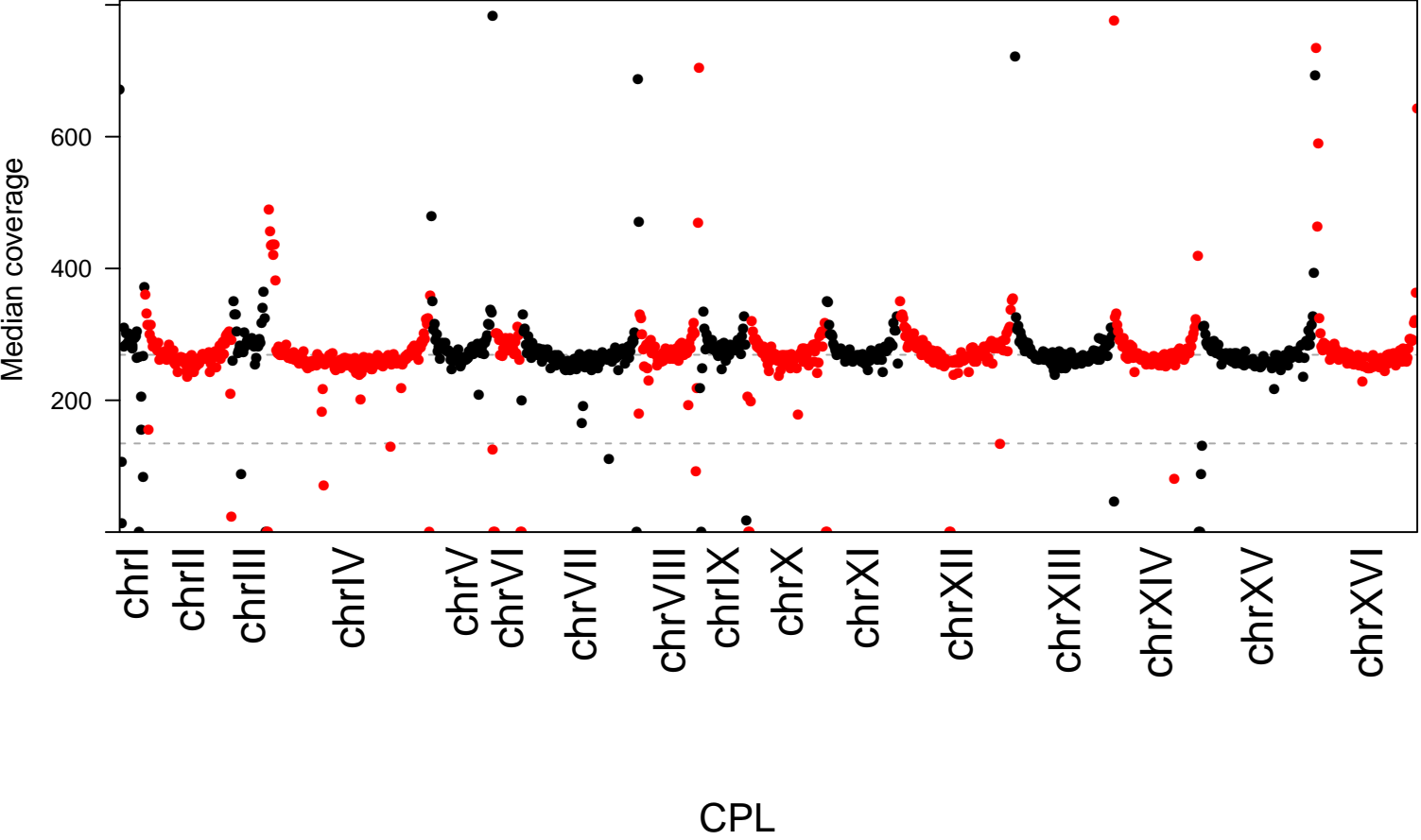
Supplementary Figure S2



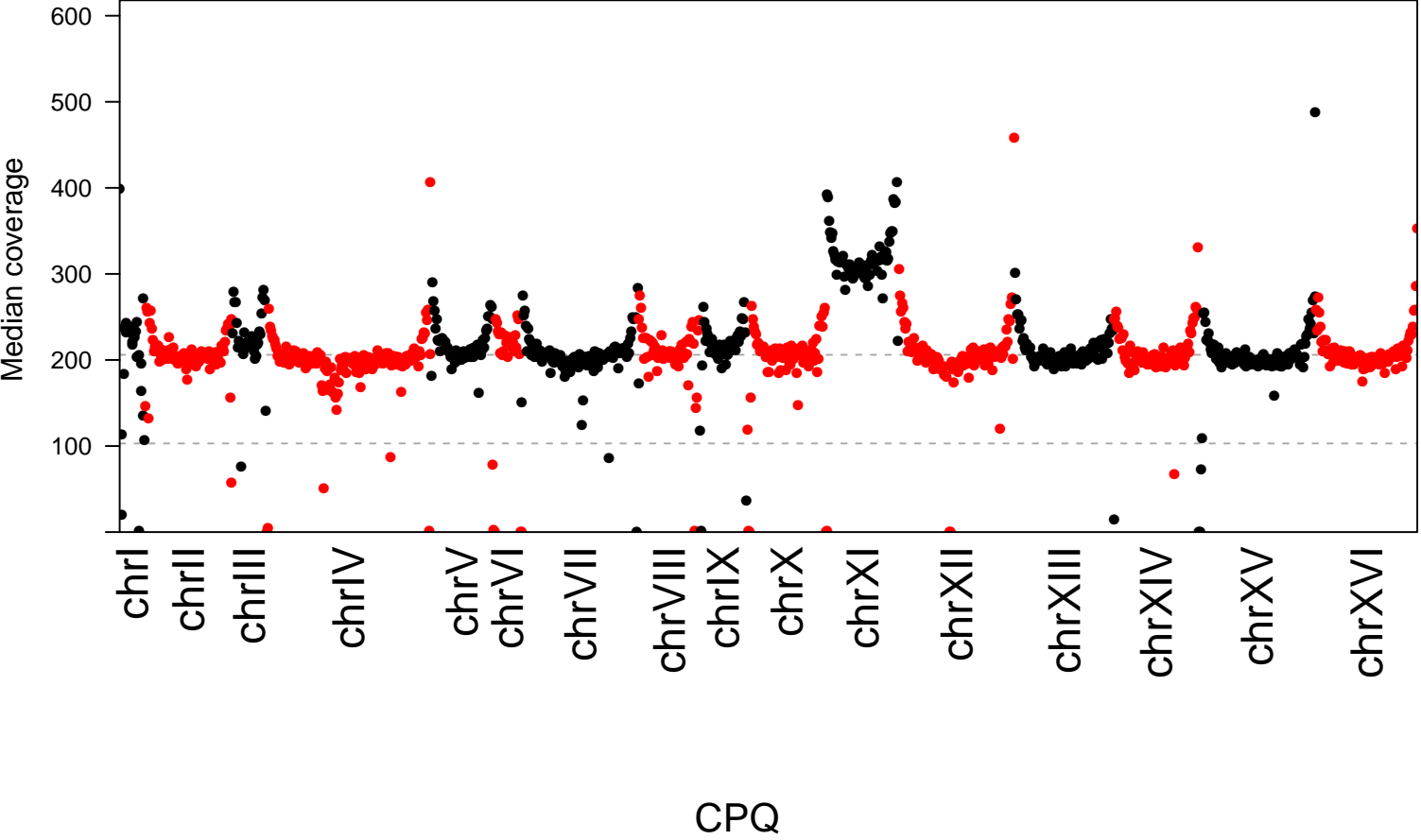
Supplementary Figure S2



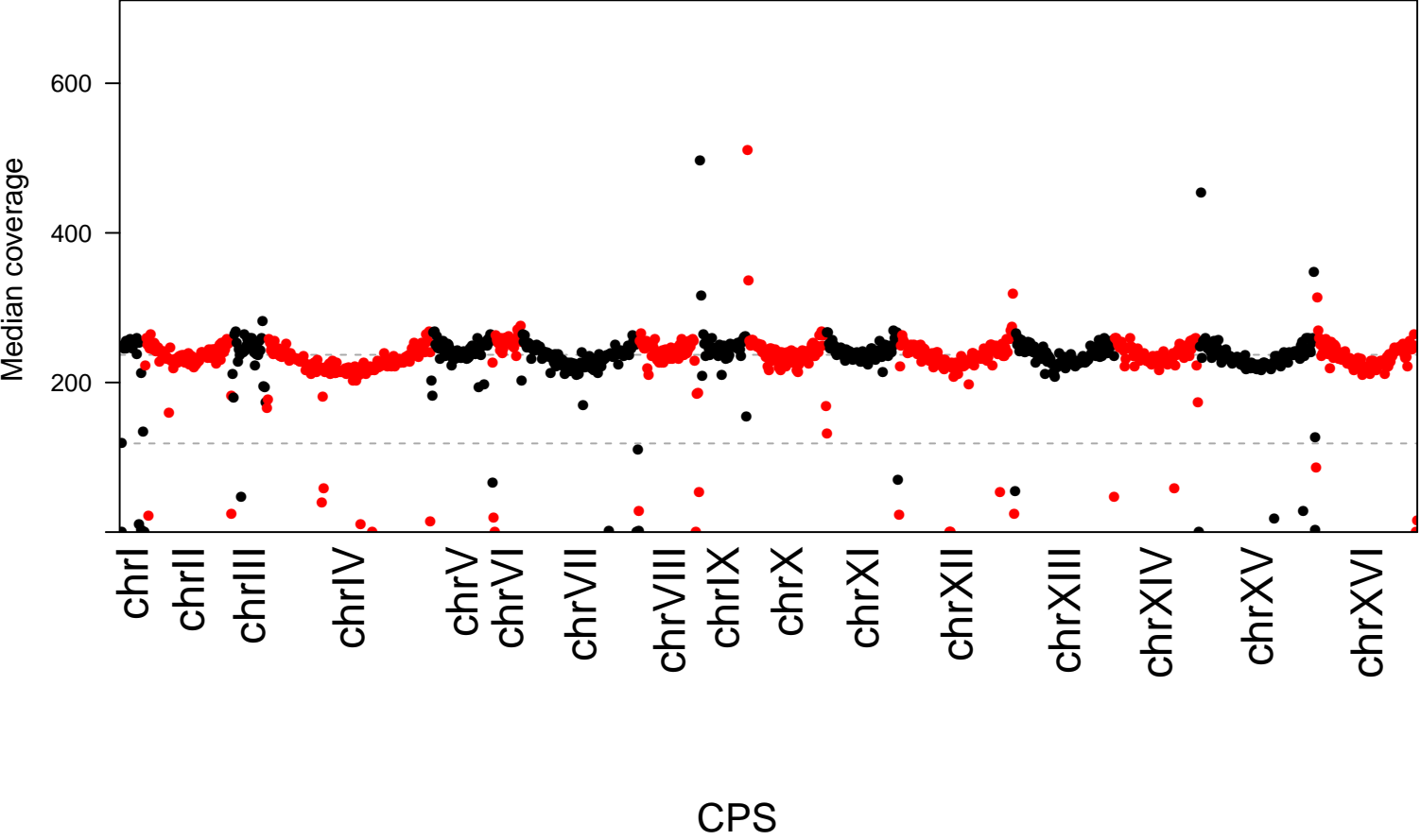
Supplementary Figure S2



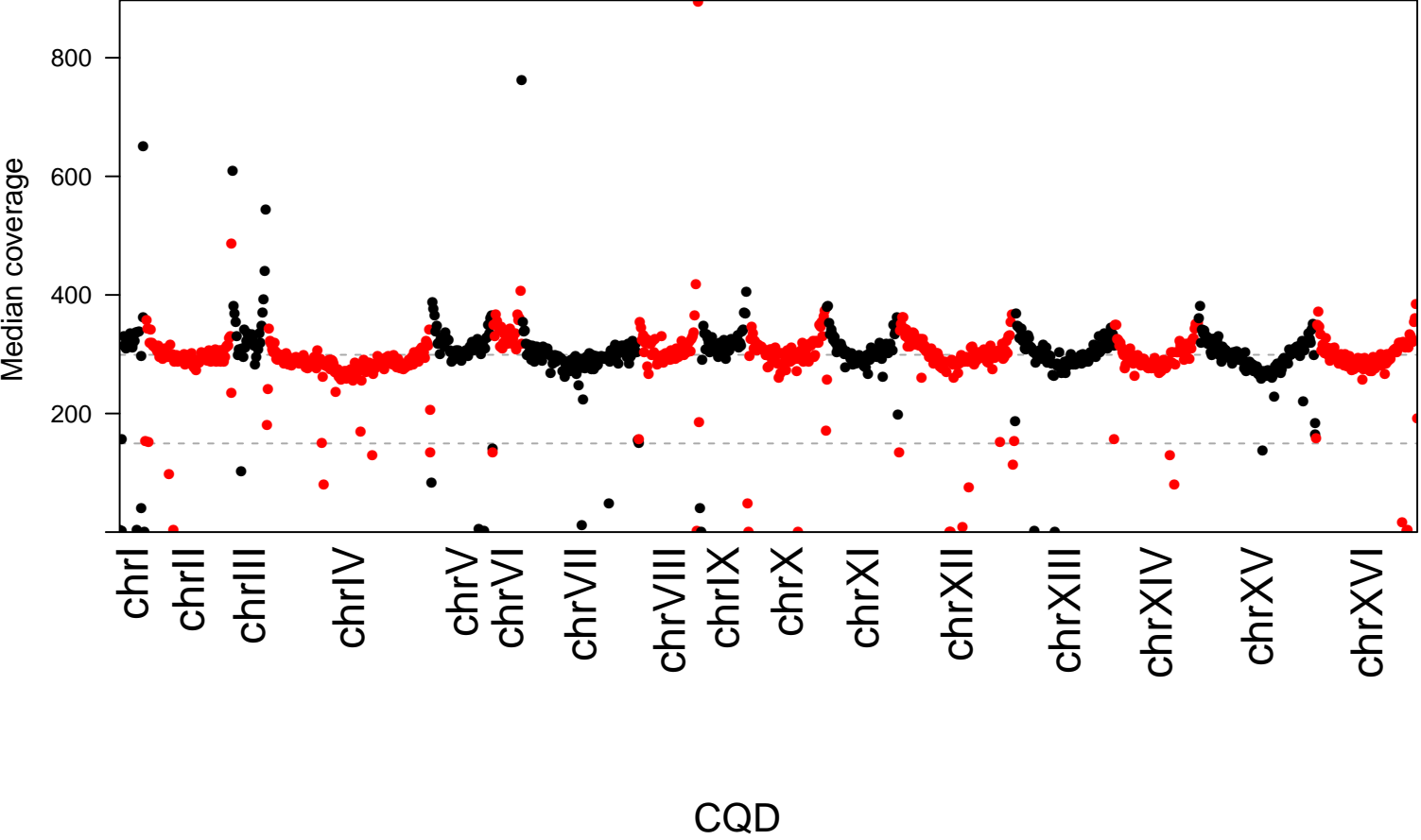
Supplementary Figure S2



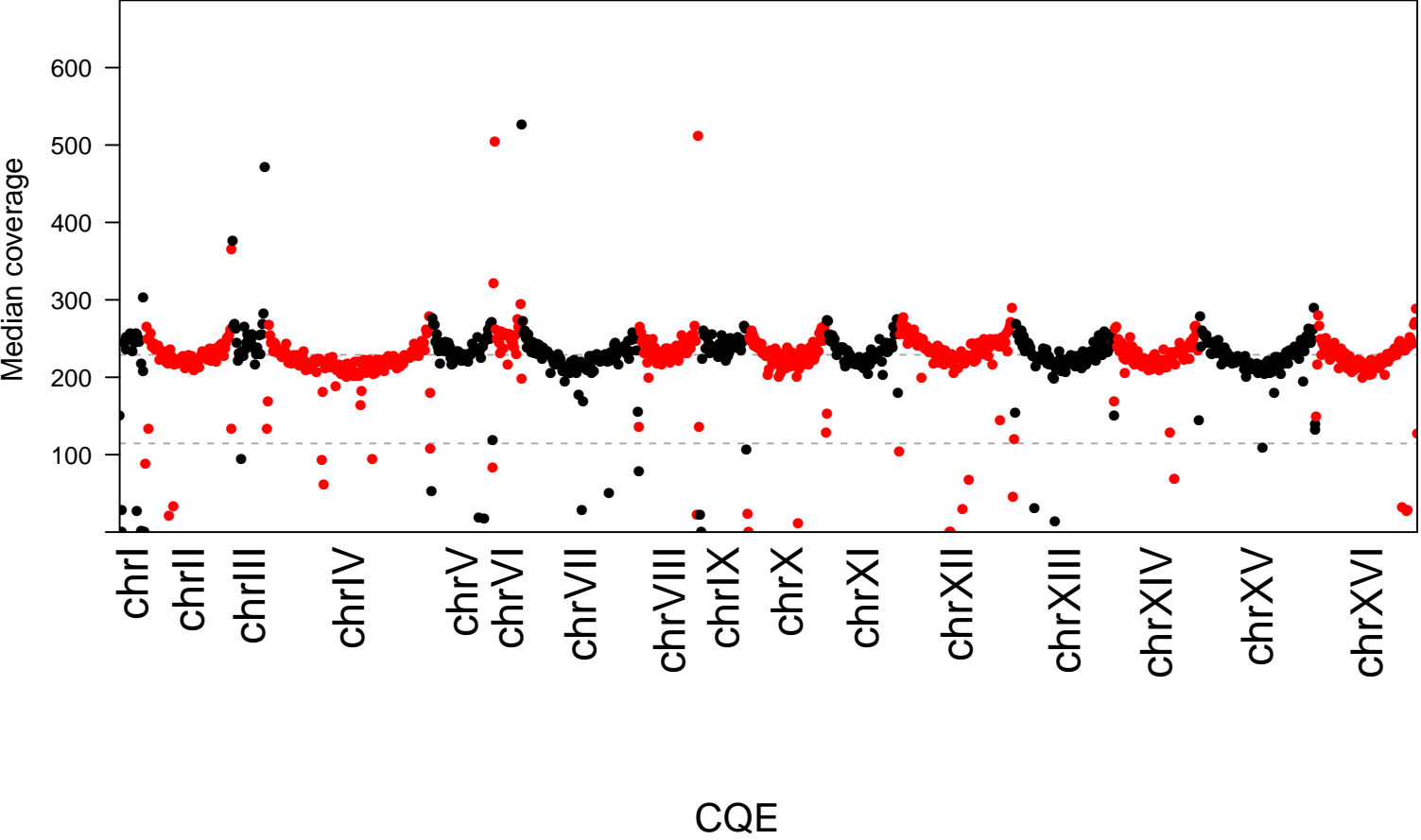
Supplementary Figure S2



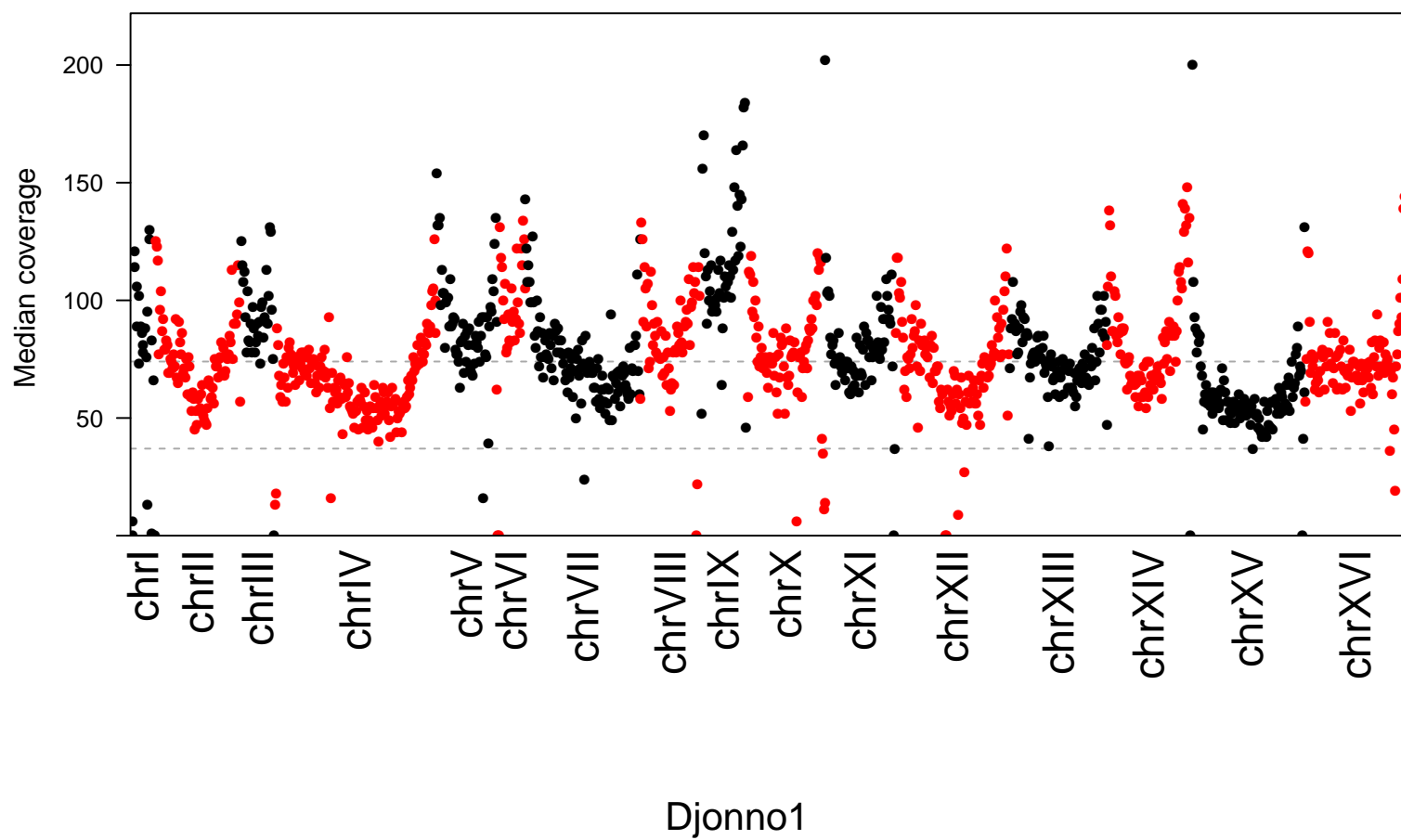
Supplementary Figure S2



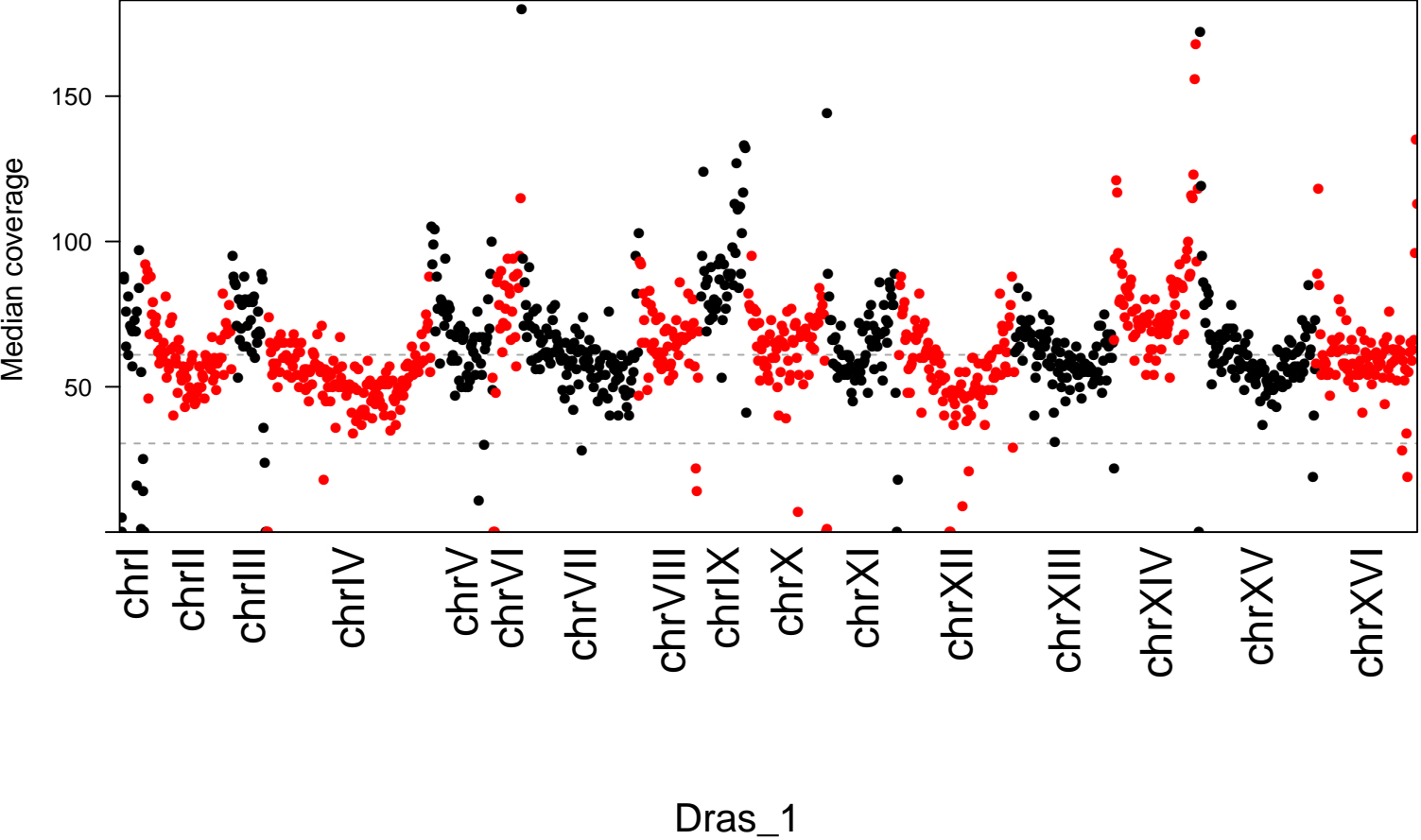
Supplementary Figure S2



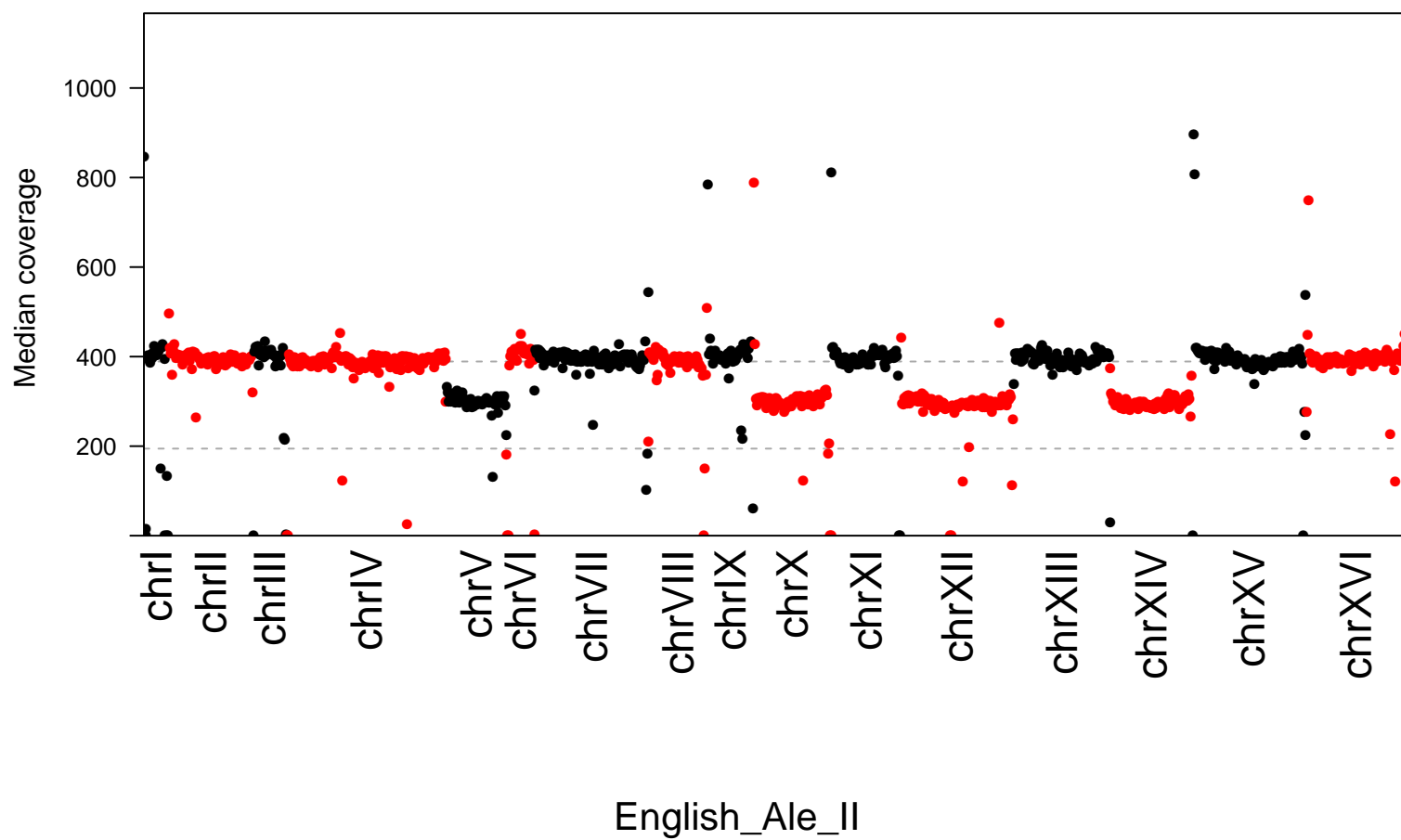
Supplementary Figure S2



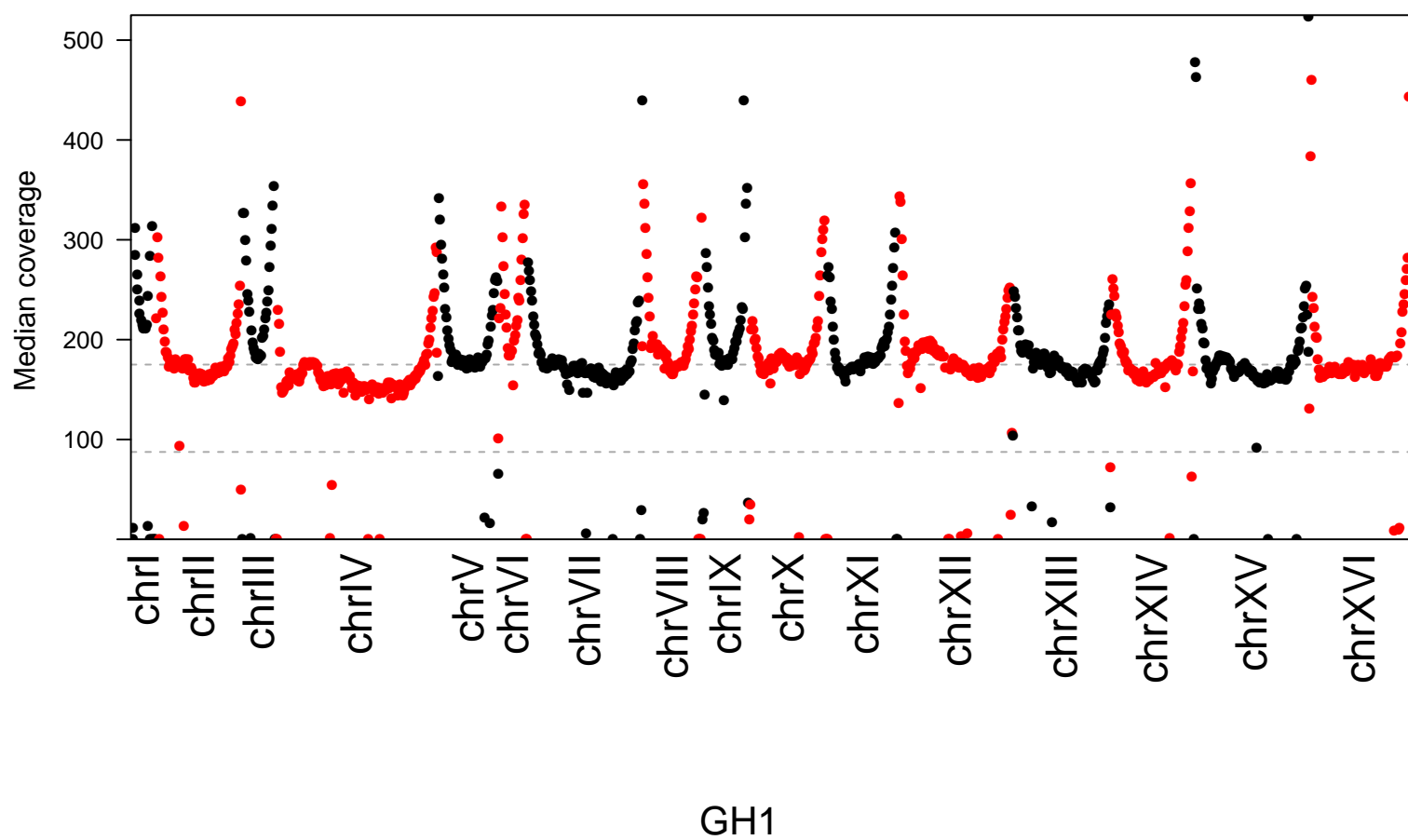
Supplementary Figure S2



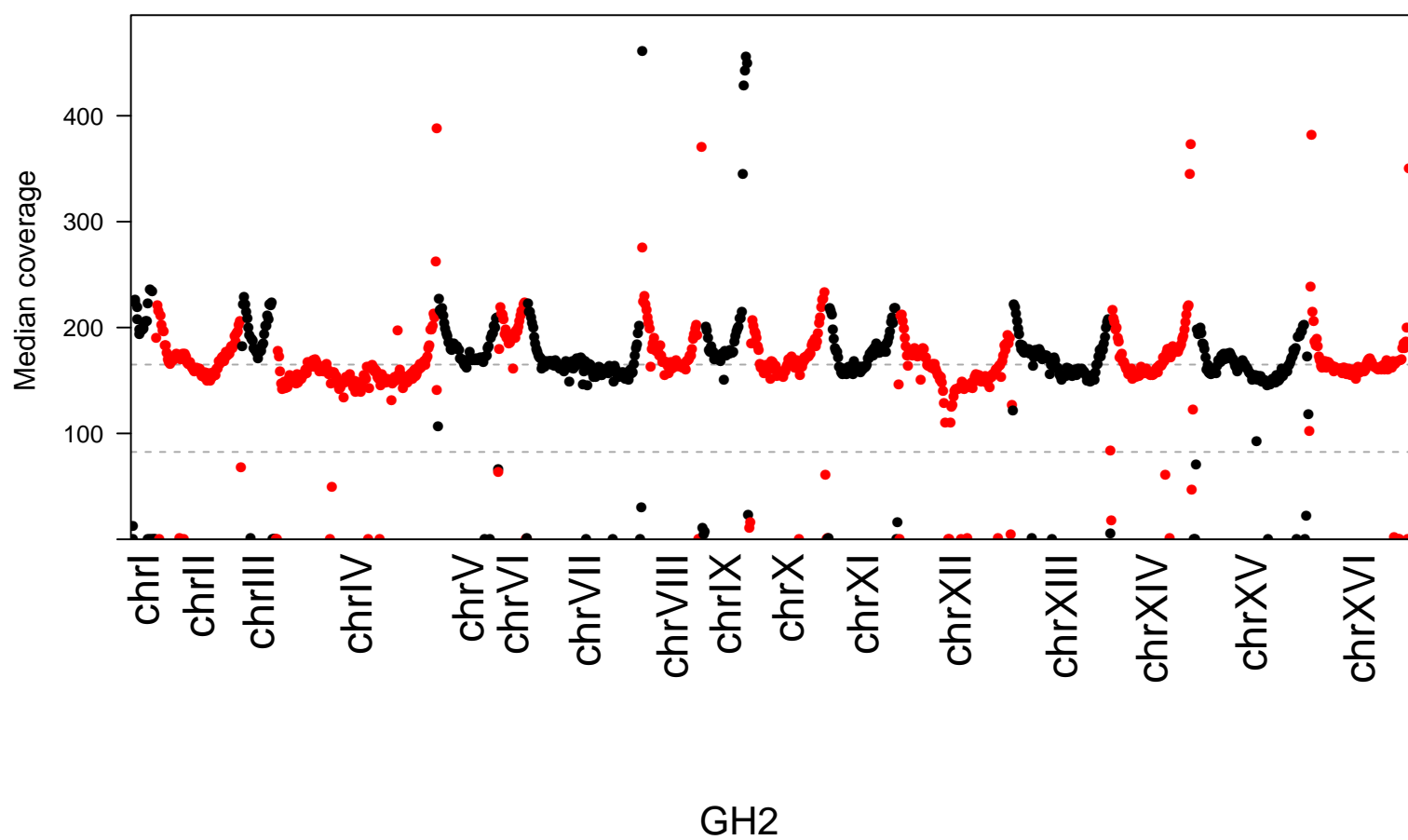
Supplementary Figure S2



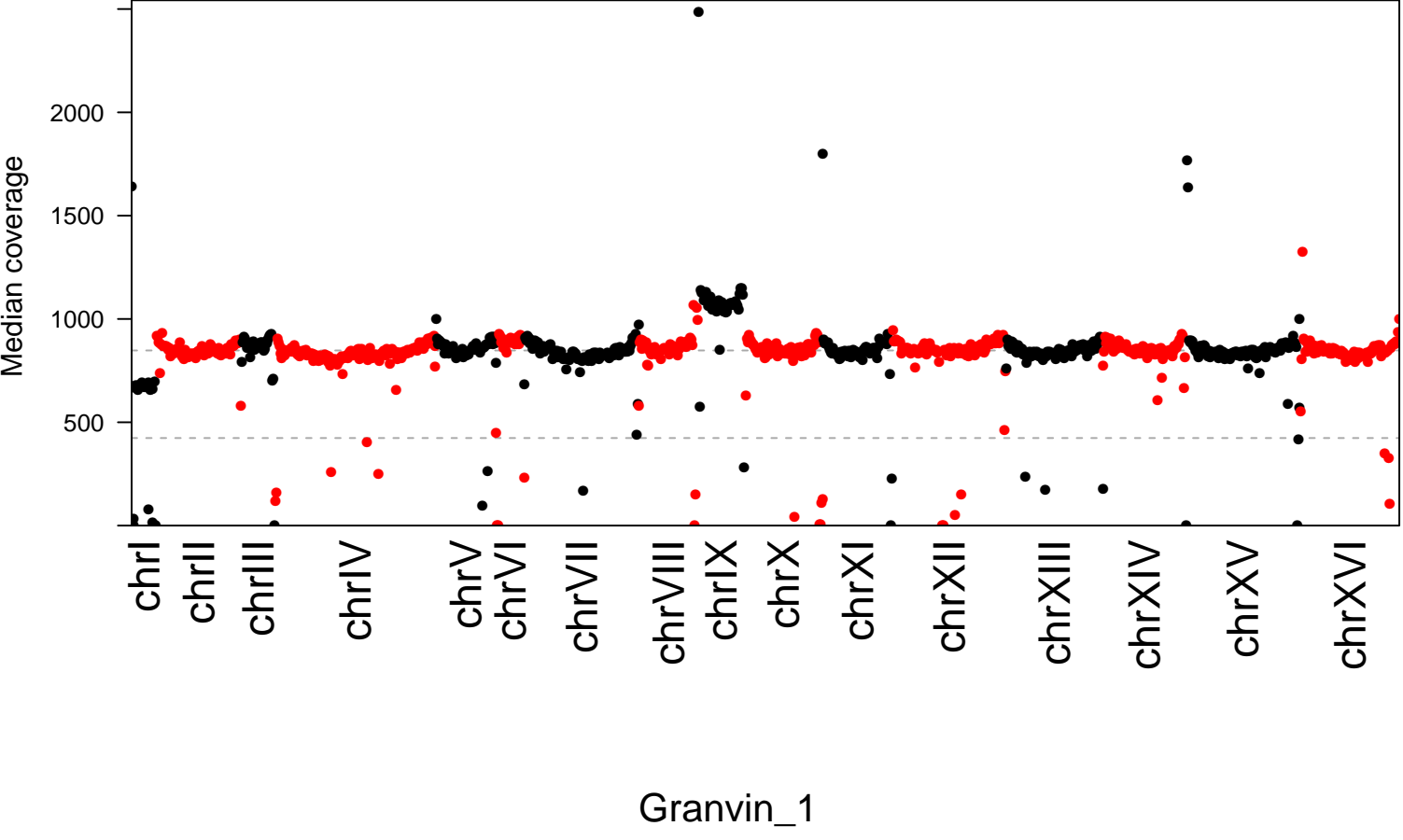
Supplementary Figure S2



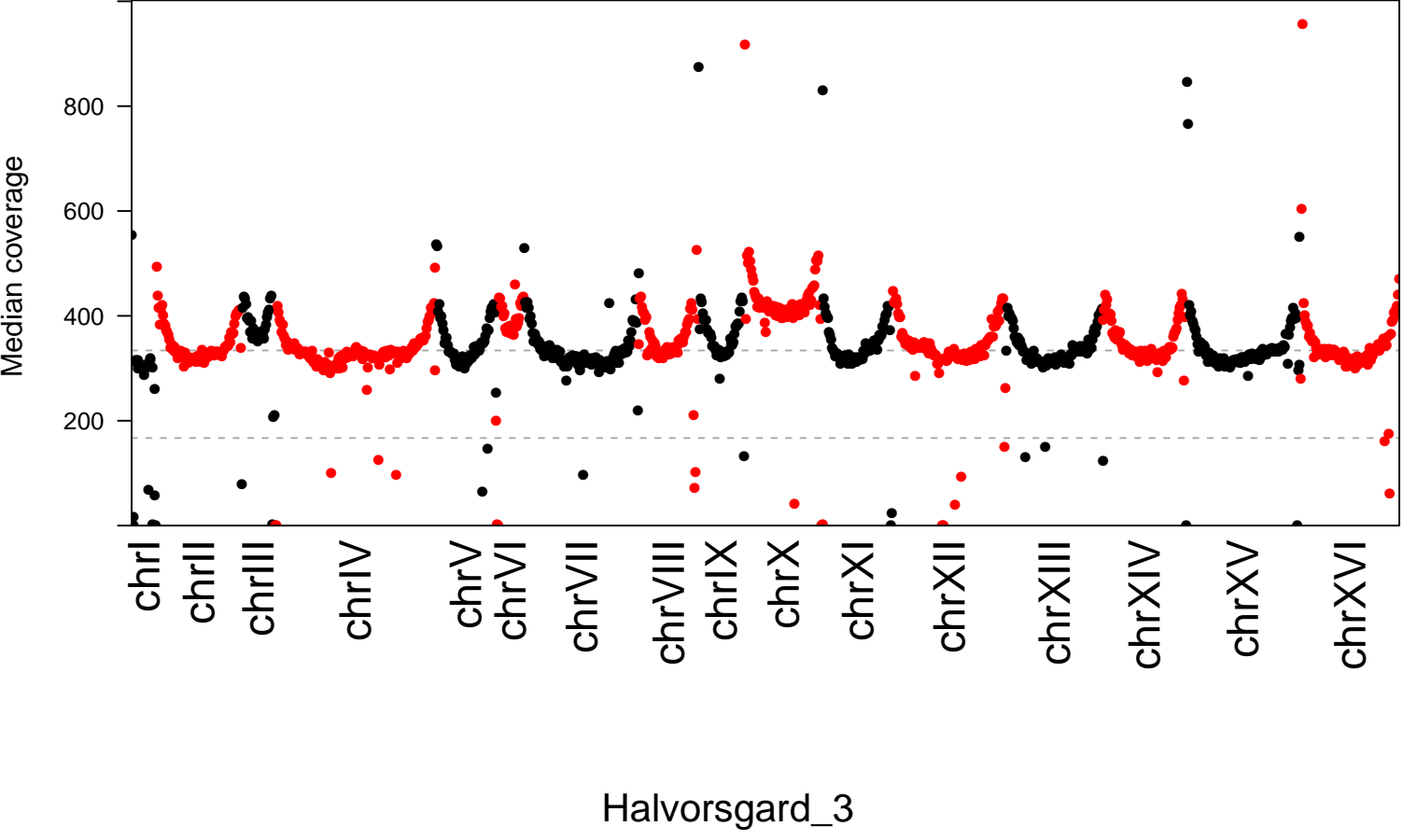
Supplementary Figure S2



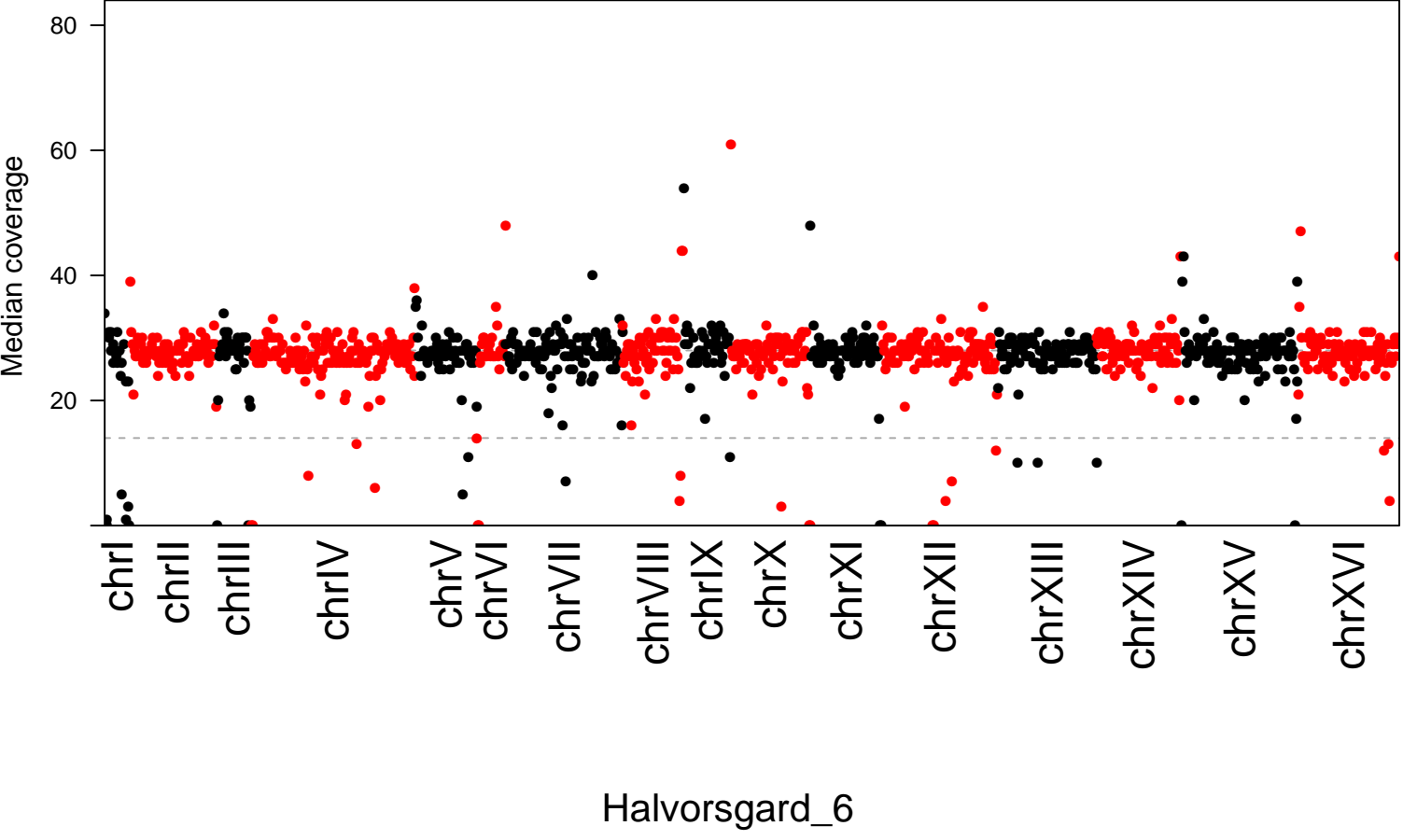
Supplementary Figure S2



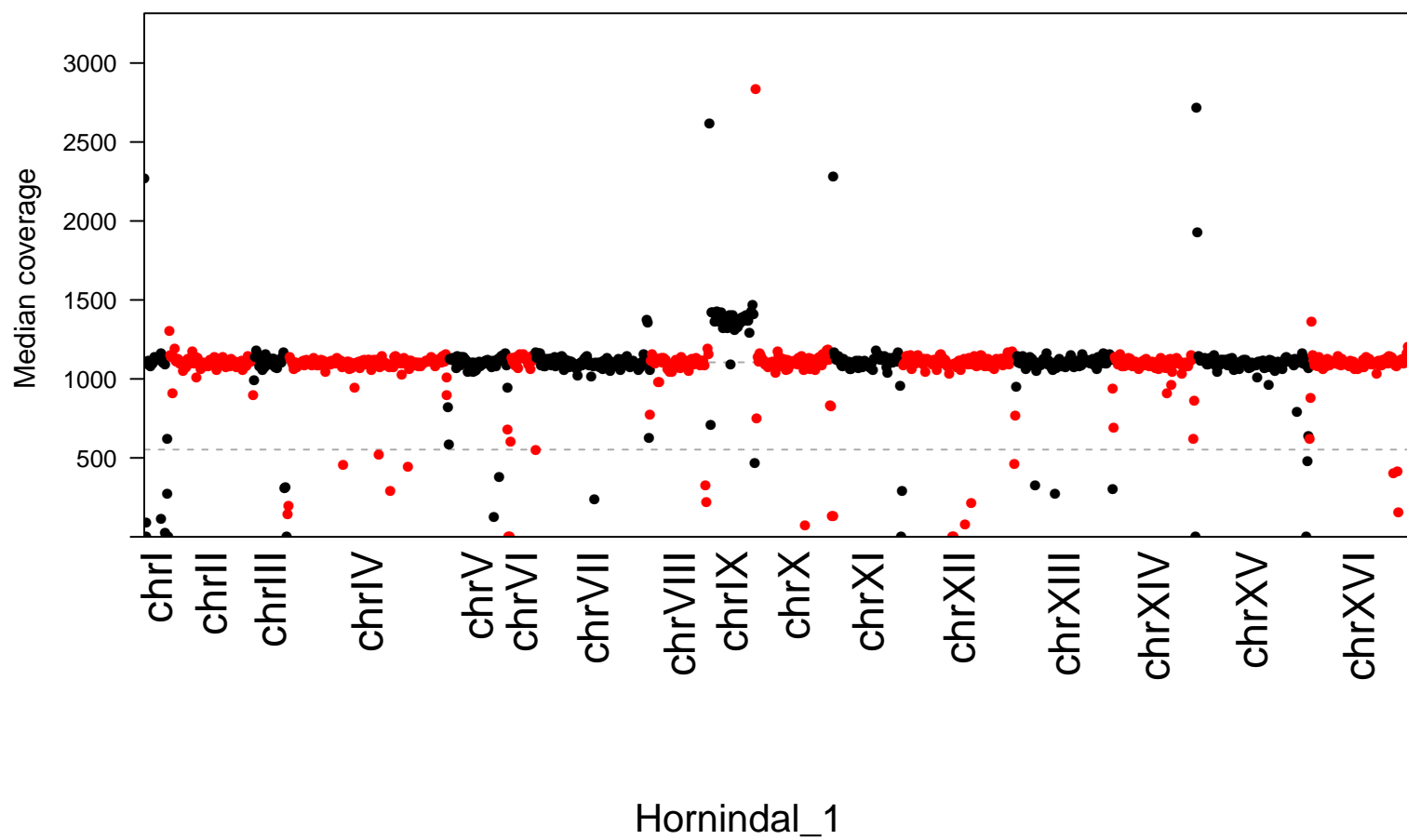
Supplementary Figure S2



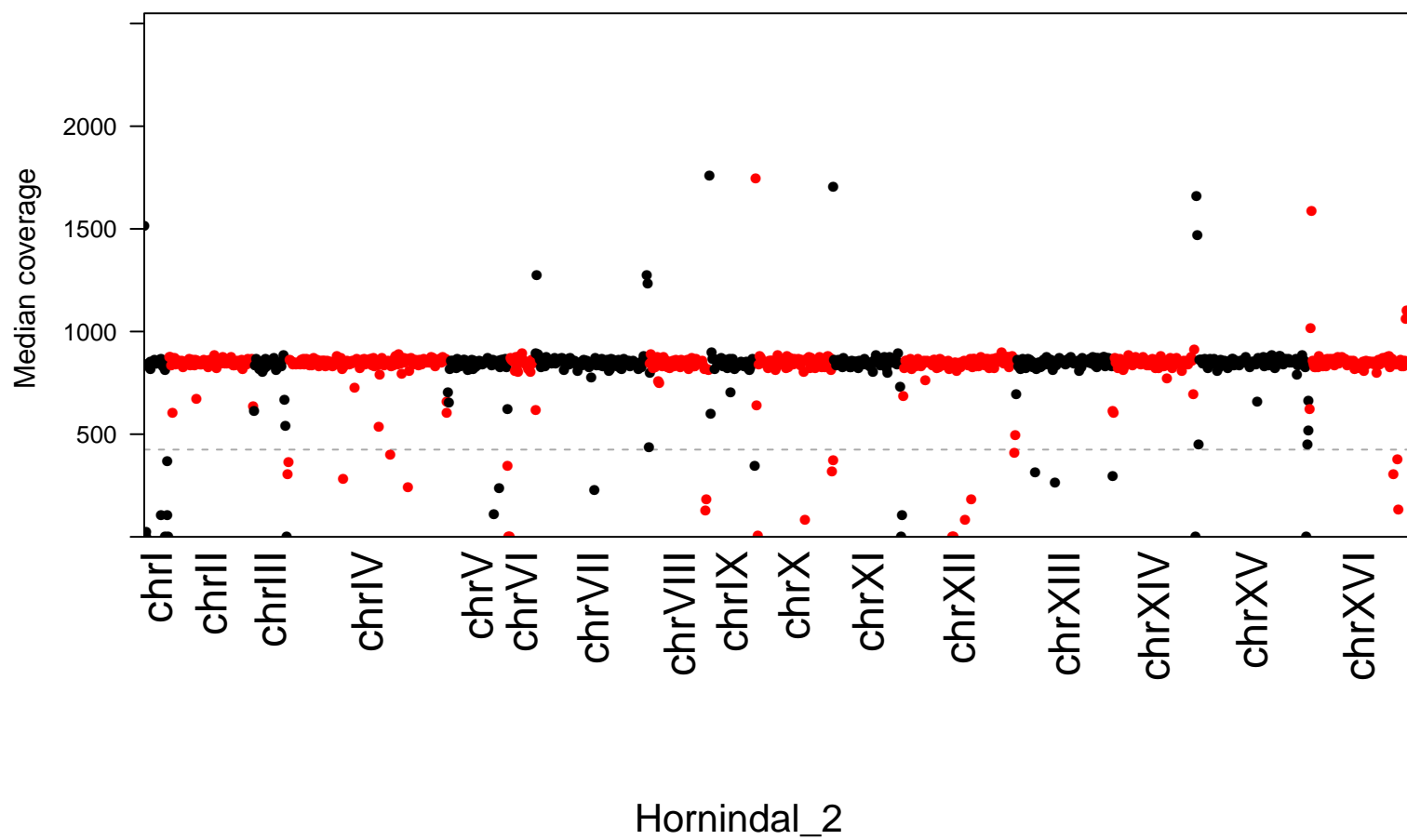
Supplementary Figure S2



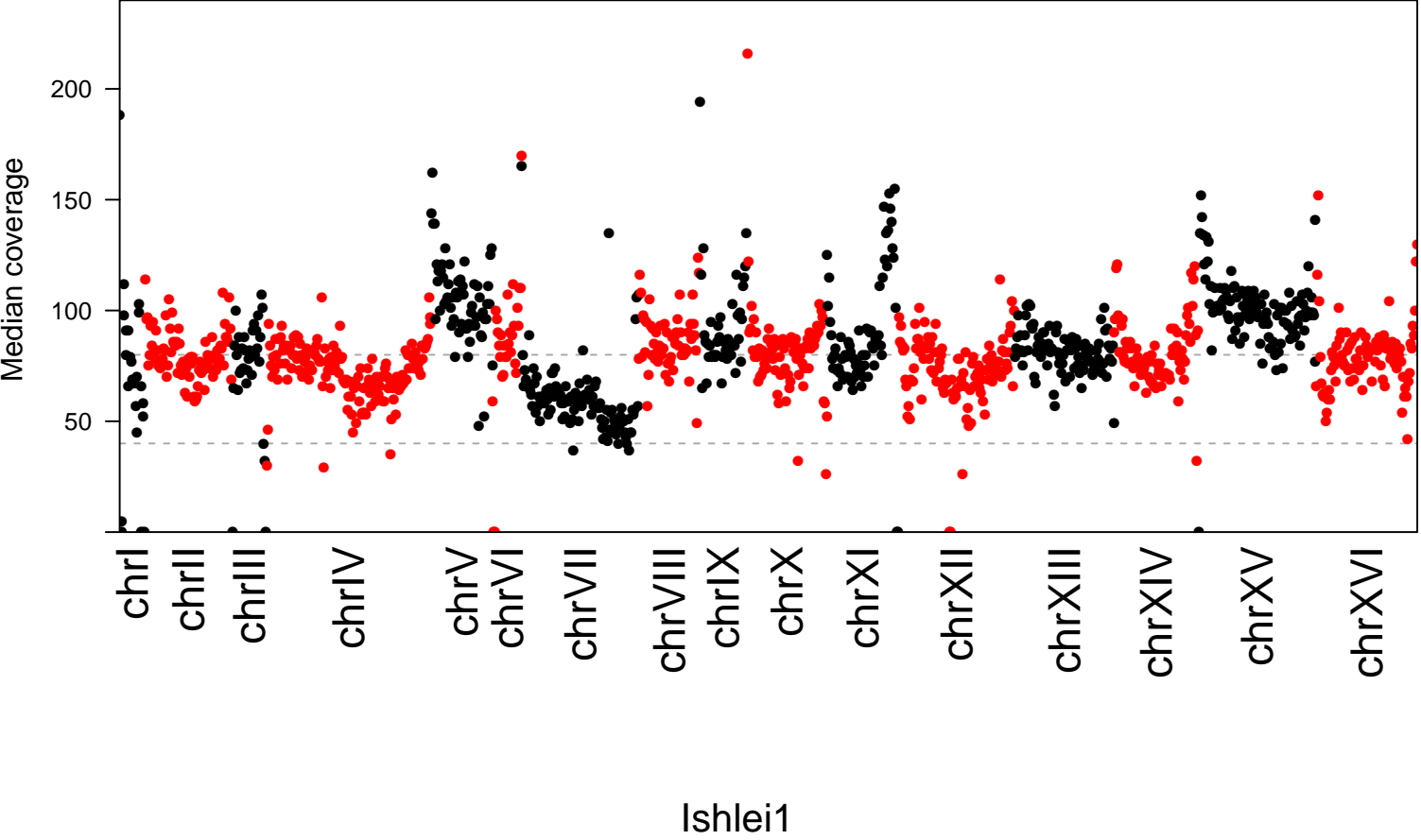
Supplementary Figure S2



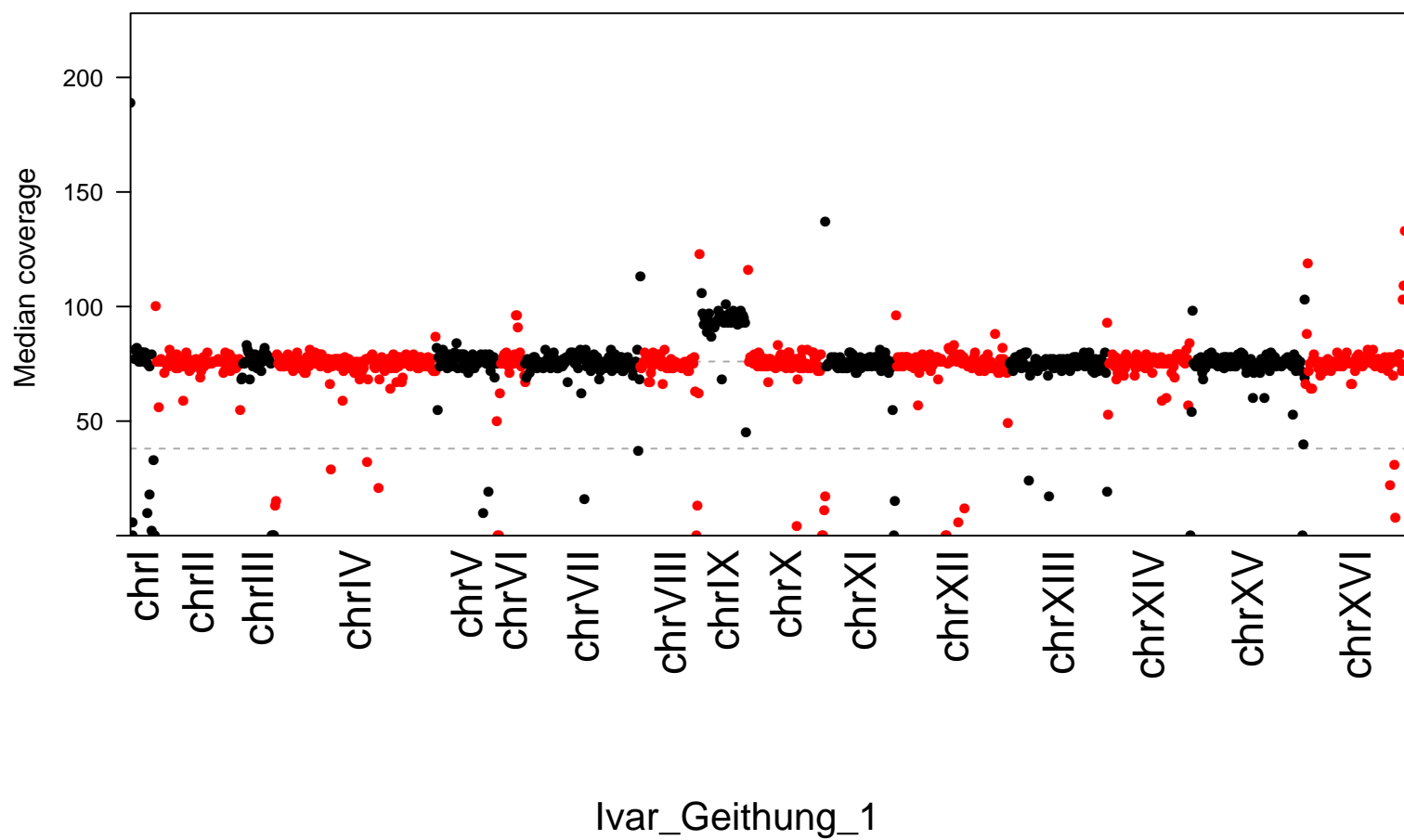
Supplementary Figure S2



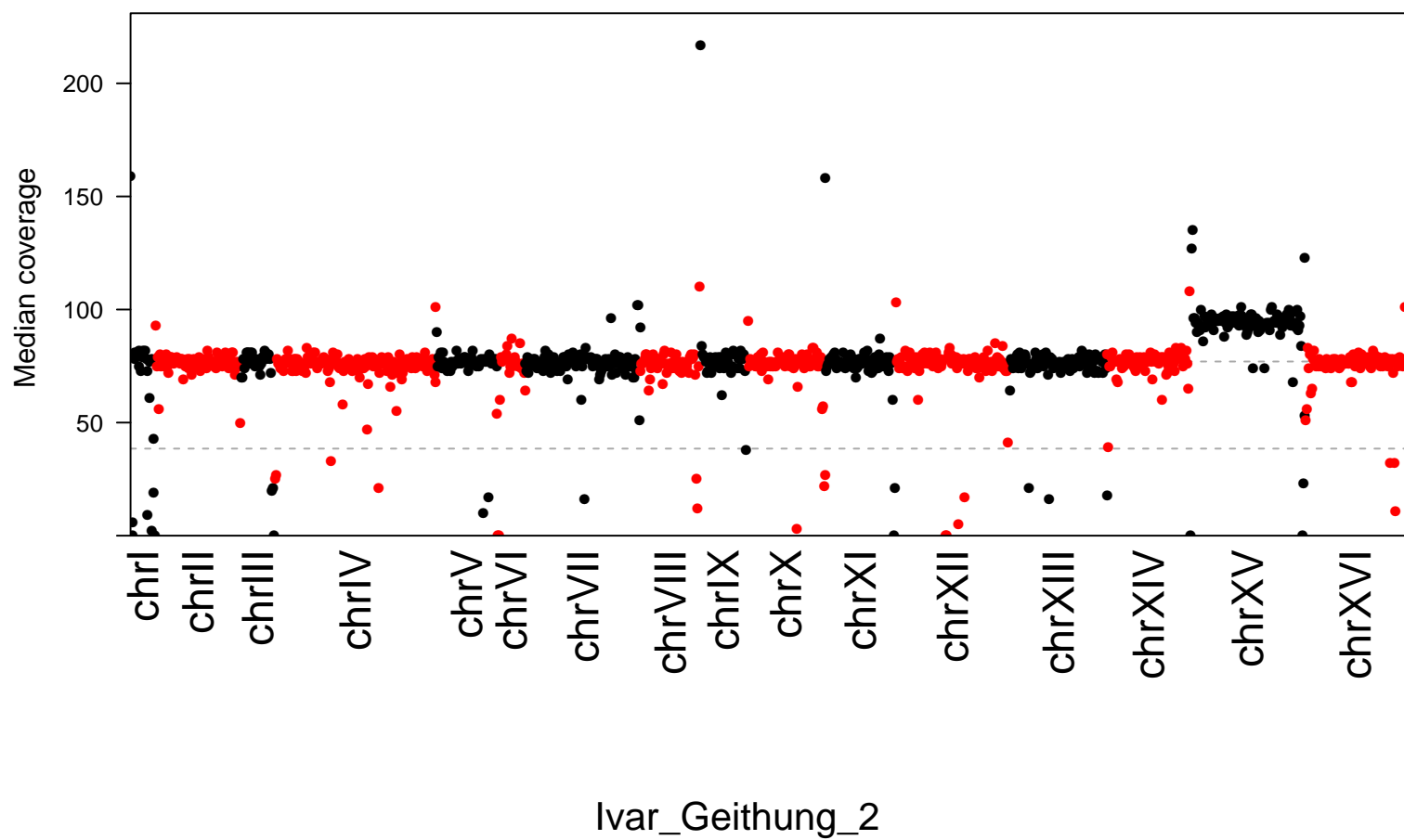
Supplementary Figure S2



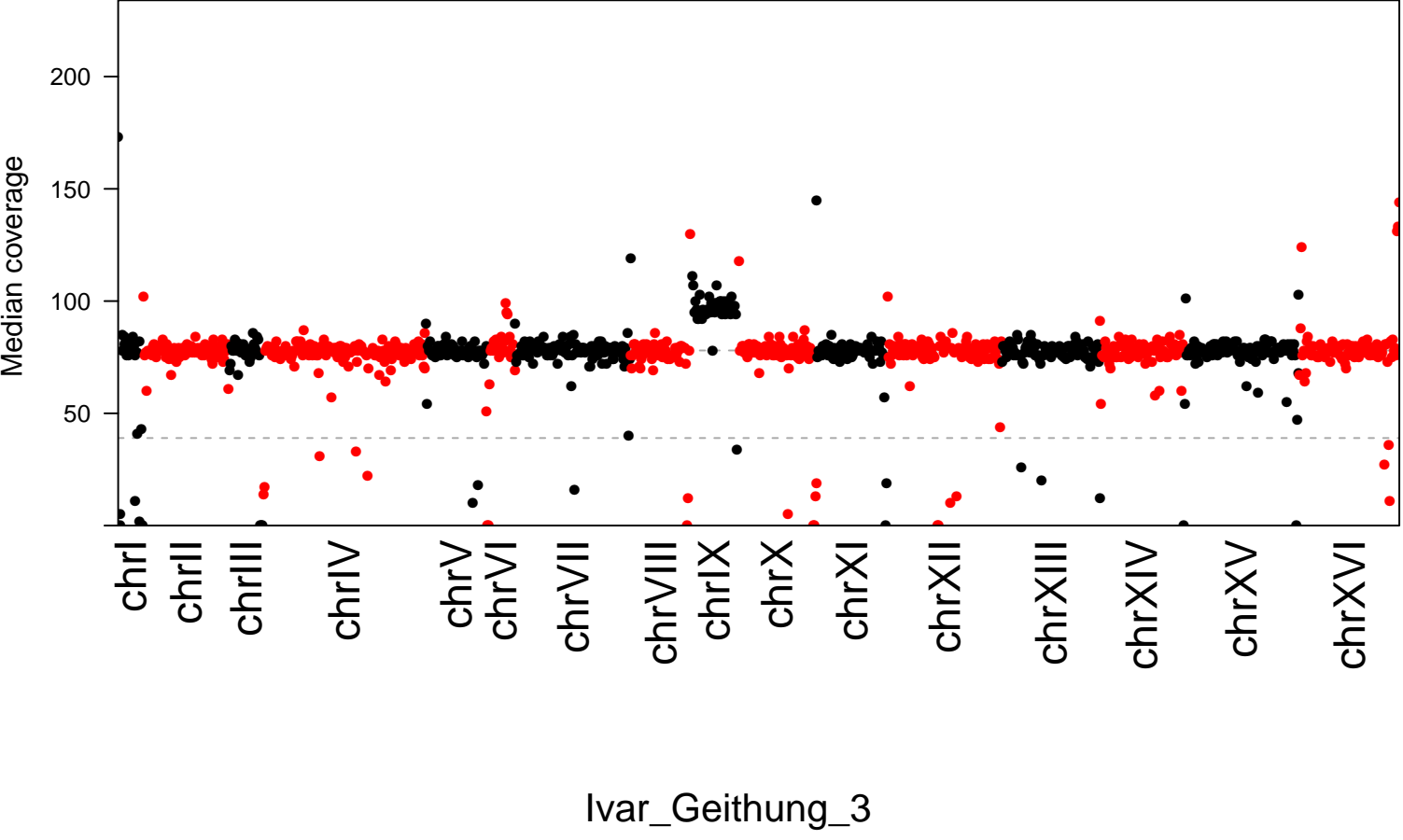
Supplementary Figure S2



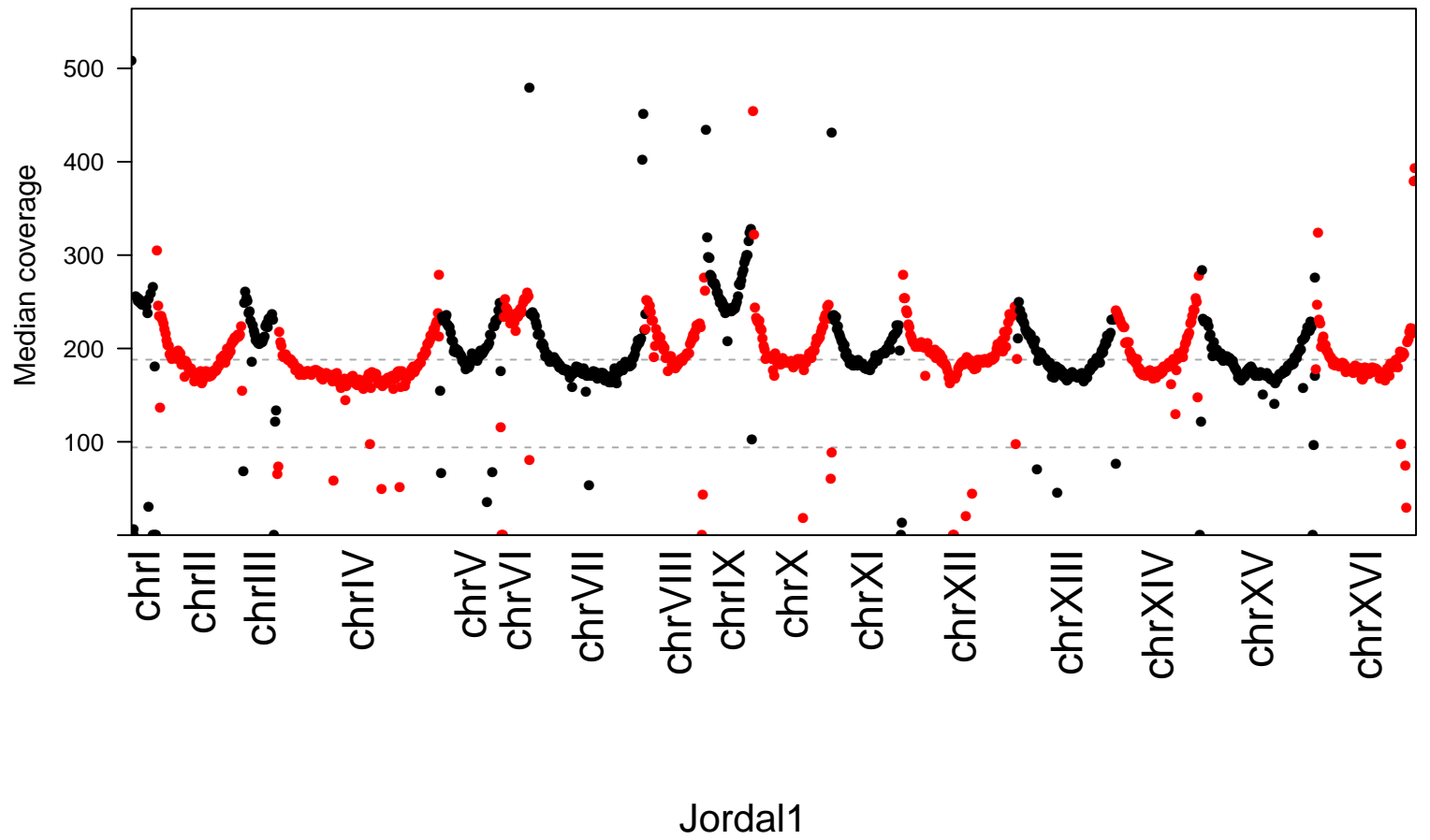
Supplementary Figure S2



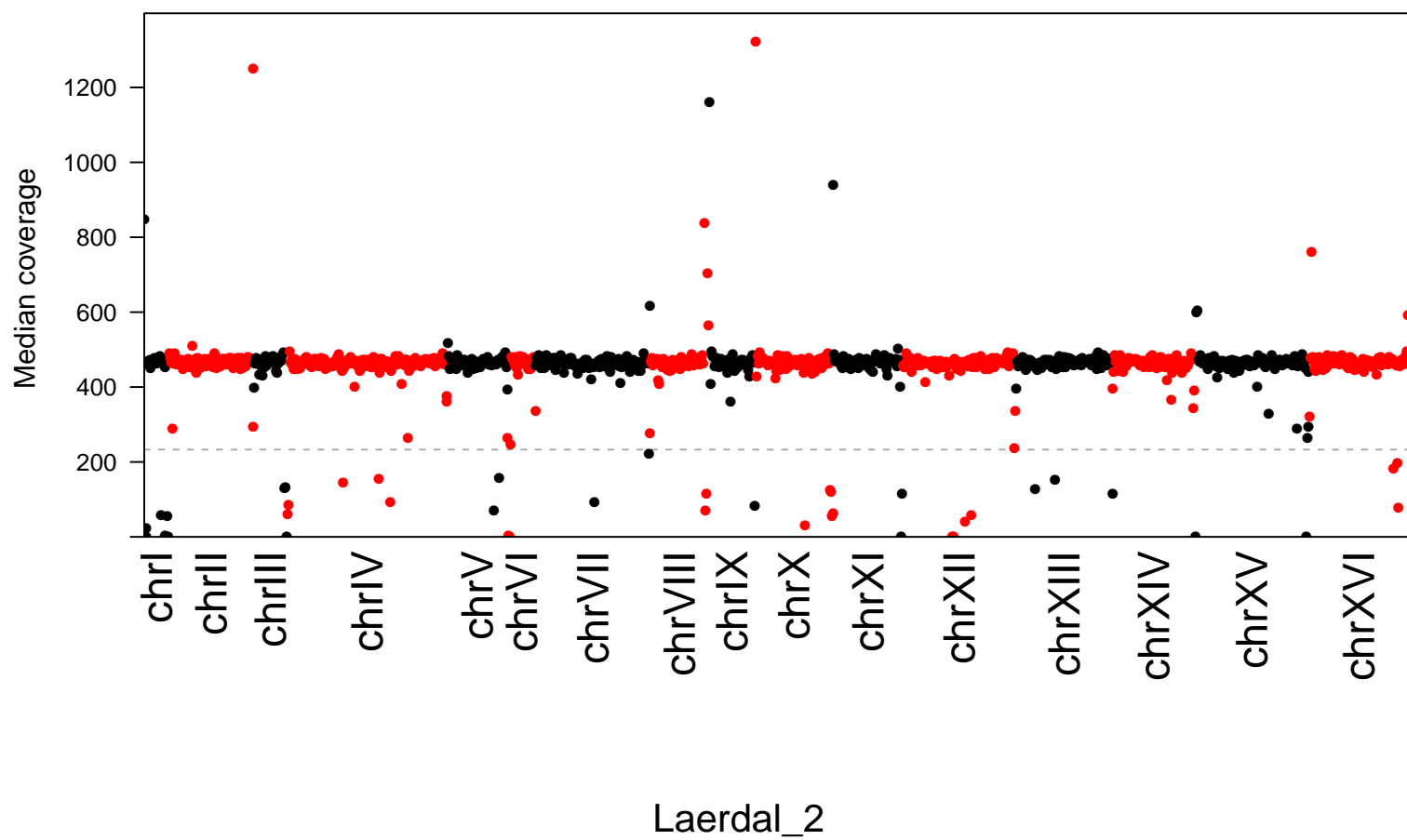
Supplementary Figure S2



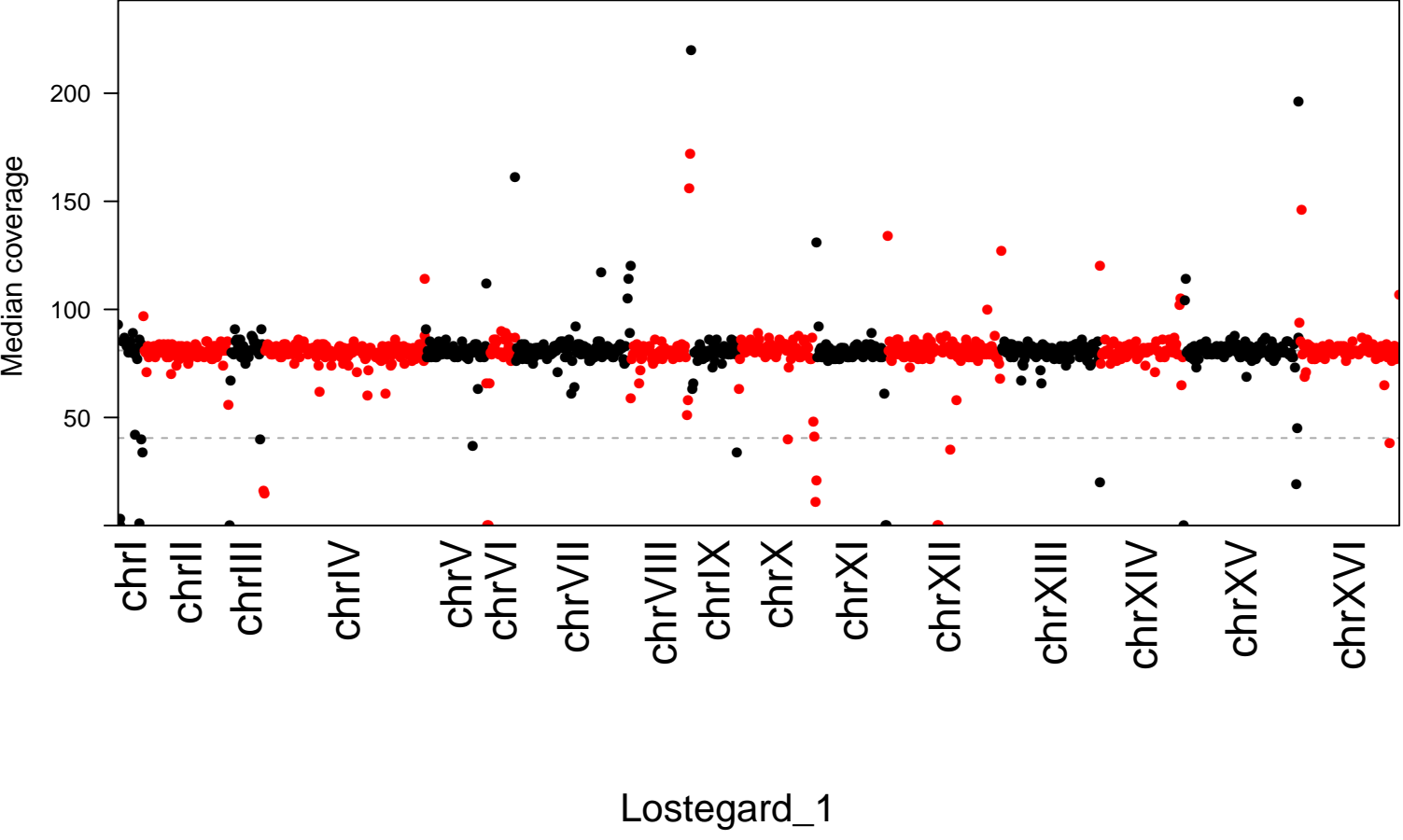
Supplementary Figure S2



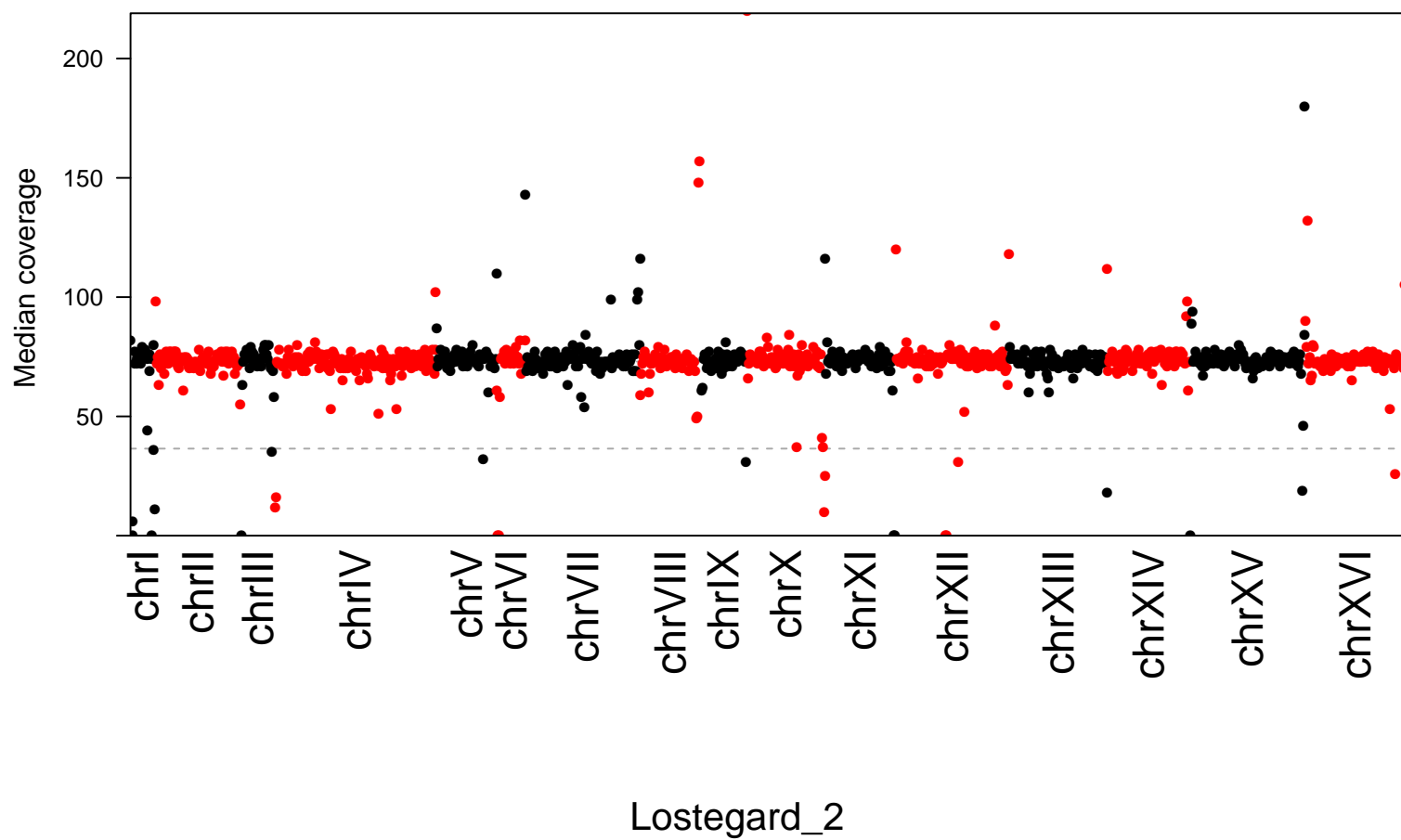
Supplementary Figure S2



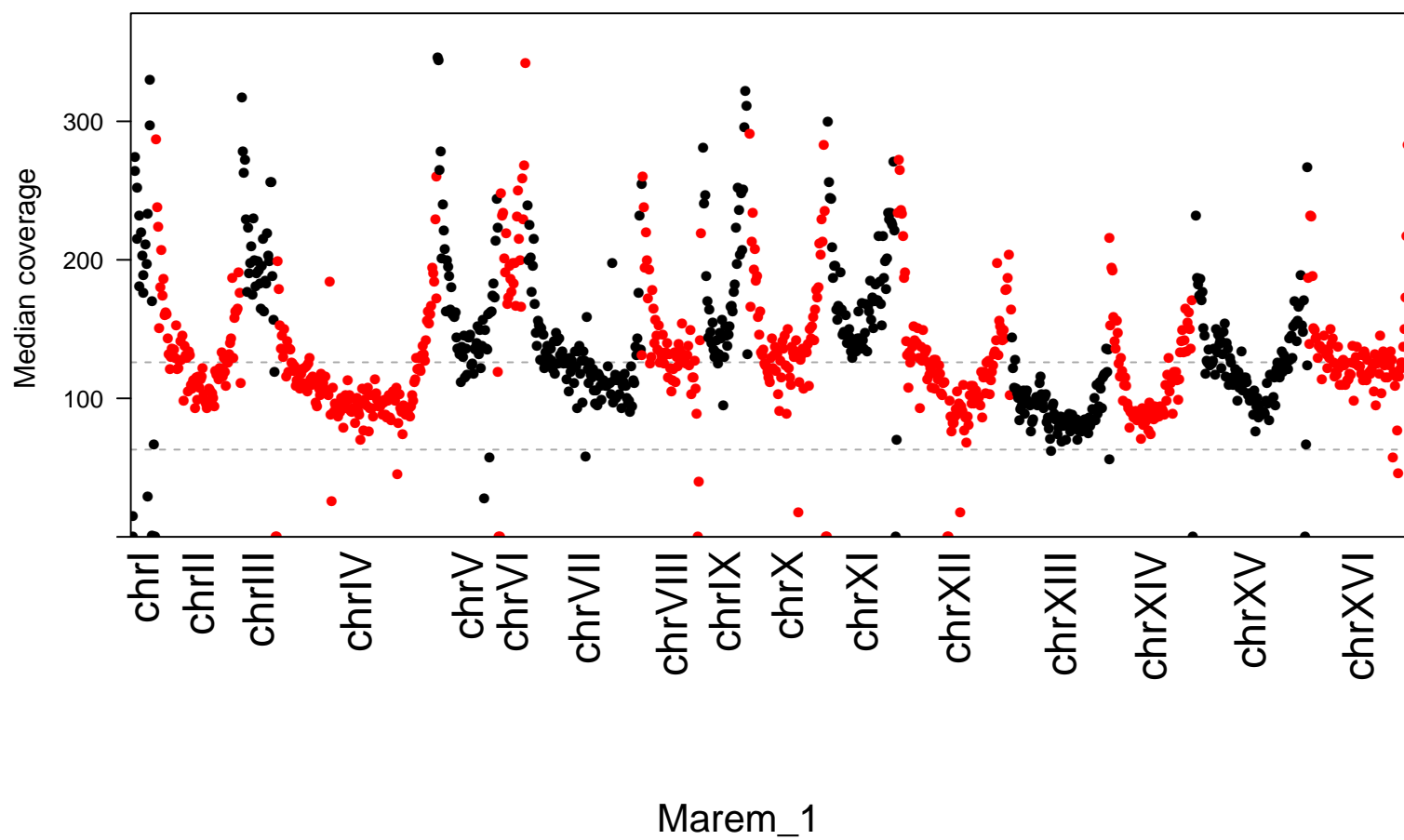
Supplementary Figure S2



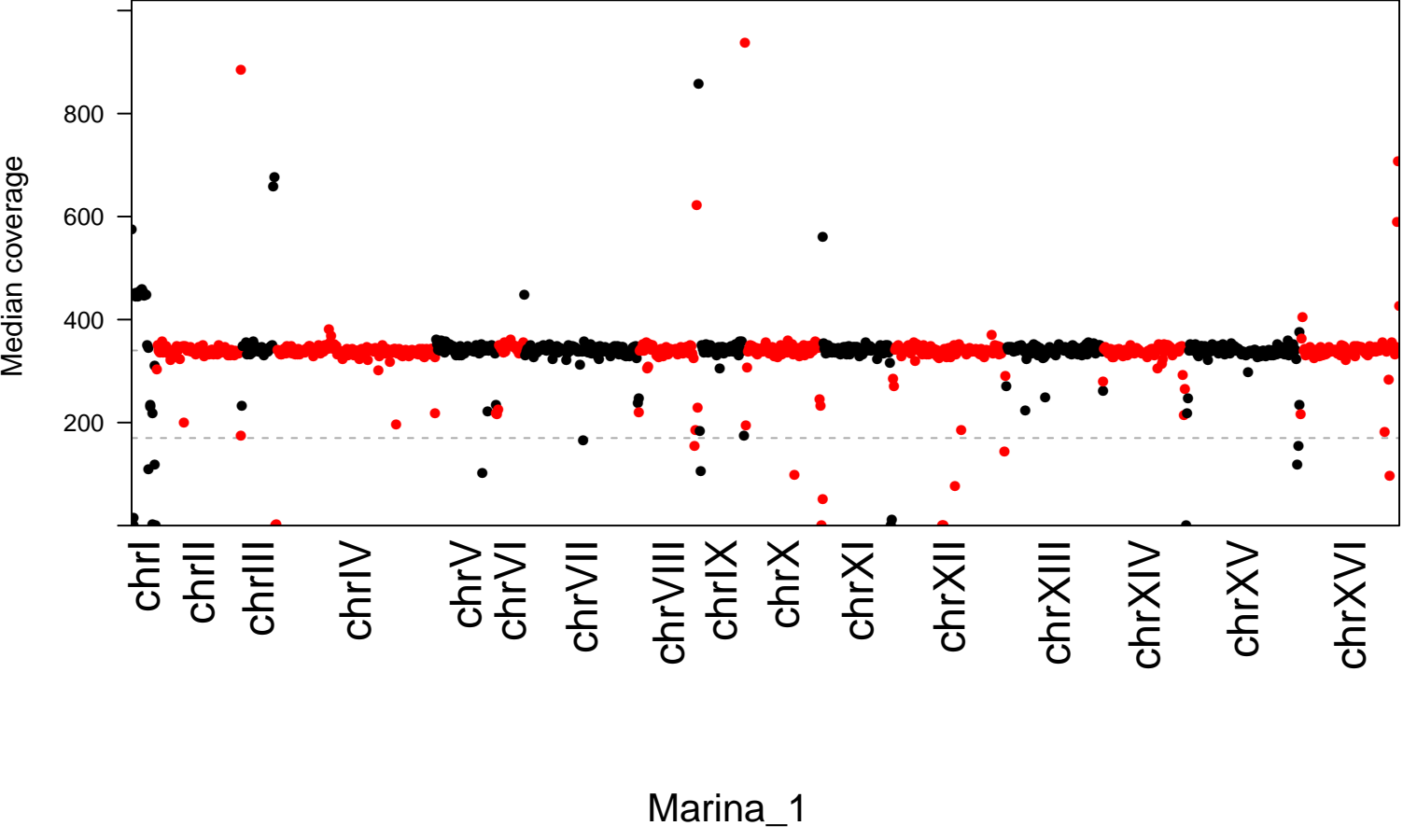
Supplementary Figure S2



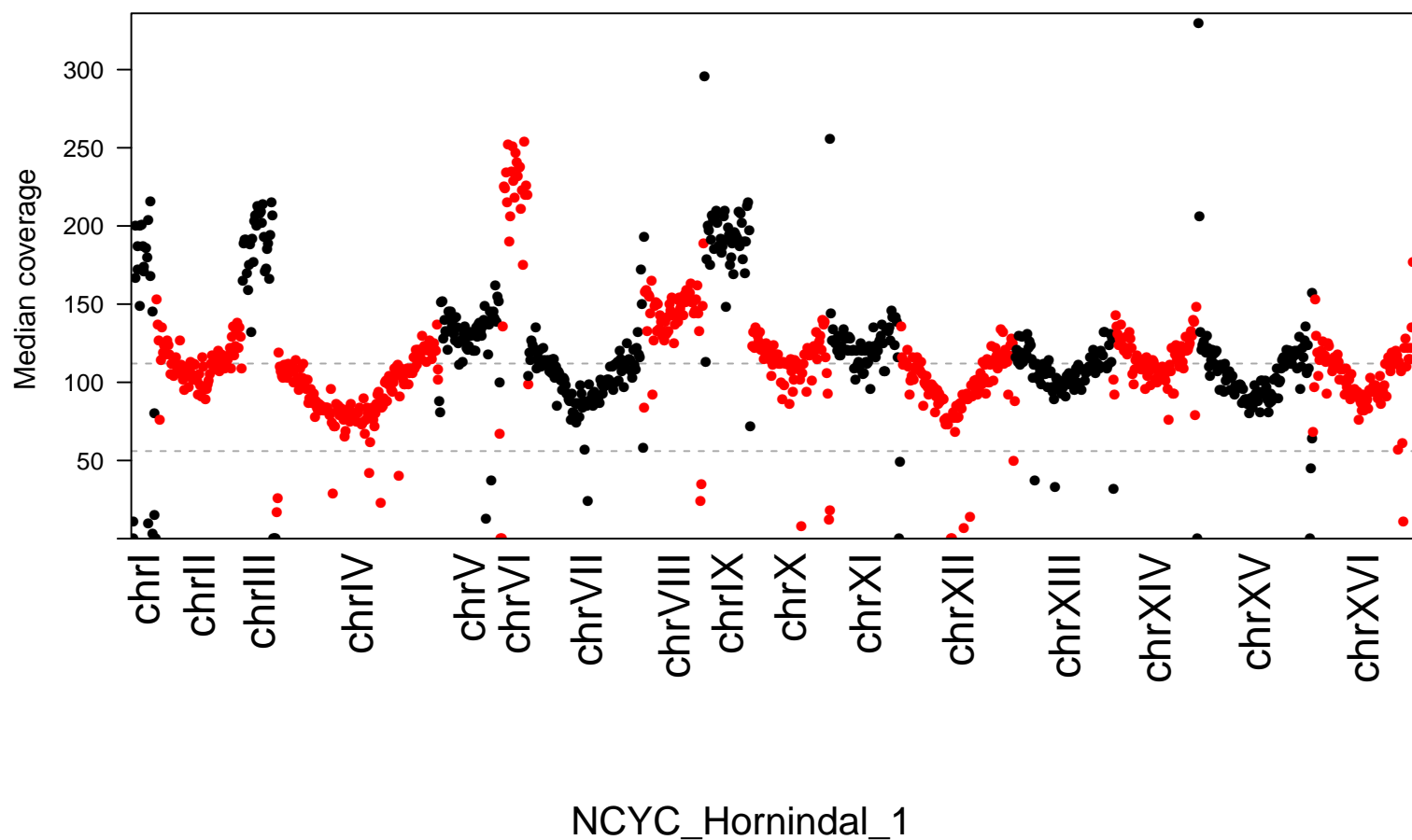
Supplementary Figure S2



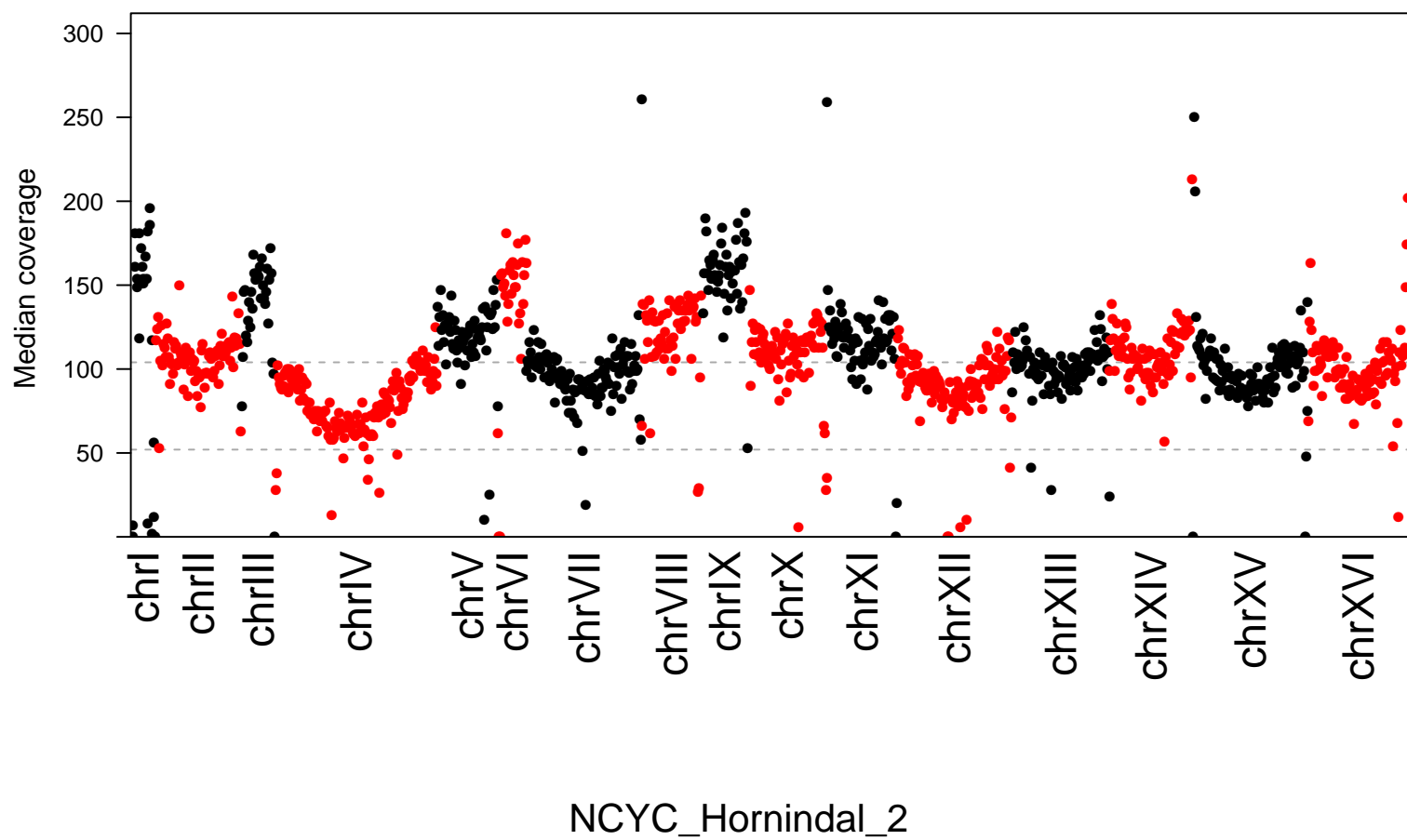
Supplementary Figure S2



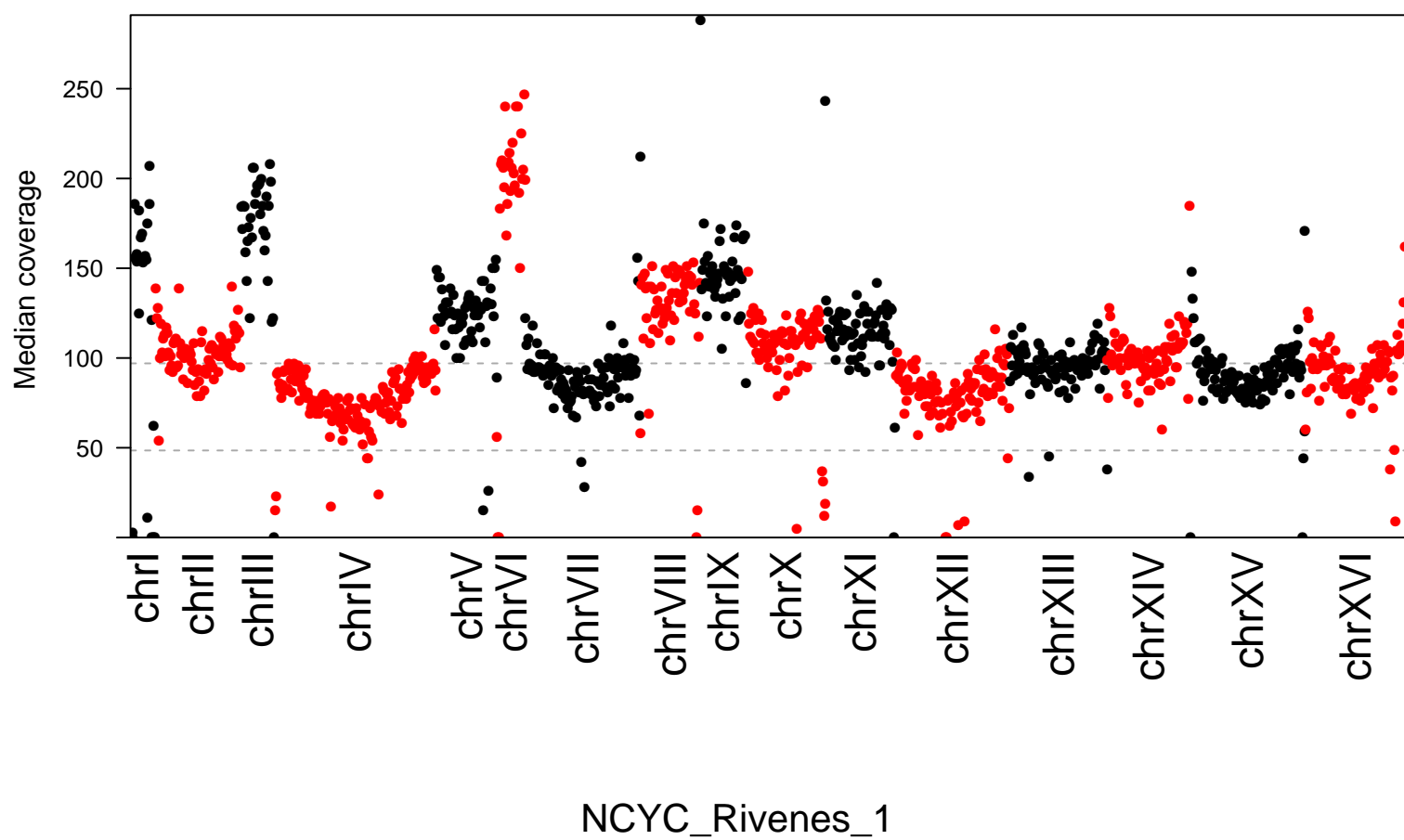
Supplementary Figure S2



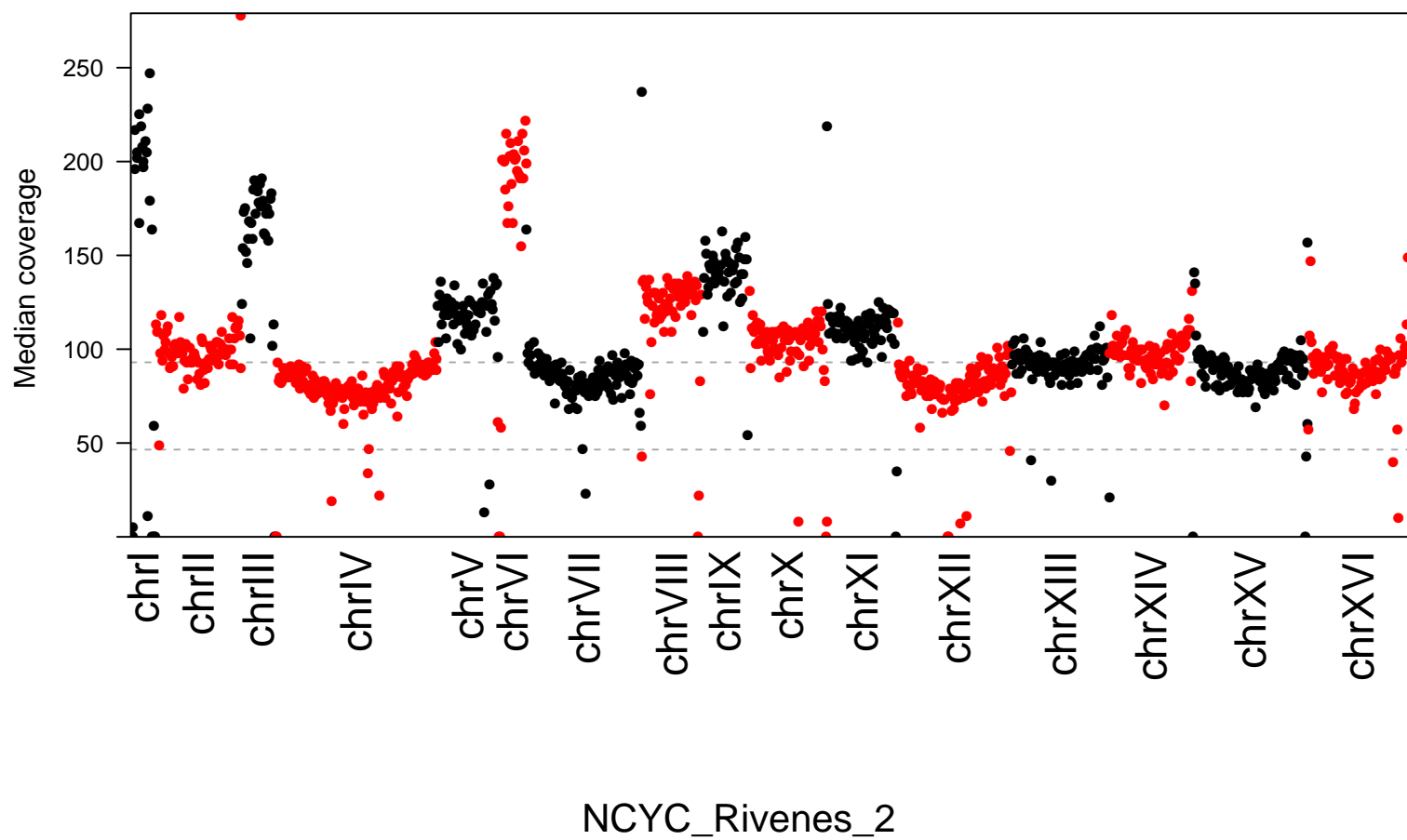
Supplementary Figure S2



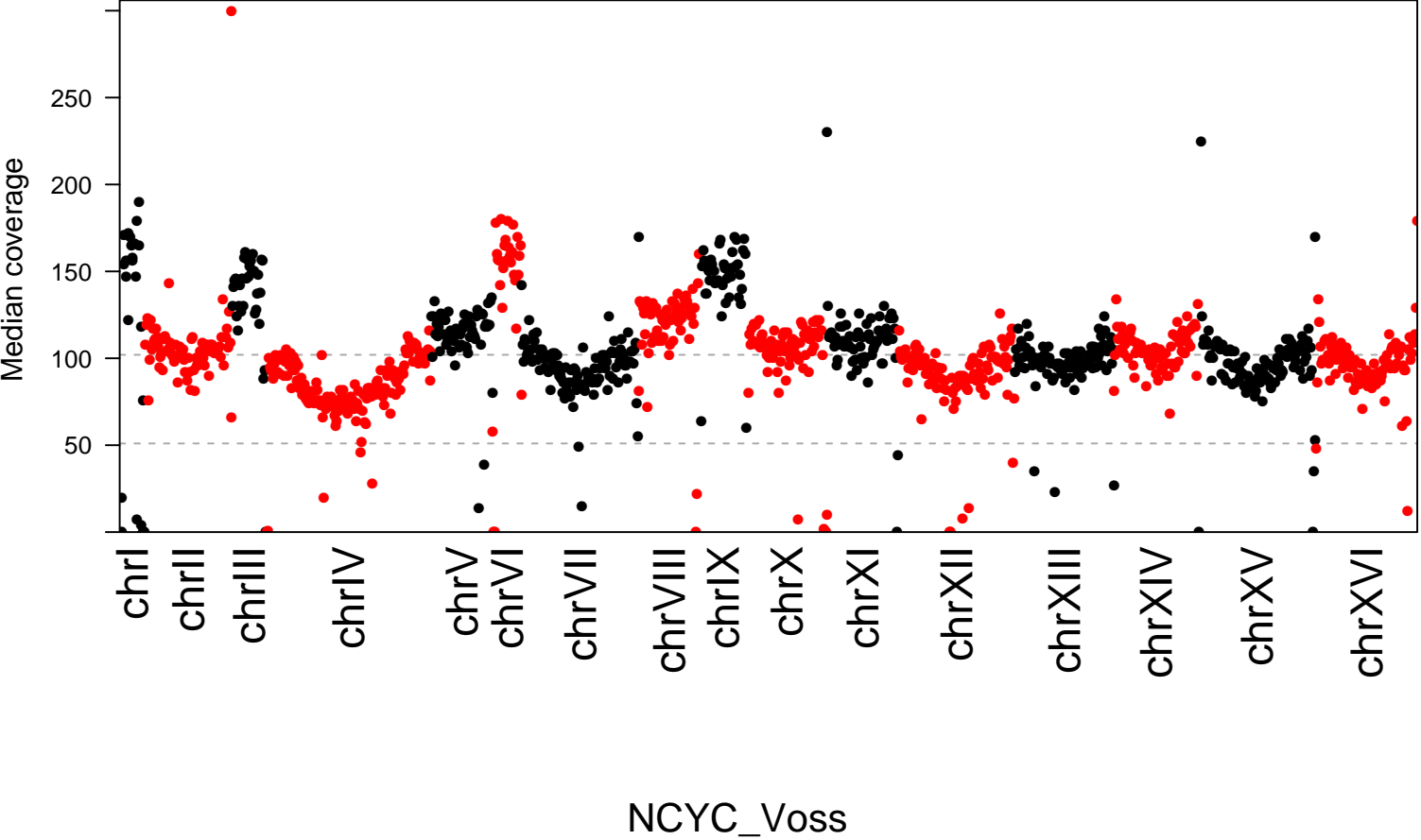
Supplementary Figure S2



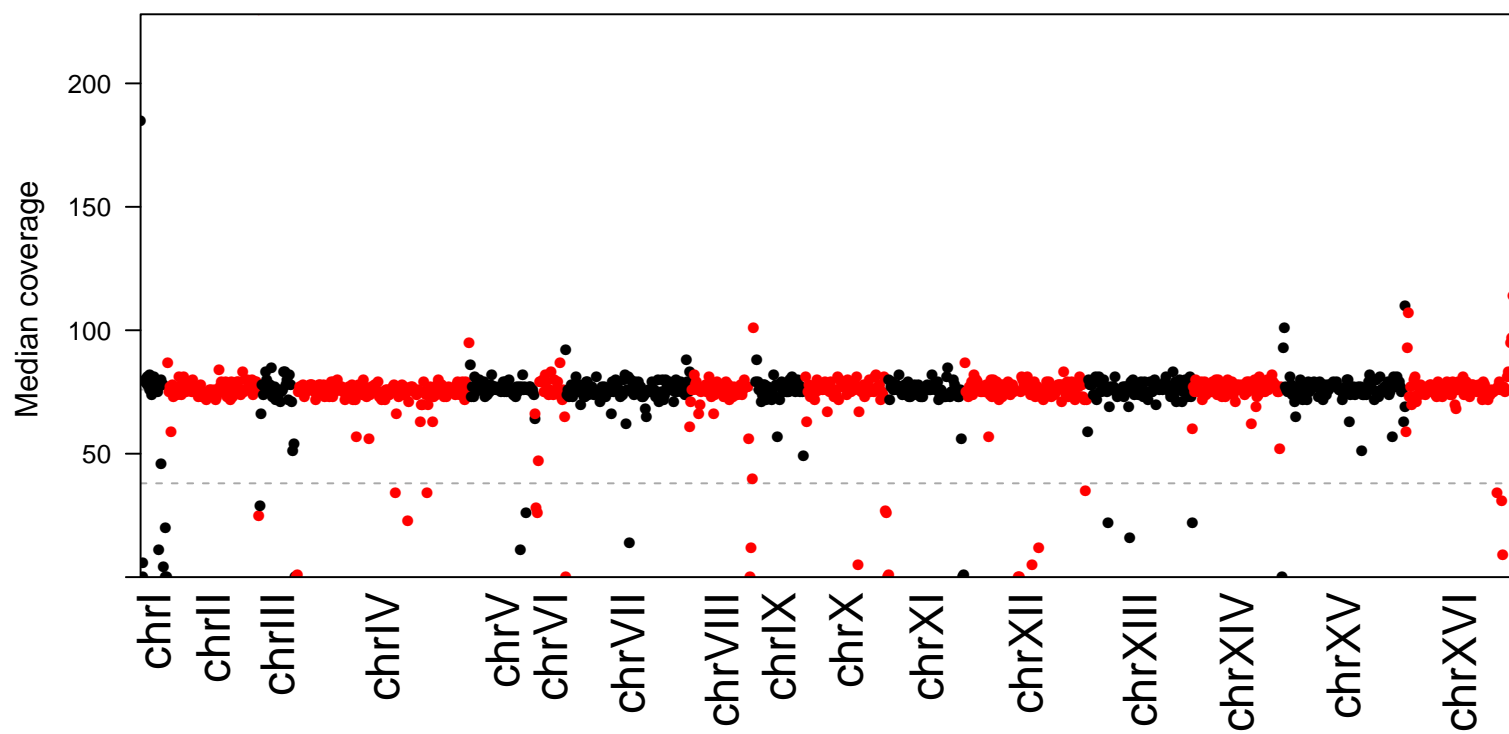
Supplementary Figure S2



Supplementary Figure S2

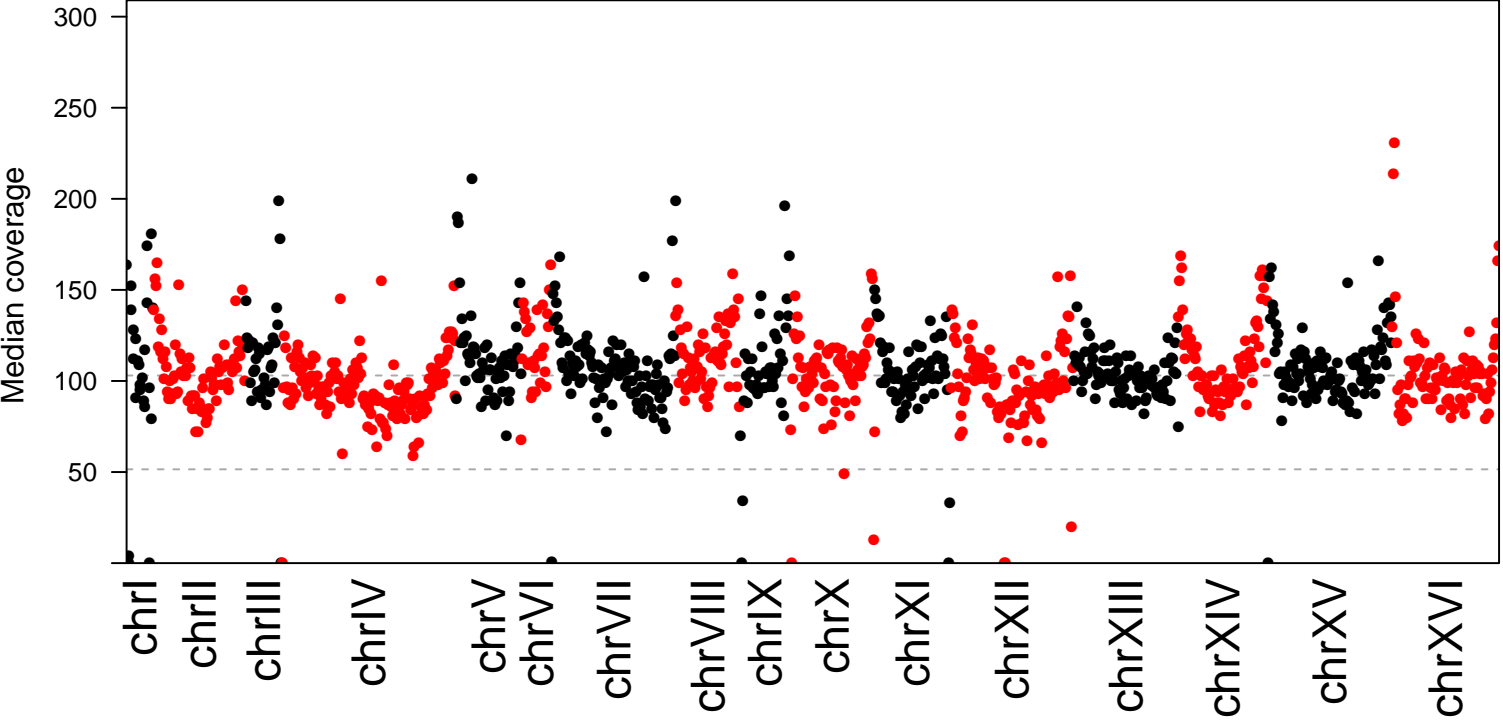


Supplementary Figure S2



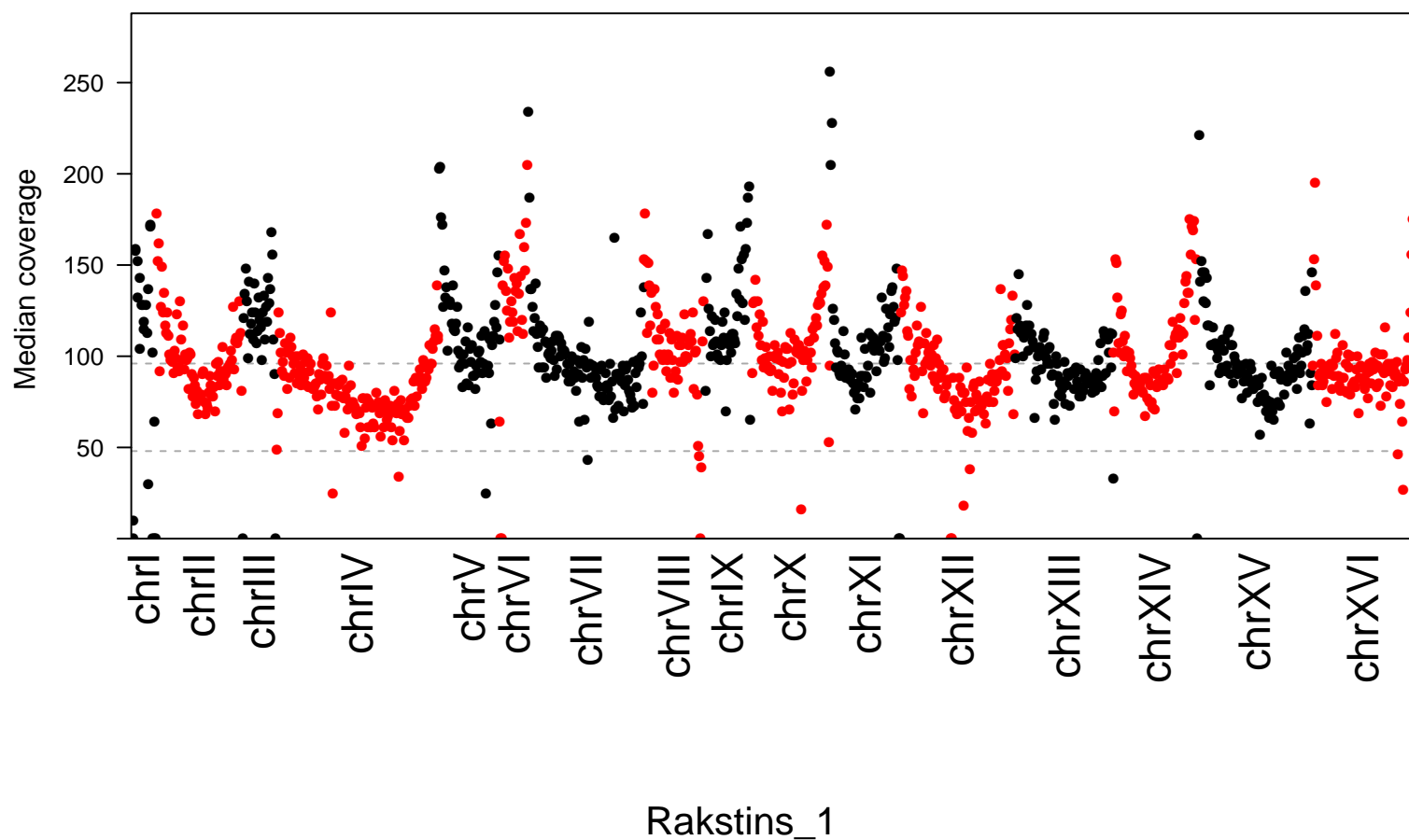
Pundurs_2

Supplementary Figure S2

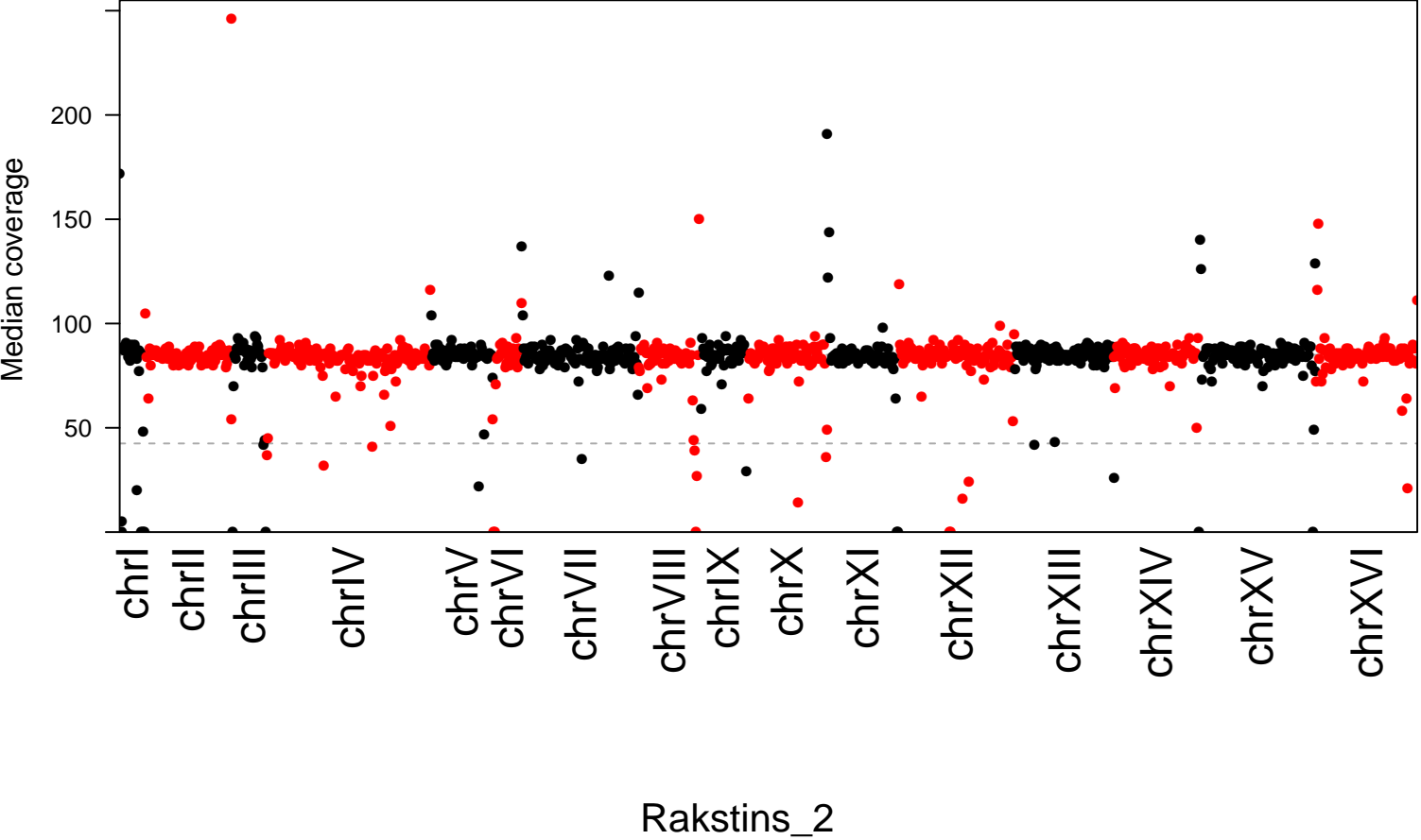


Pundurs1

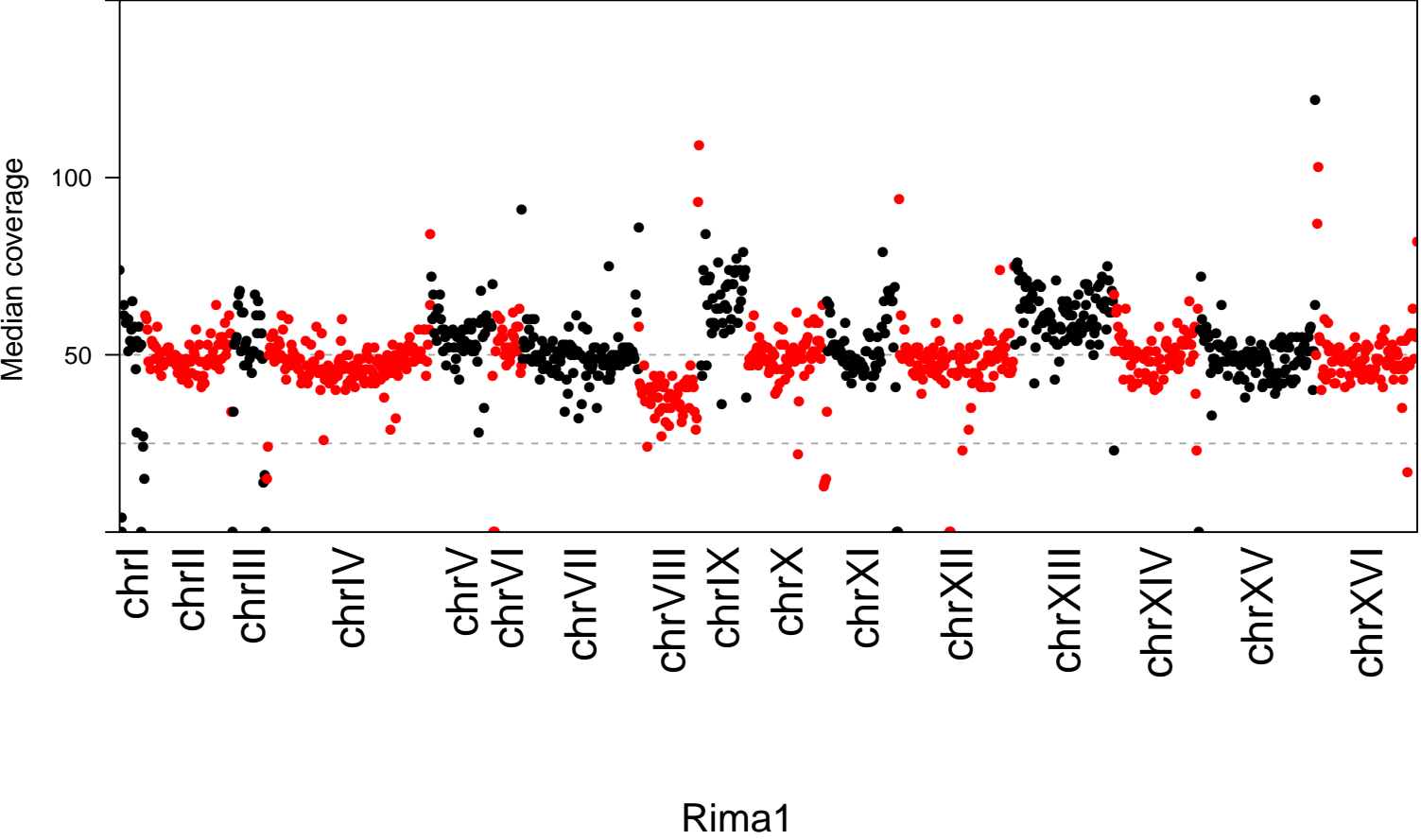
Supplementary Figure S2



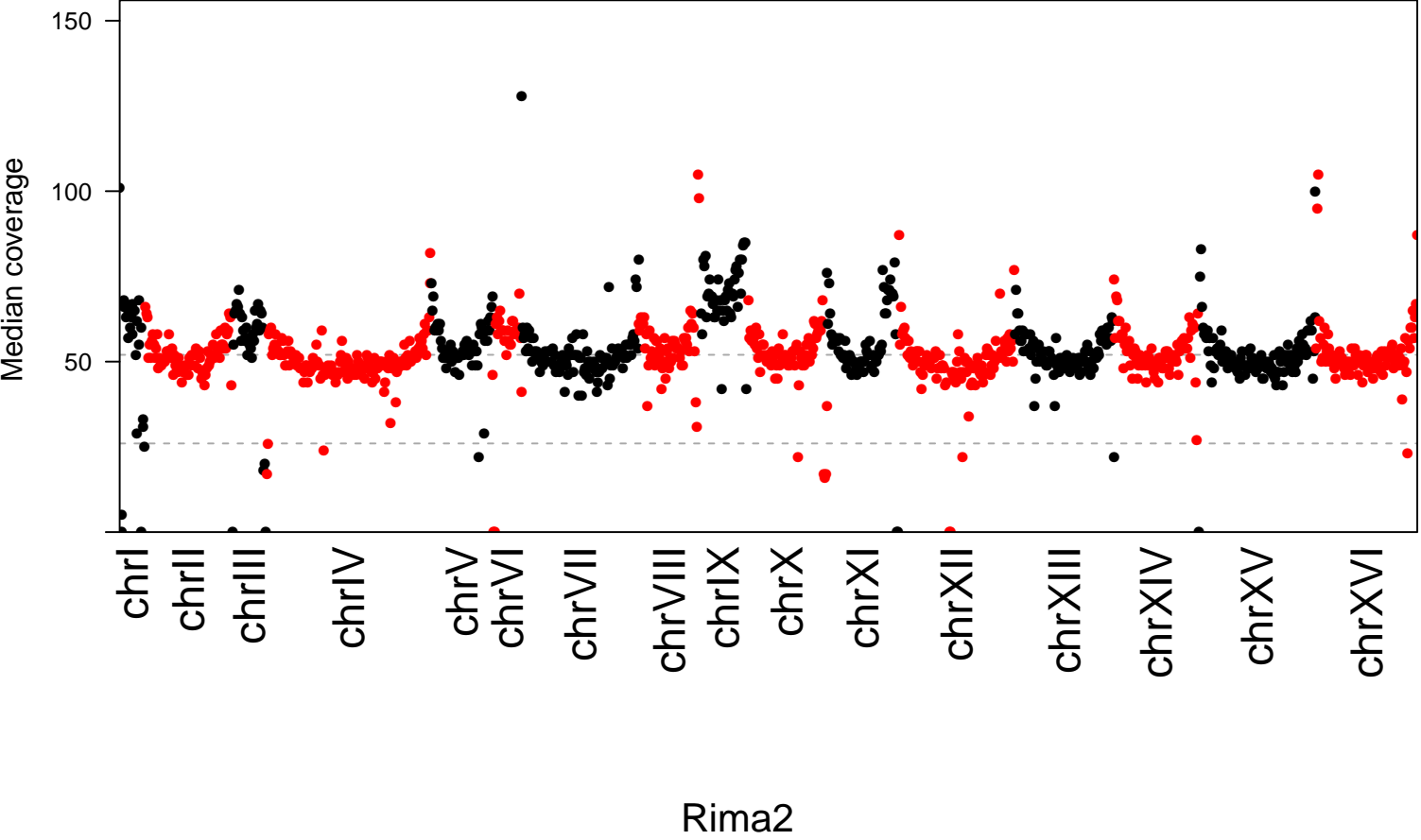
Supplementary Figure S2



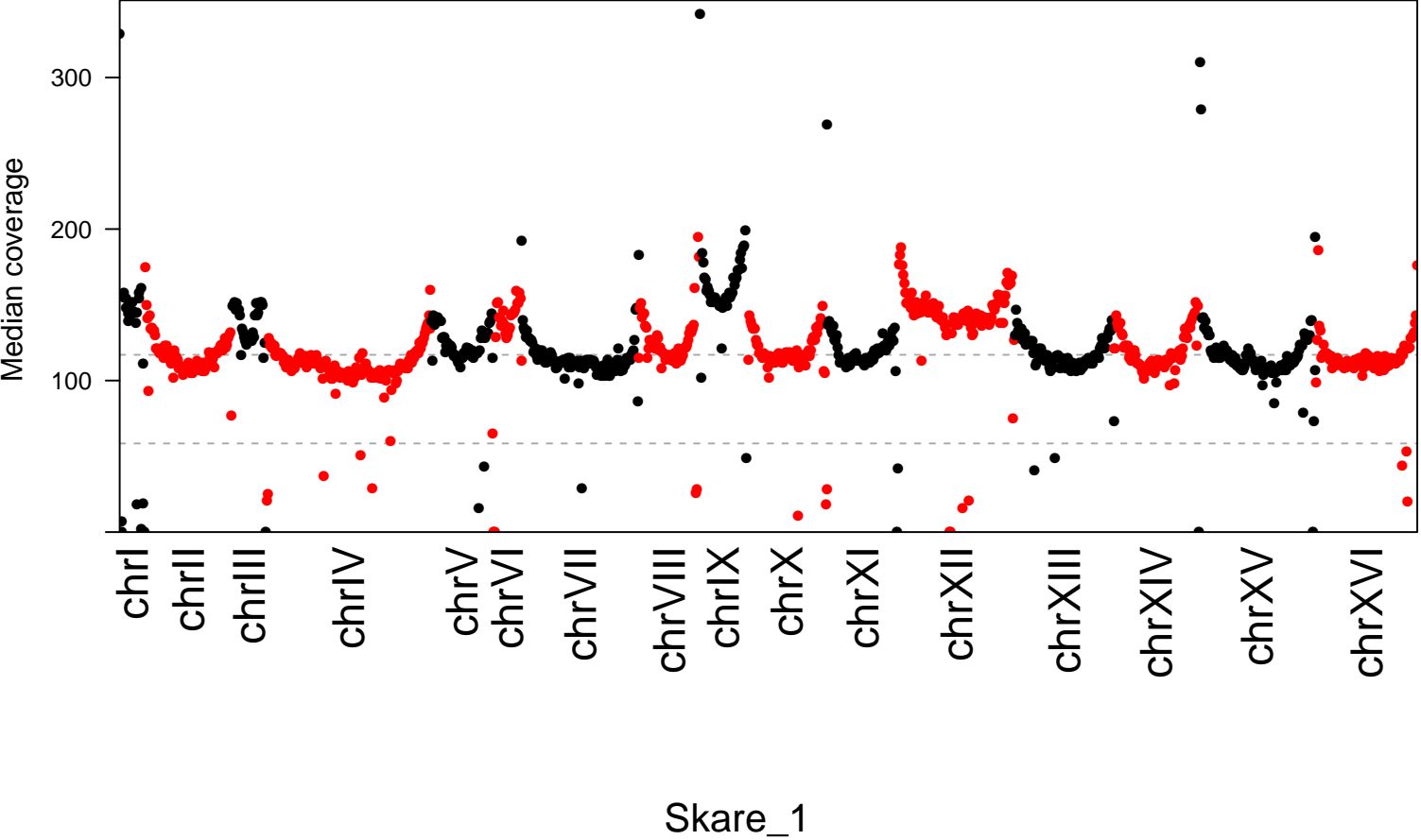
Supplementary Figure S2



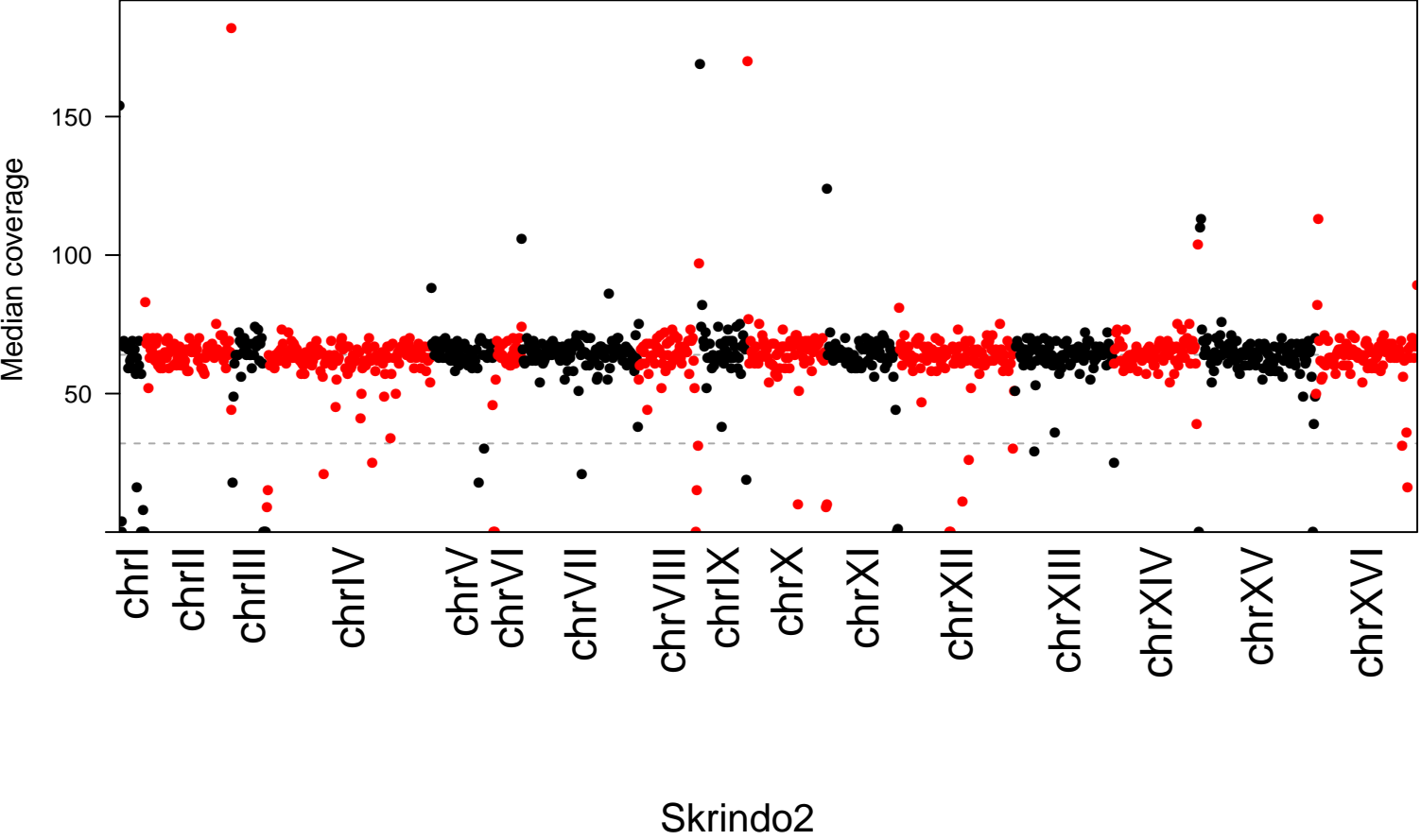
Supplementary Figure S2



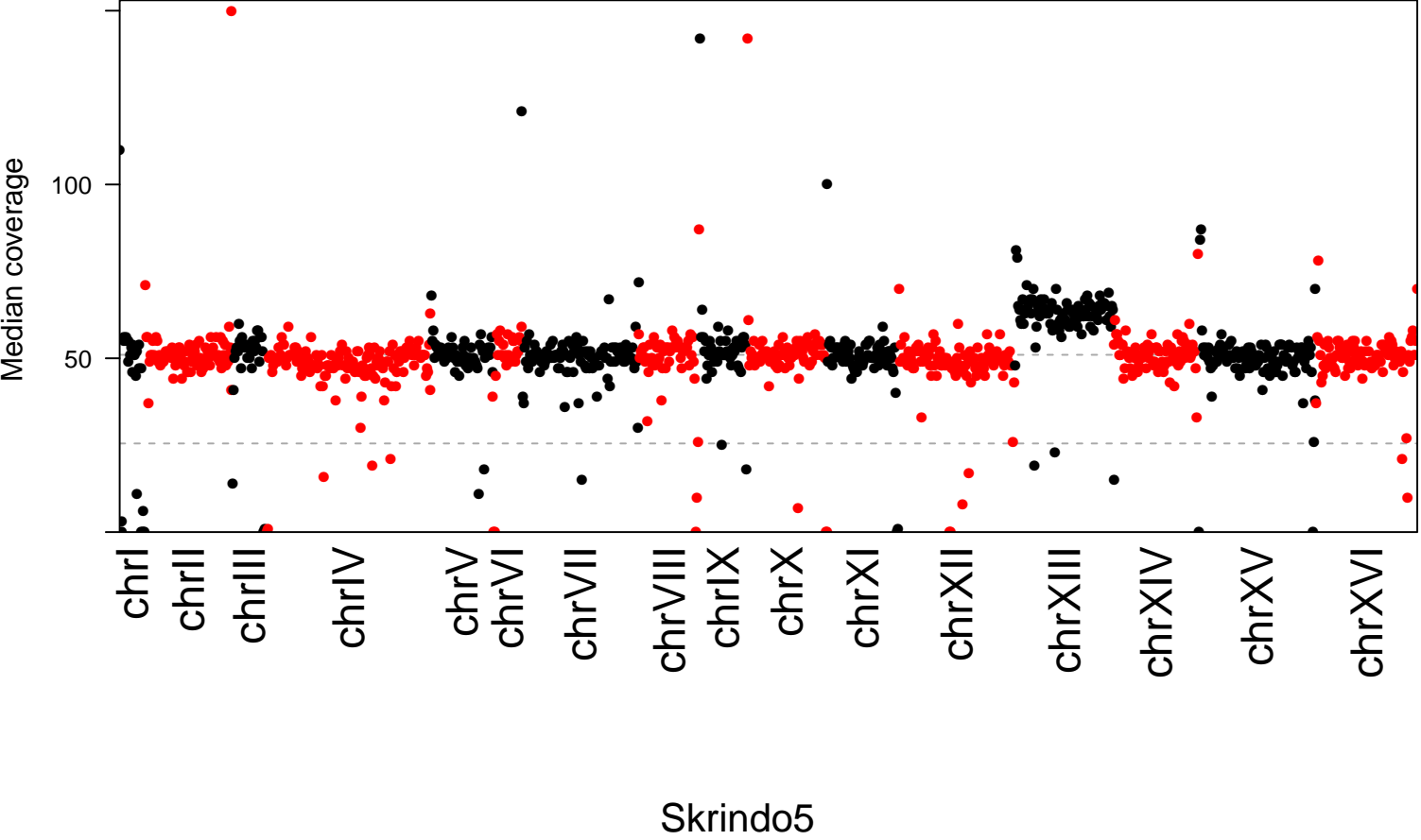
Supplementary Figure S2



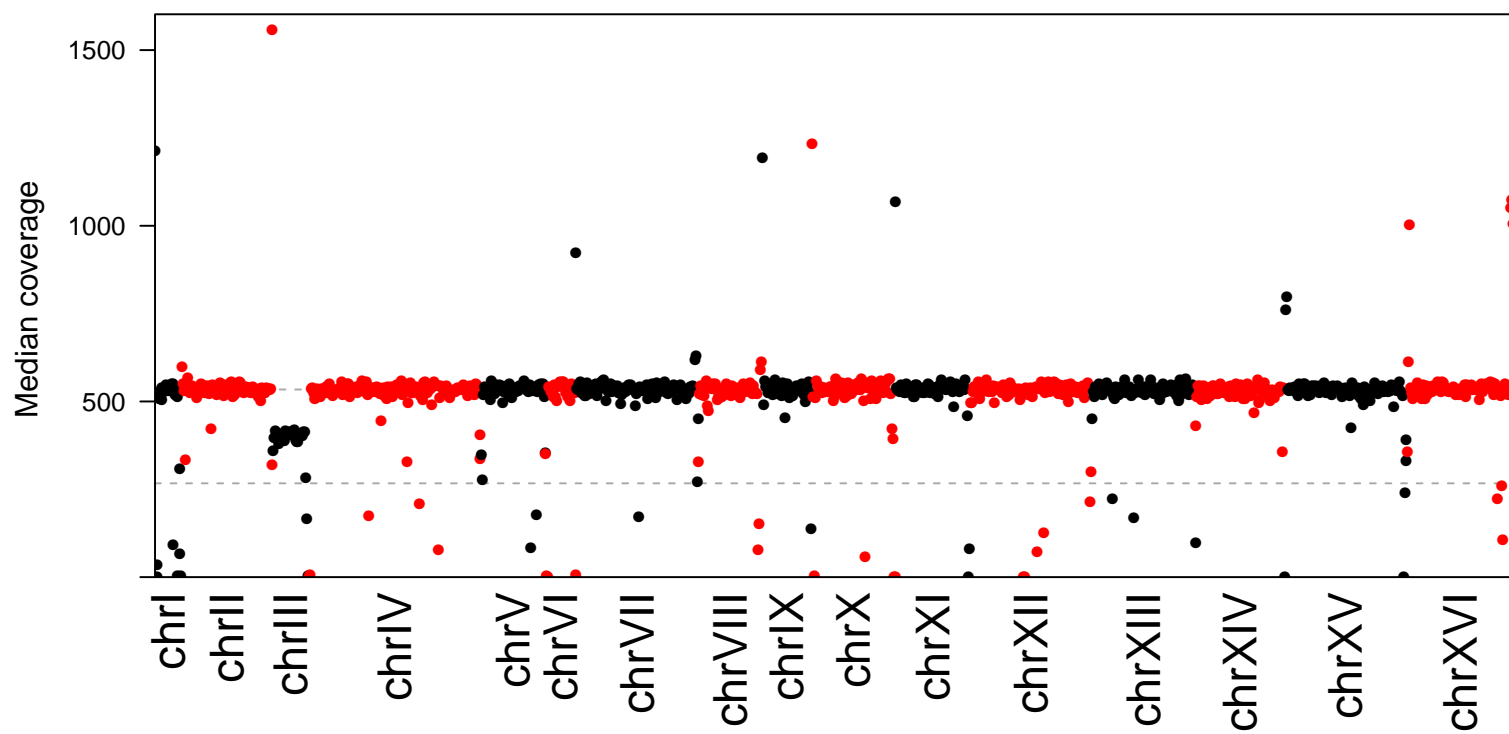
Supplementary Figure S2



Supplementary Figure S2

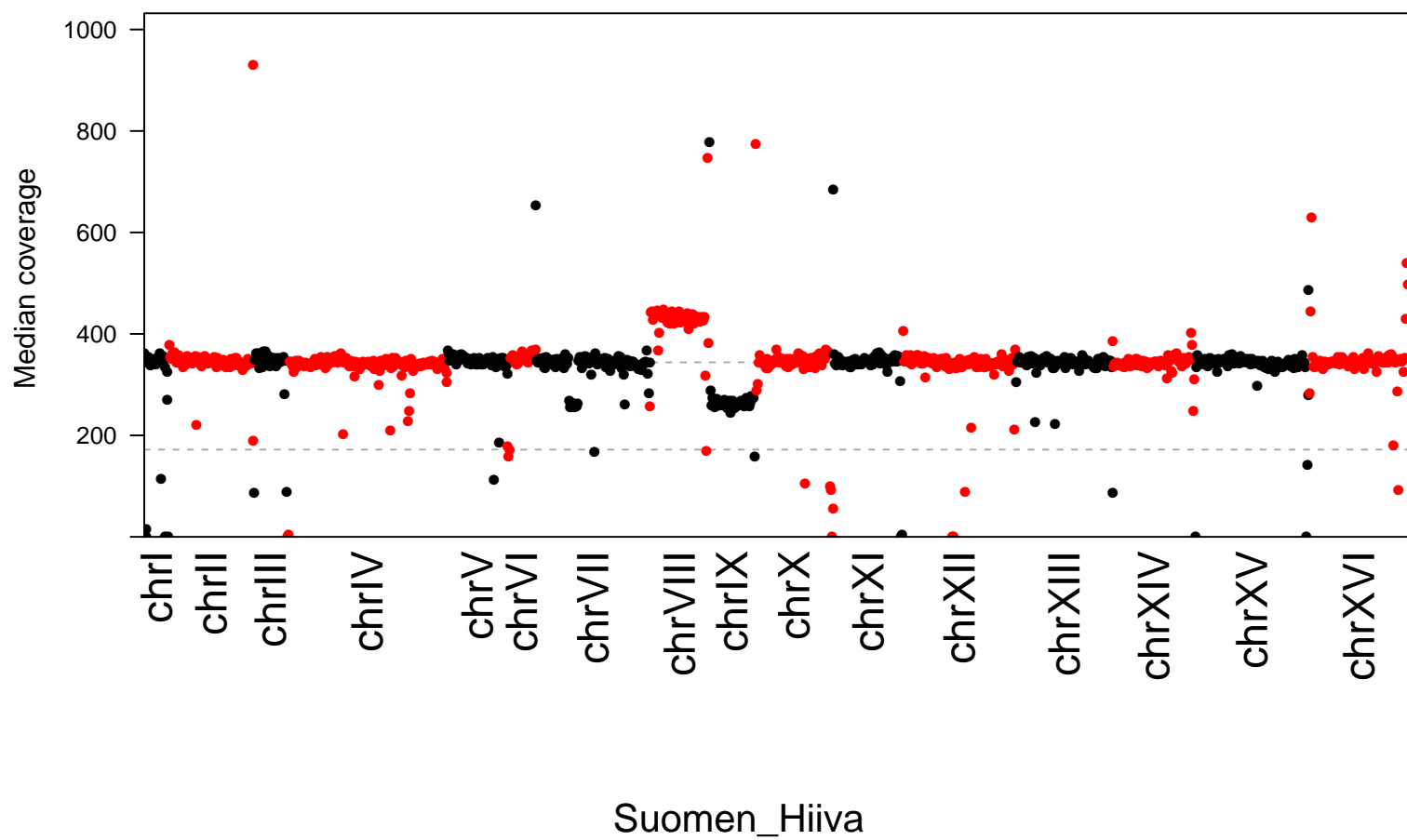


Supplementary Figure S2

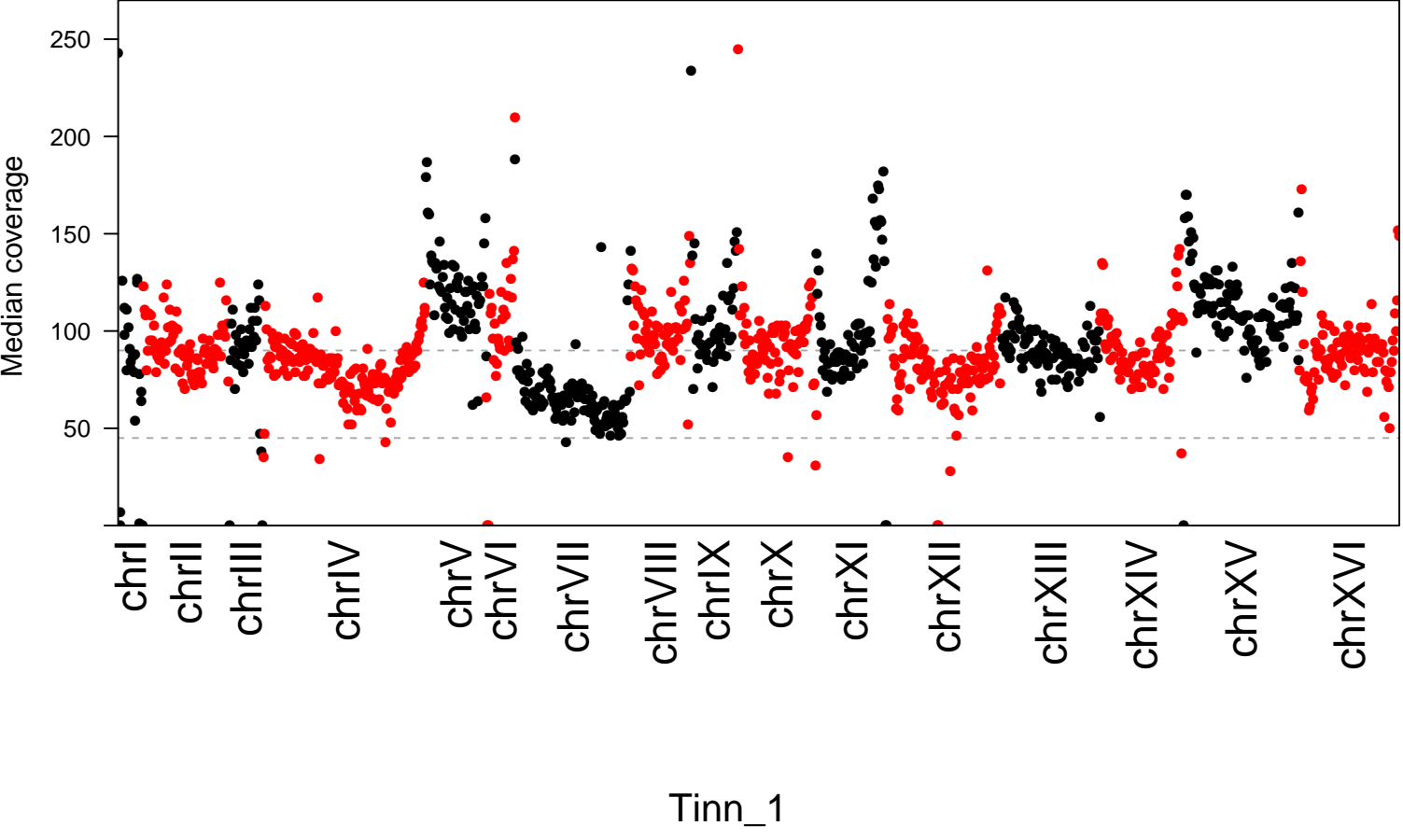


Stordal_Ebbegarden_1

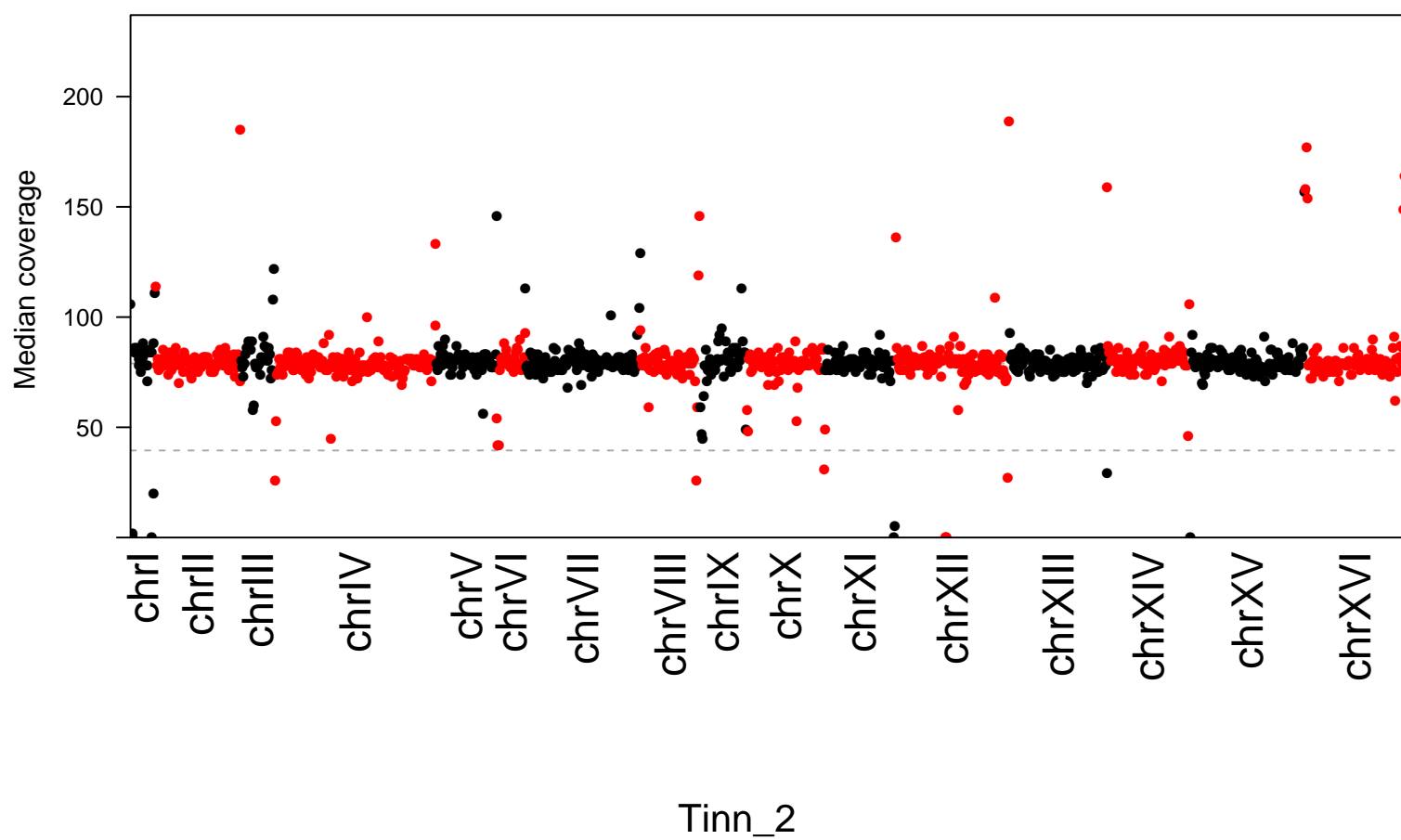
Supplementary Figure S2



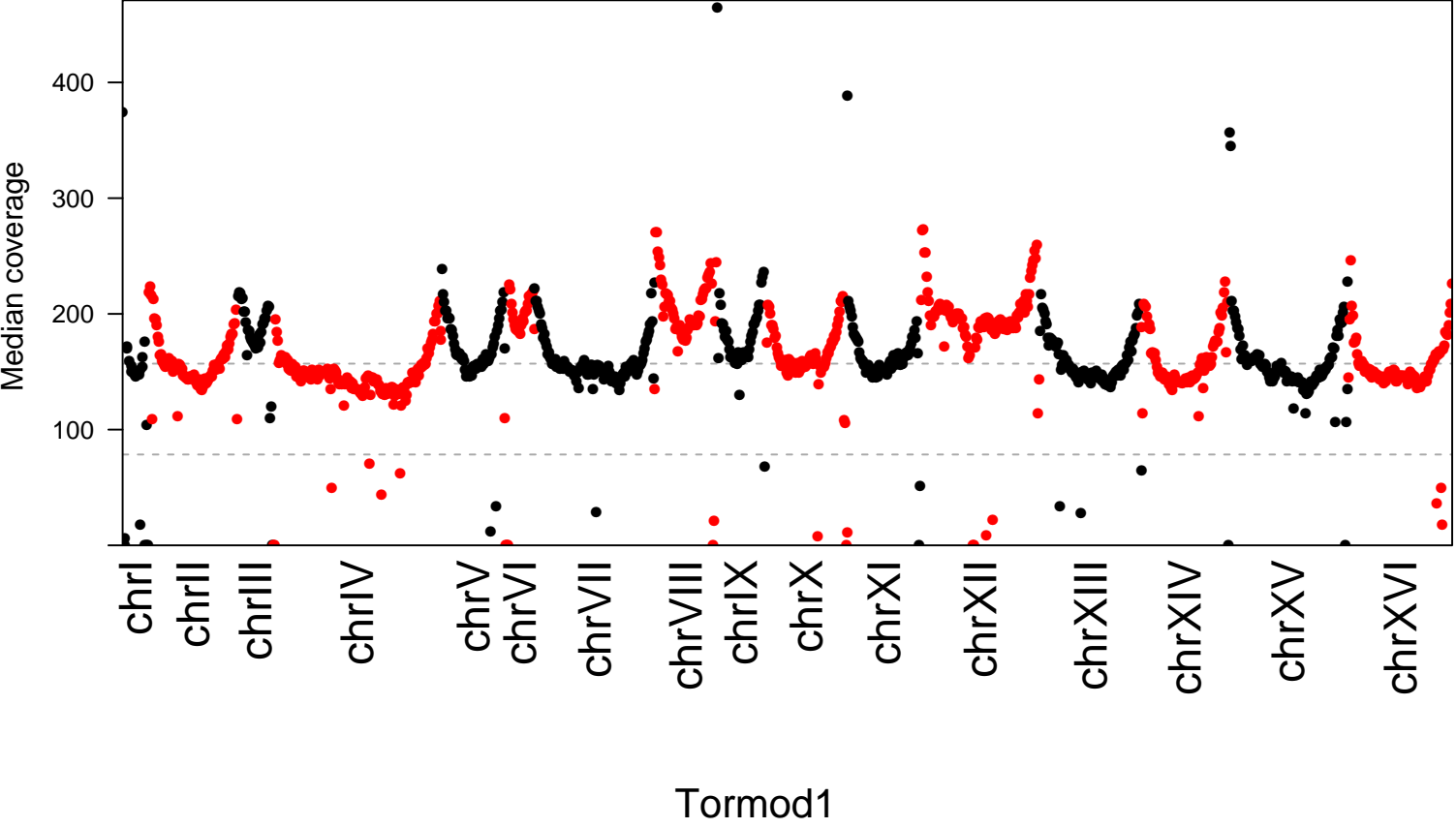
Supplementary Figure S2



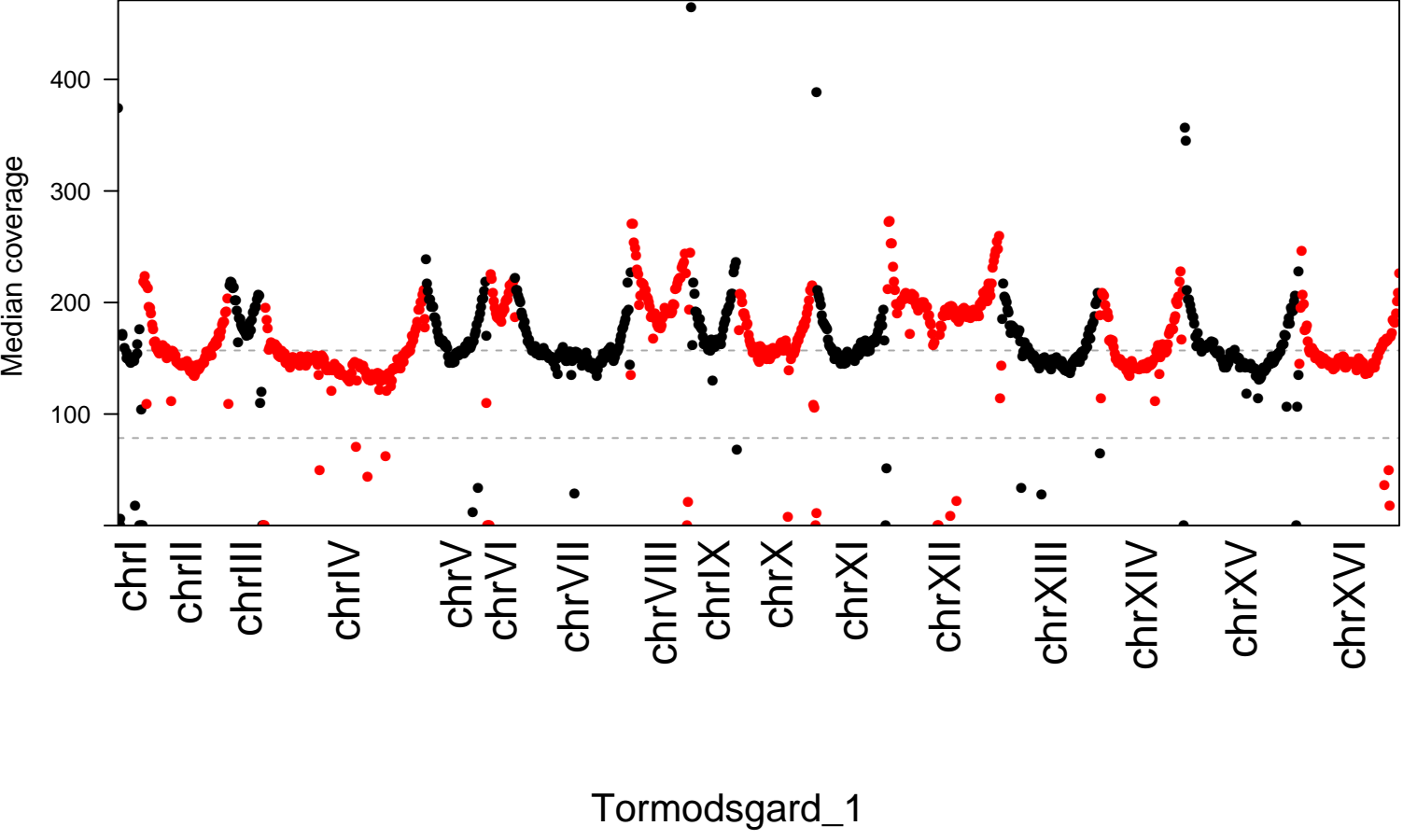
Supplementary Figure S2



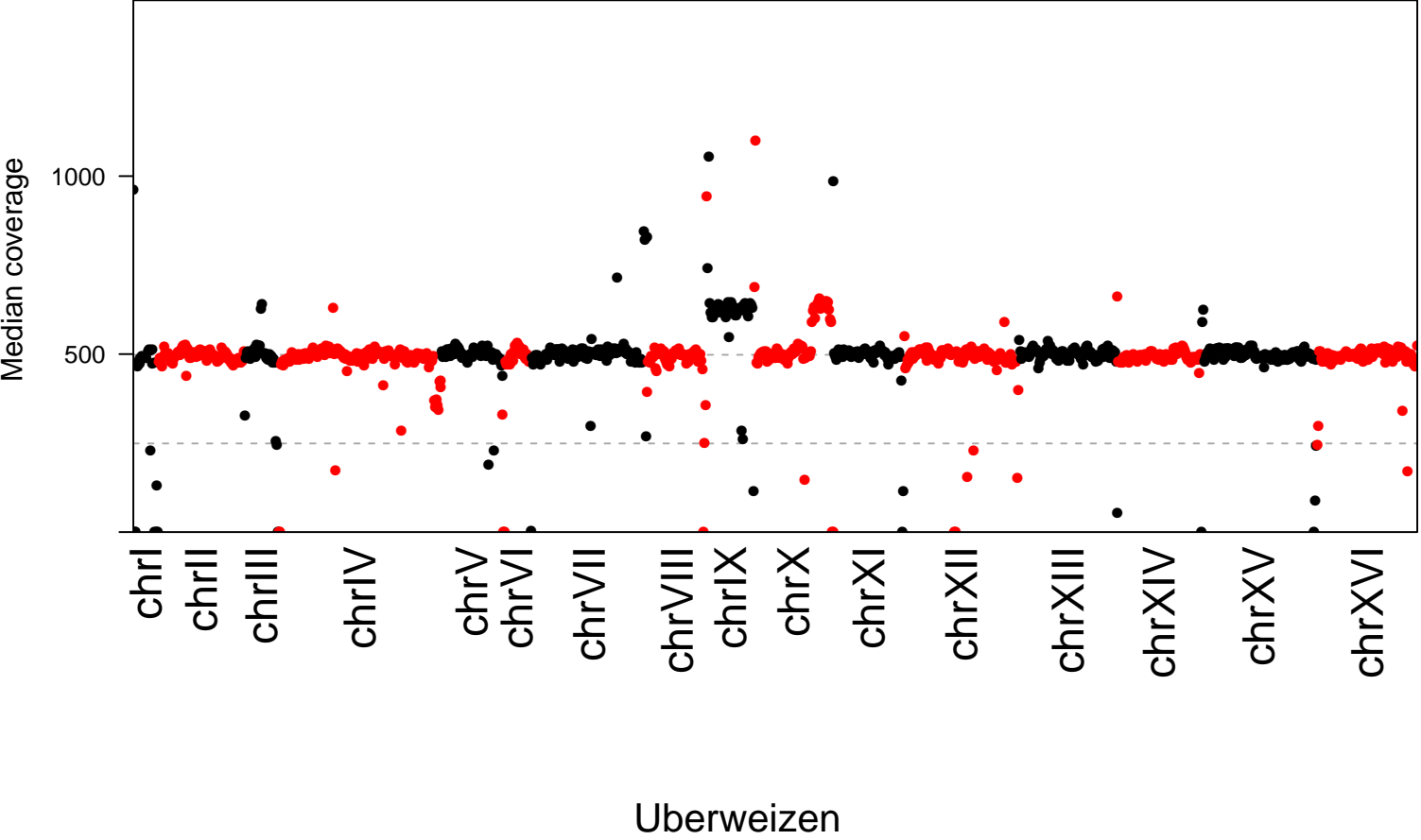
Supplementary Figure S2



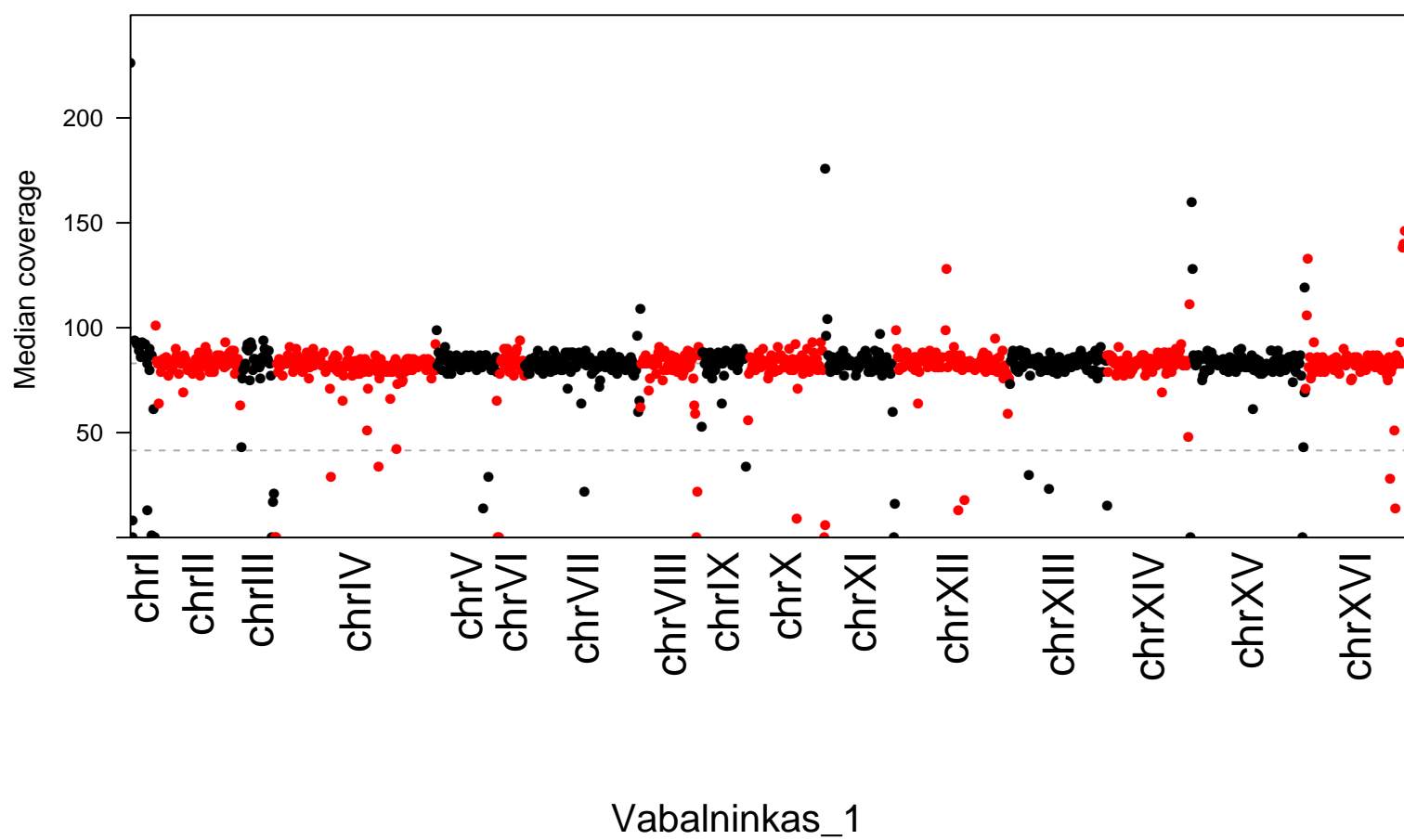
Supplementary Figure S2



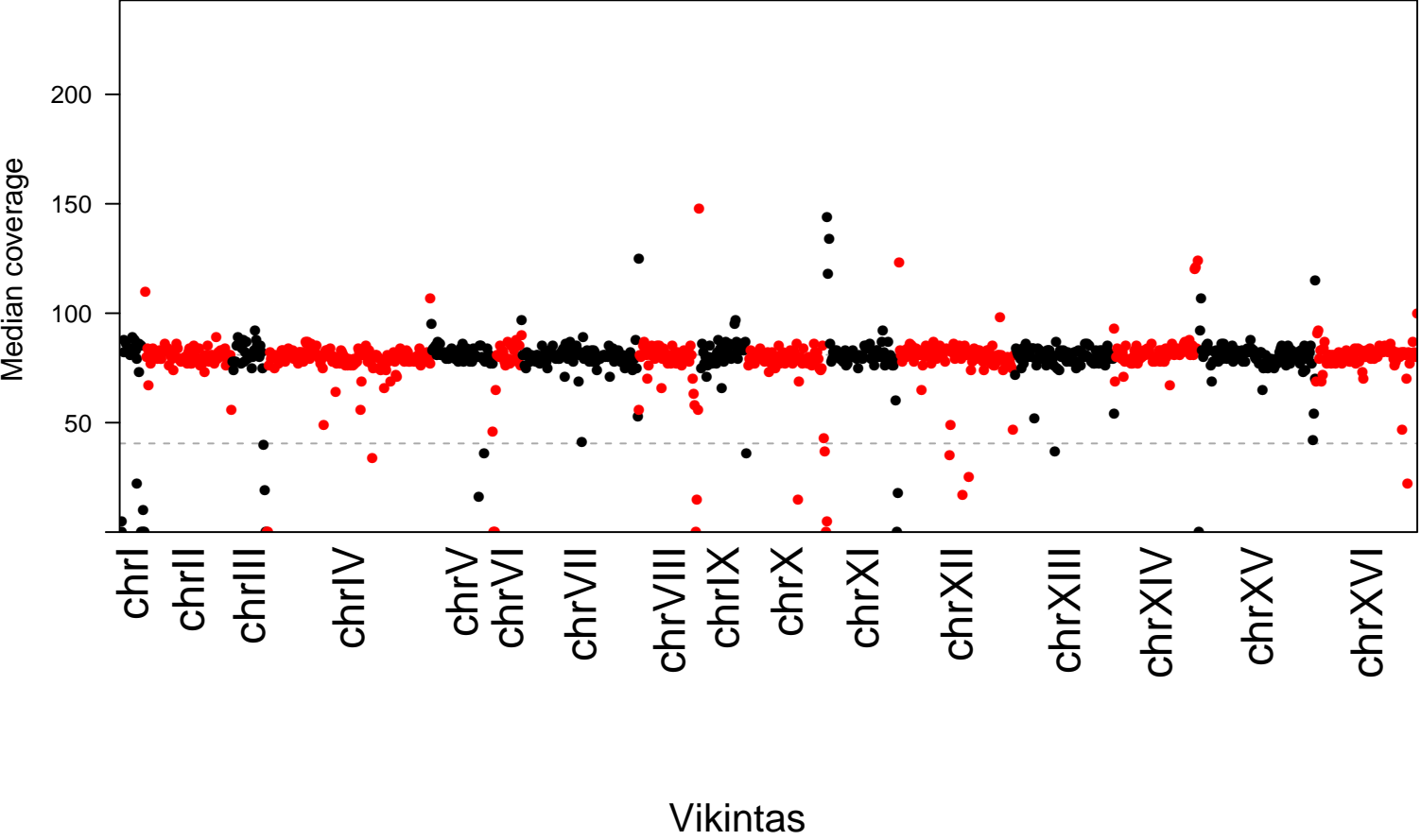
Supplementary Figure S2



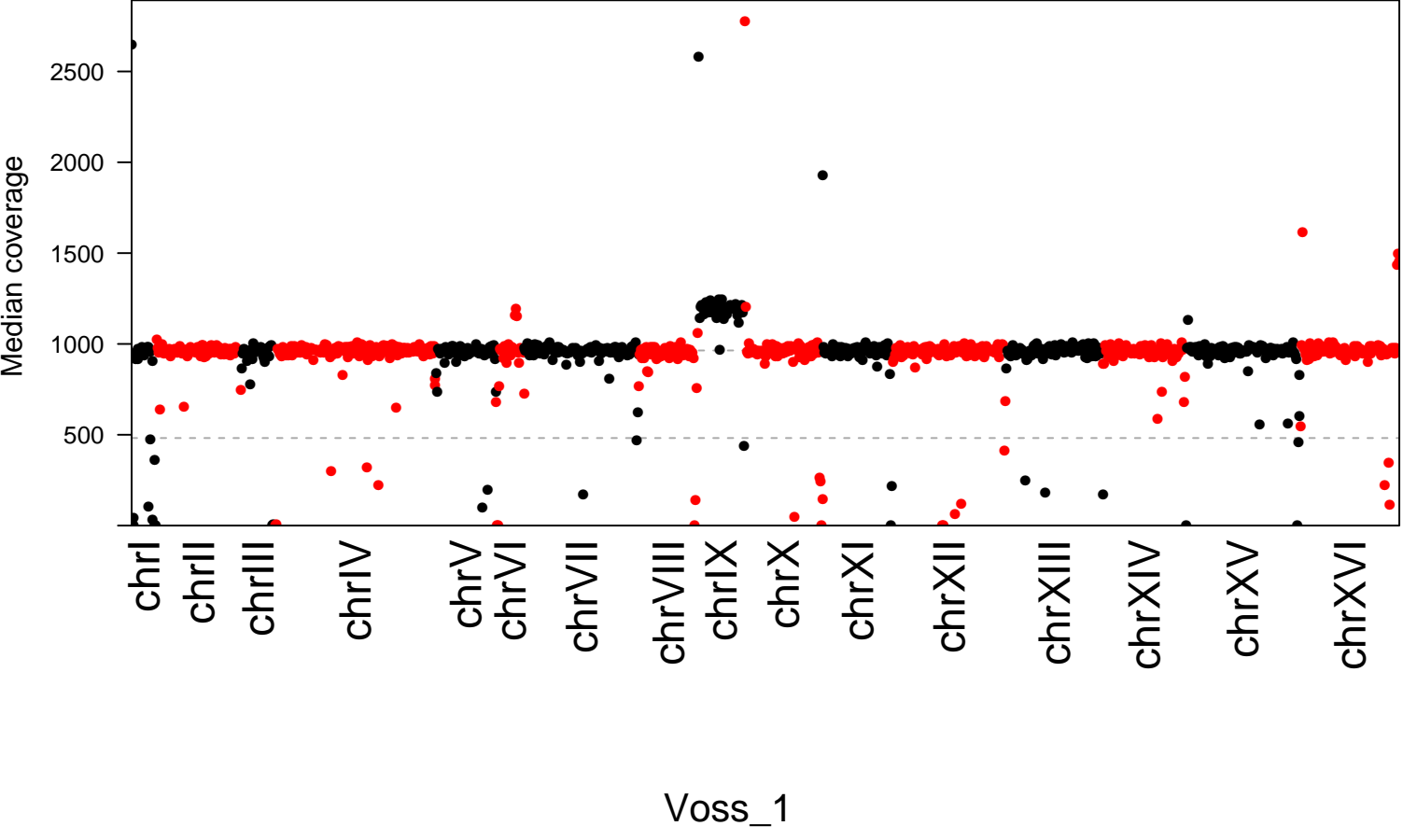
Supplementary Figure S2



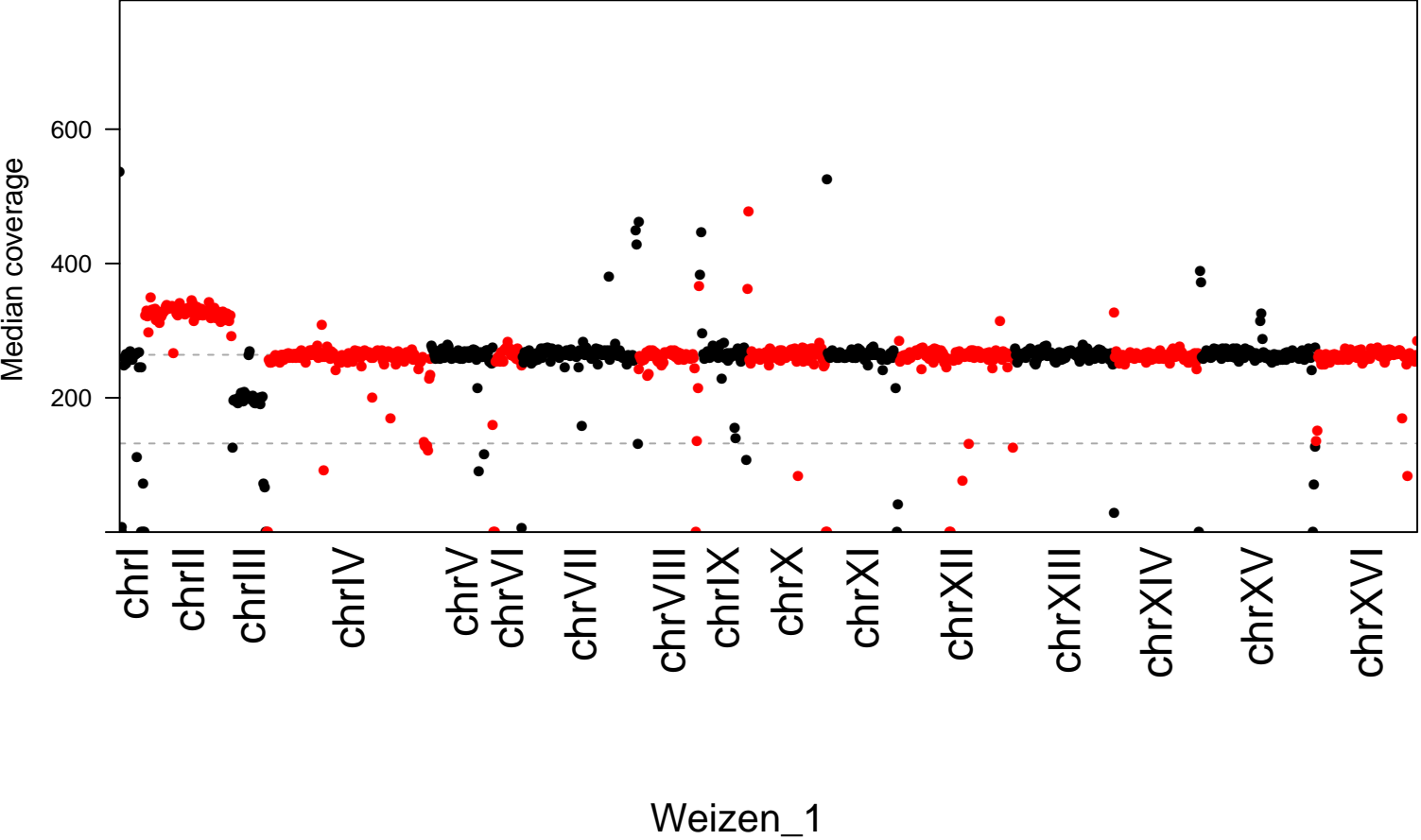
Supplementary Figure S2



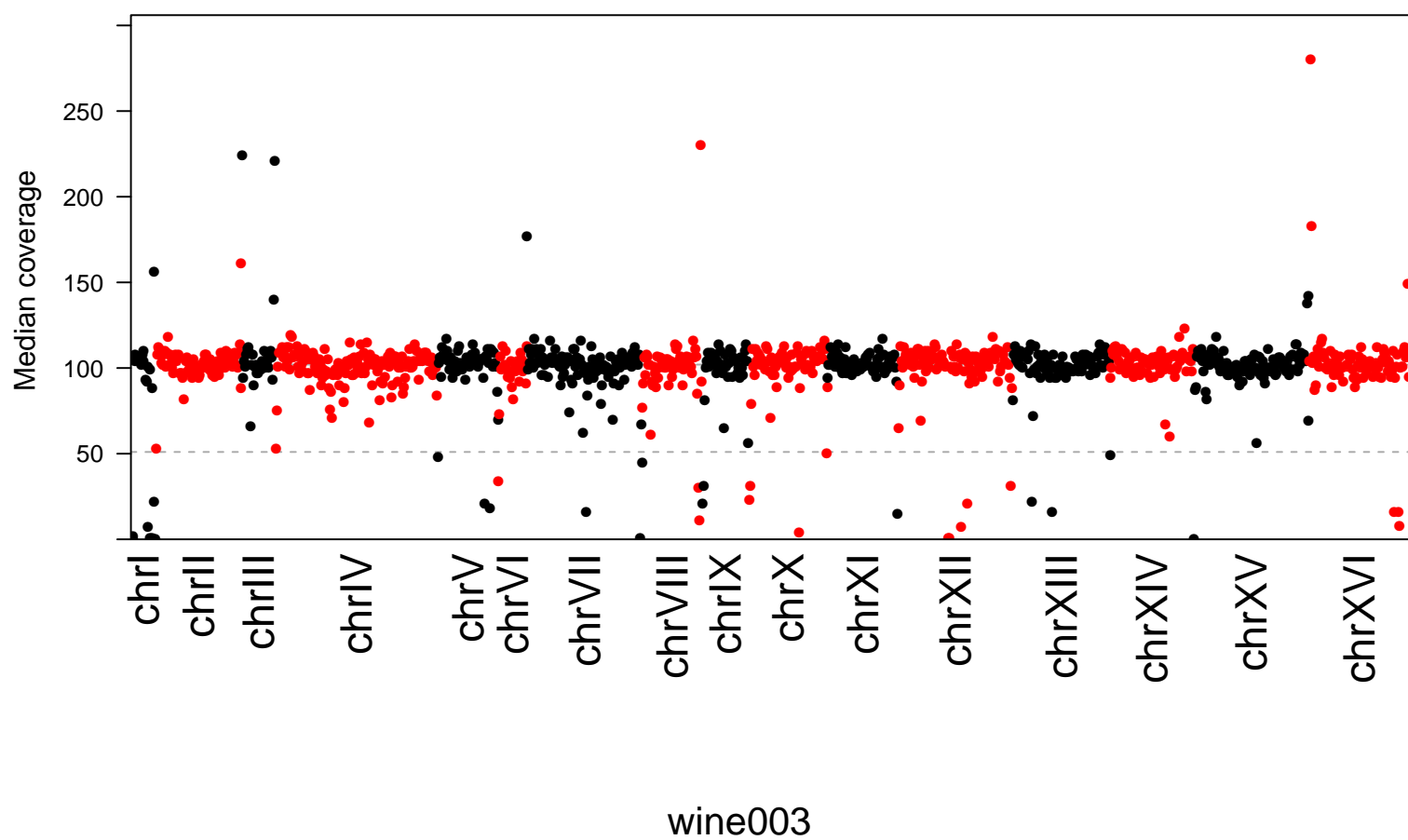
Supplementary Figure S2



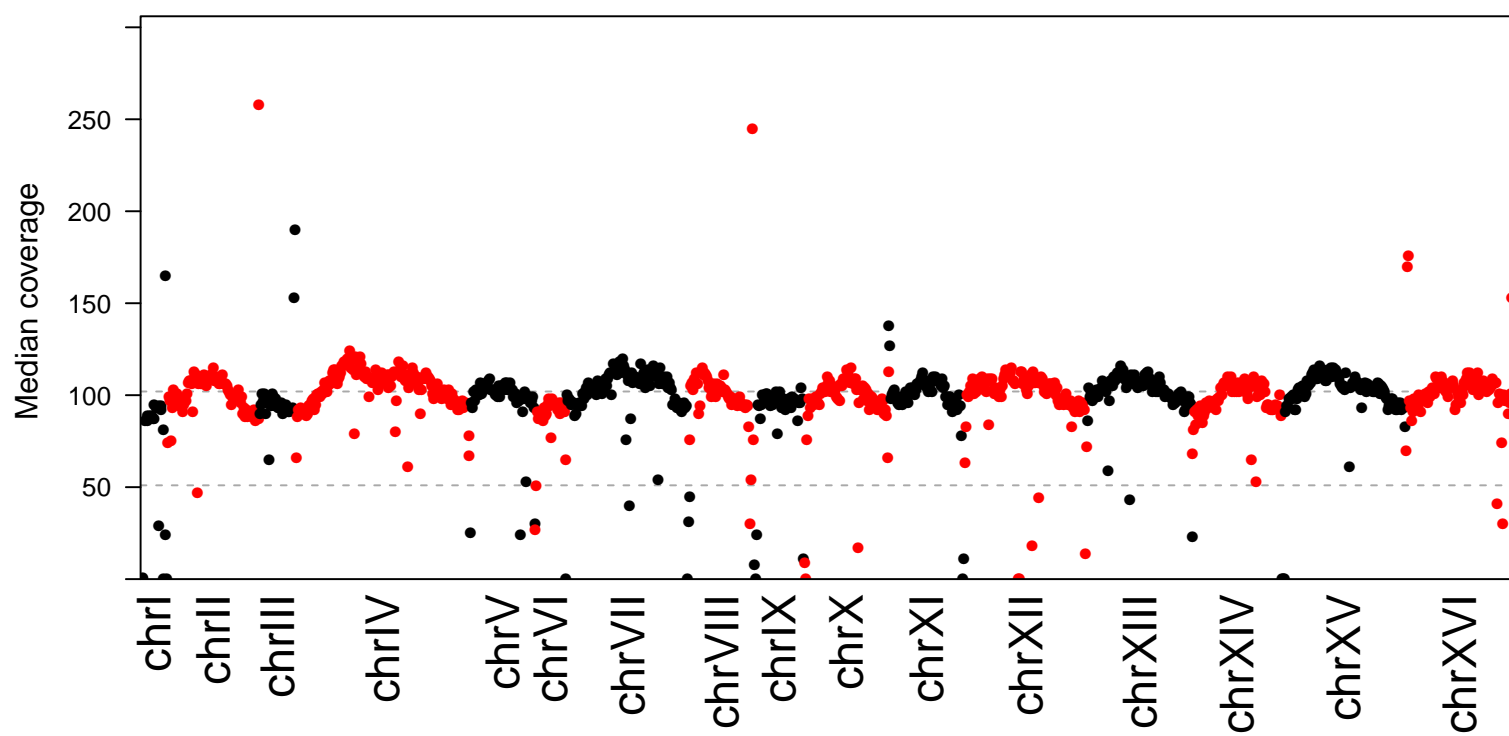
Supplementary Figure S2



Supplementary Figure S2

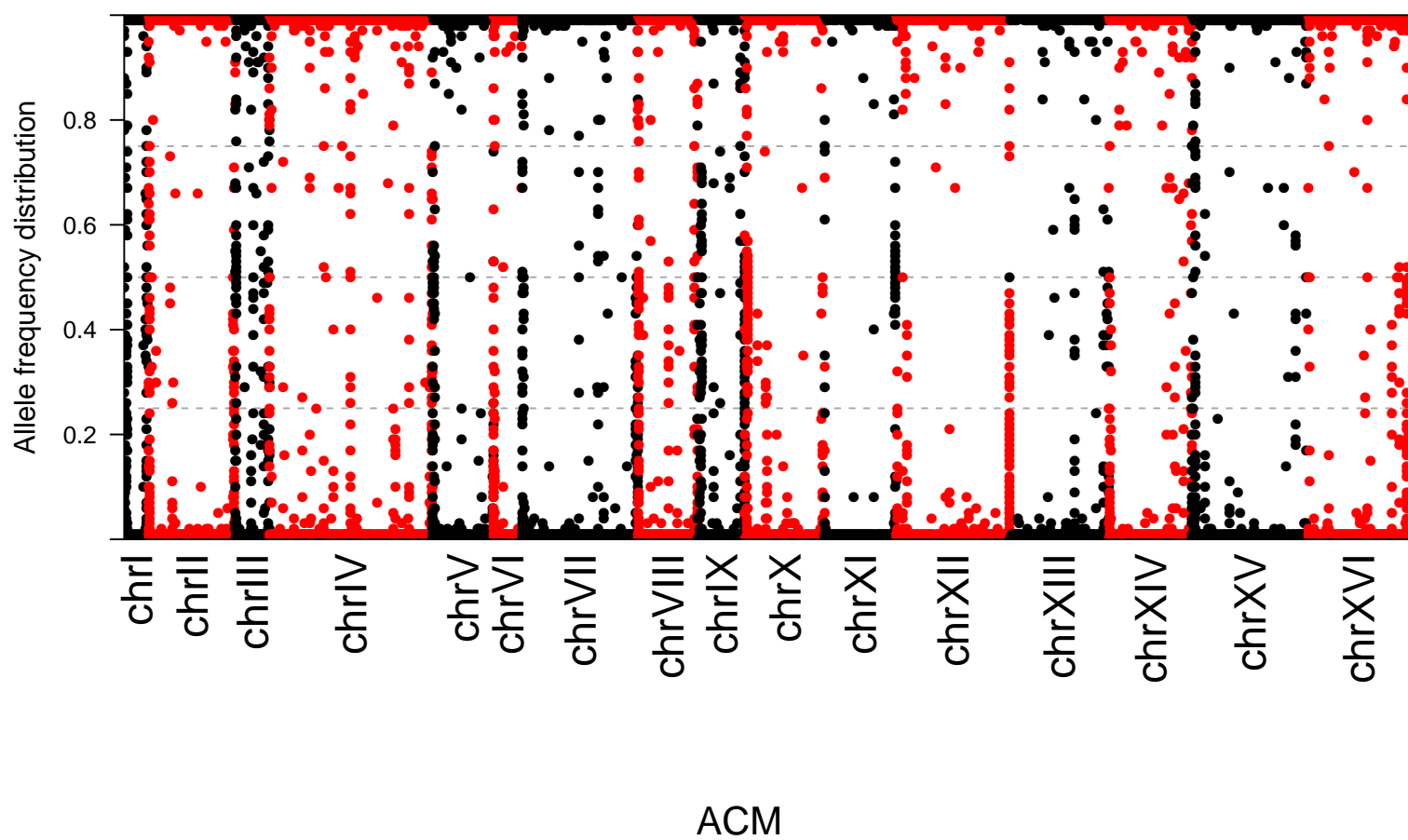


Supplementary Figure S2

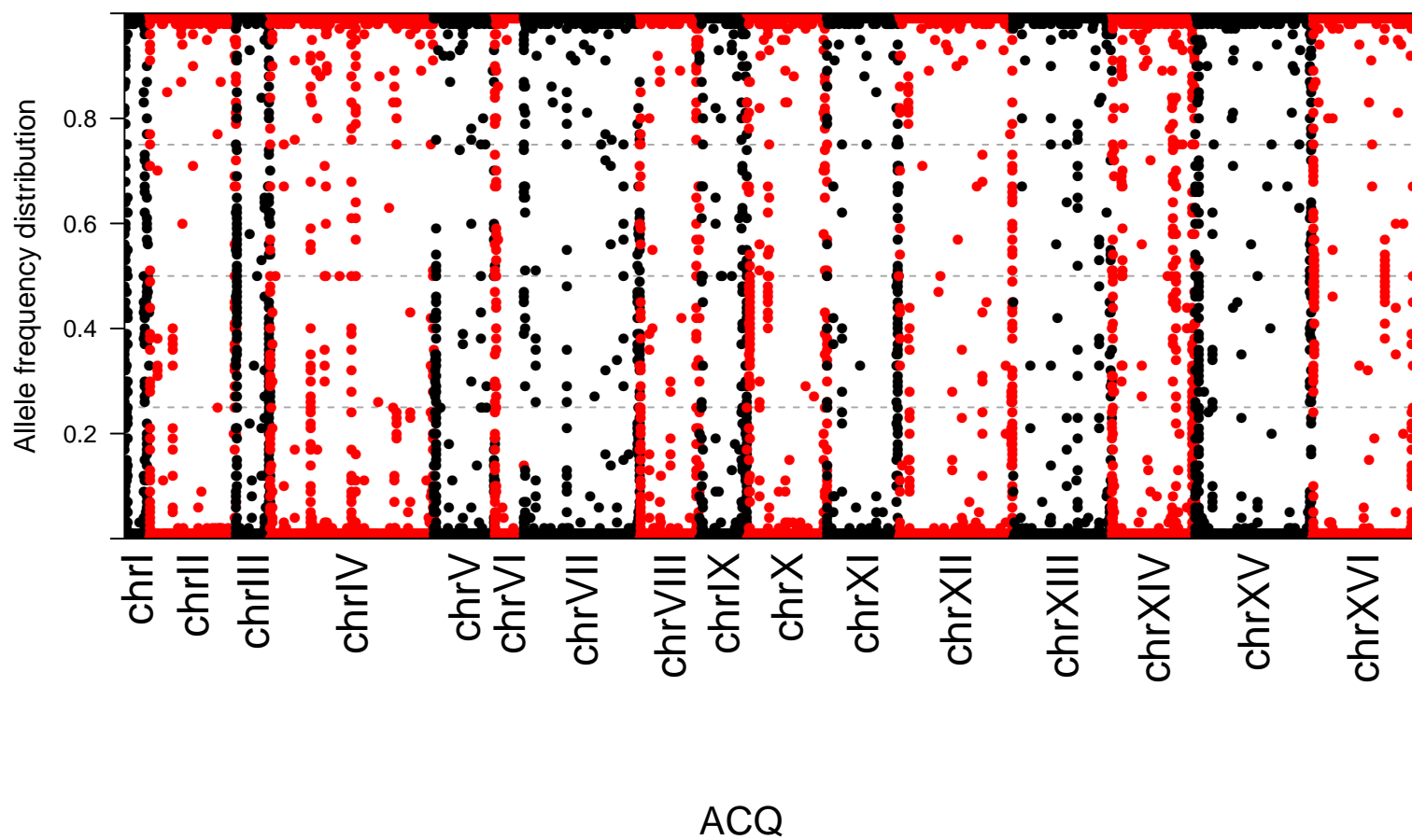


wine005

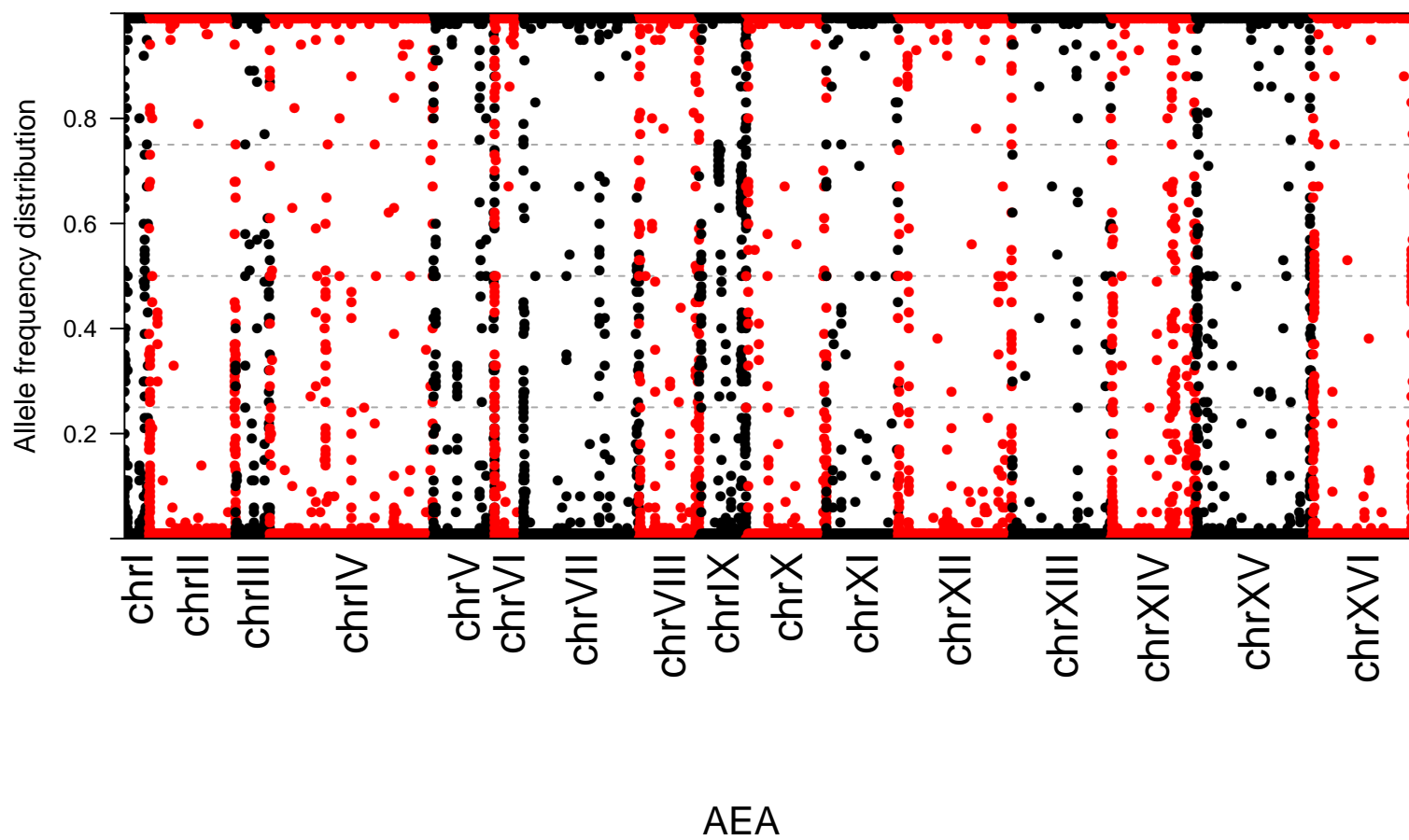
Supplementary Figure S3



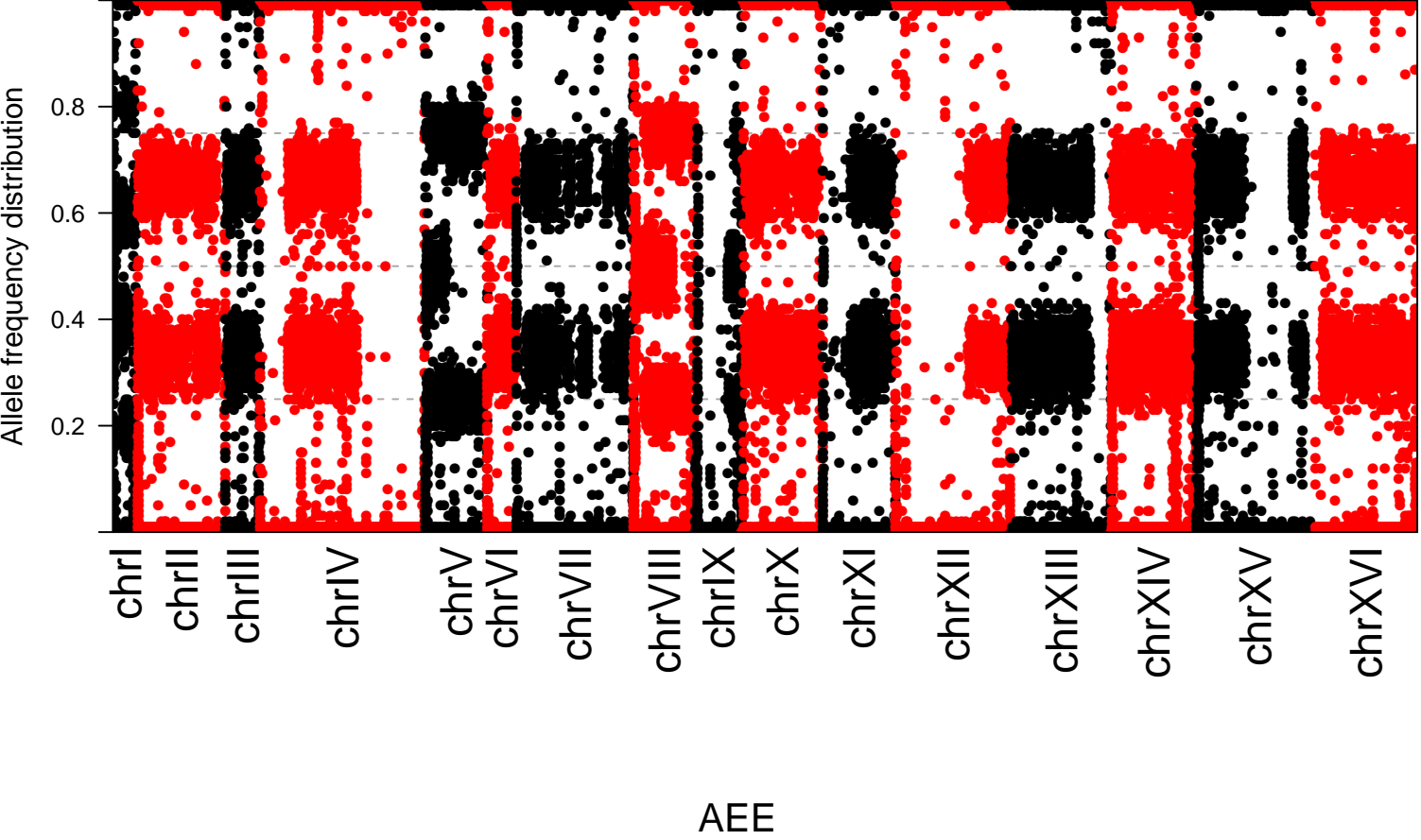
Supplementary Figure S3



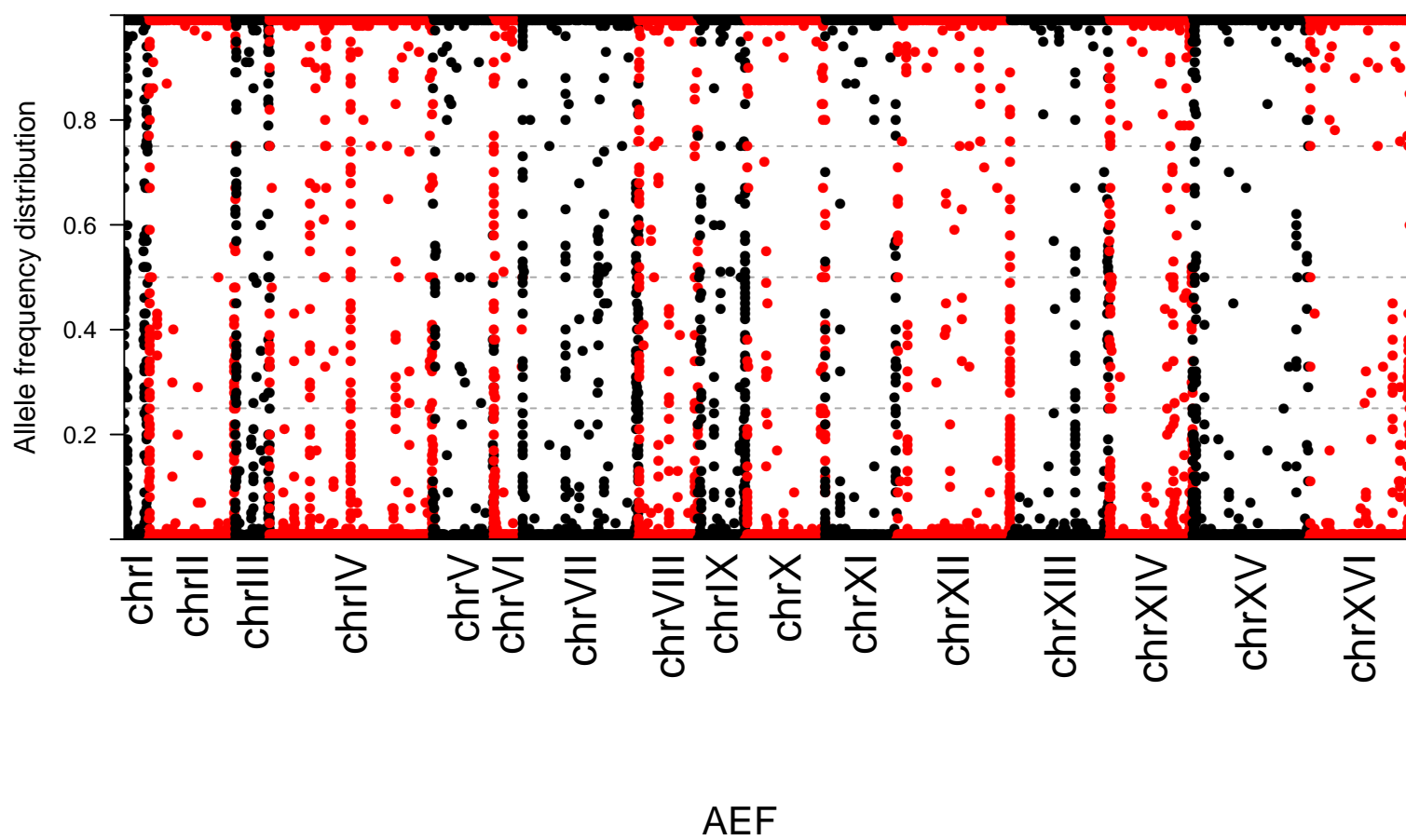
Supplementary Figure S3



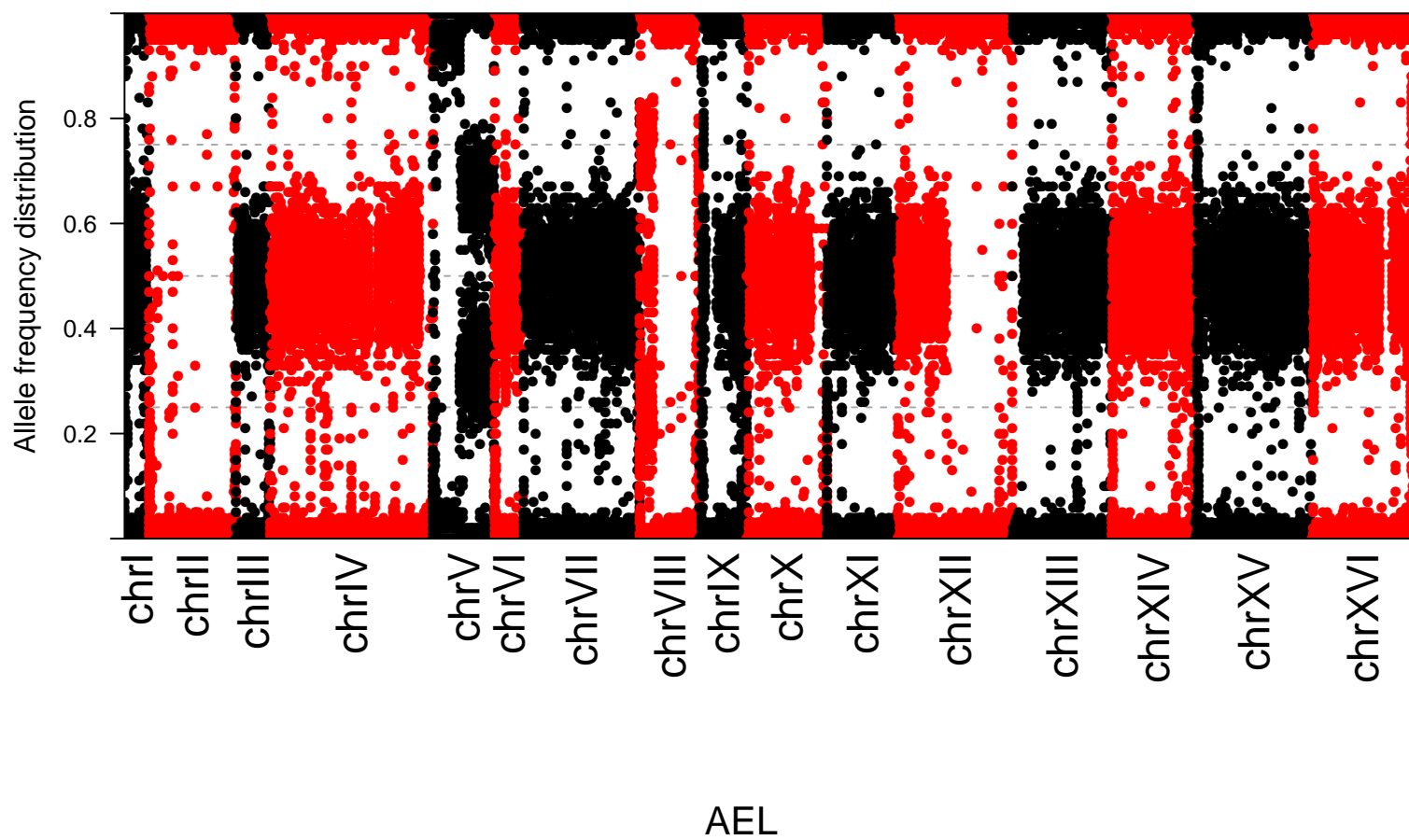
Supplementary Figure S3



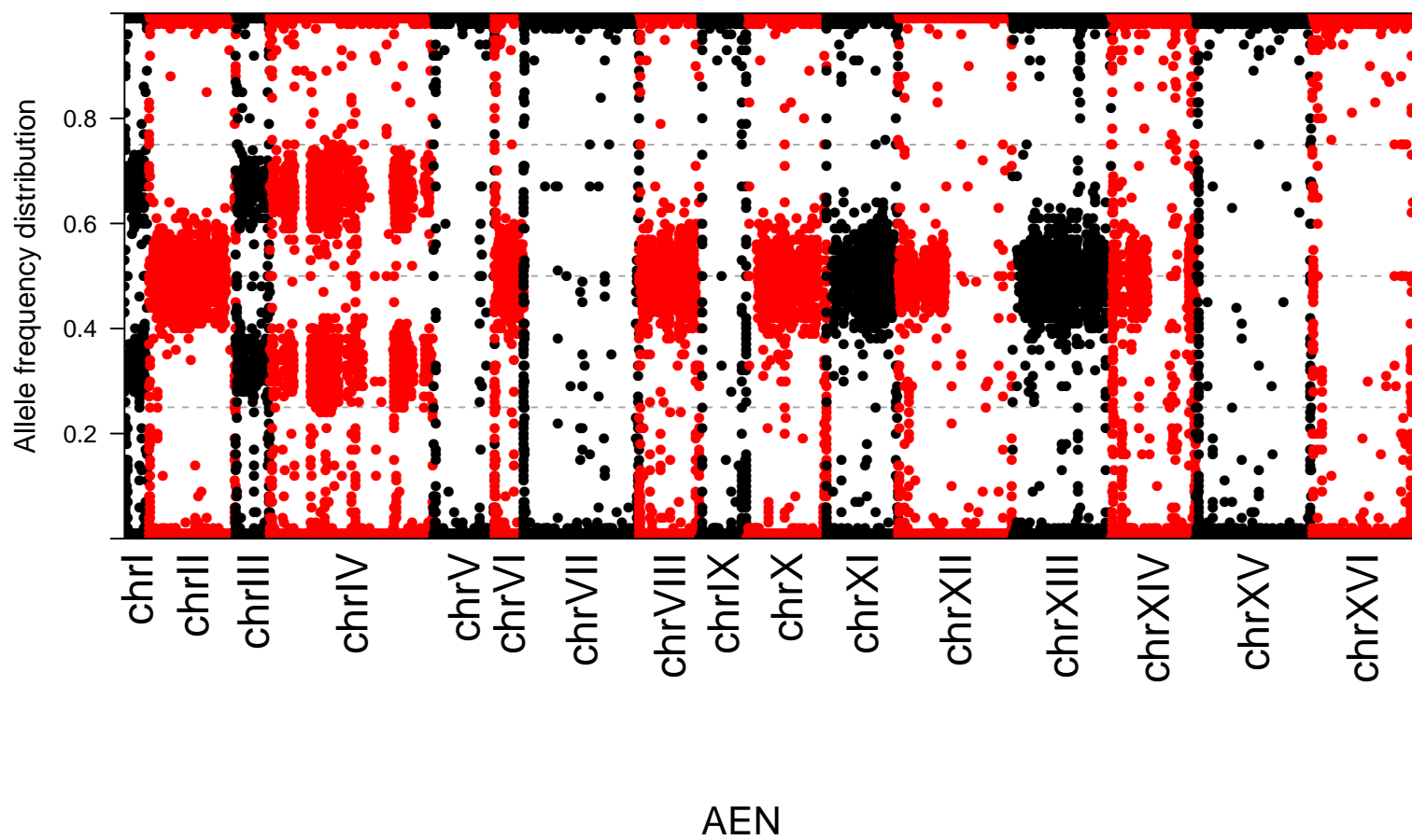
Supplementary Figure S3



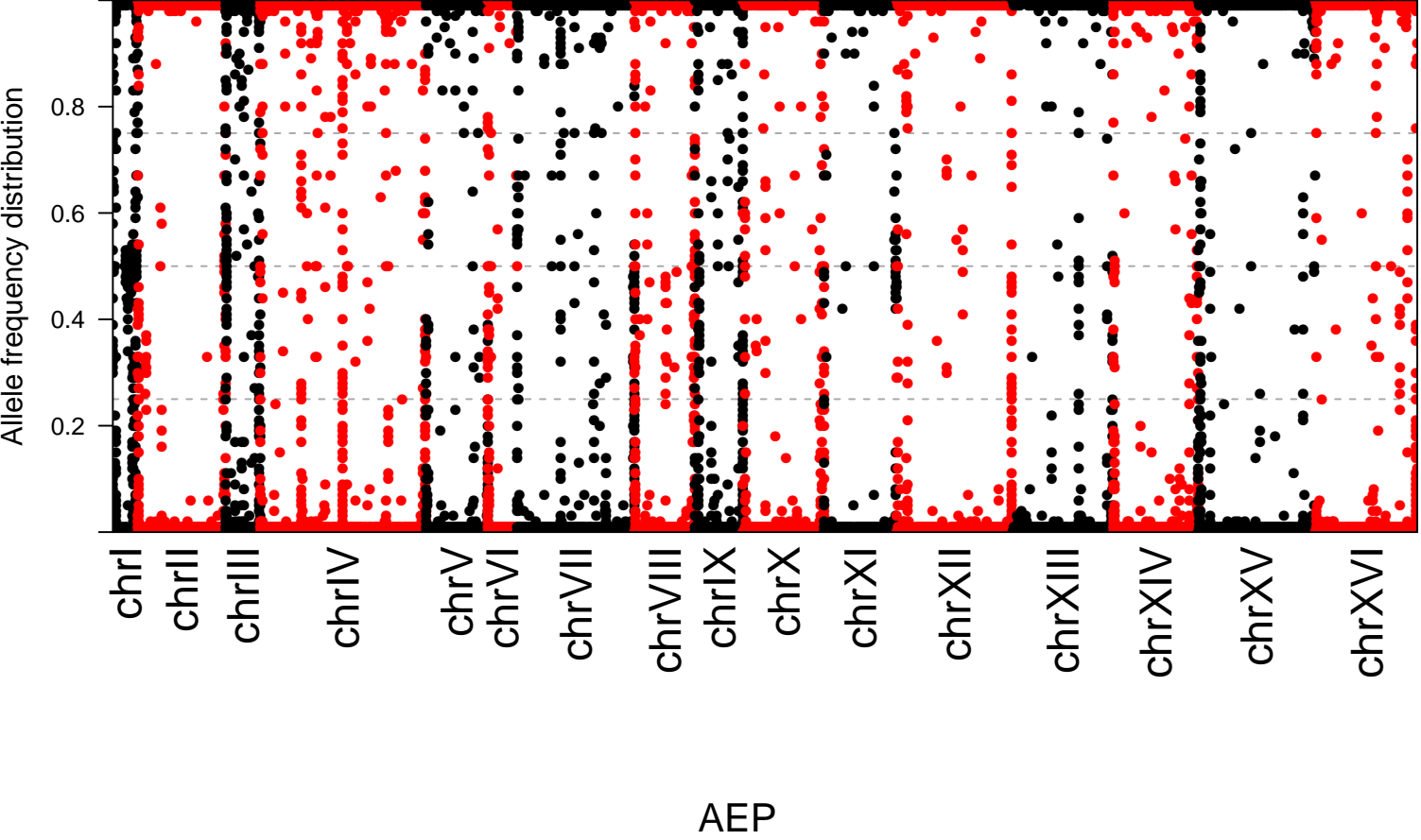
Supplementary Figure S3



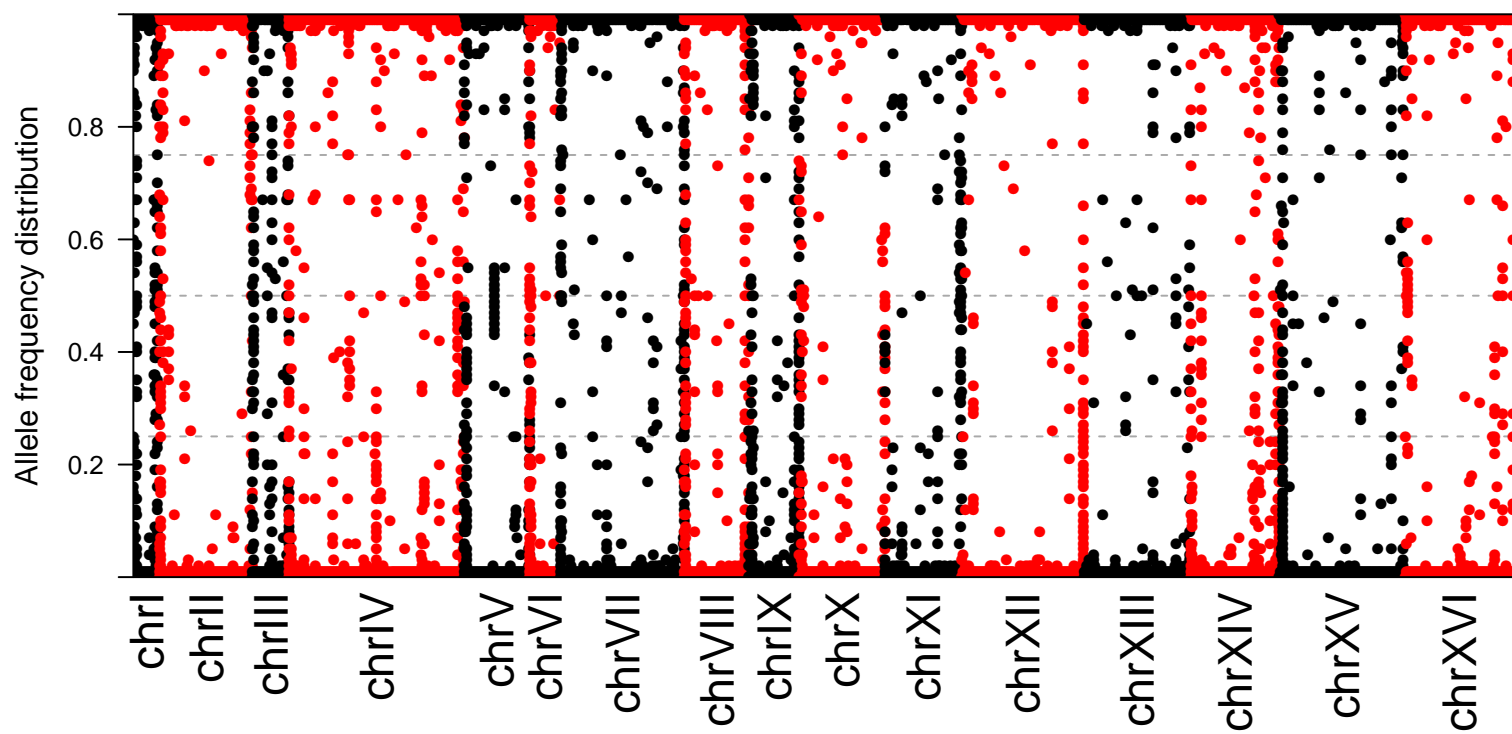
Supplementary Figure S3



Supplementary Figure S3

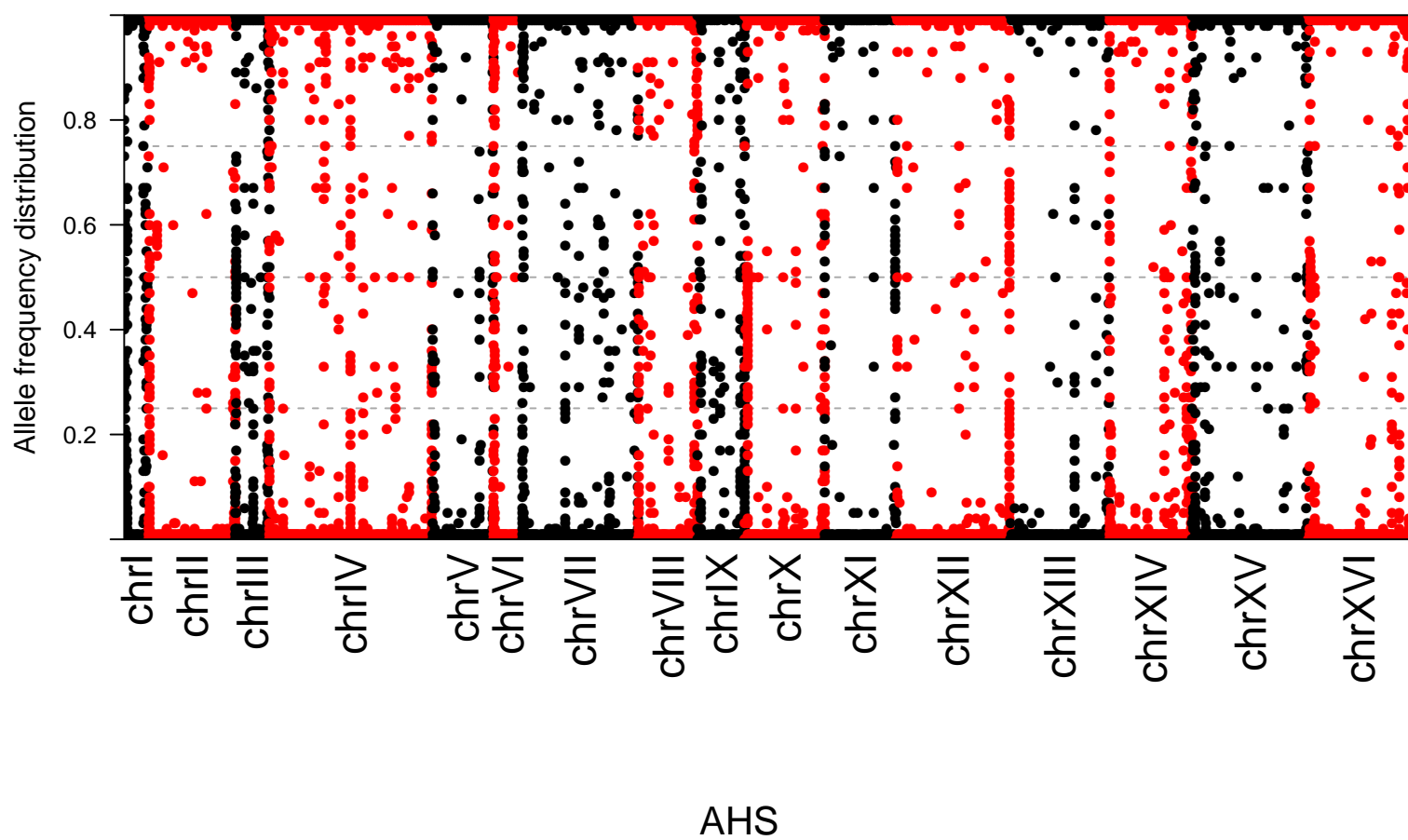


Supplementary Figure S3

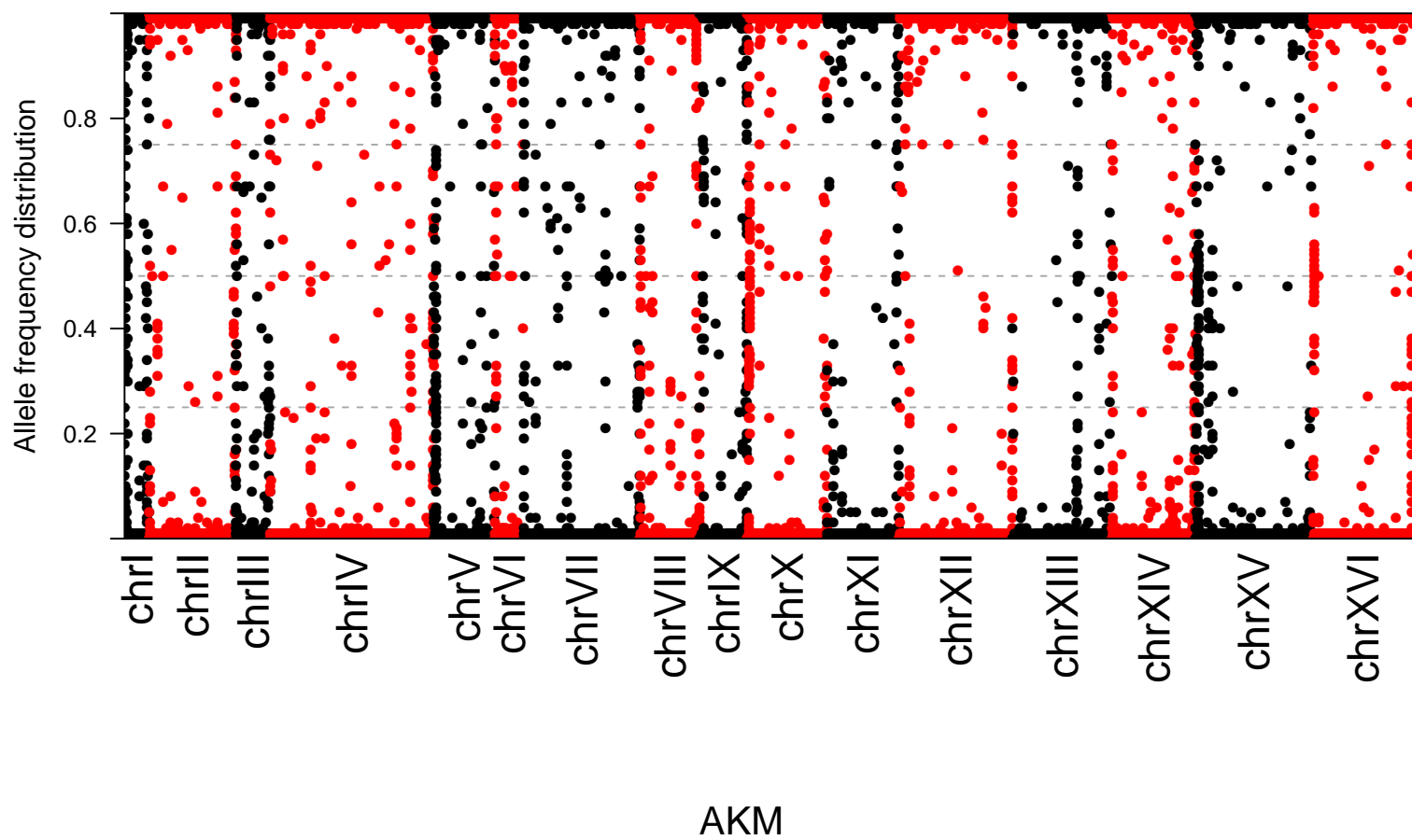


AFK

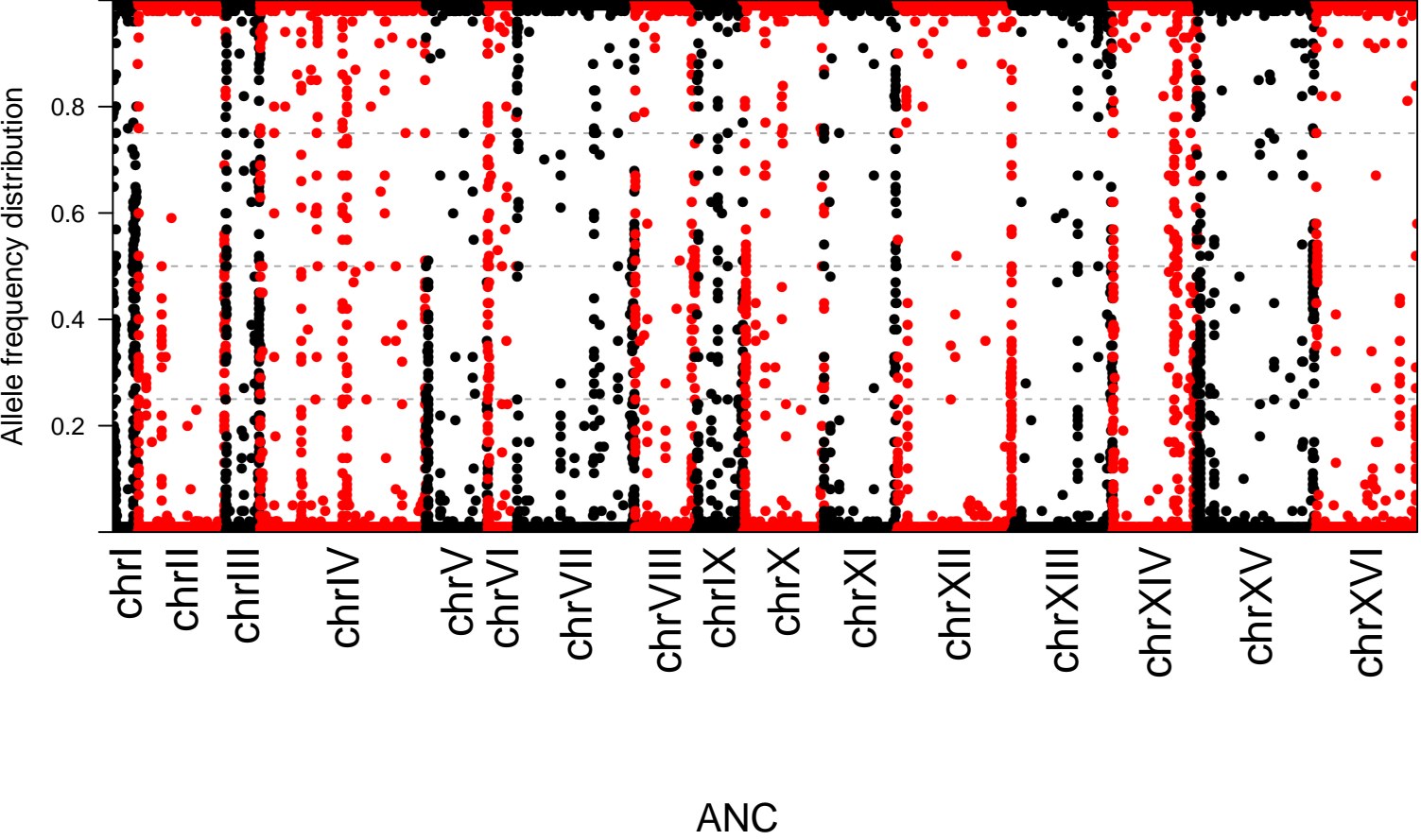
Supplementary Figure S3



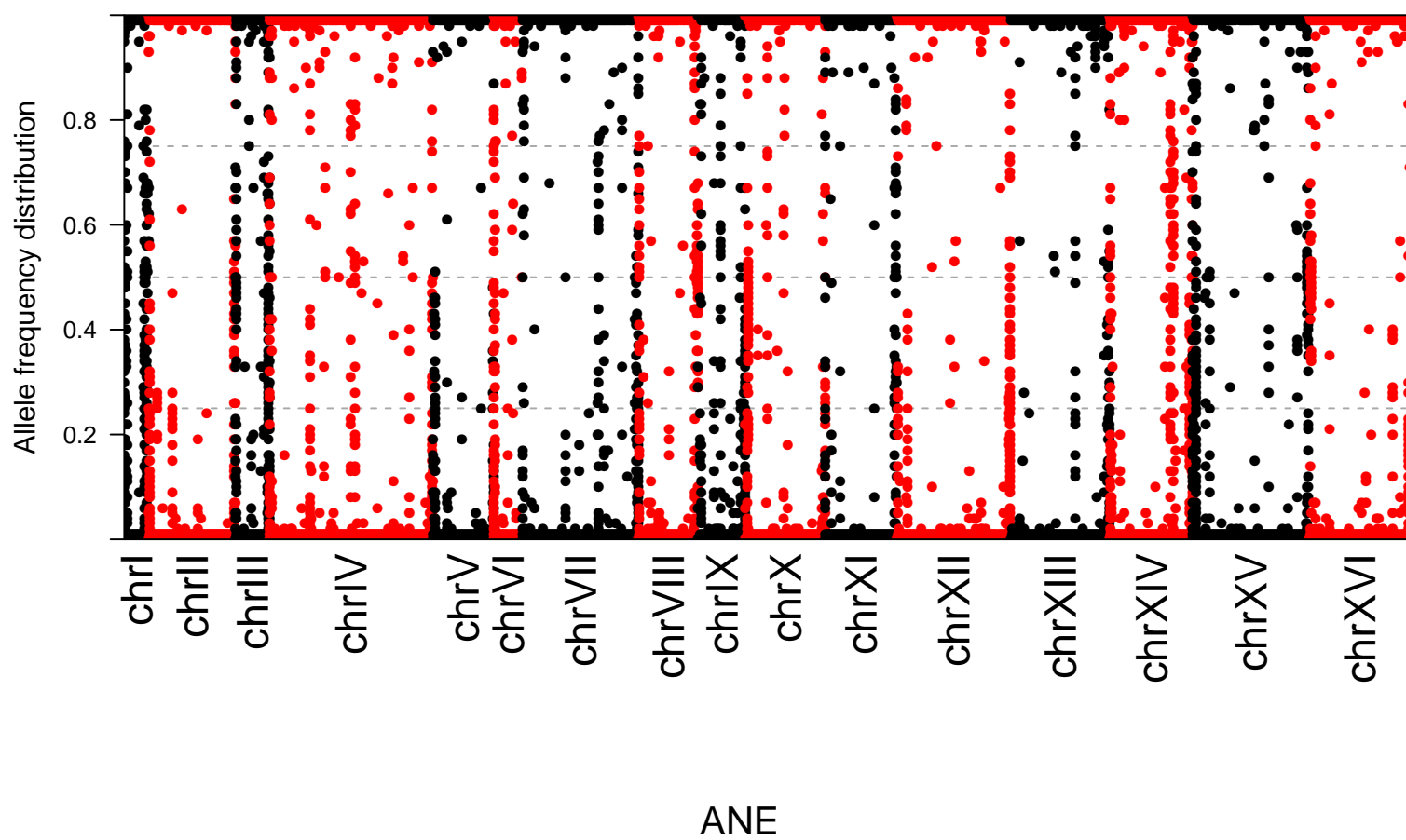
Supplementary Figure S3



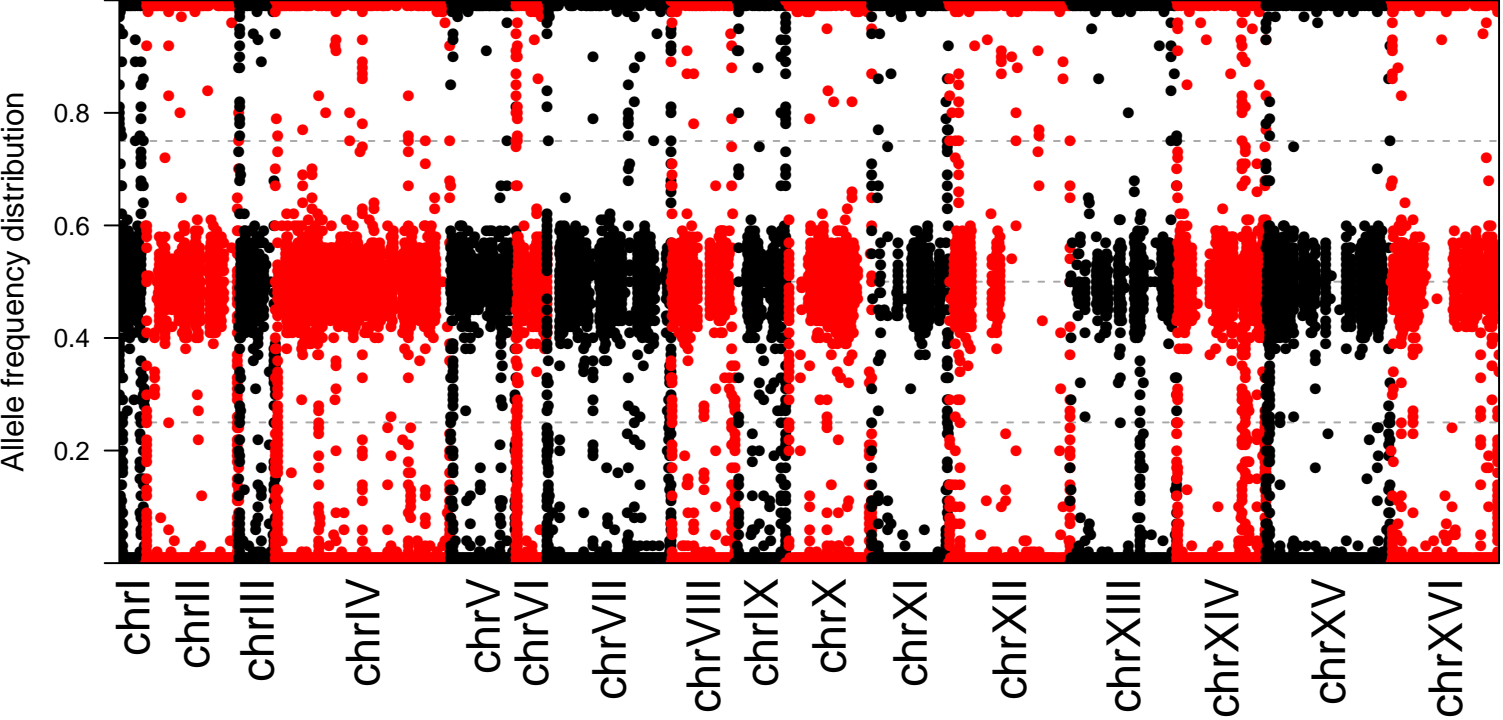
Supplementary Figure S3



Supplementary Figure S3

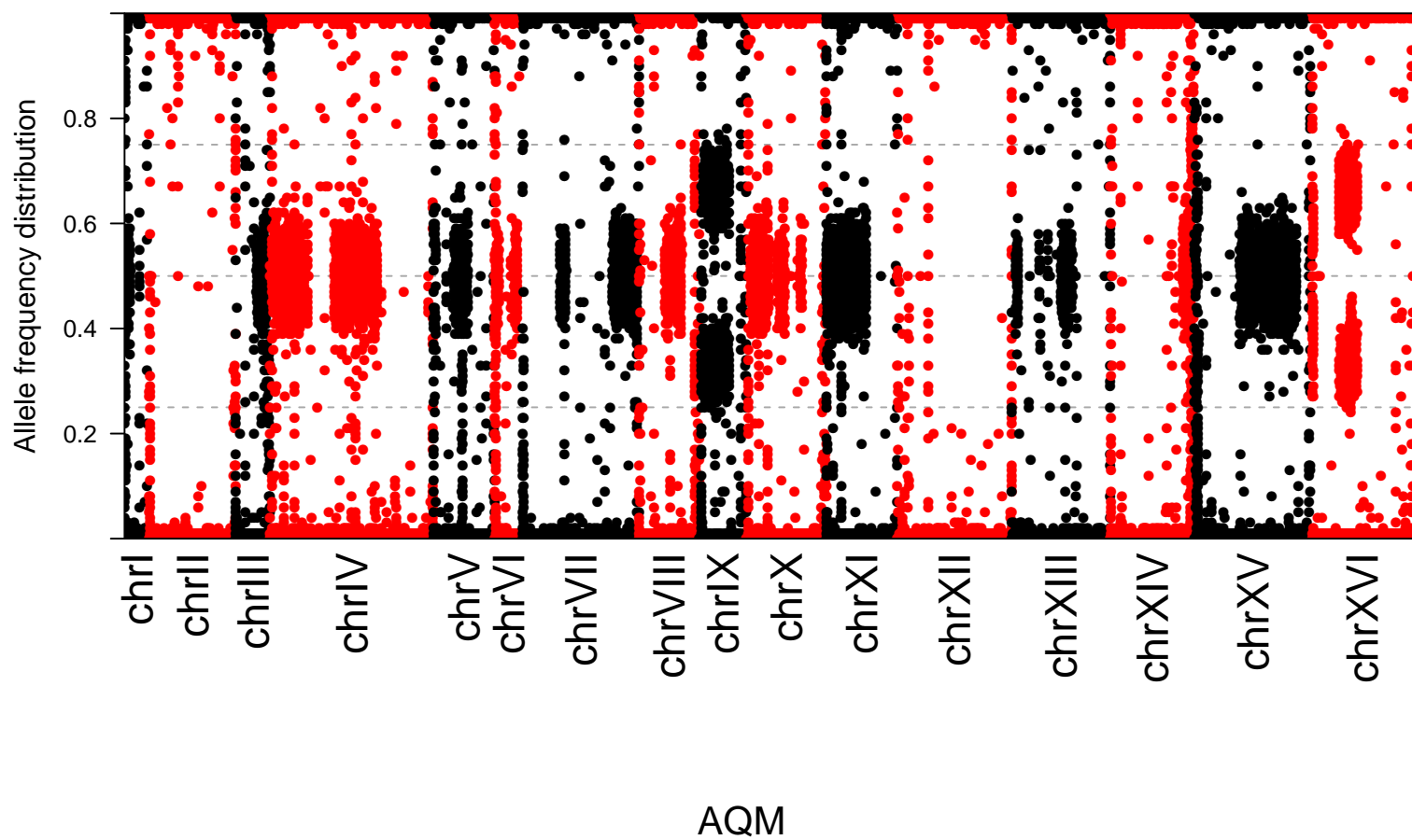


Supplementary Figure S3

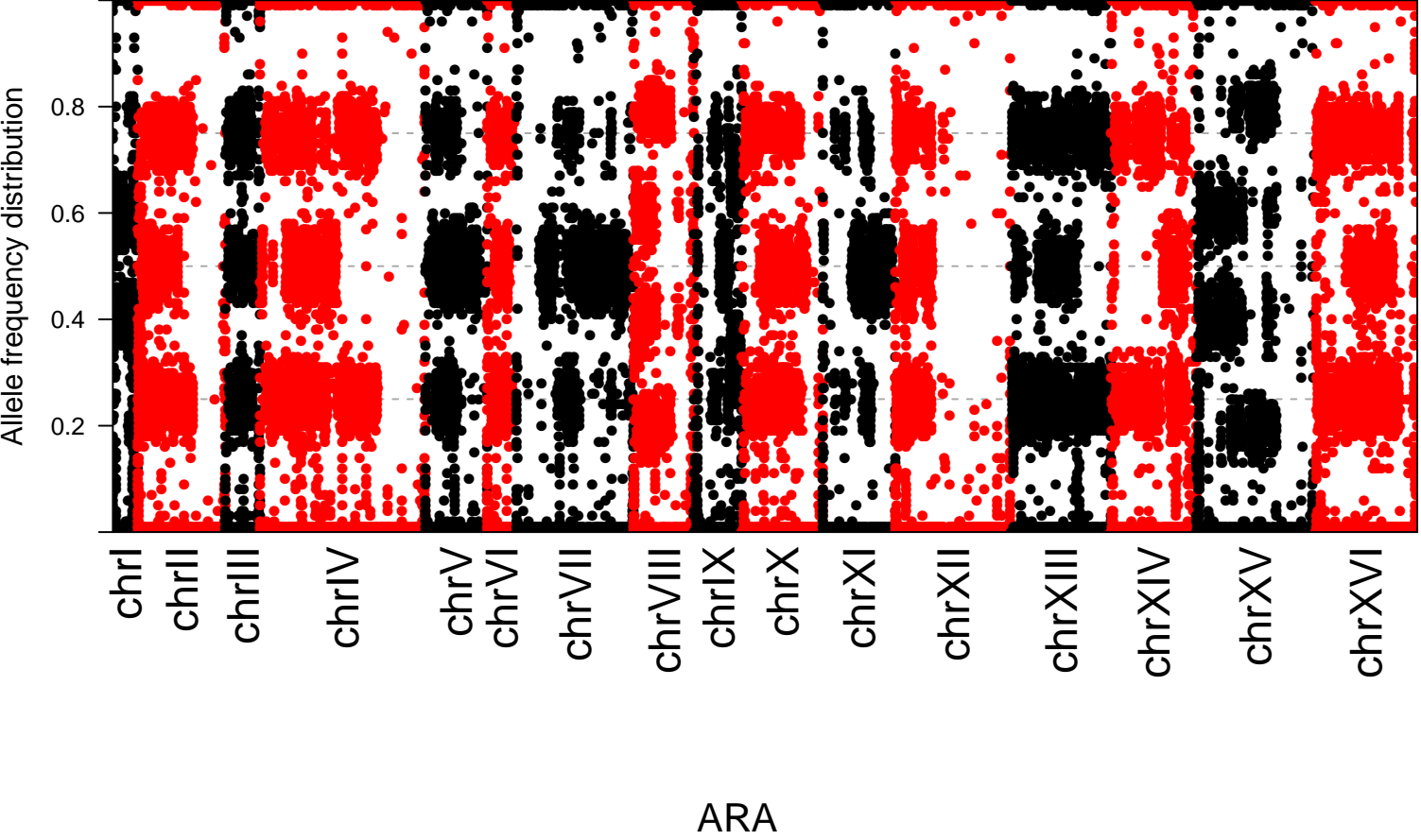


ANN

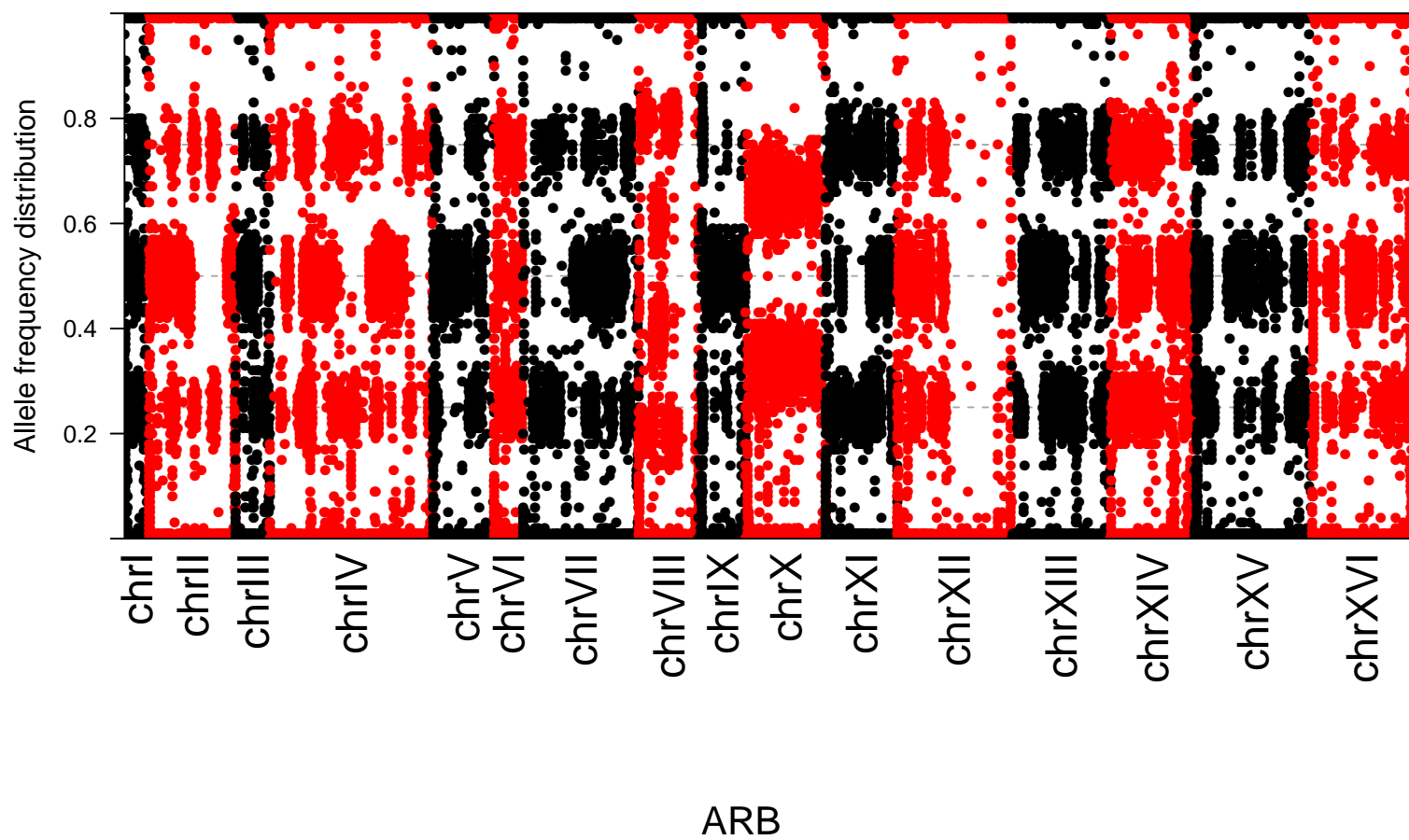
Supplementary Figure S3



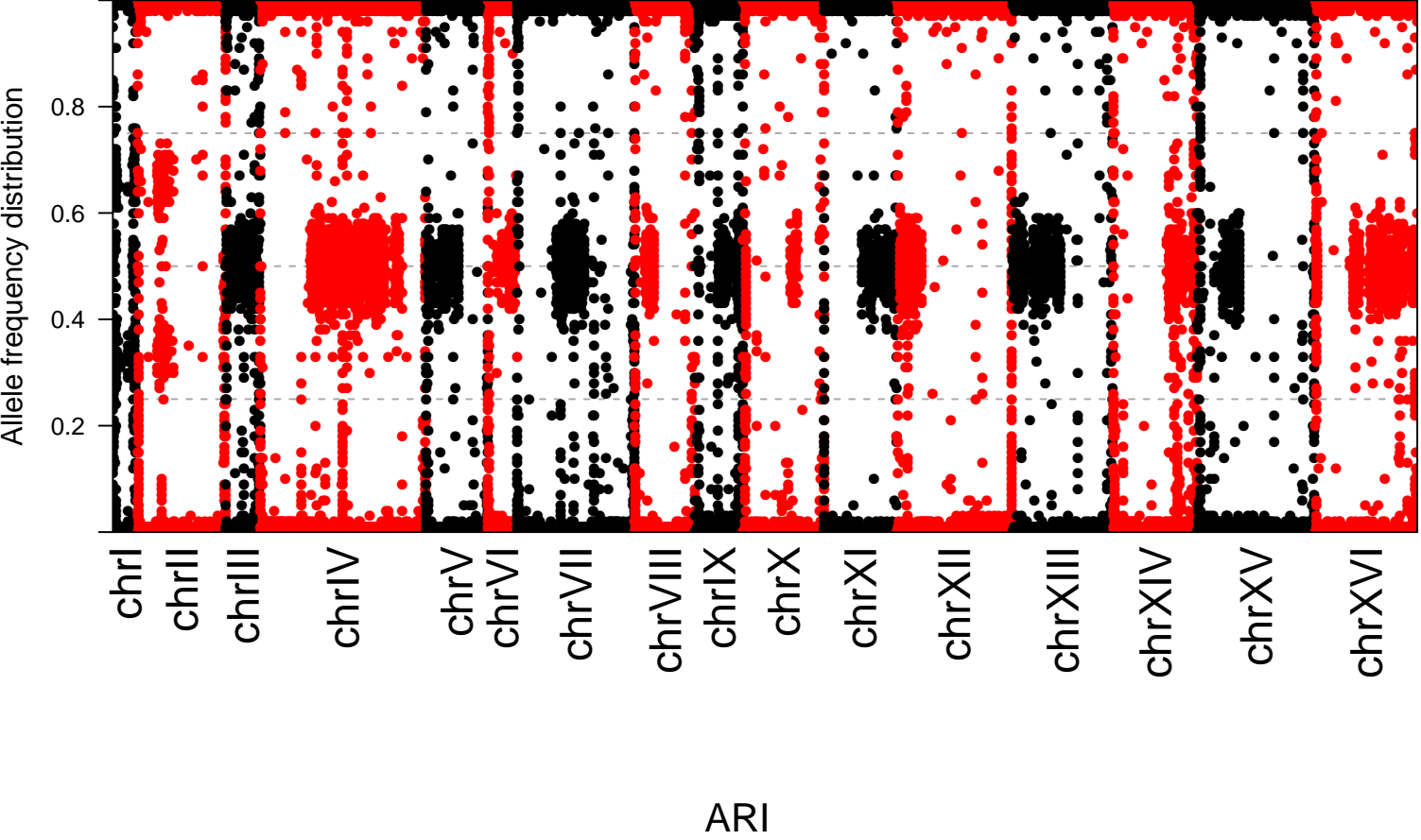
Supplementary Figure S3



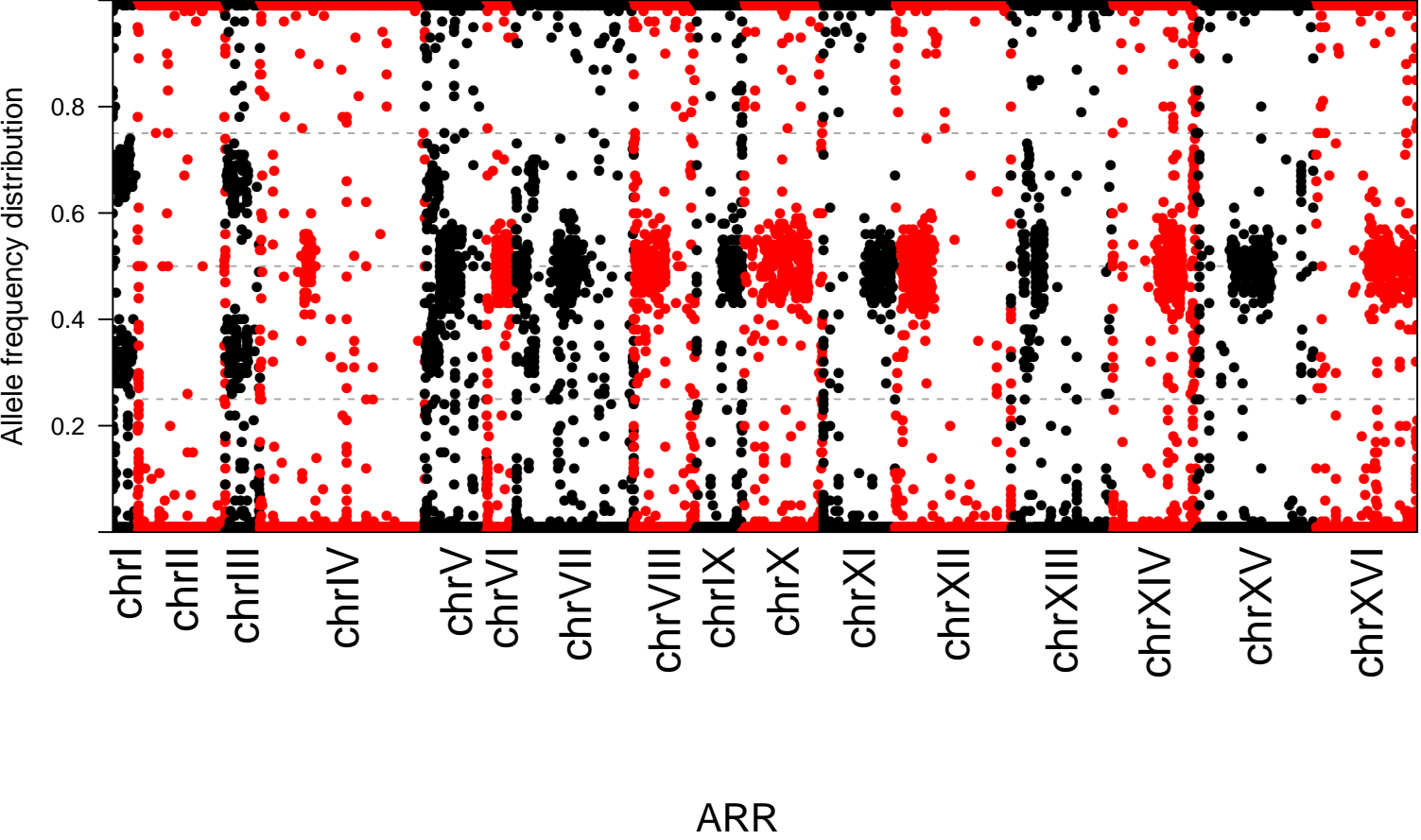
Supplementary Figure S3



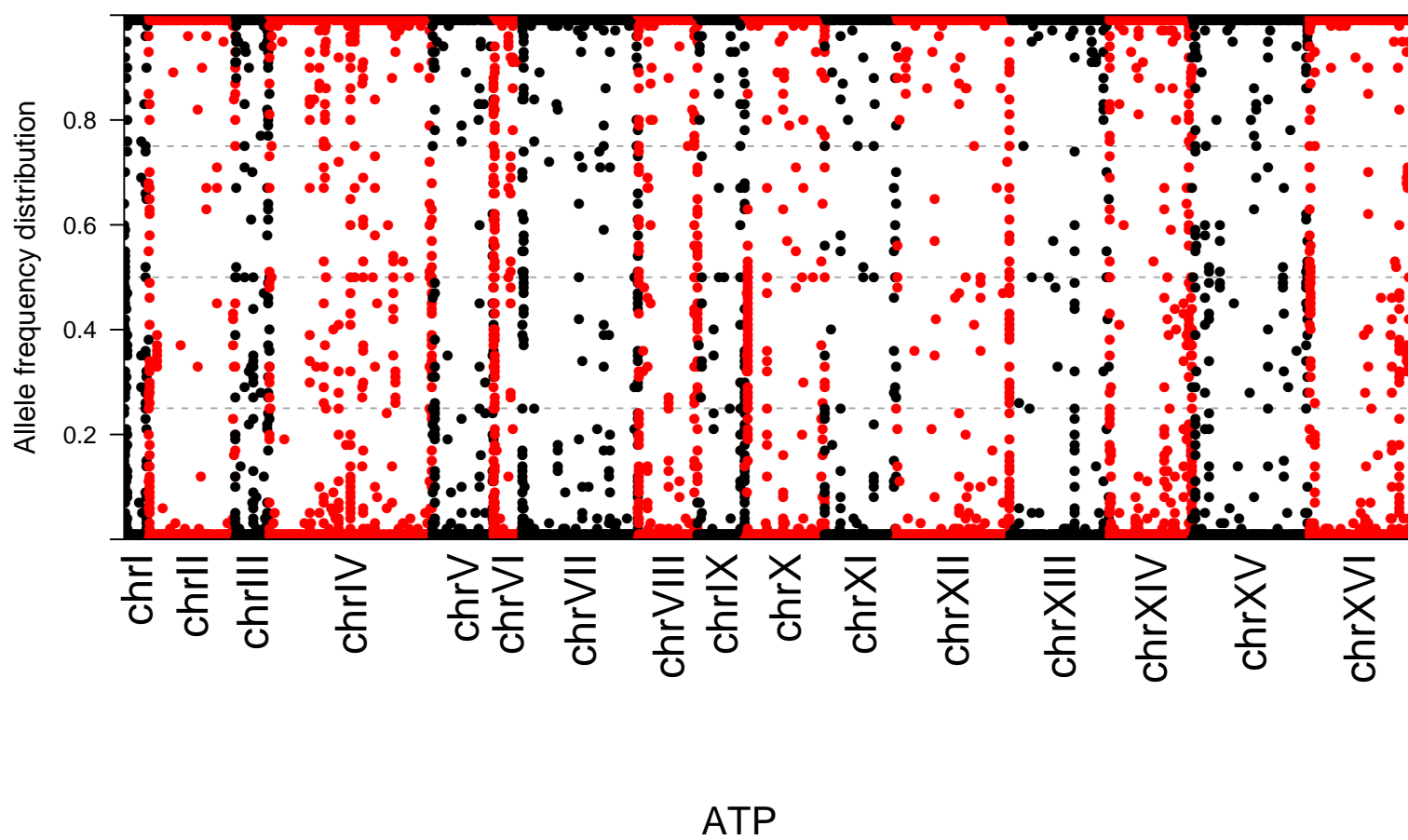
Supplementary Figure S3



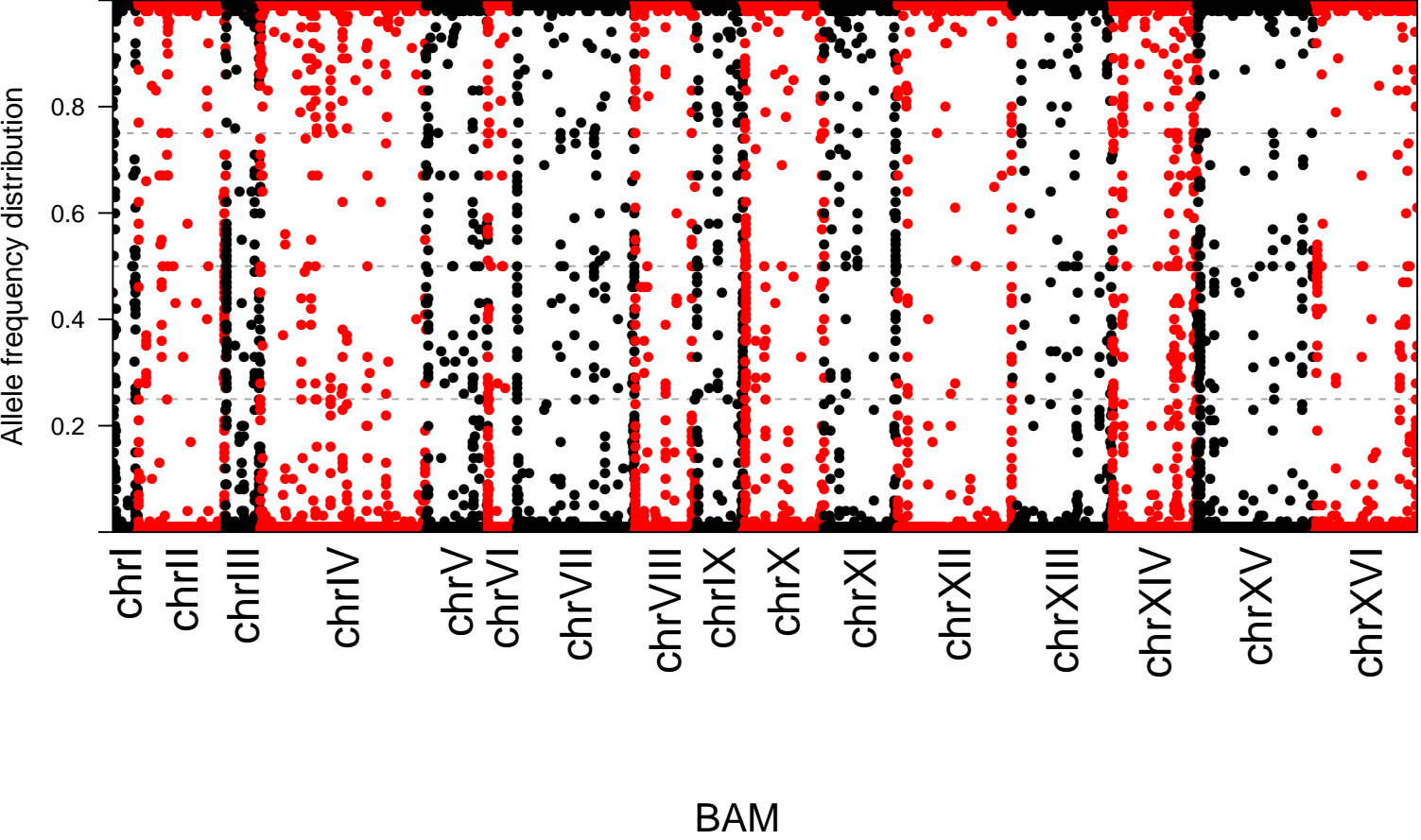
Supplementary Figure S3



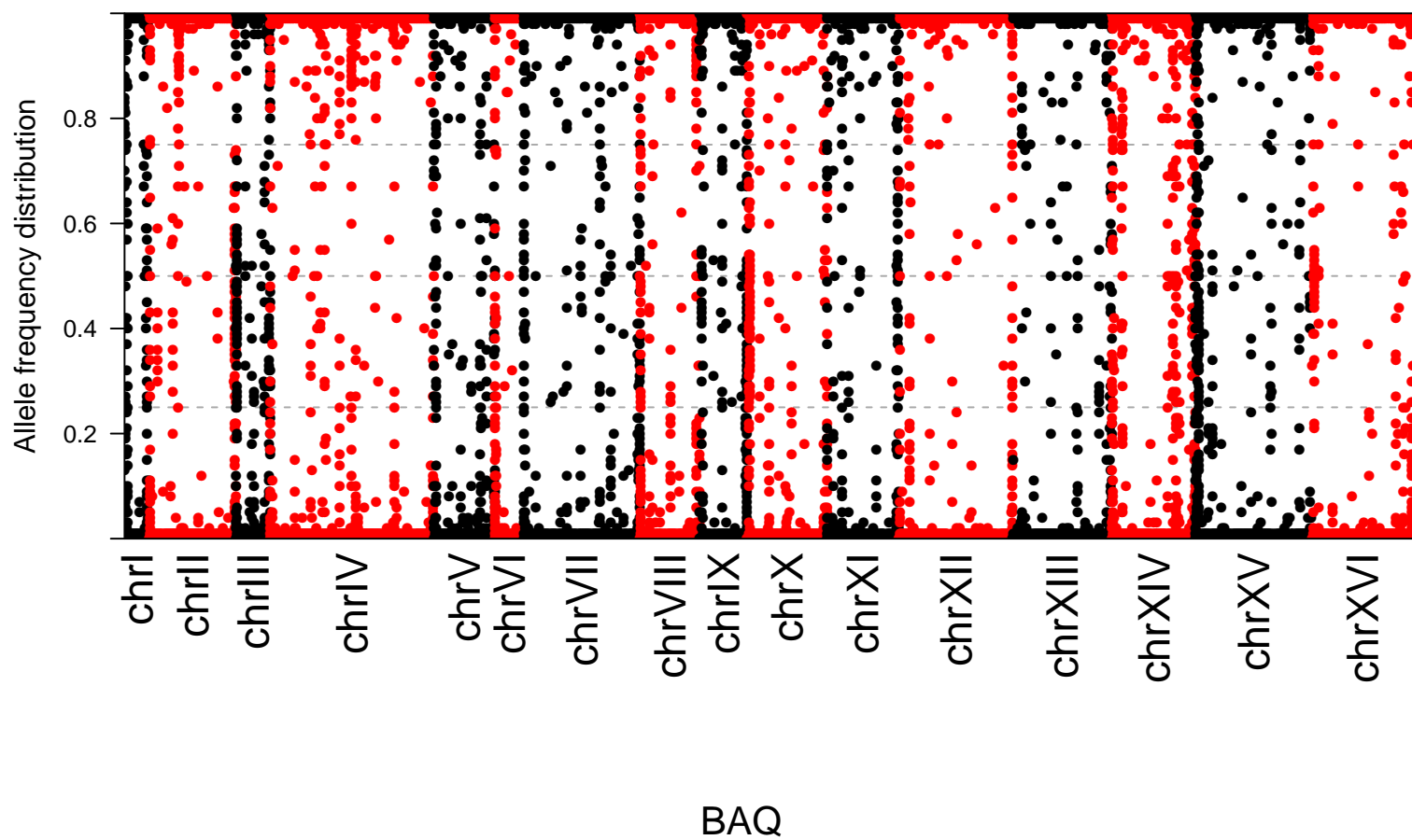
Supplementary Figure S3



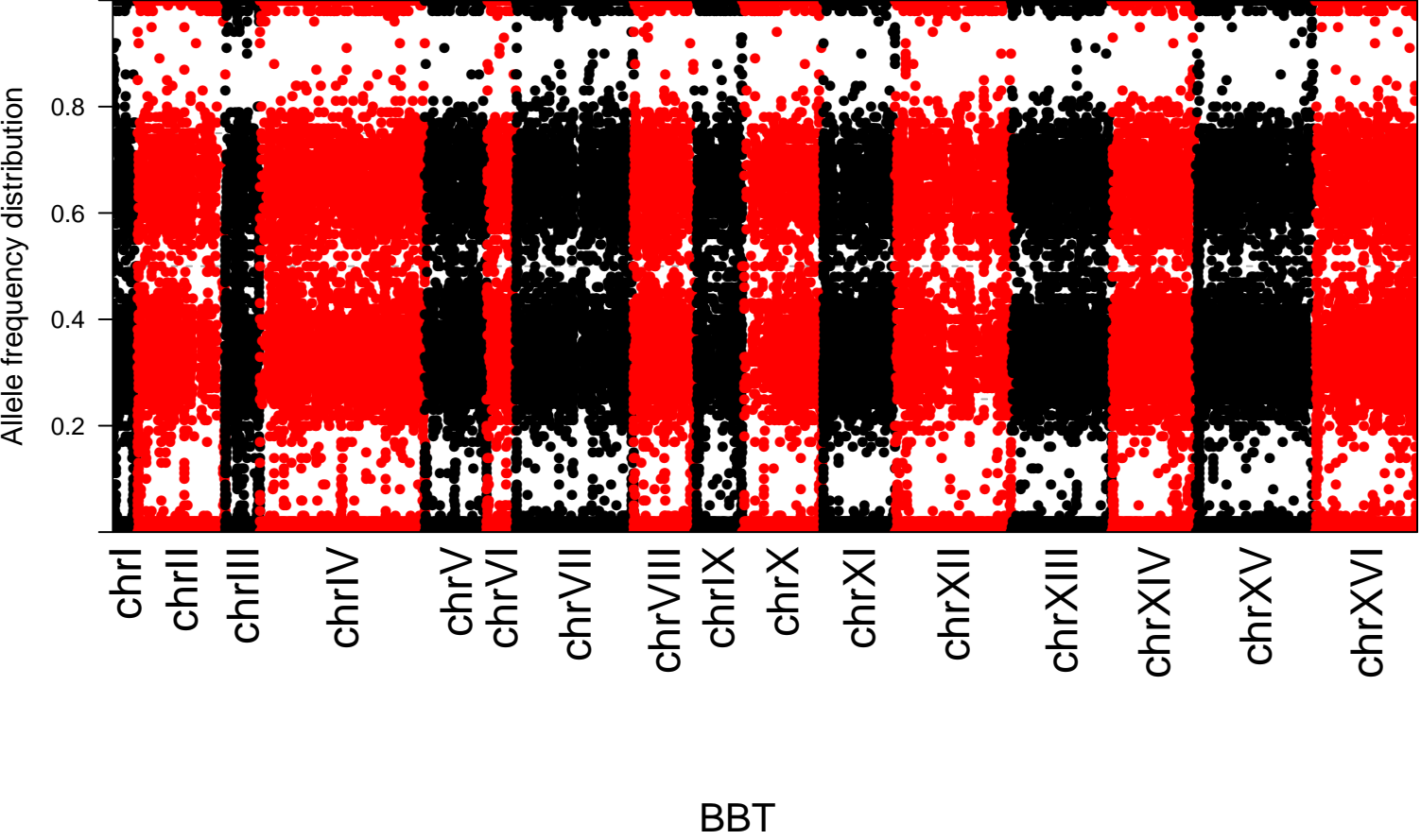
Supplementary Figure S3



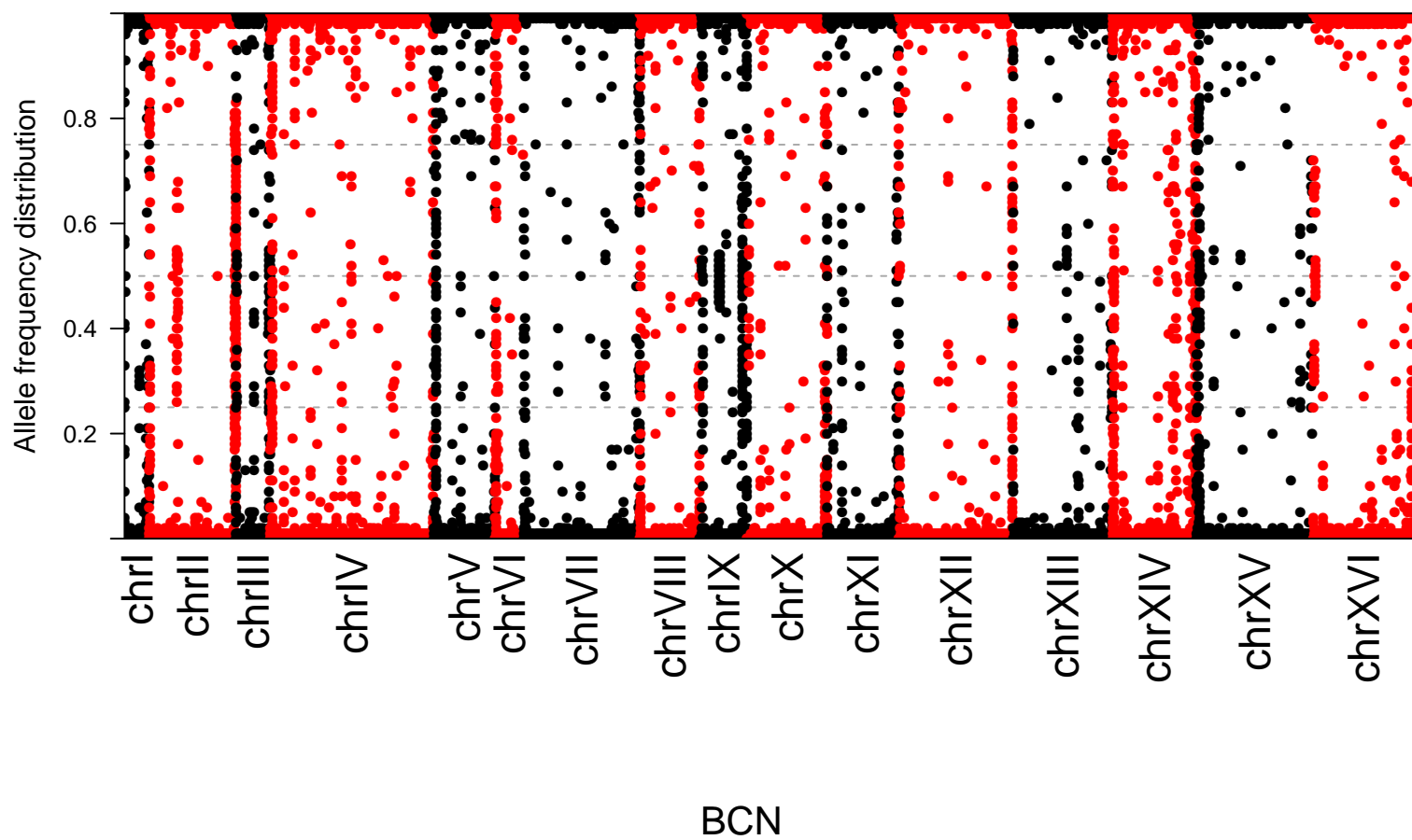
Supplementary Figure S3



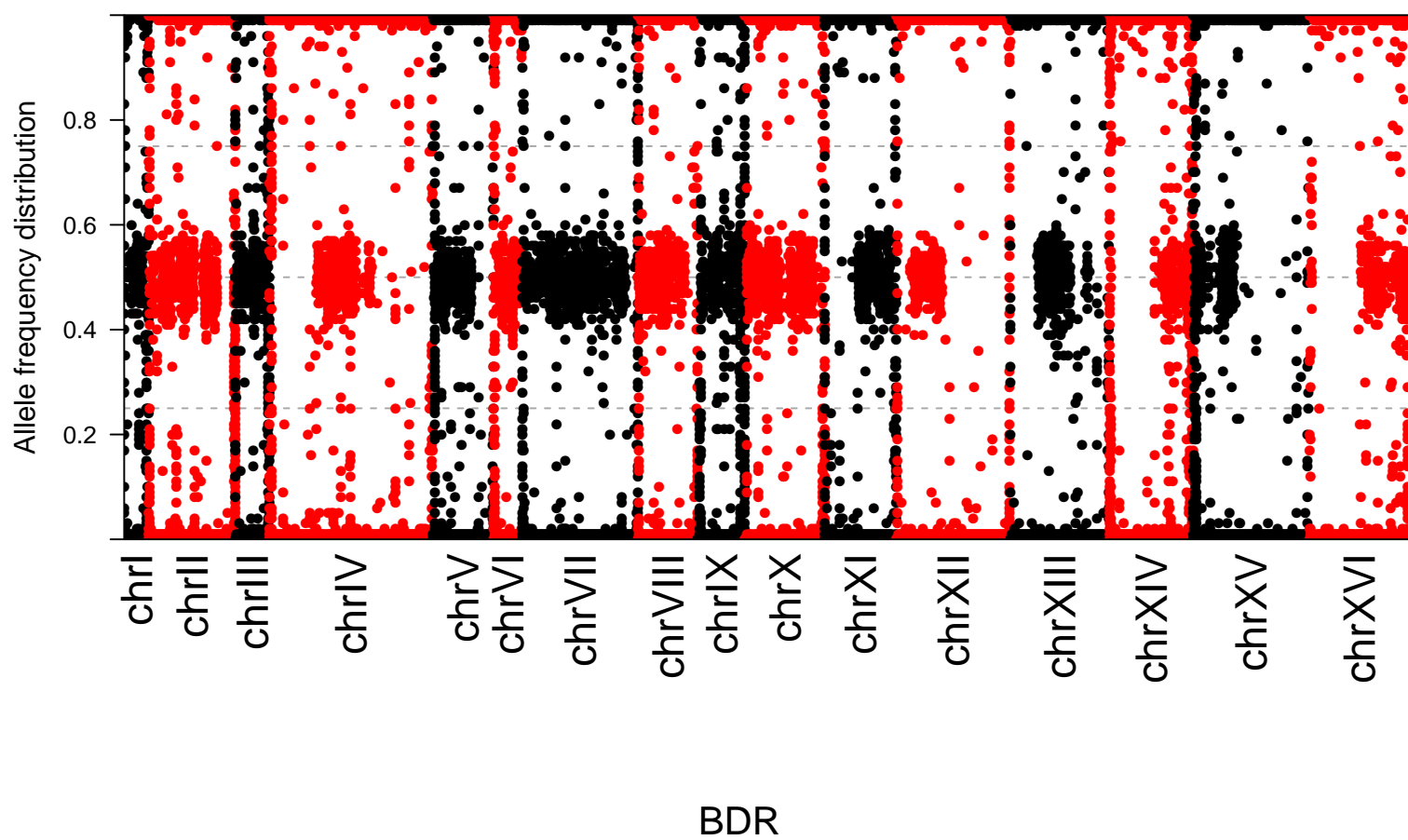
Supplementary Figure S3



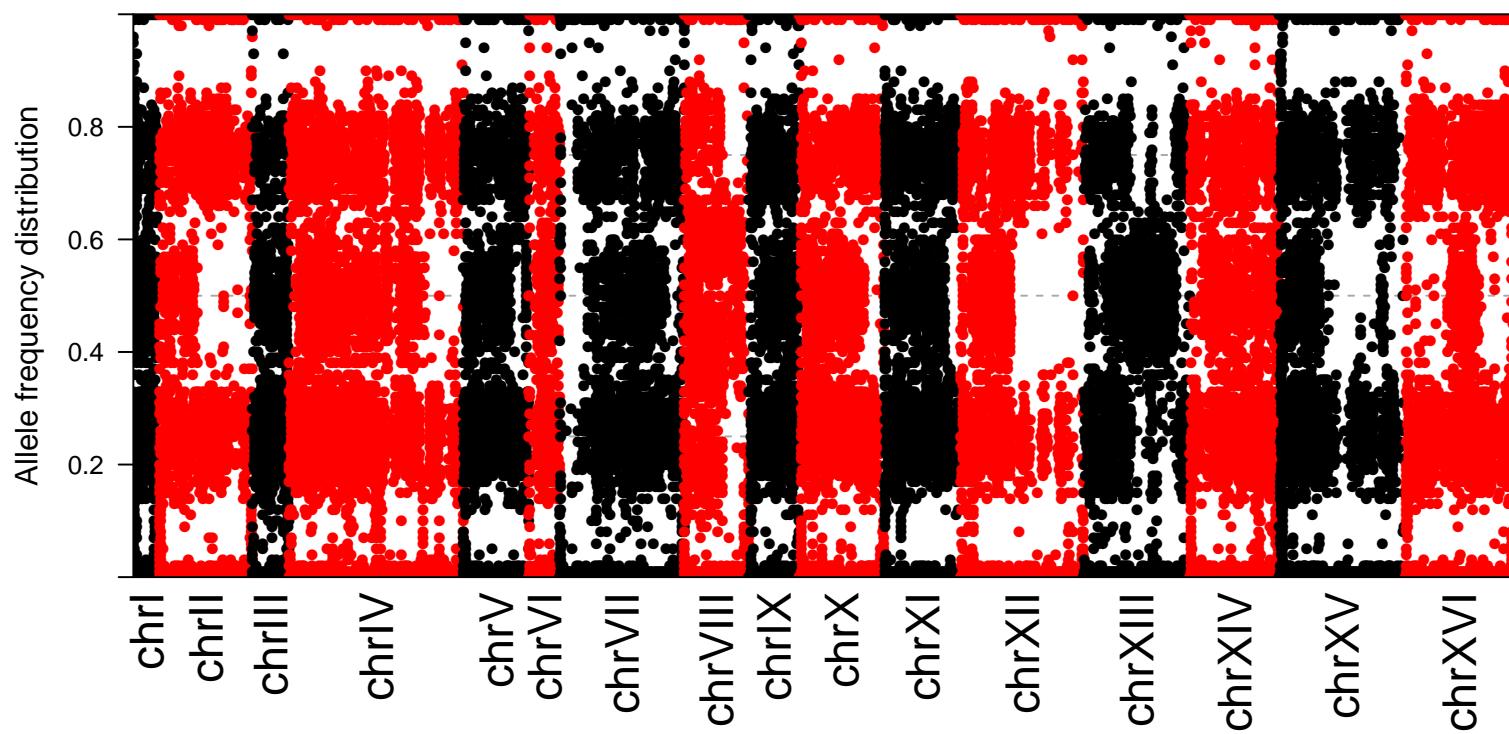
Supplementary Figure S3



Supplementary Figure S3

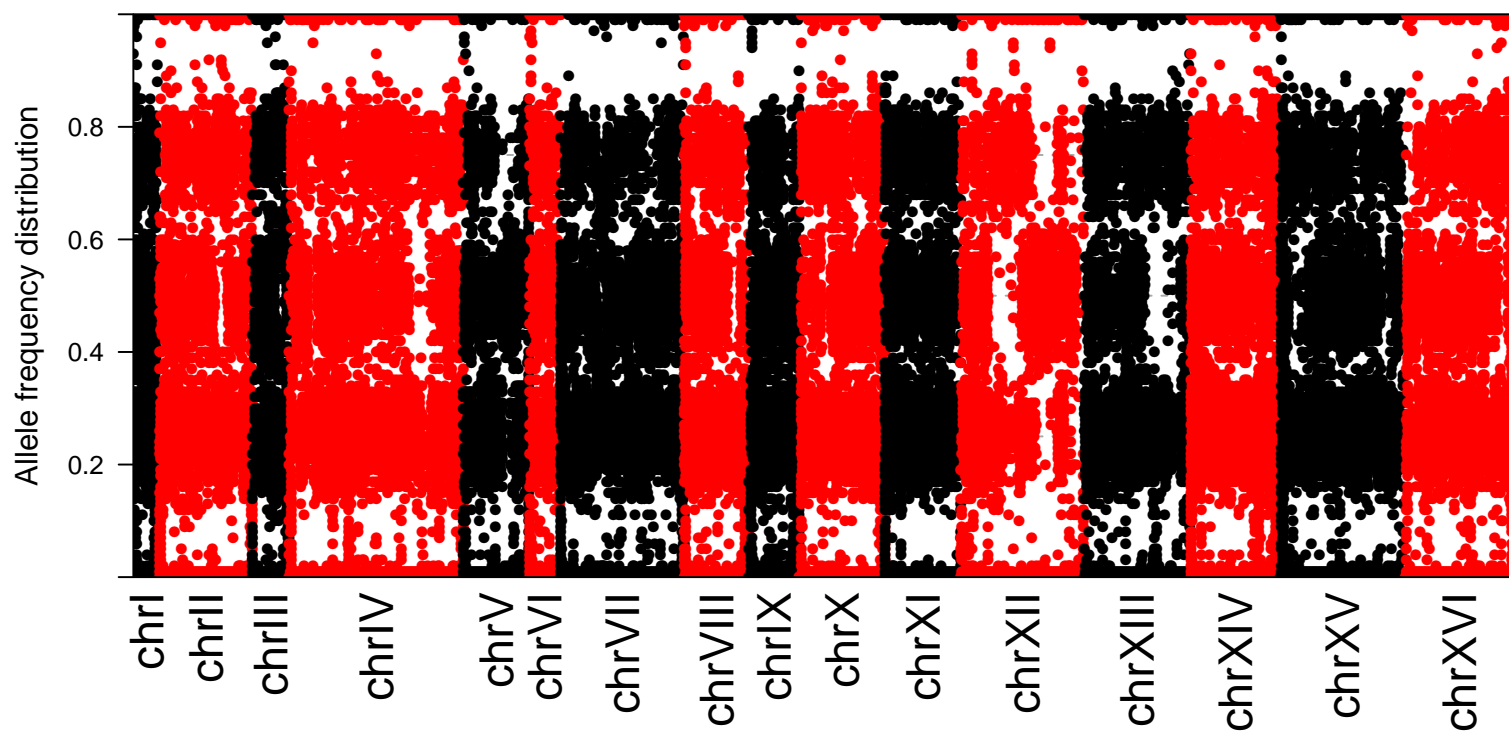


Supplementary Figure S3



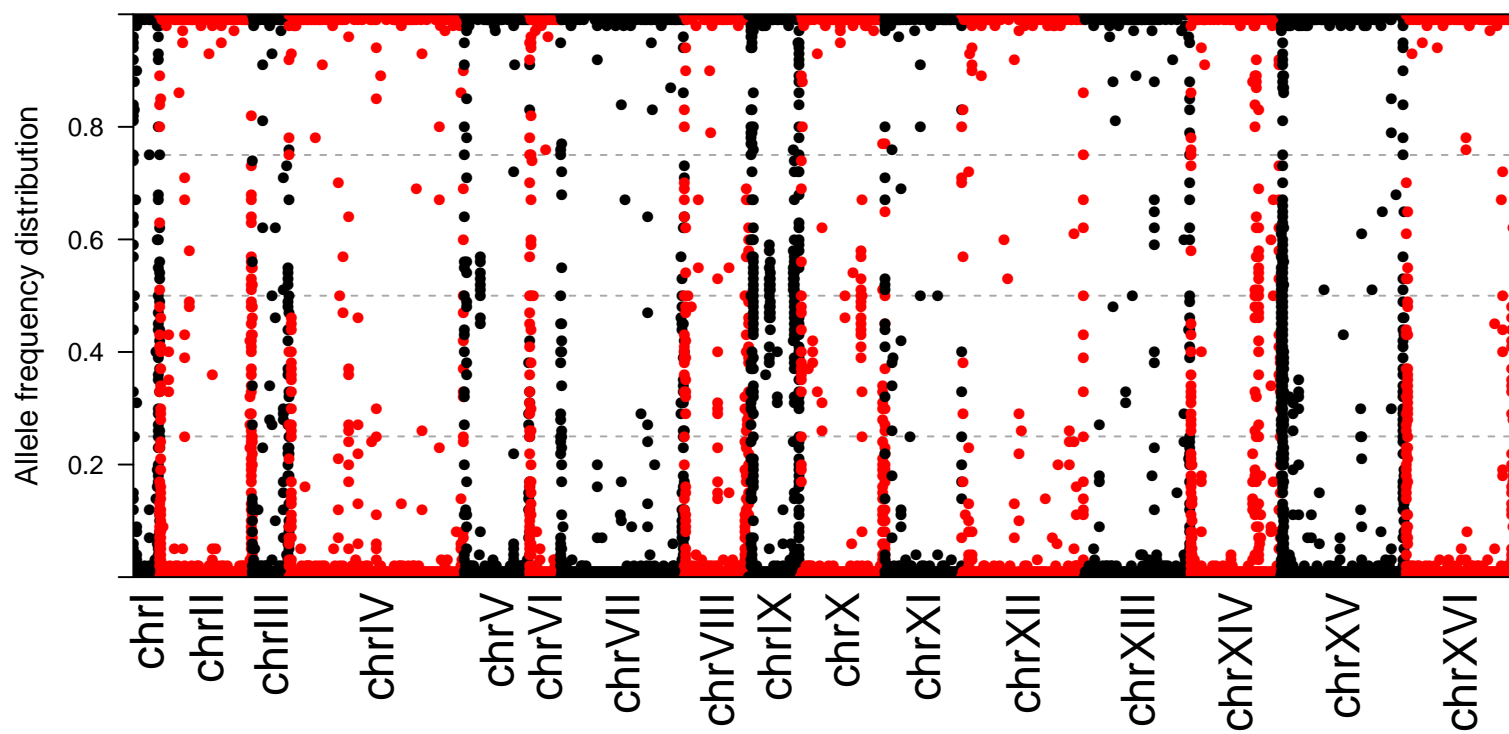
beer022

Supplementary Figure S3



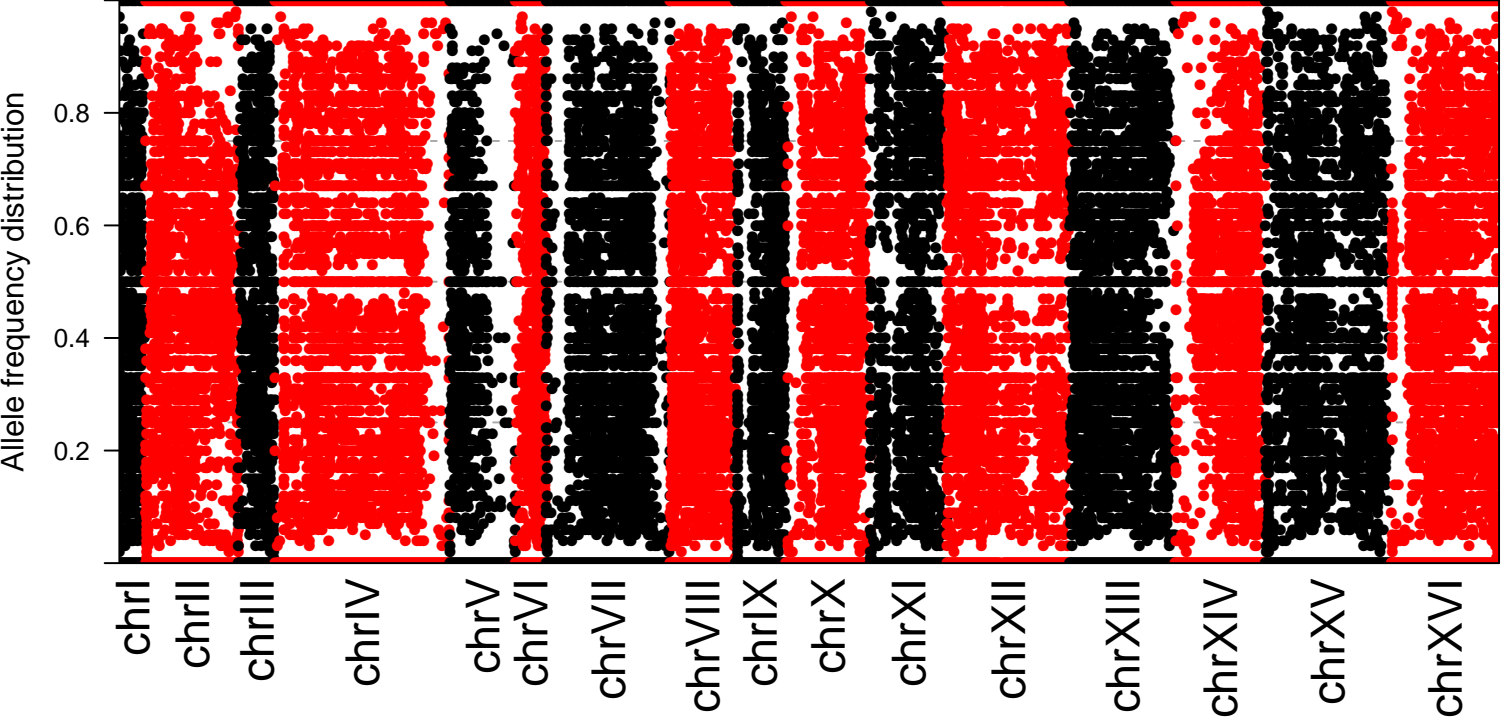
beer028

Supplementary Figure S3



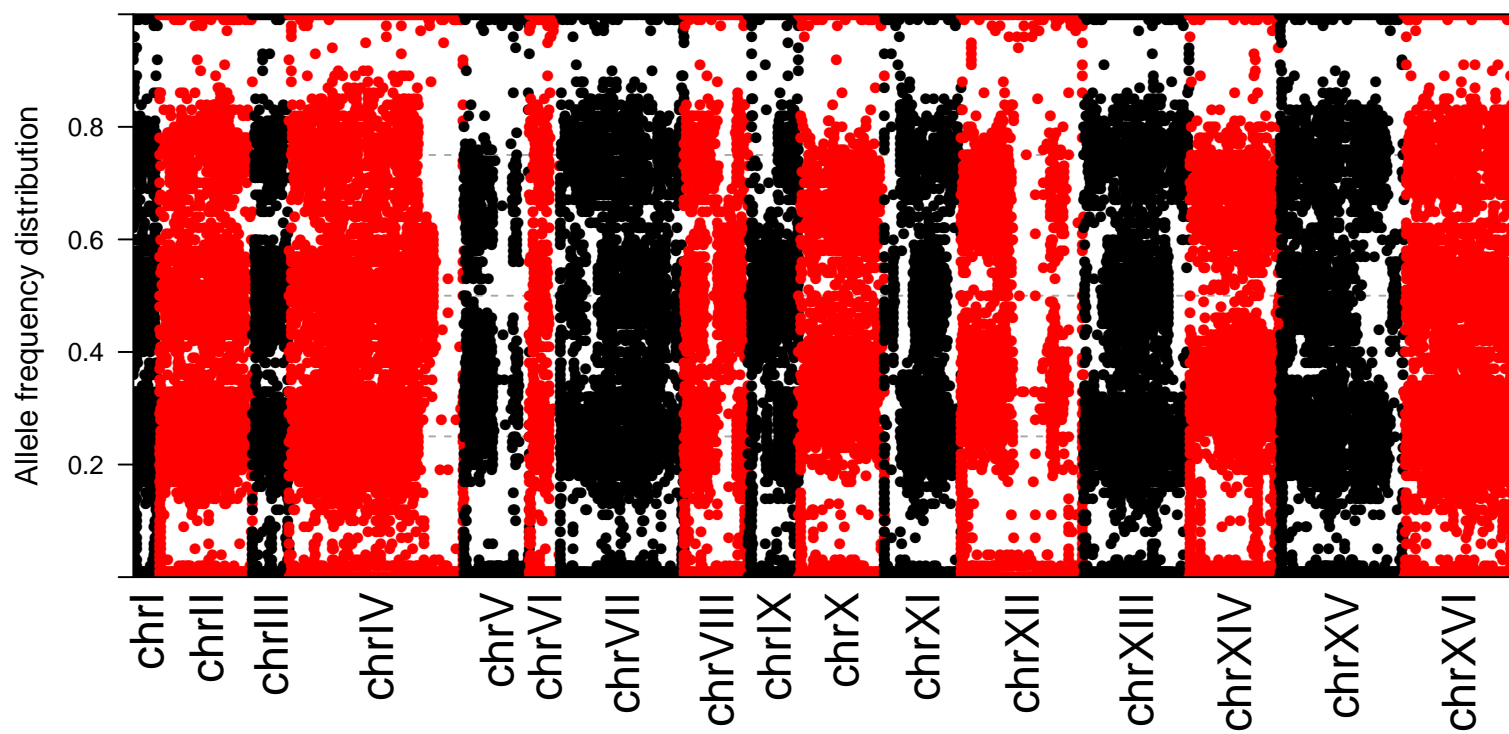
beer034

Supplementary Figure S3



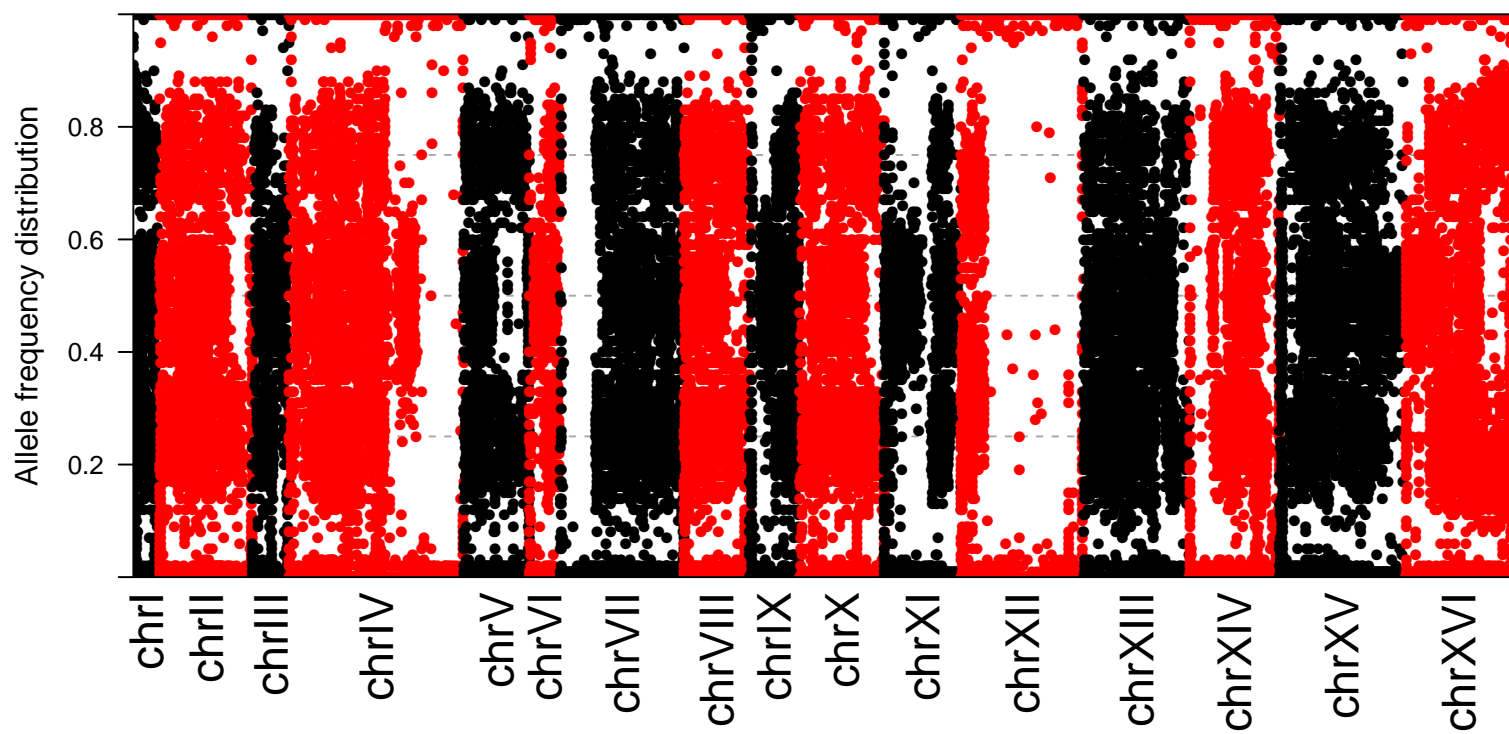
beer044

Supplementary Figure S3



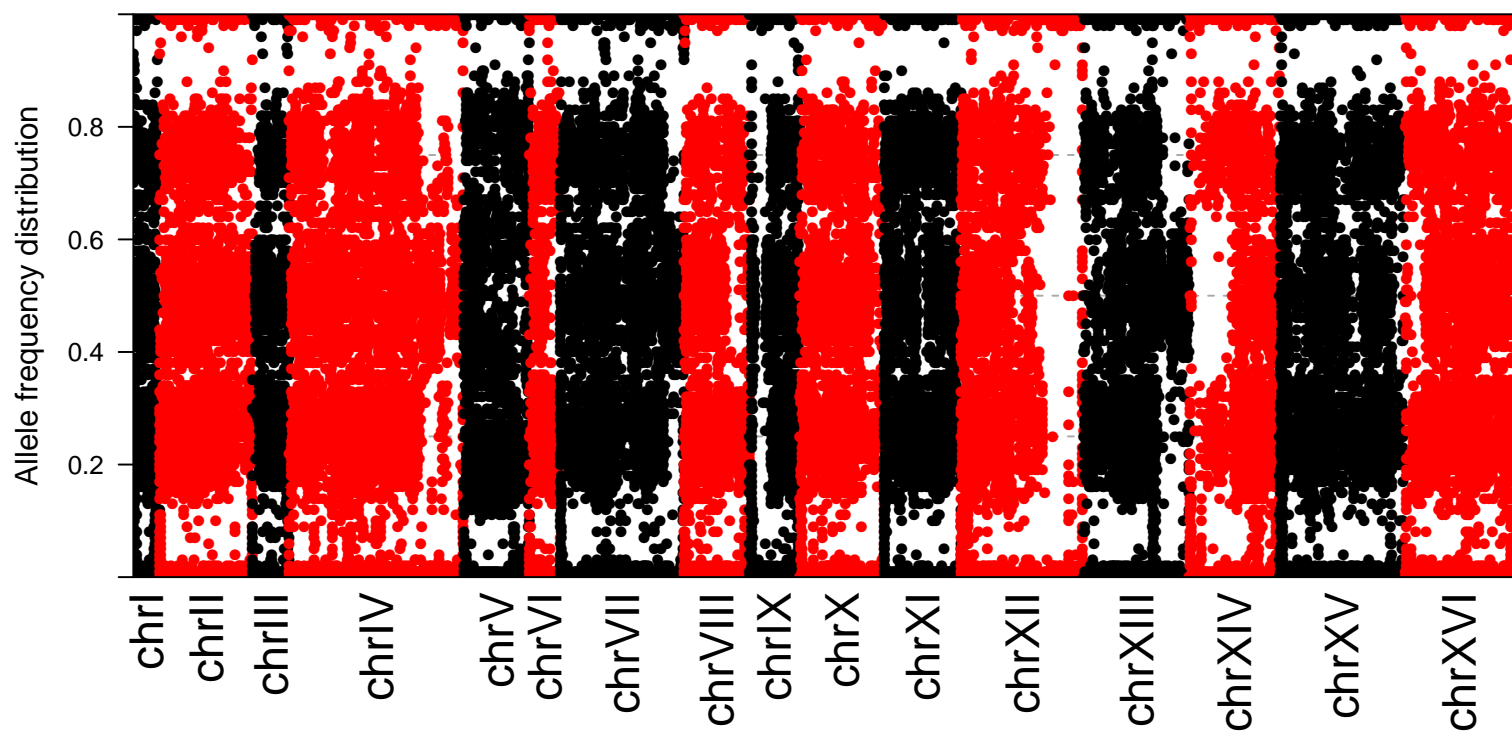
beer045

Supplementary Figure S3



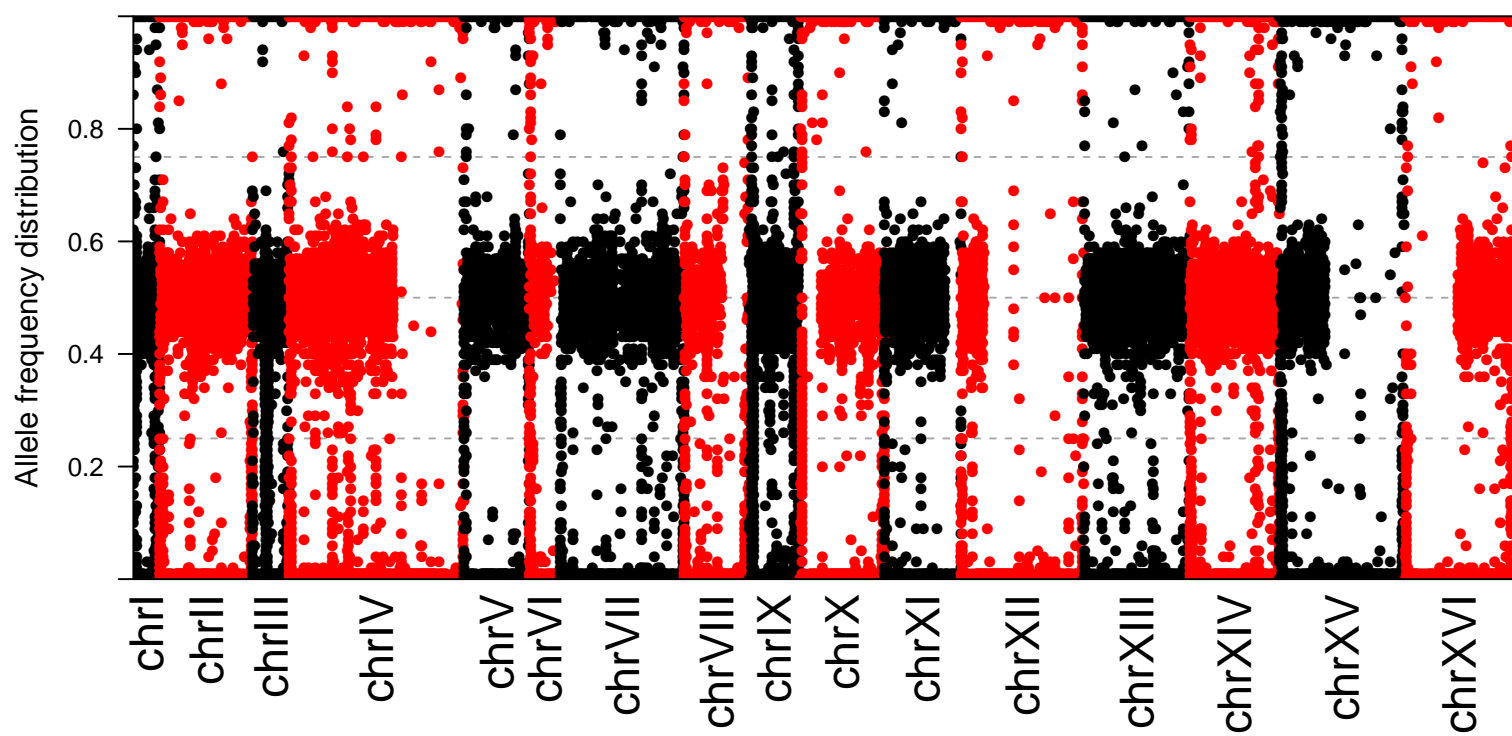
beer046

Supplementary Figure S3



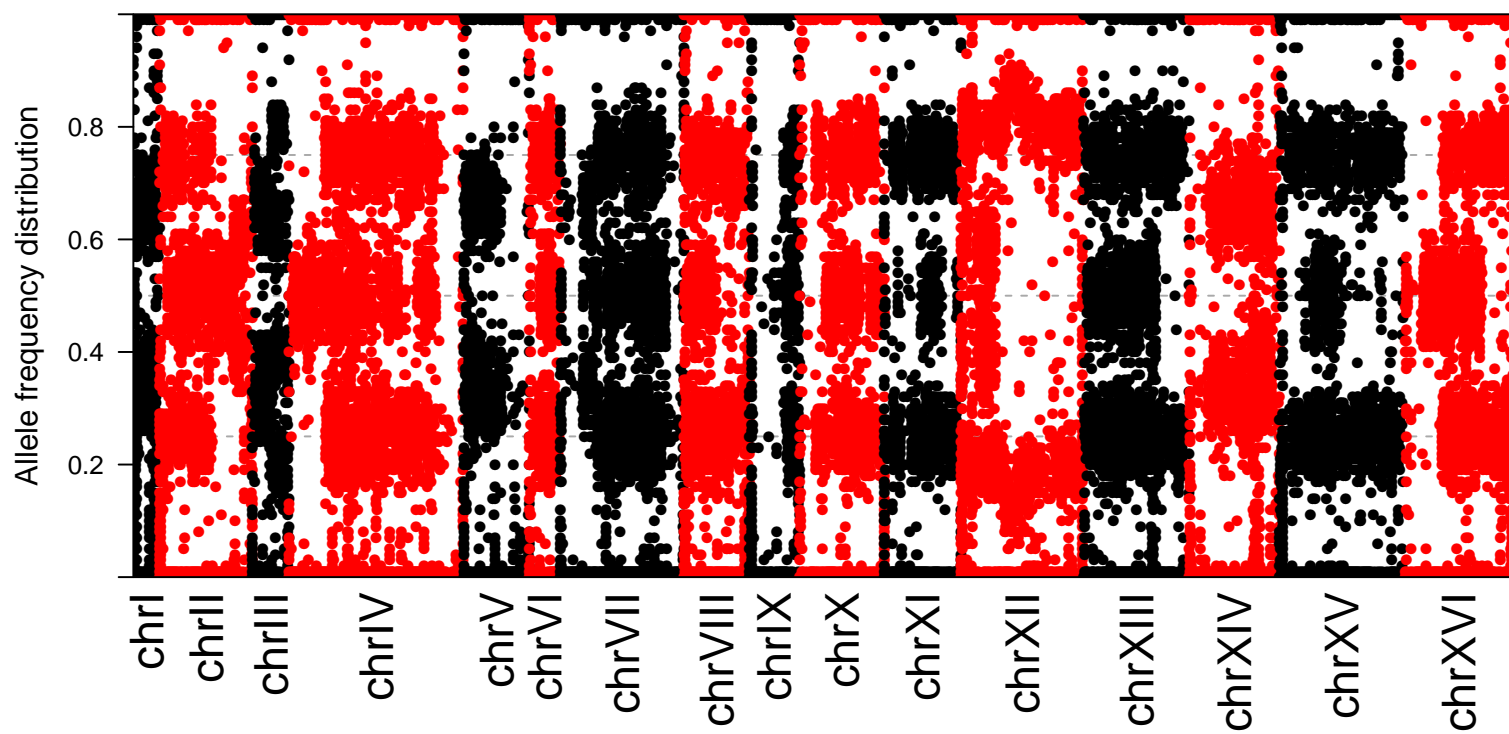
beer079

Supplementary Figure S3



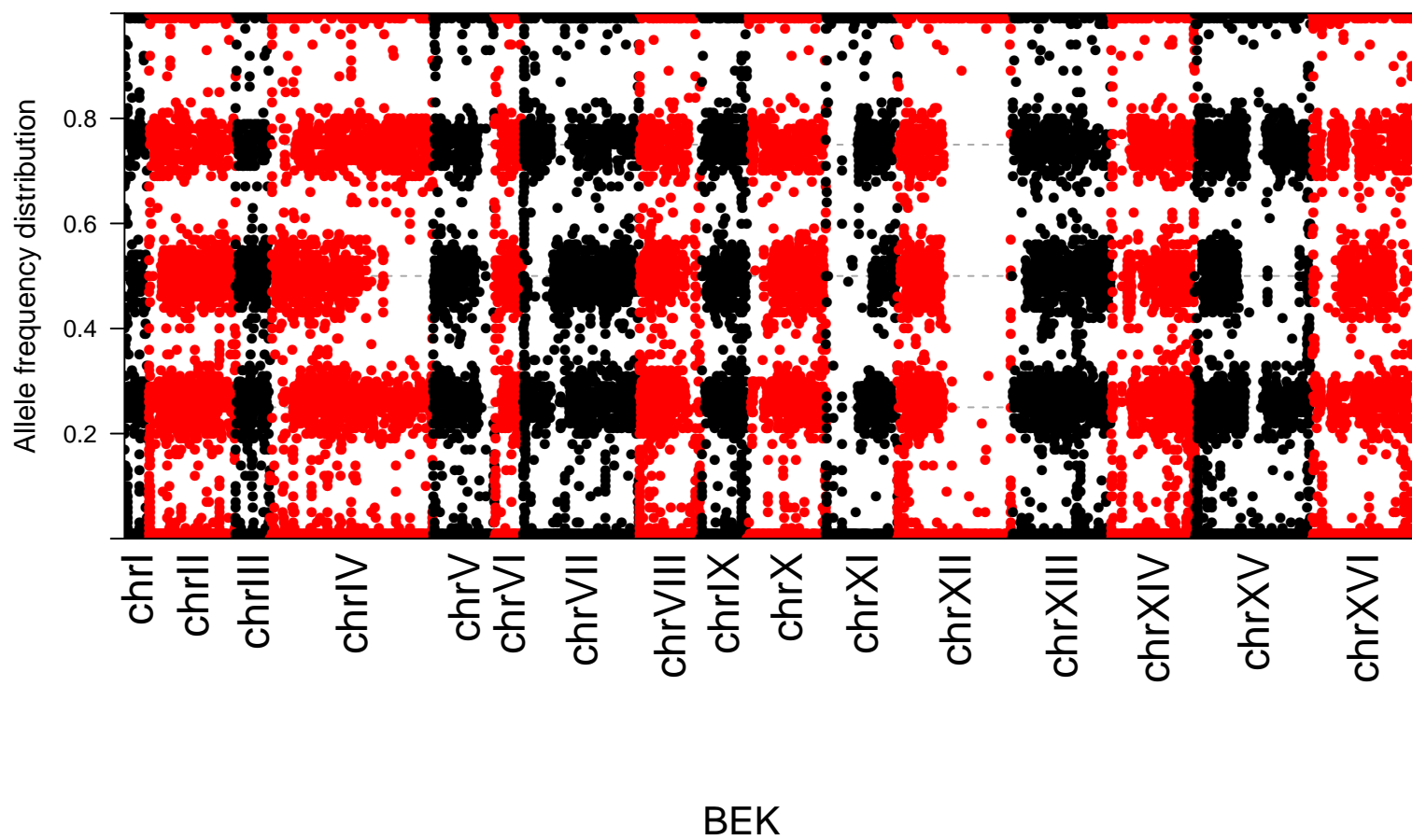
beer085

Supplementary Figure S3

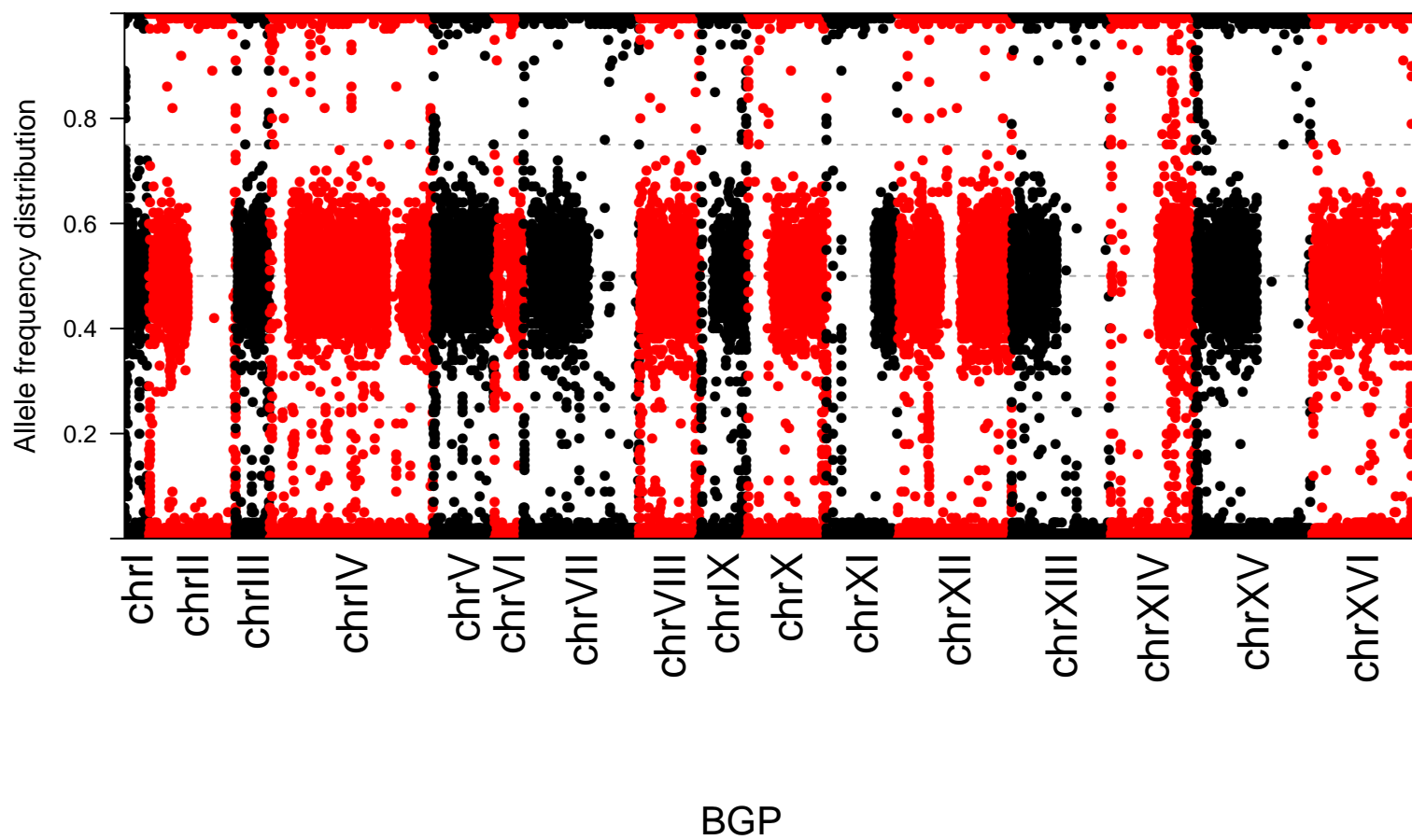


beer098

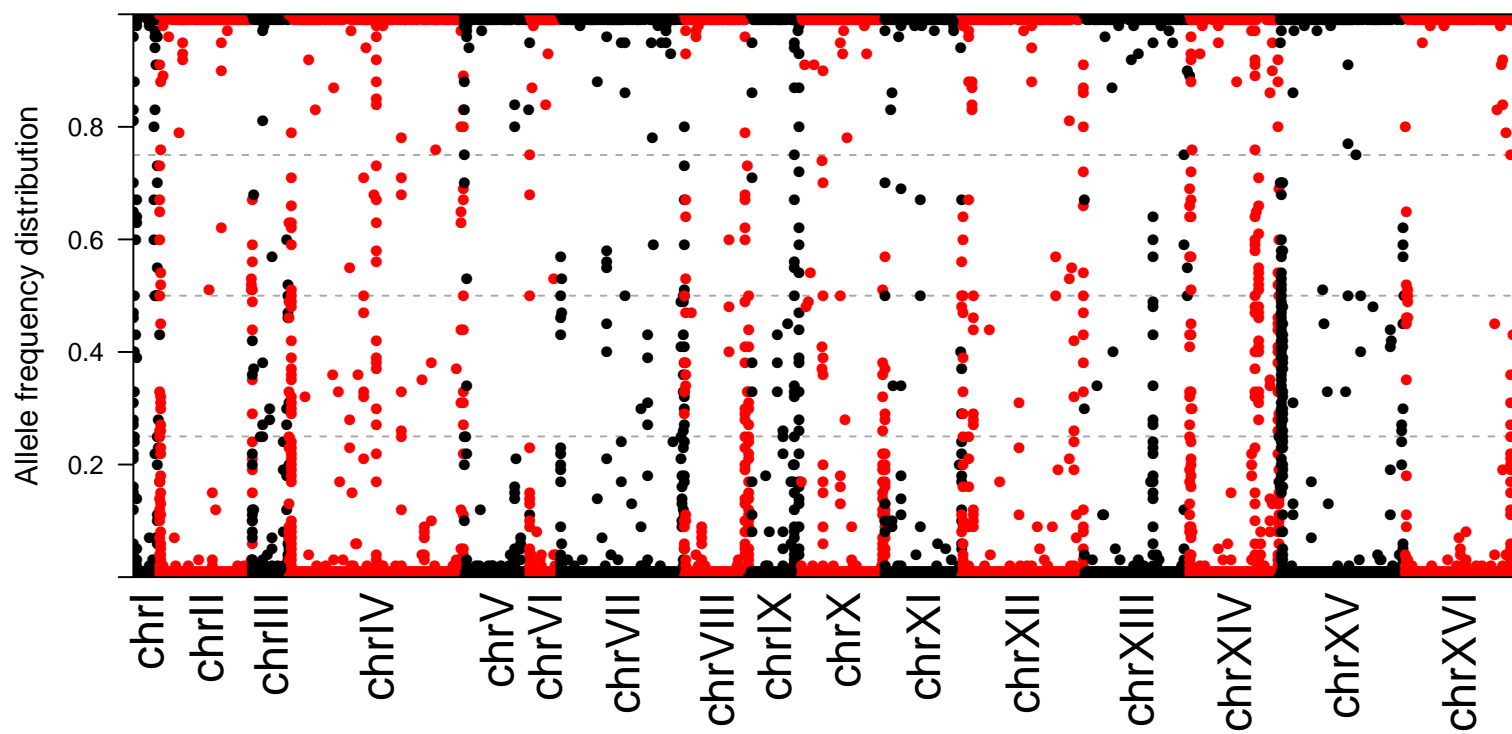
Supplementary Figure S3



Supplementary Figure S3

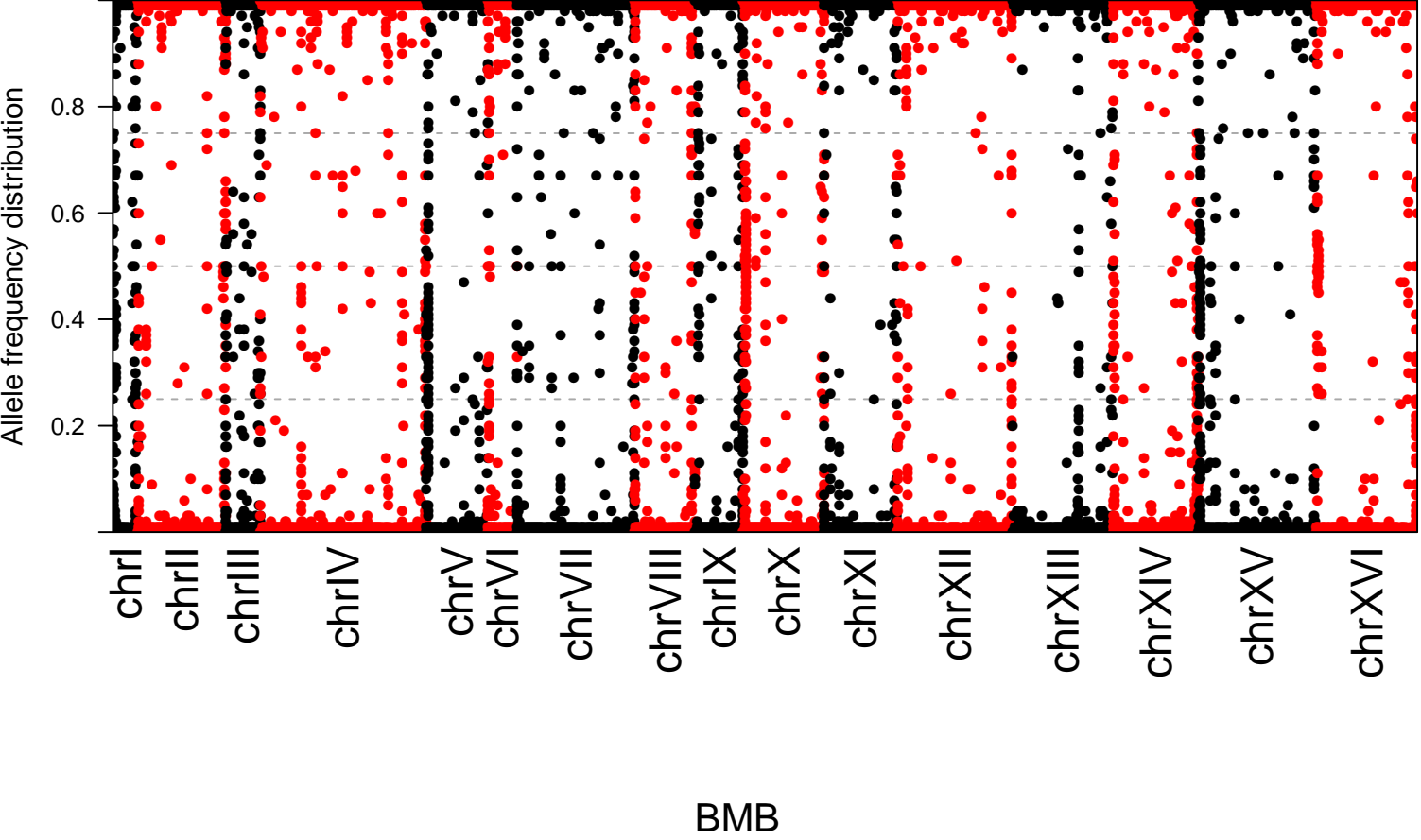


Supplementary Figure S3

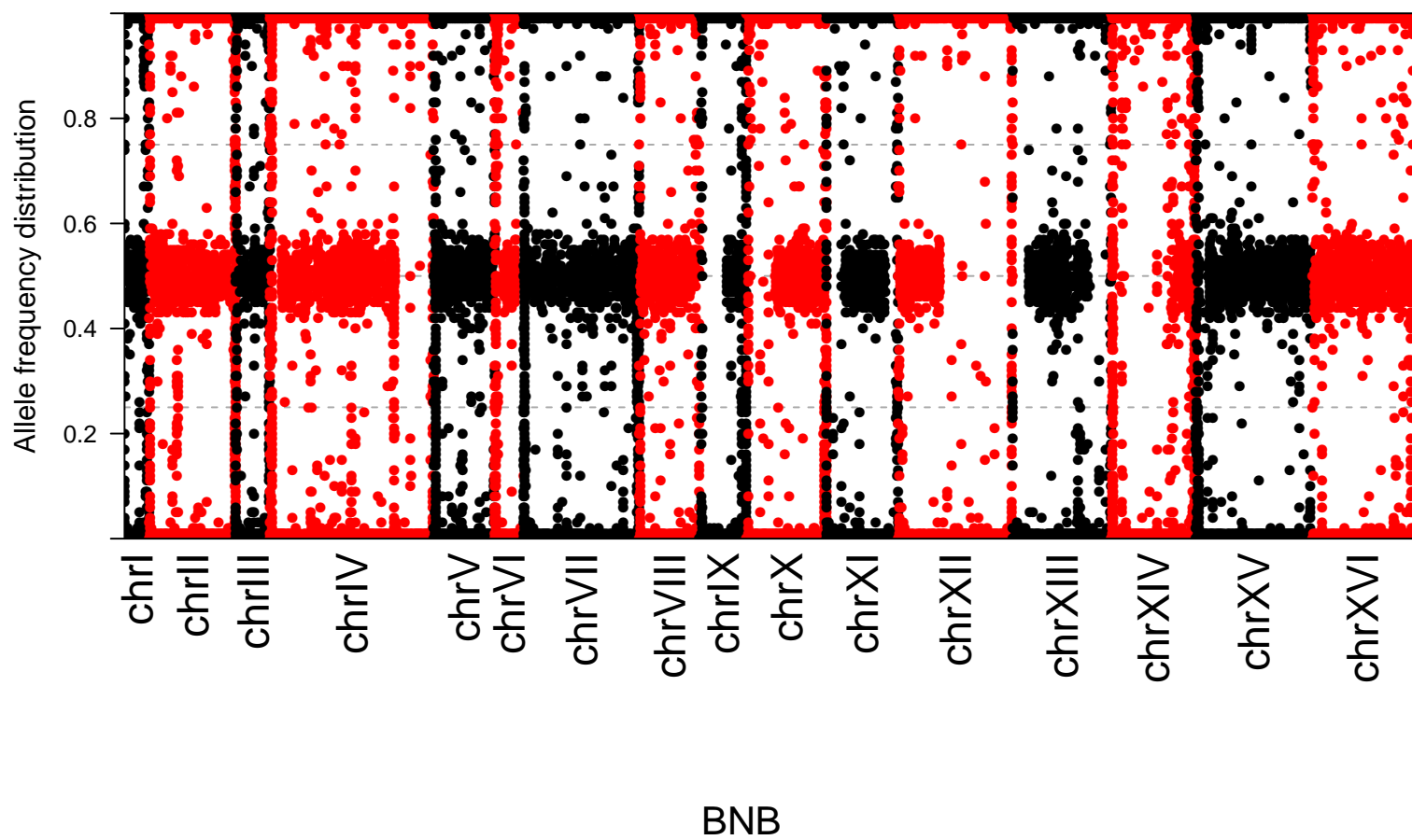


BIA

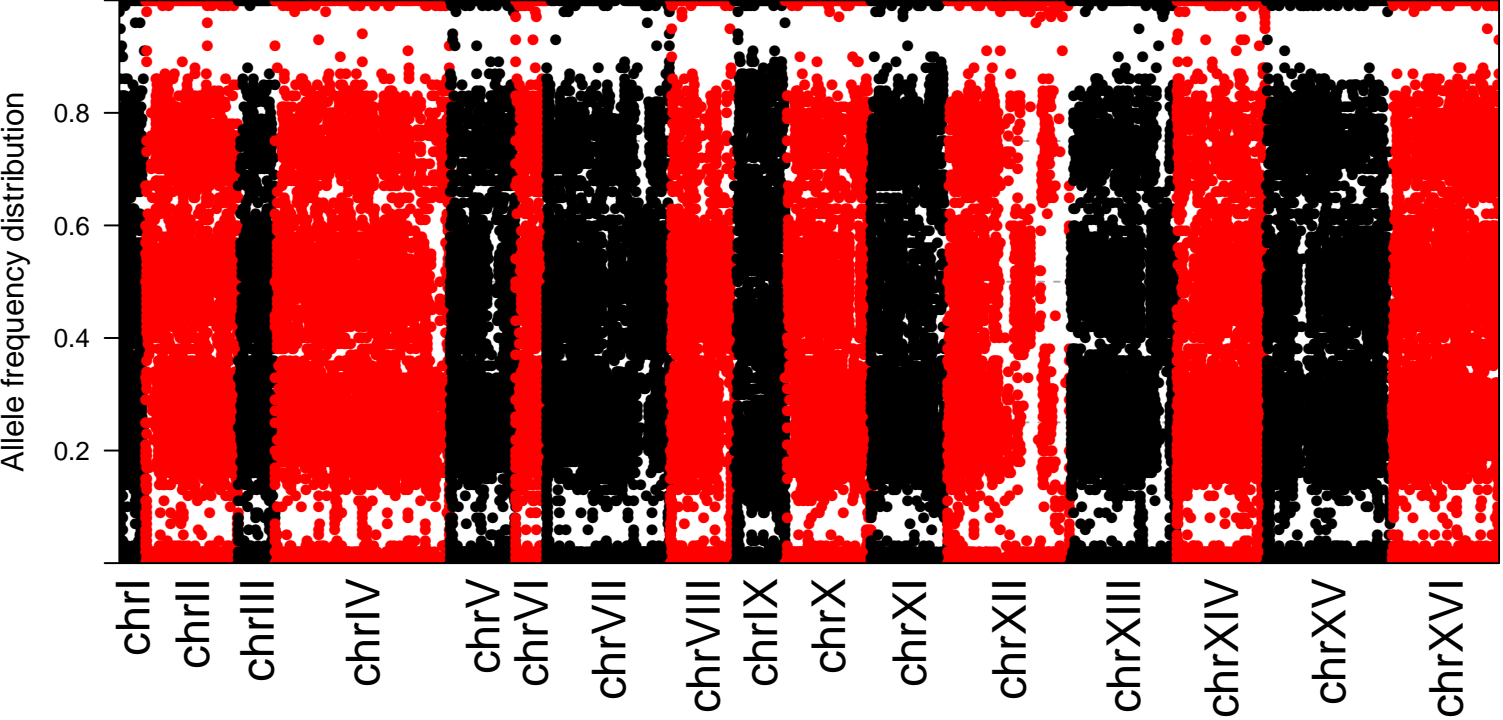
Supplementary Figure S3



Supplementary Figure S3

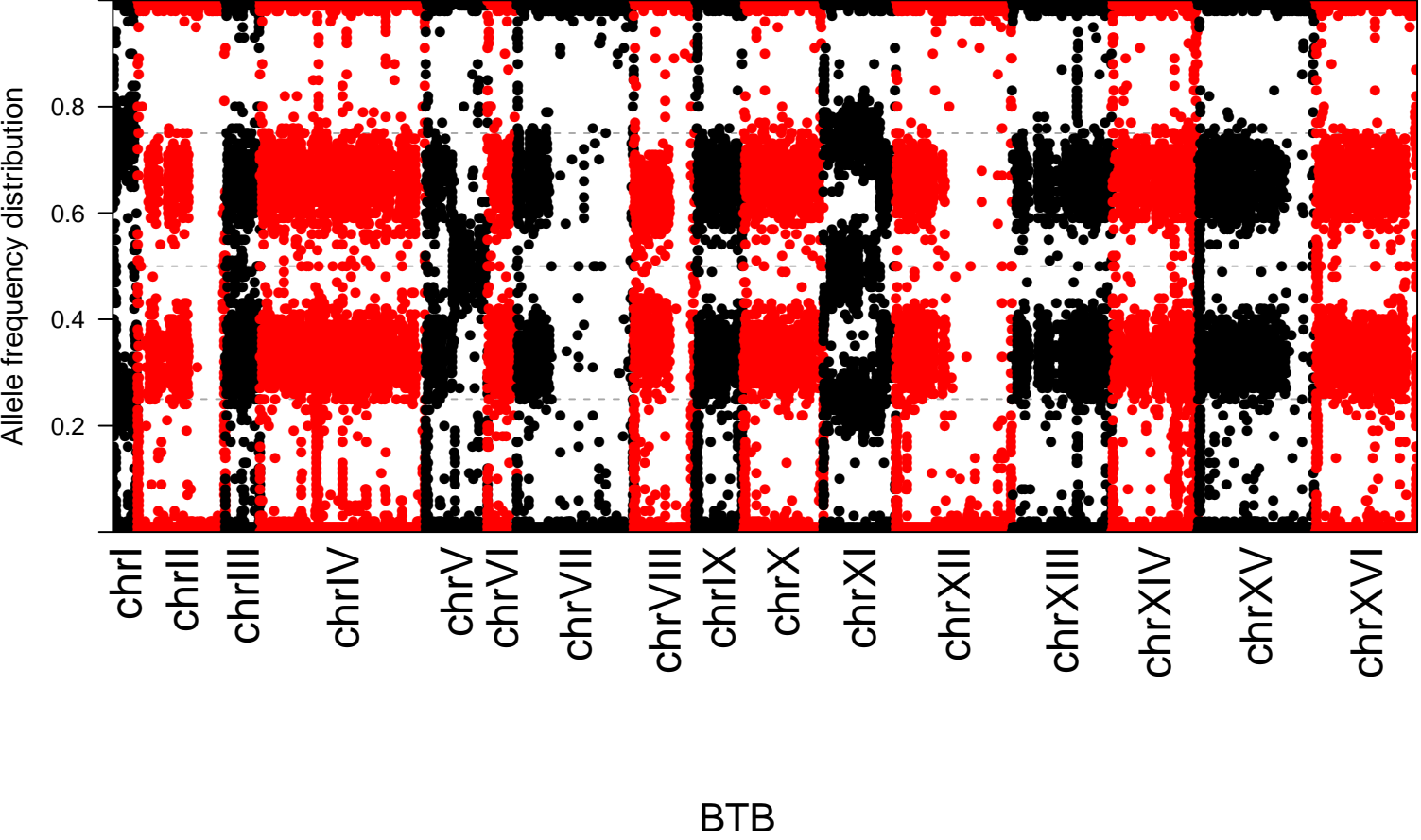


Supplementary Figure S3

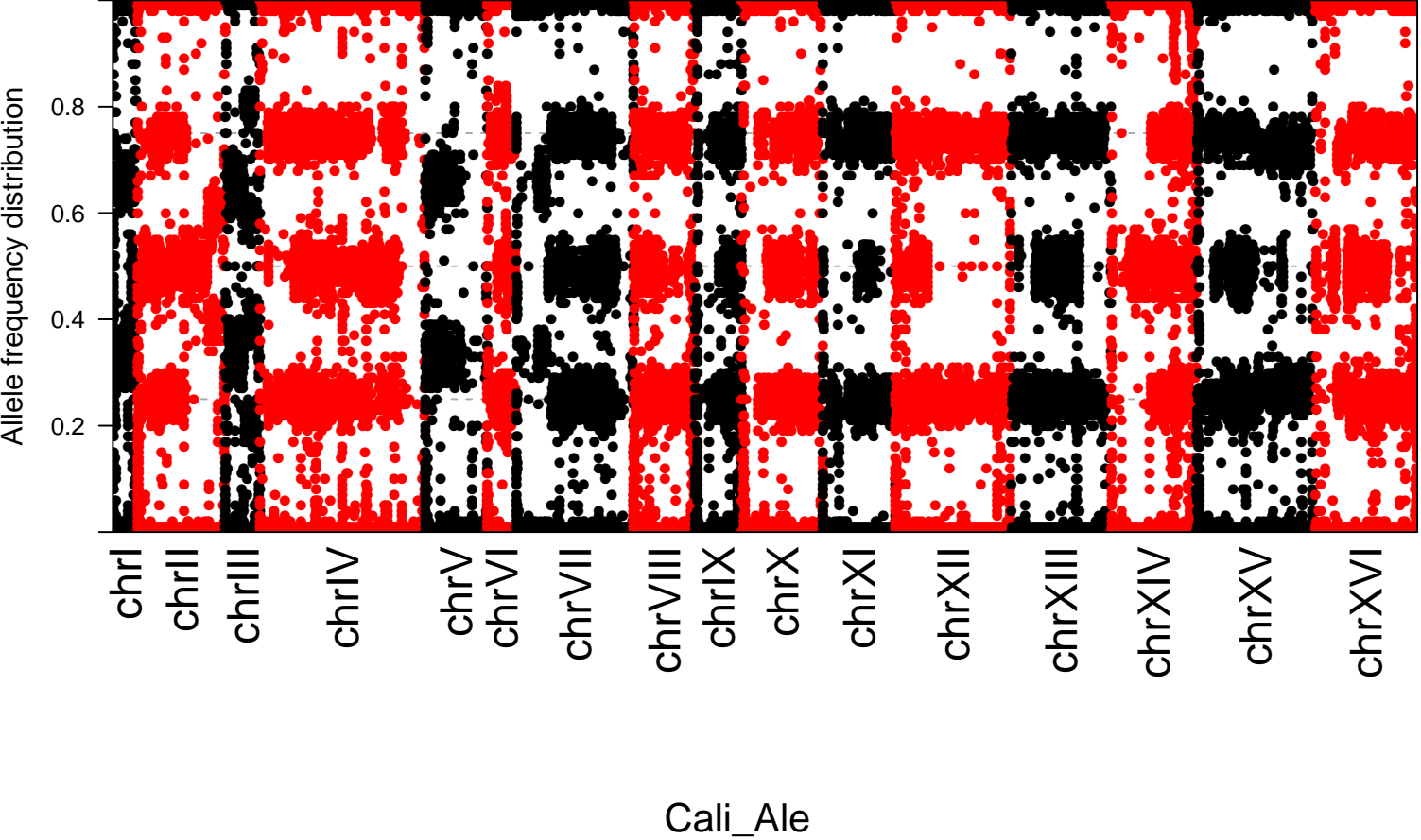


bread004

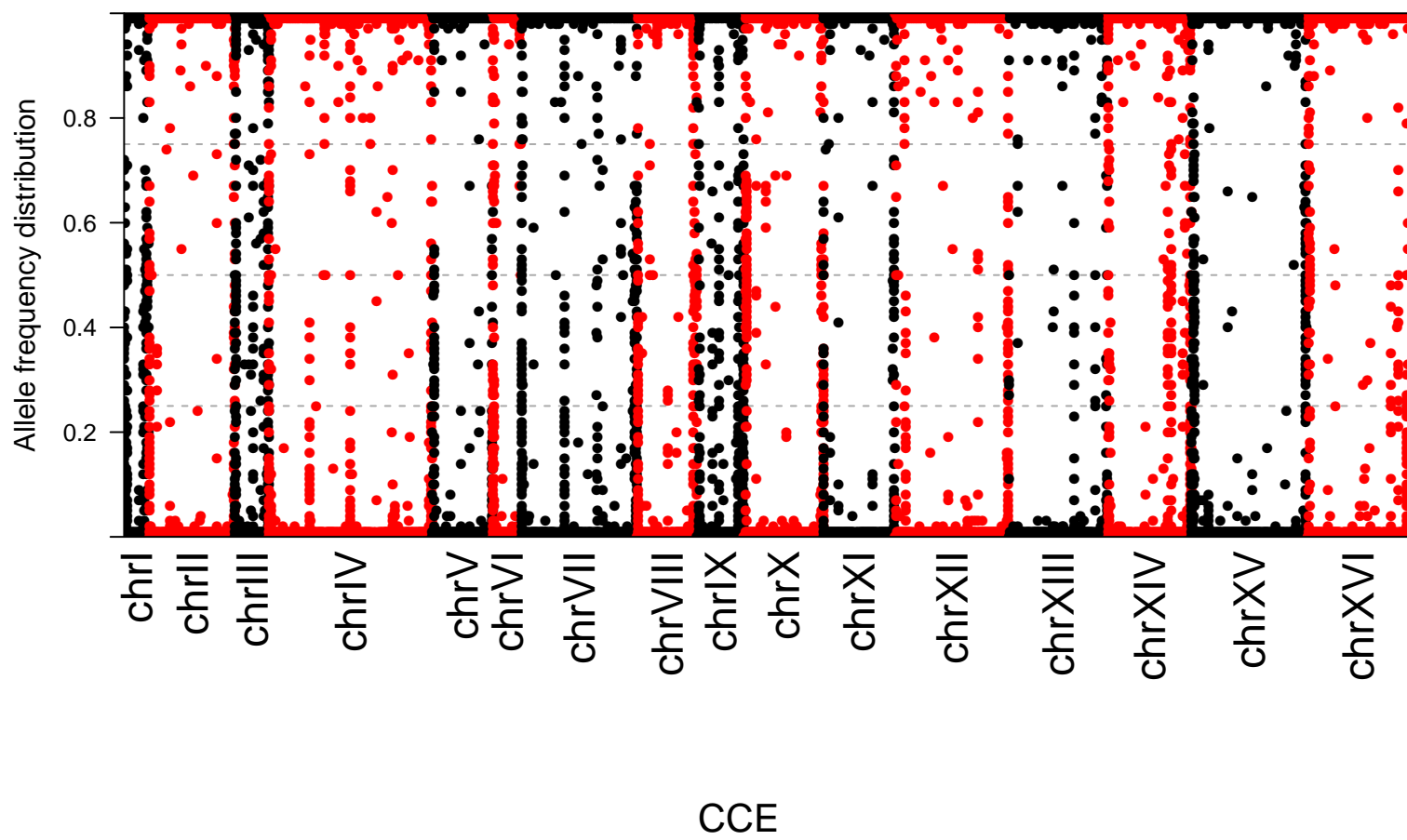
Supplementary Figure S3



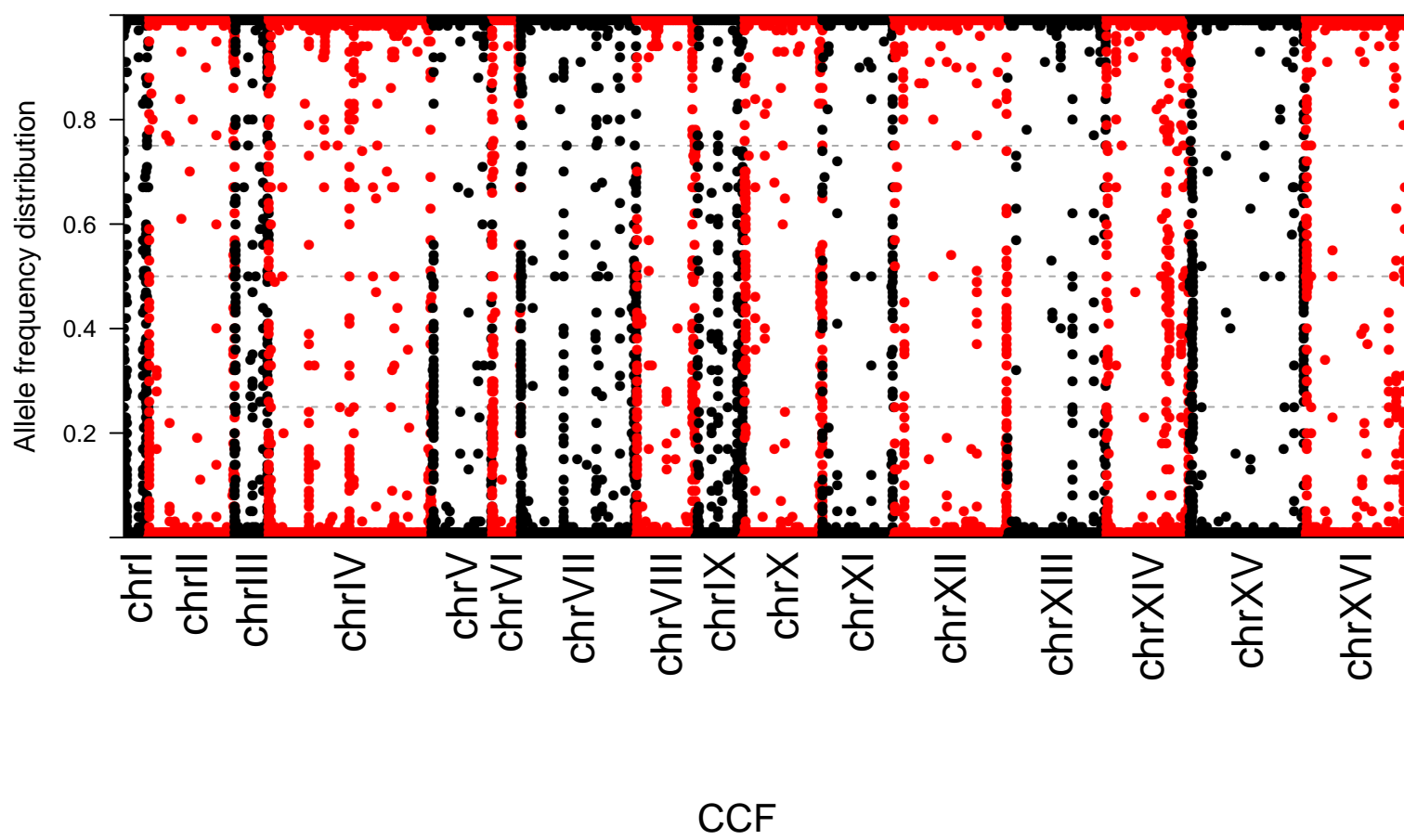
Supplementary Figure S3



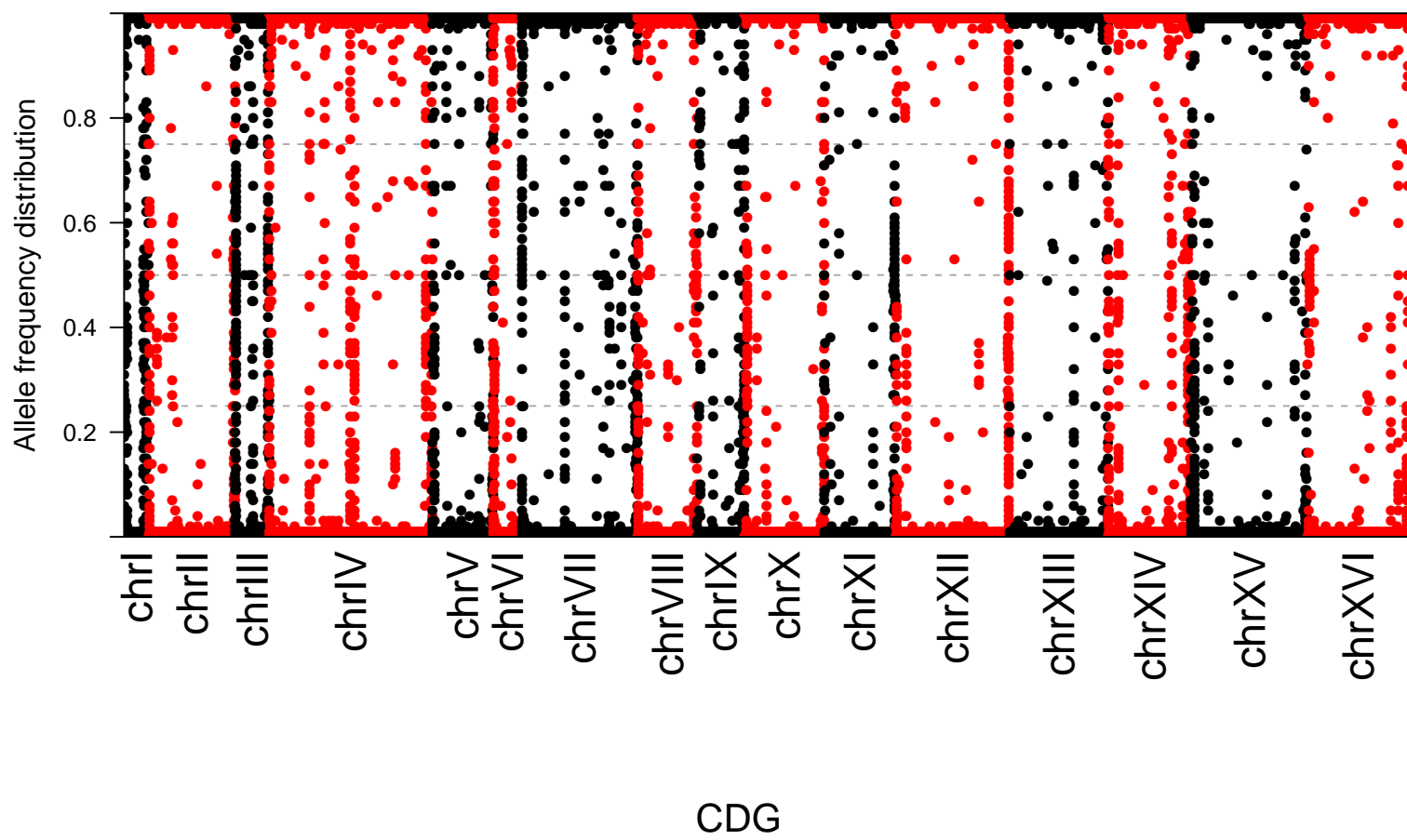
Supplementary Figure S3



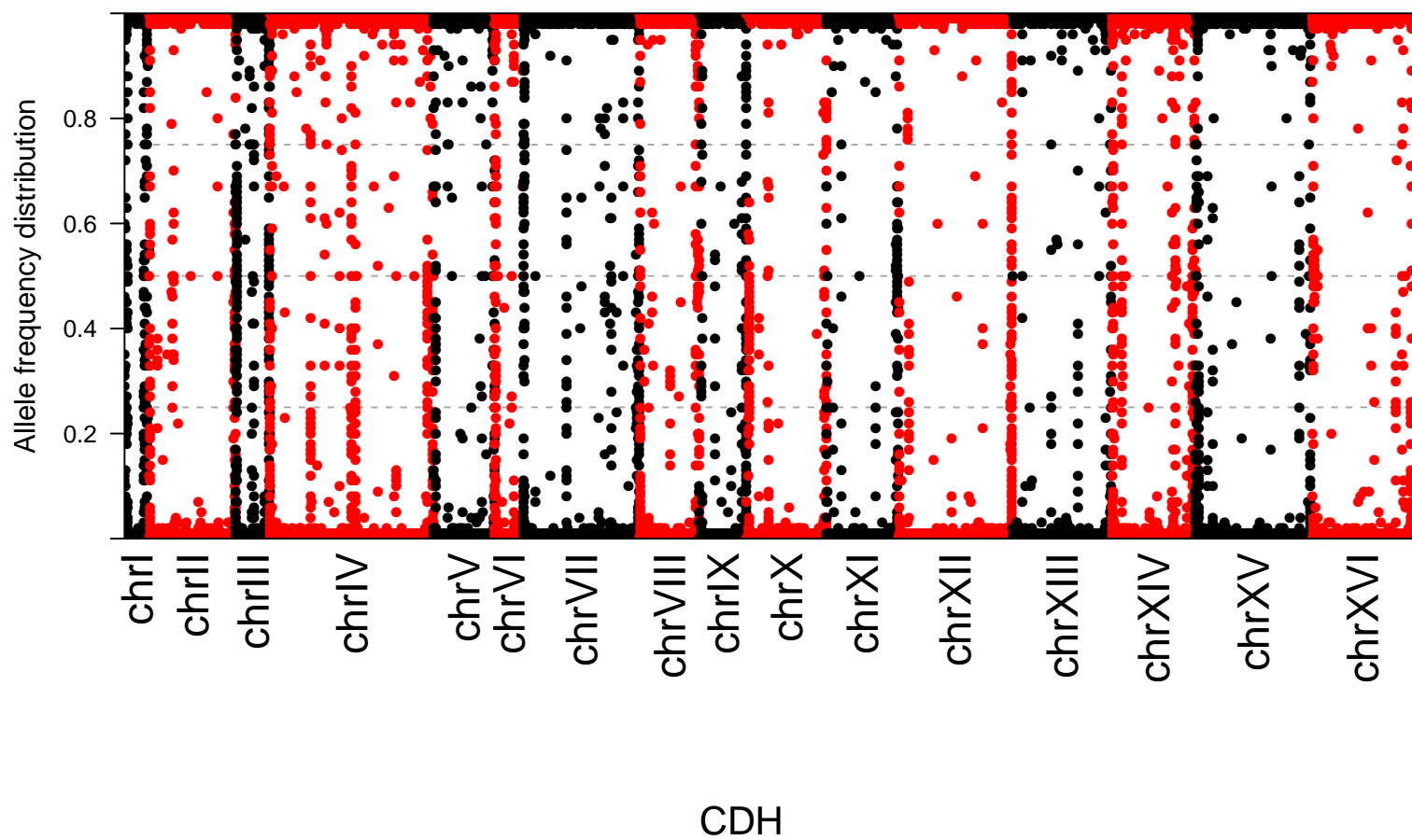
Supplementary Figure S3



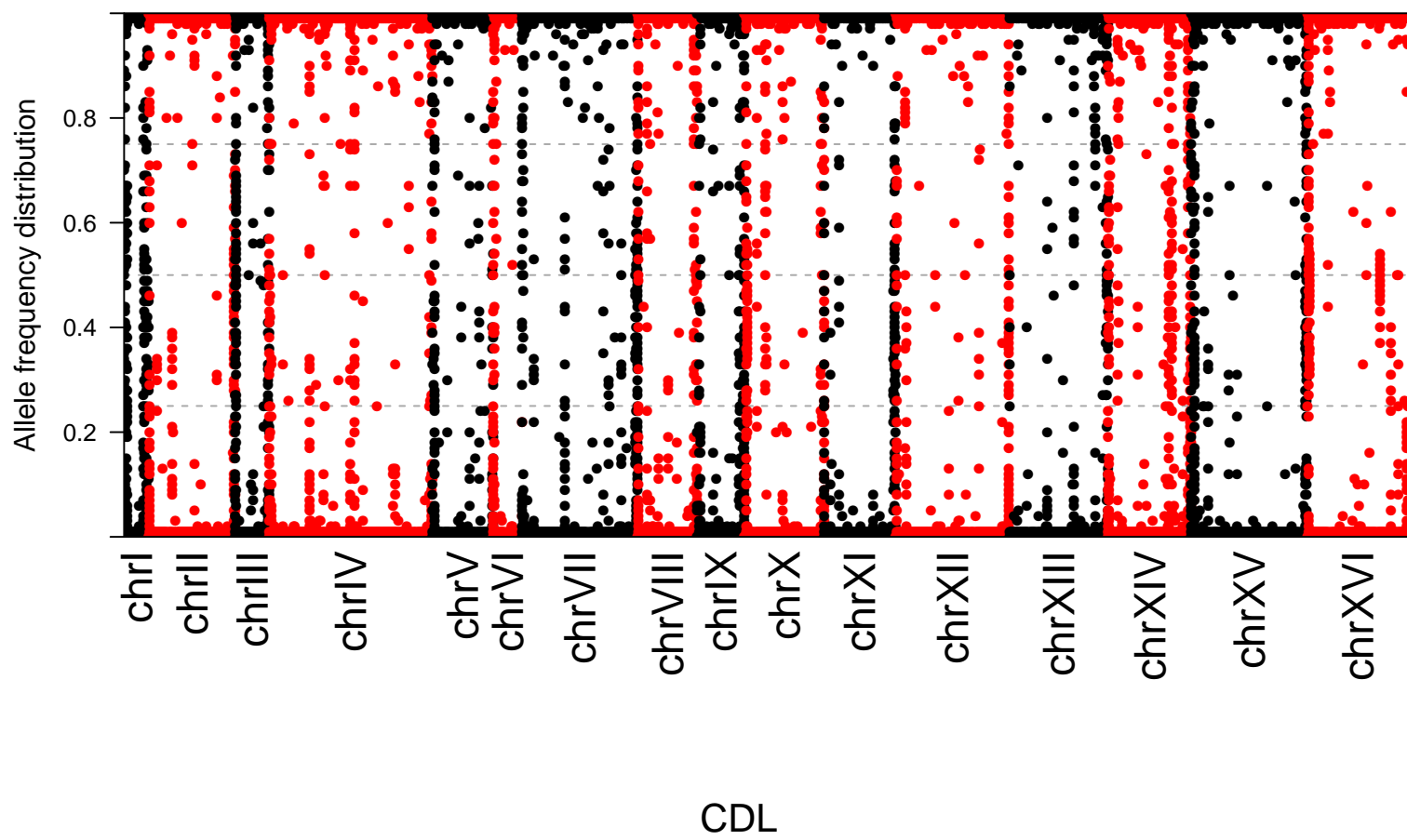
Supplementary Figure S3



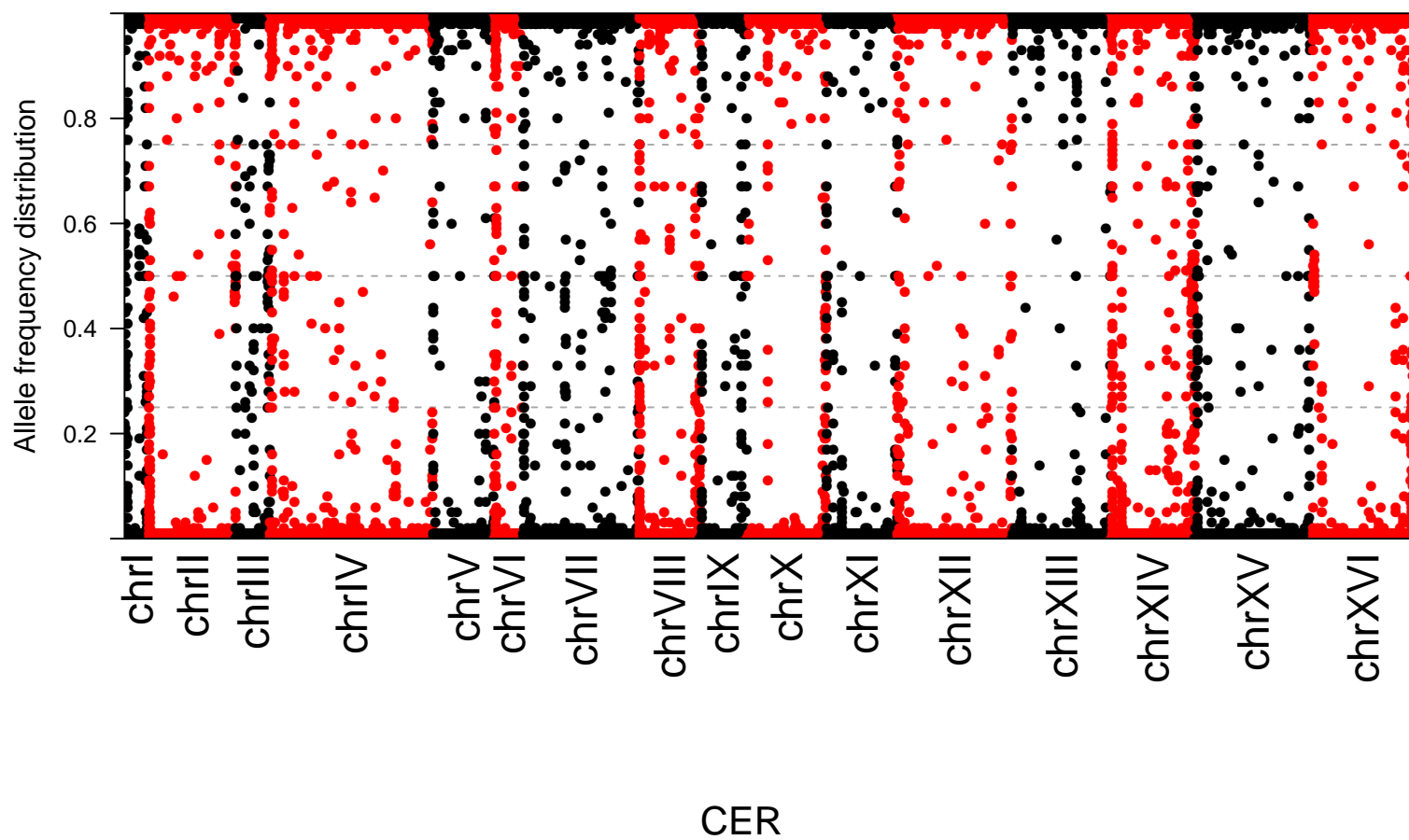
Supplementary Figure S3



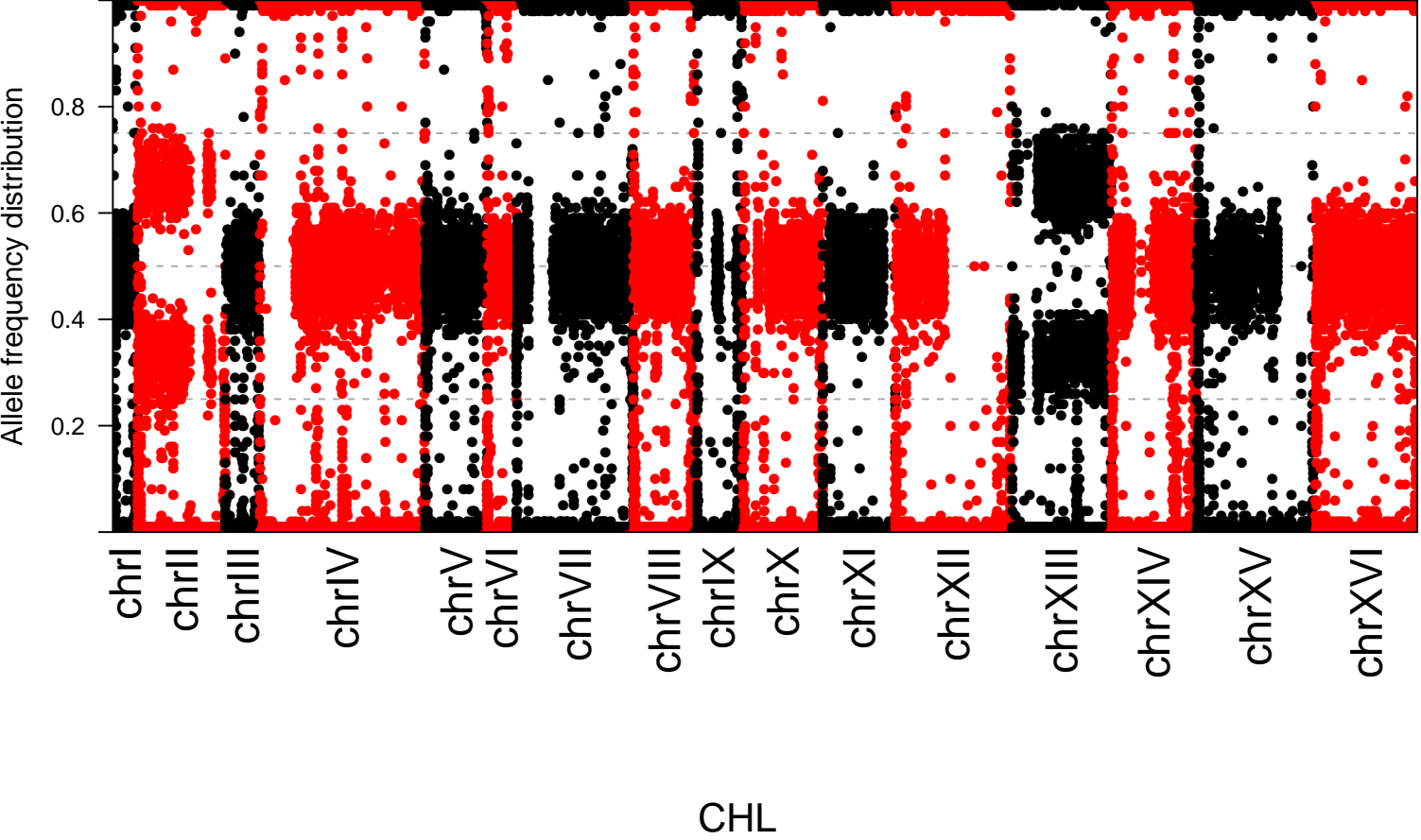
Supplementary Figure S3



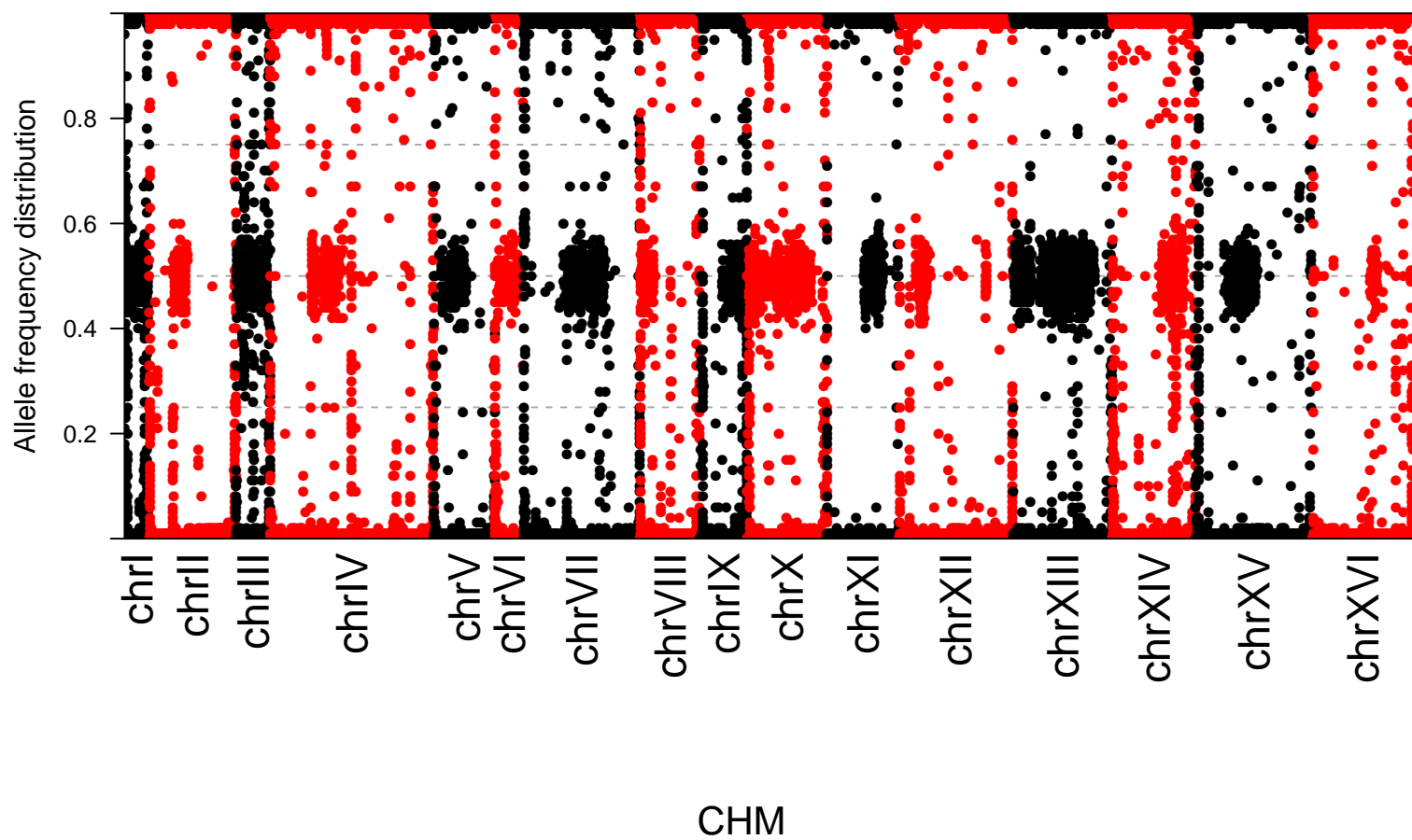
Supplementary Figure S3



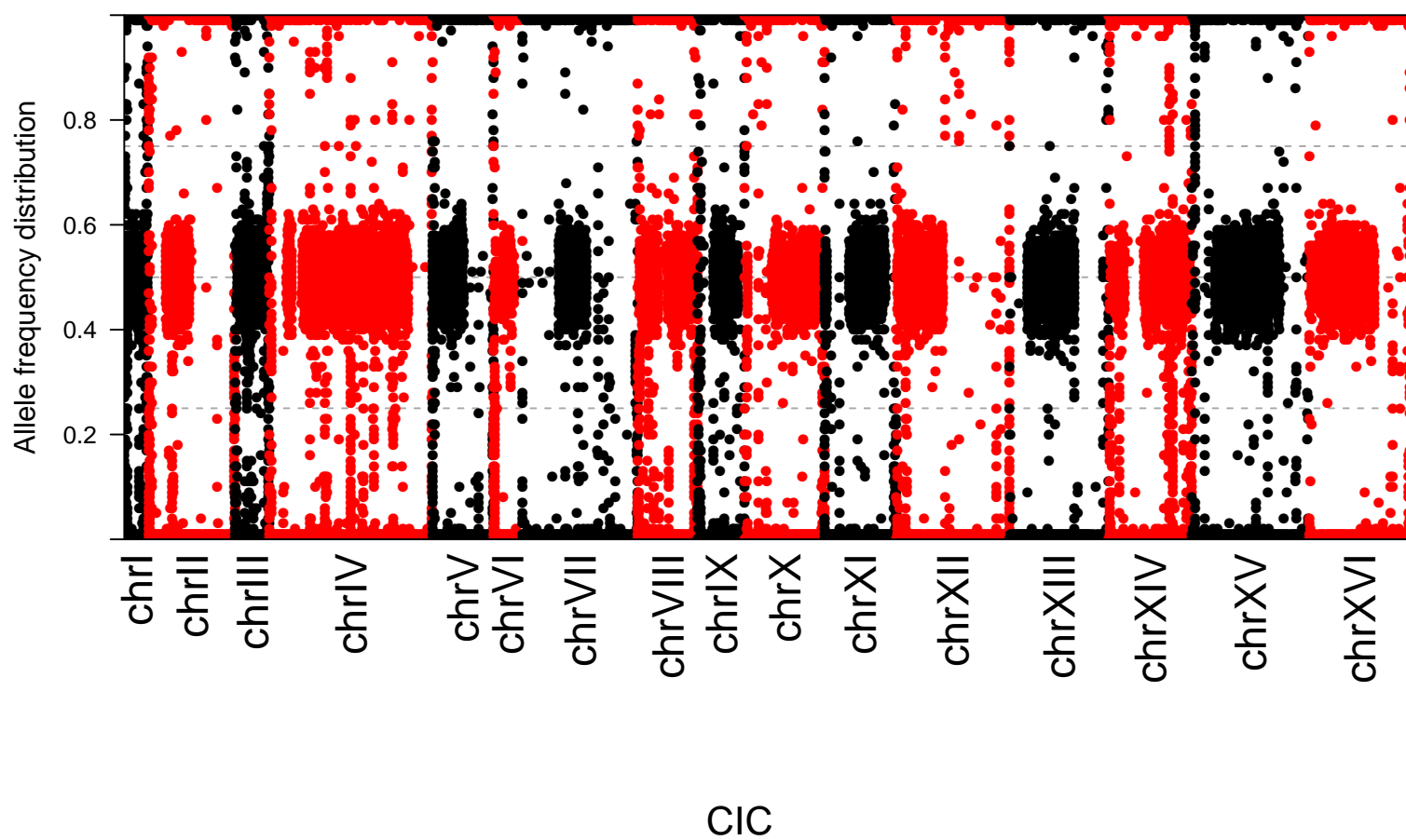
Supplementary Figure S3



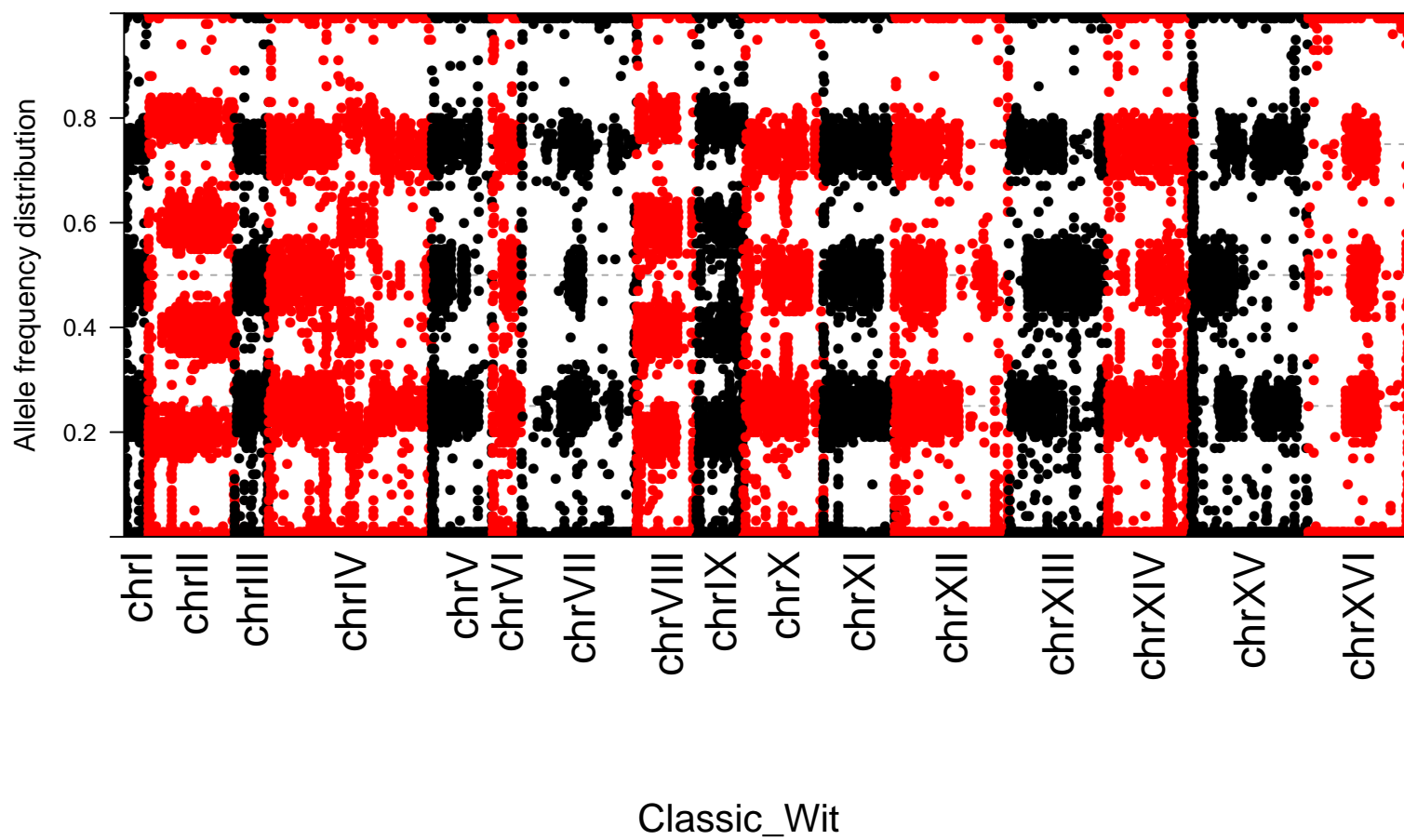
Supplementary Figure S3



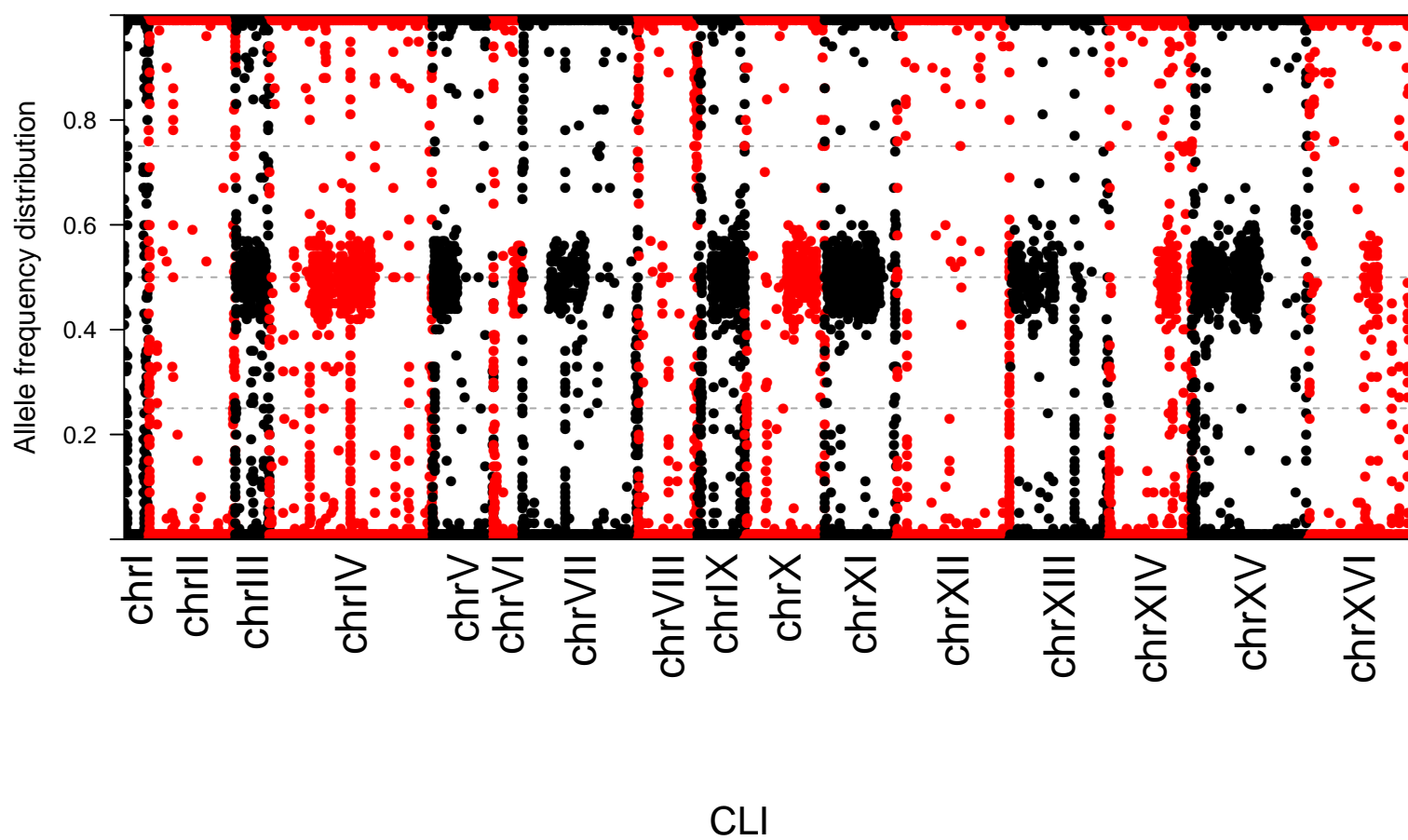
Supplementary Figure S3



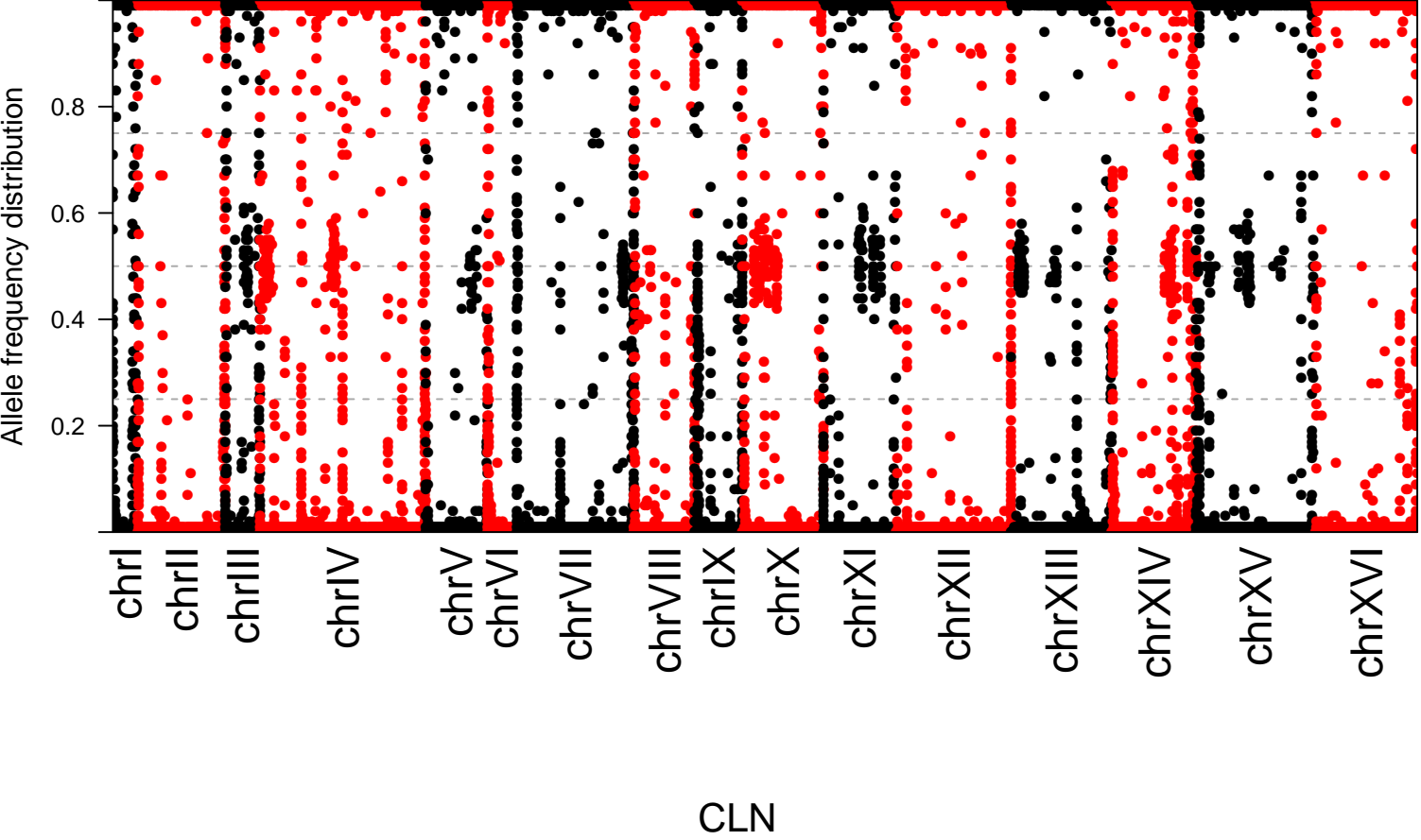
Supplementary Figure S3



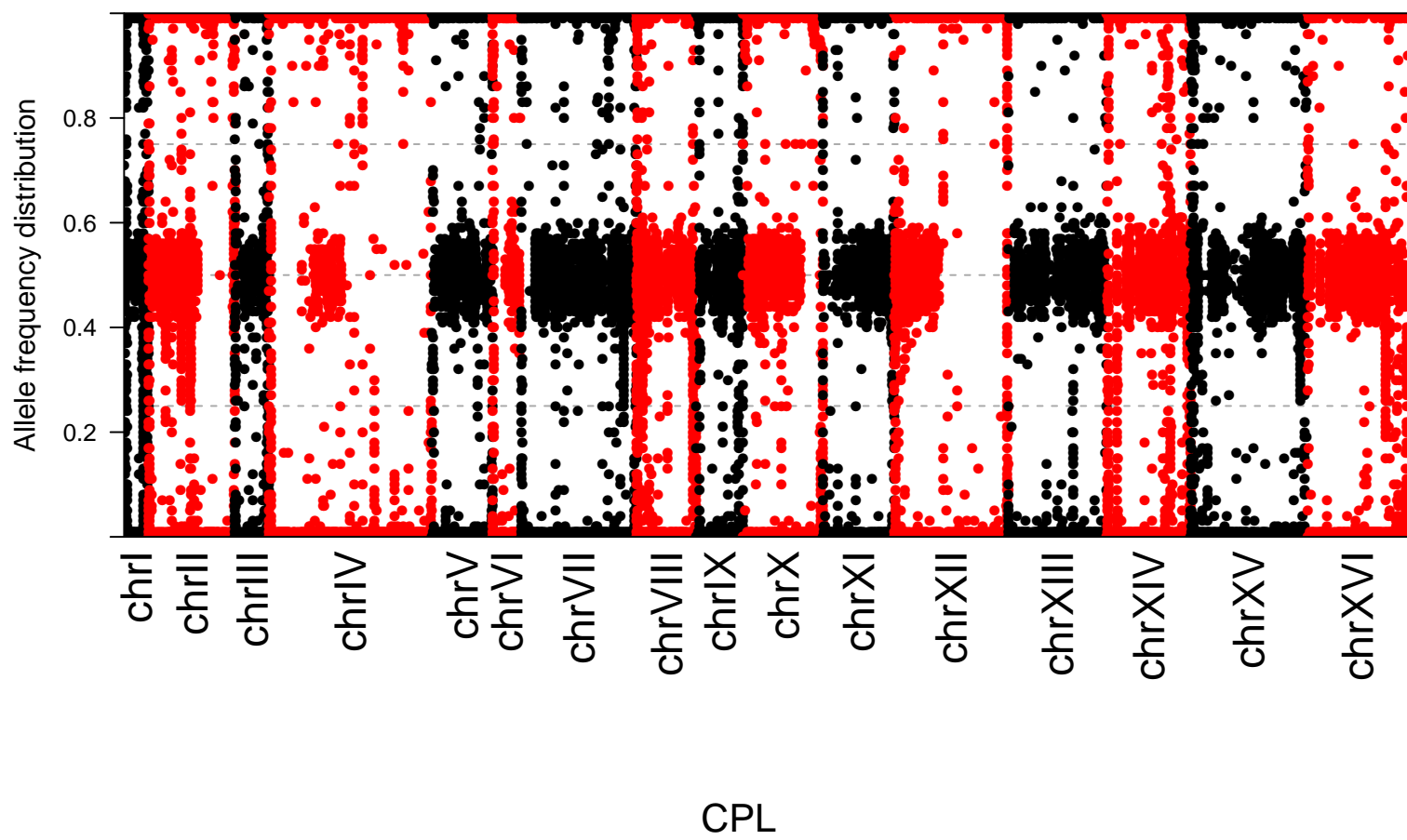
Supplementary Figure S3



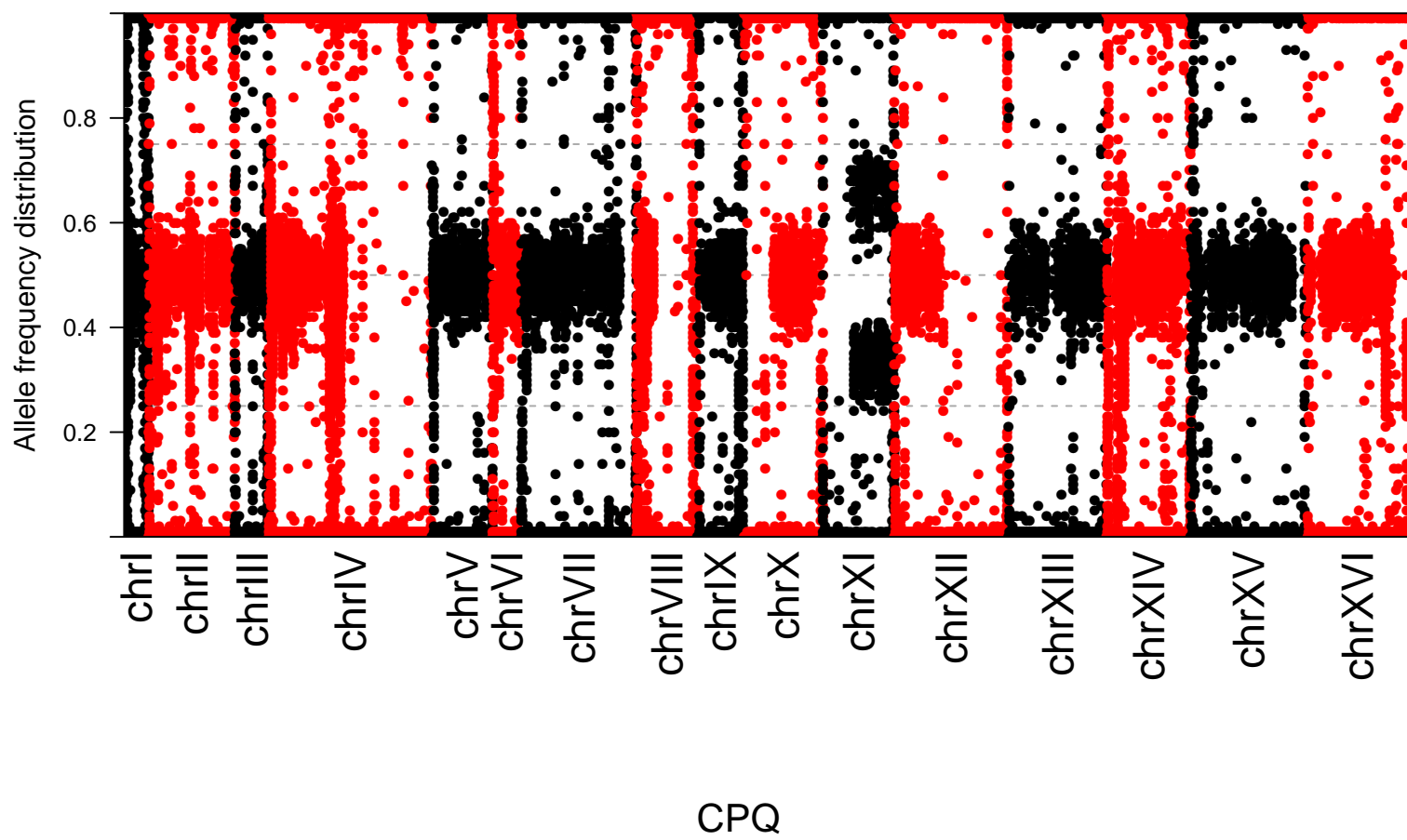
Supplementary Figure S3



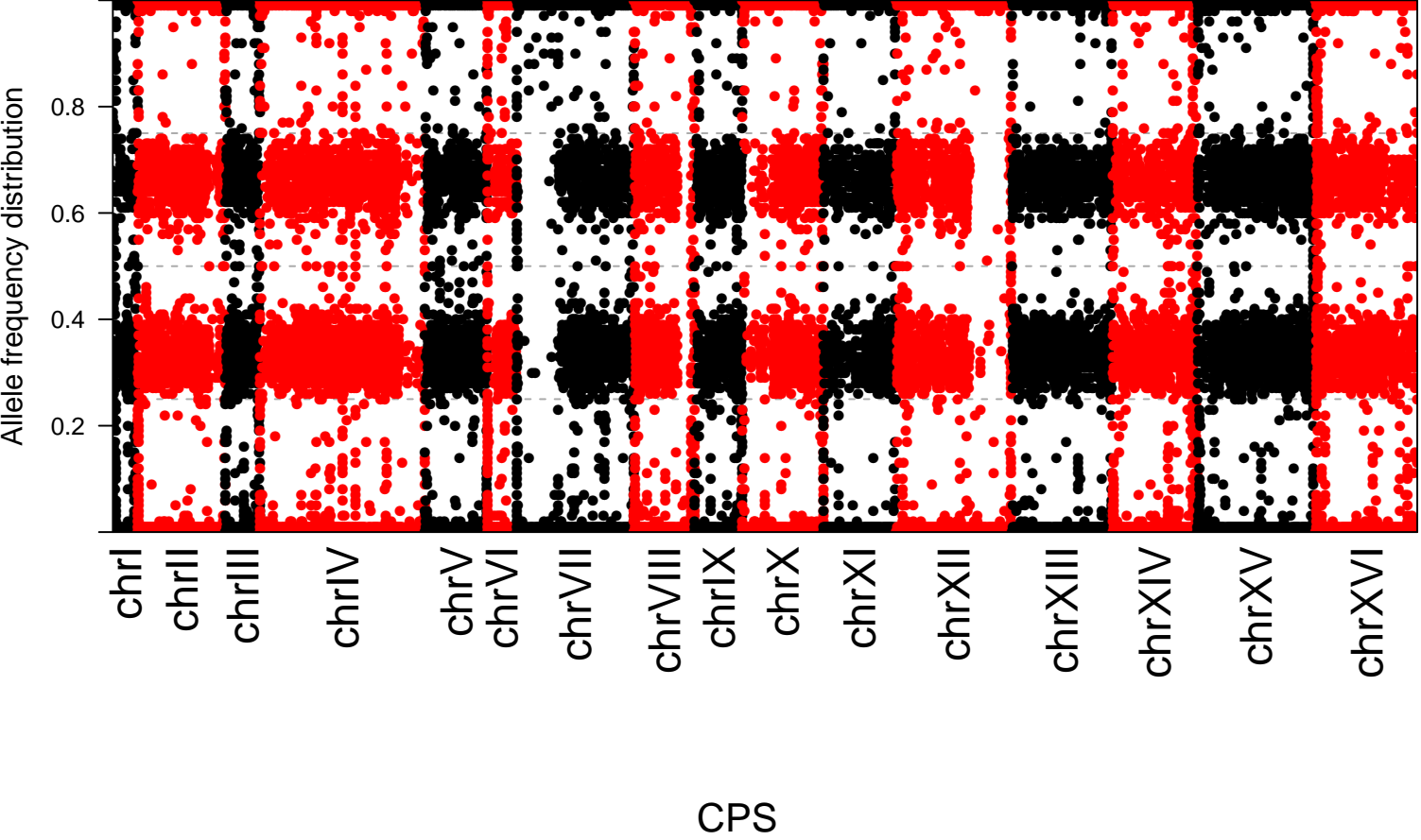
Supplementary Figure S3



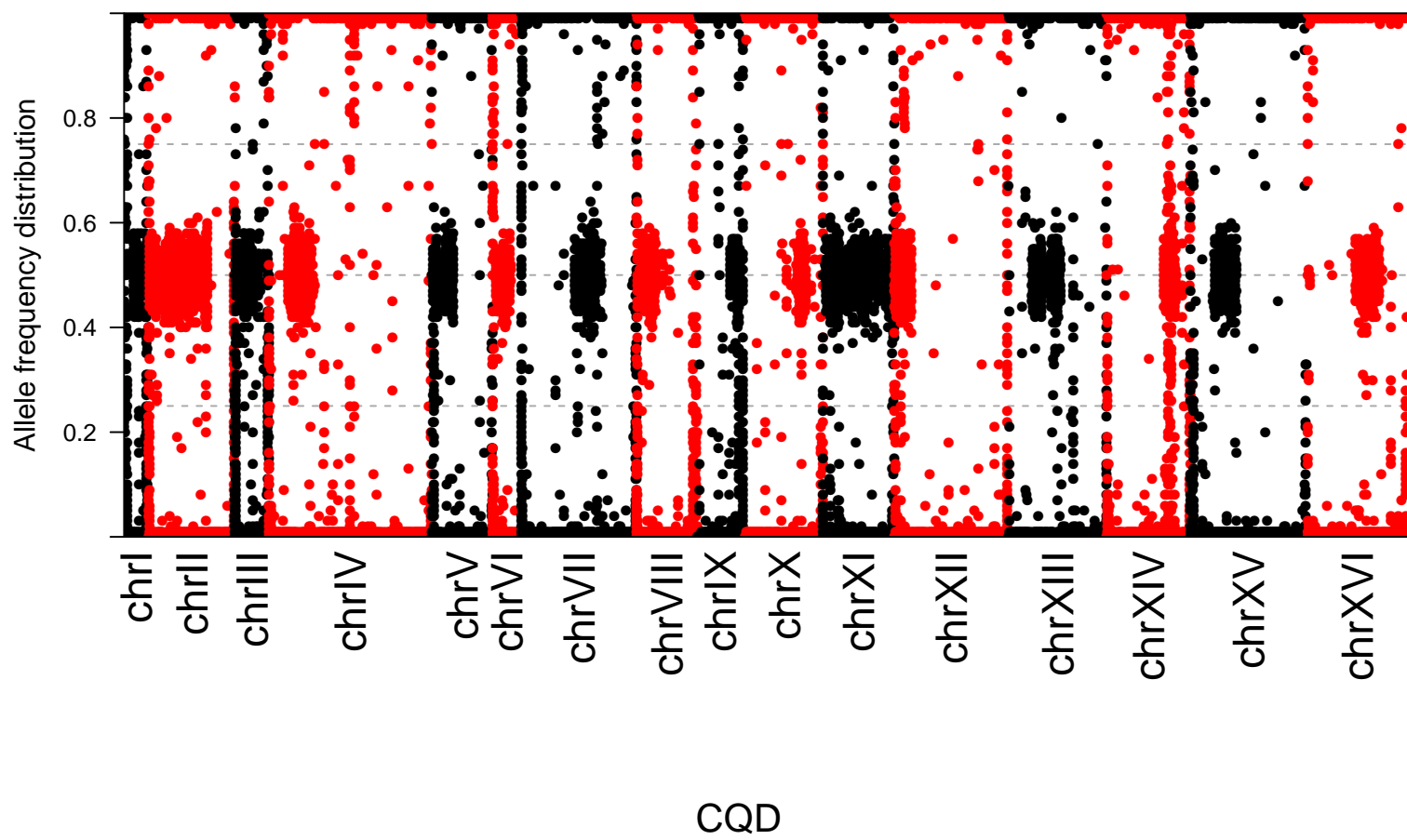
Supplementary Figure S3



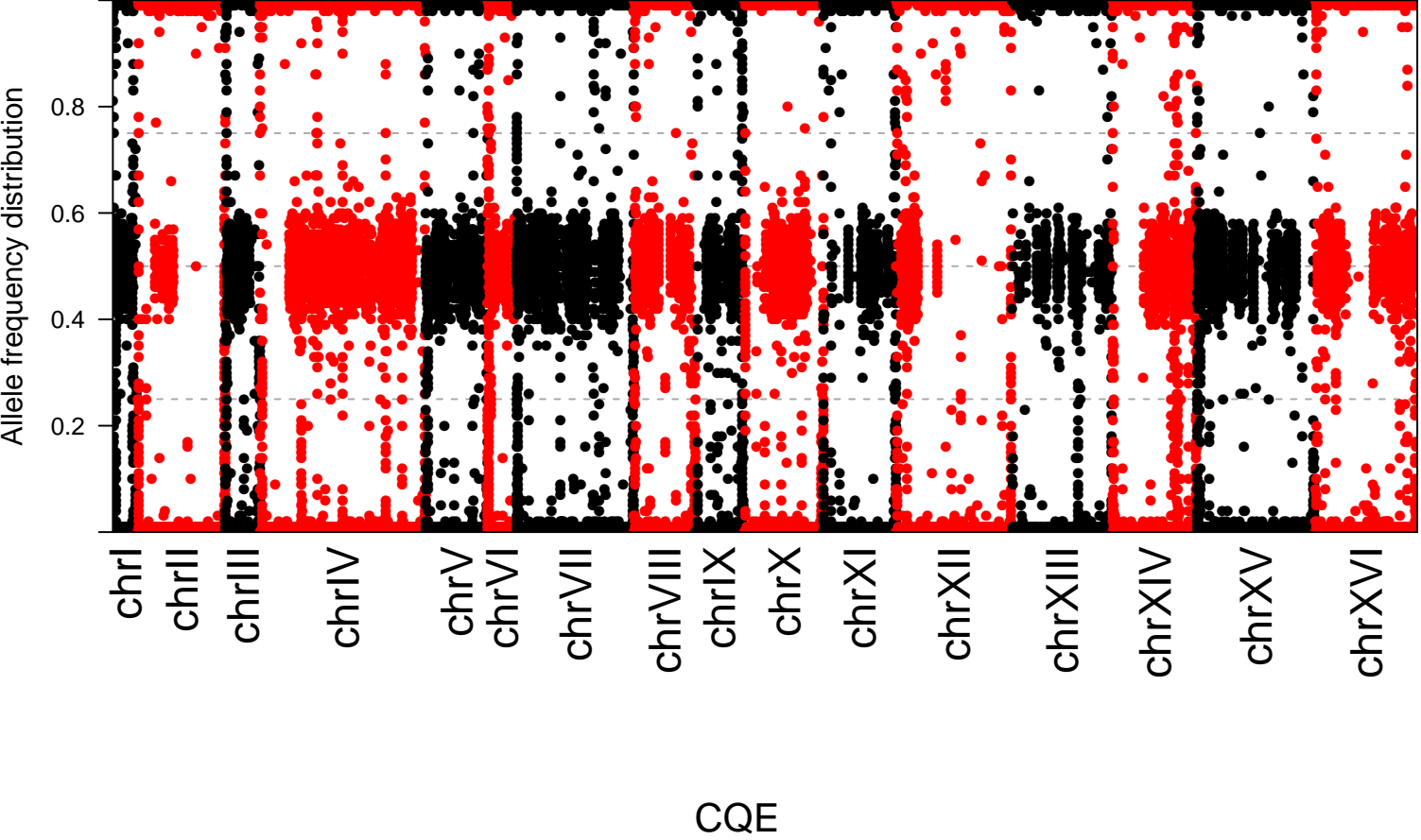
Supplementary Figure S3



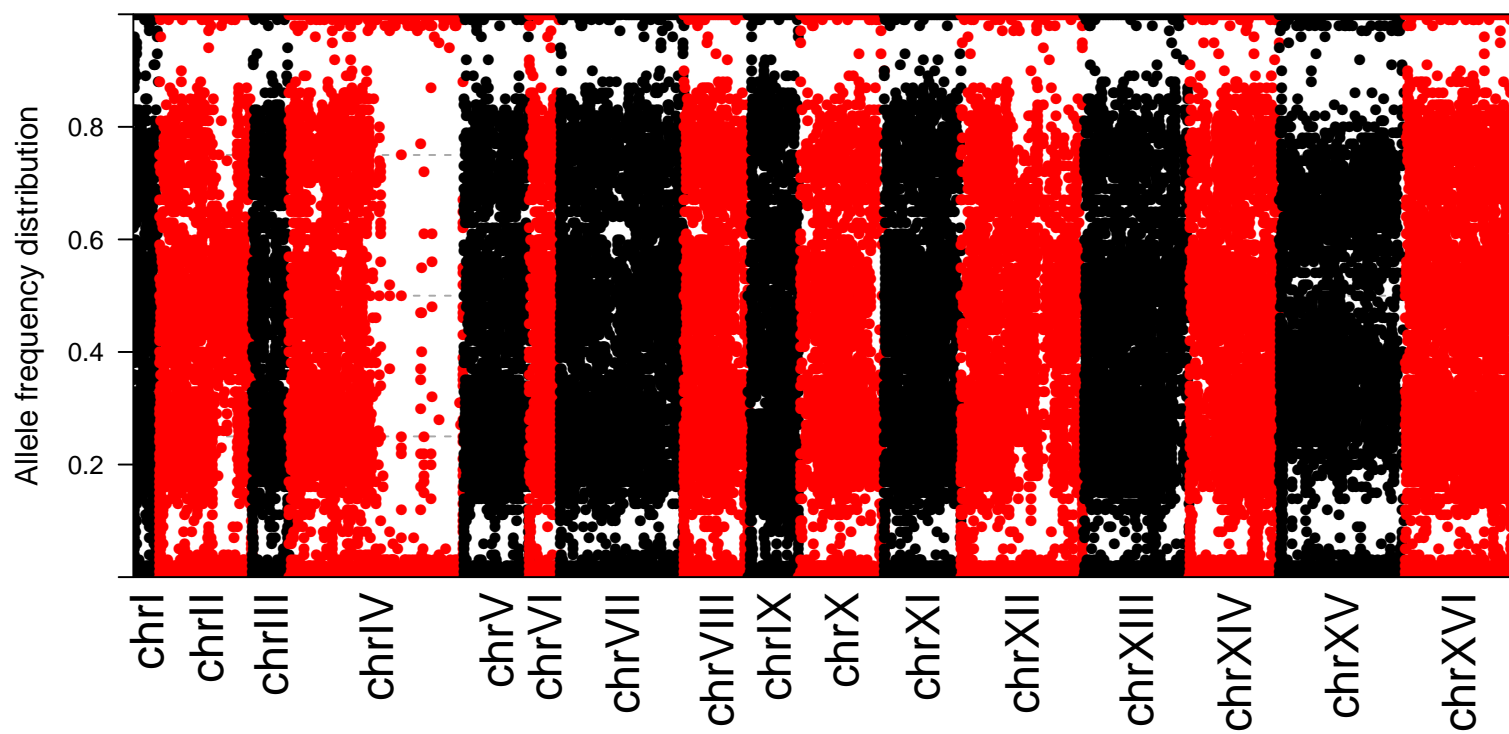
Supplementary Figure S3



Supplementary Figure S3

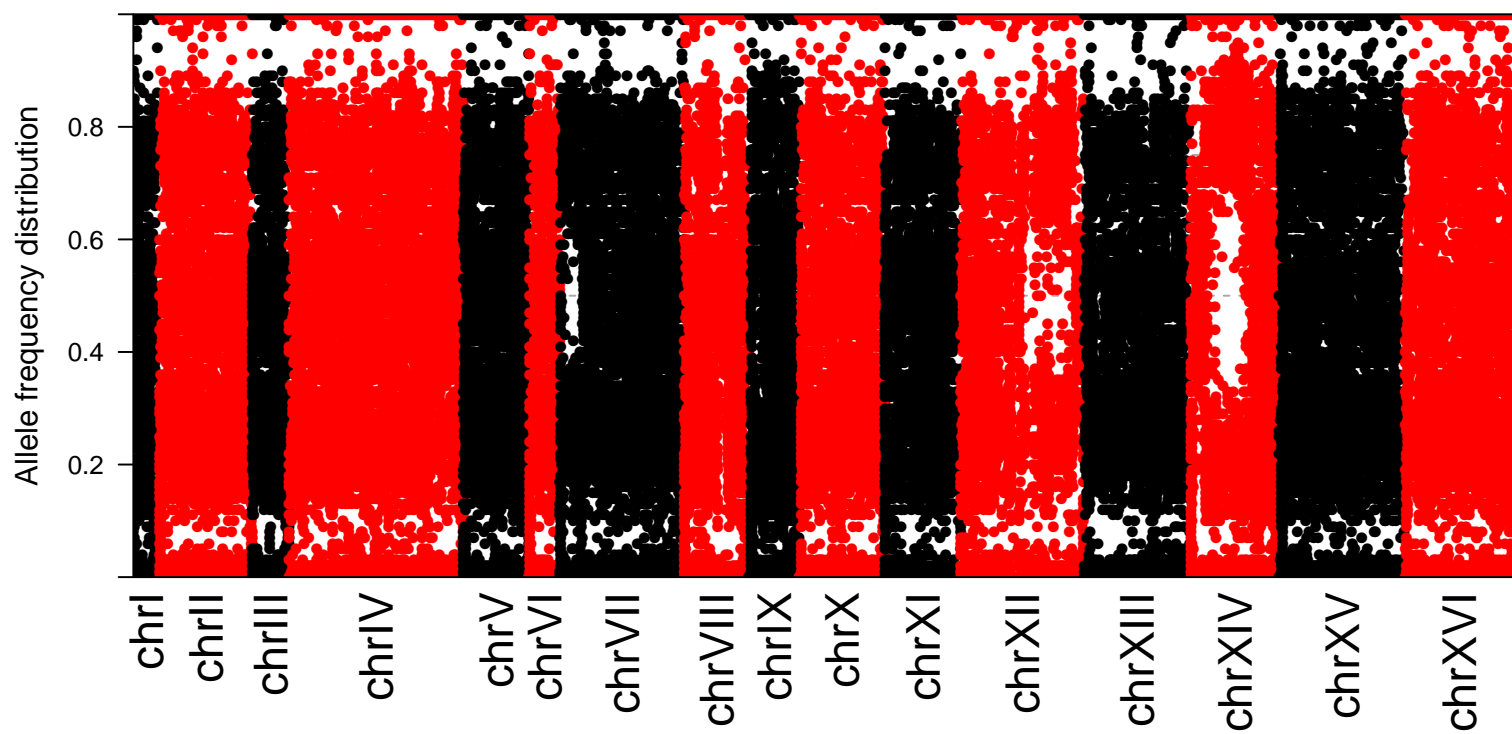


Supplementary Figure S3



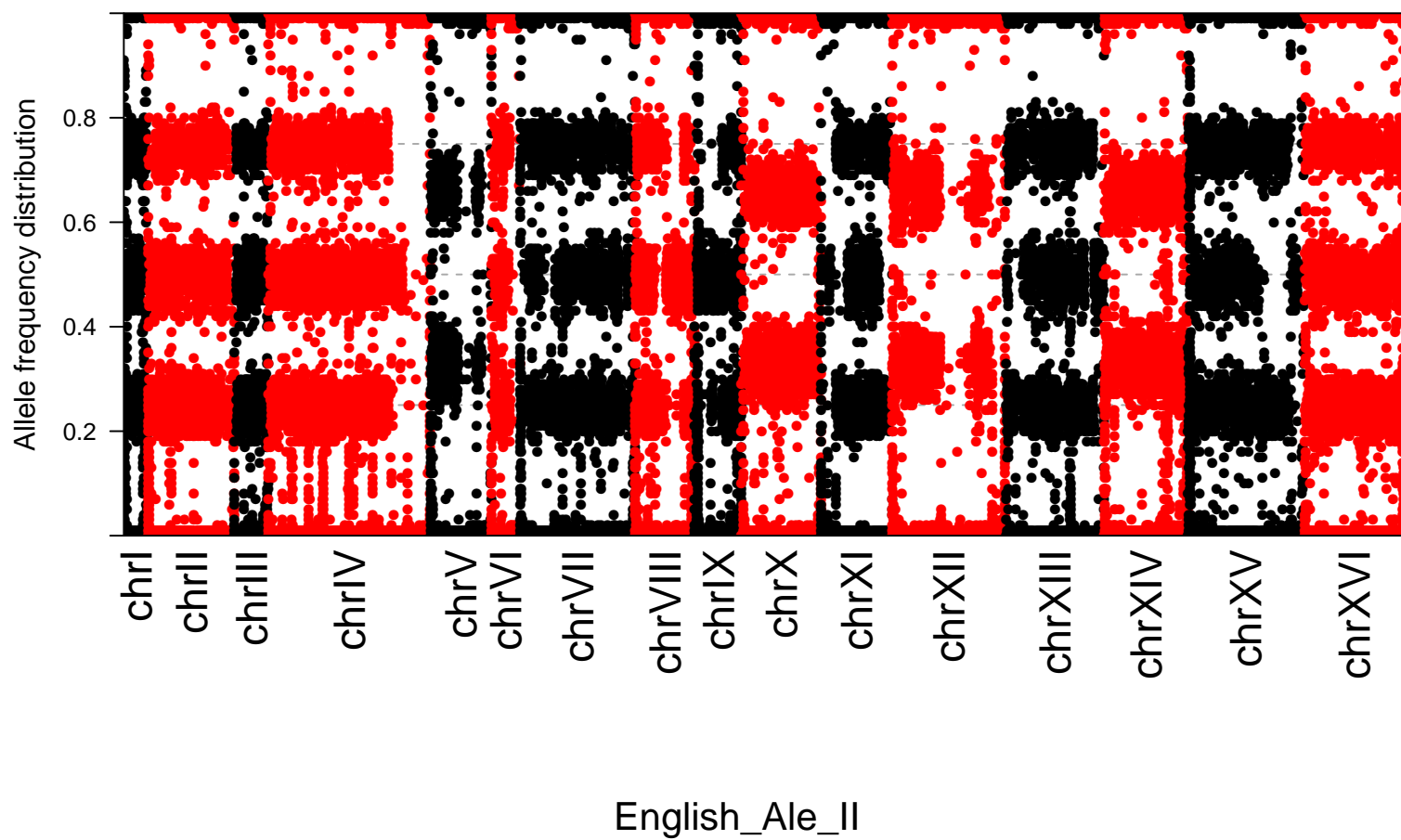
Djonno1

Supplementary Figure S3

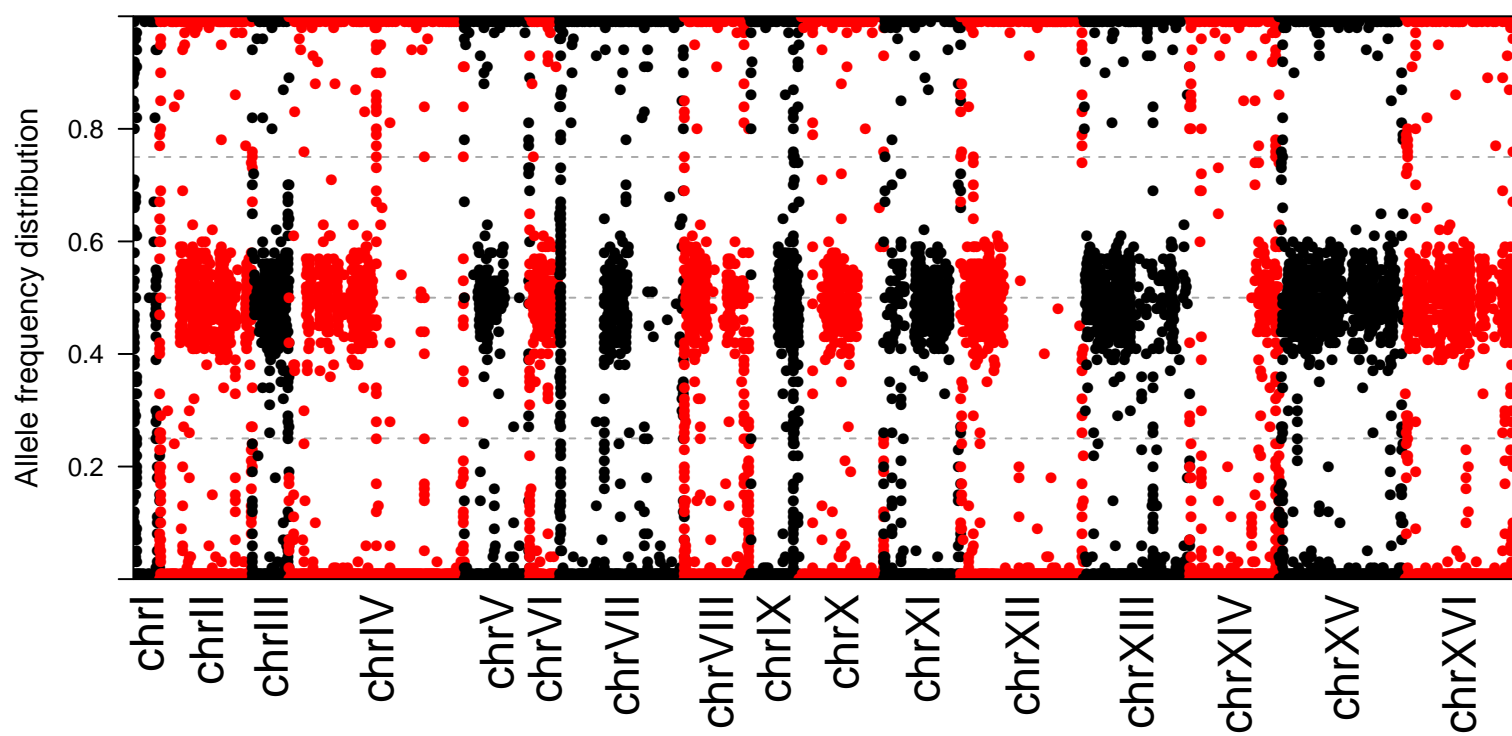


Dras_1

Supplementary Figure S3

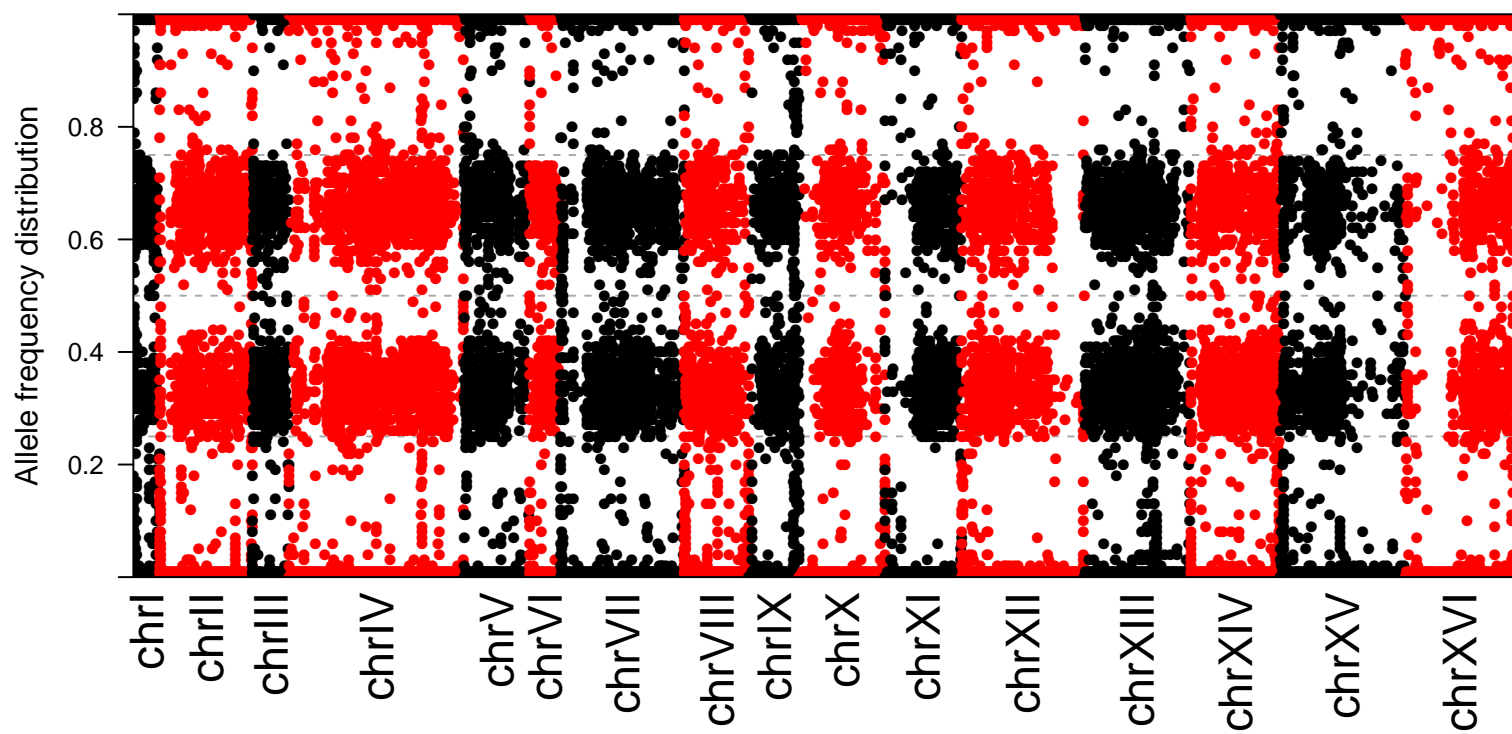


Supplementary Figure S3



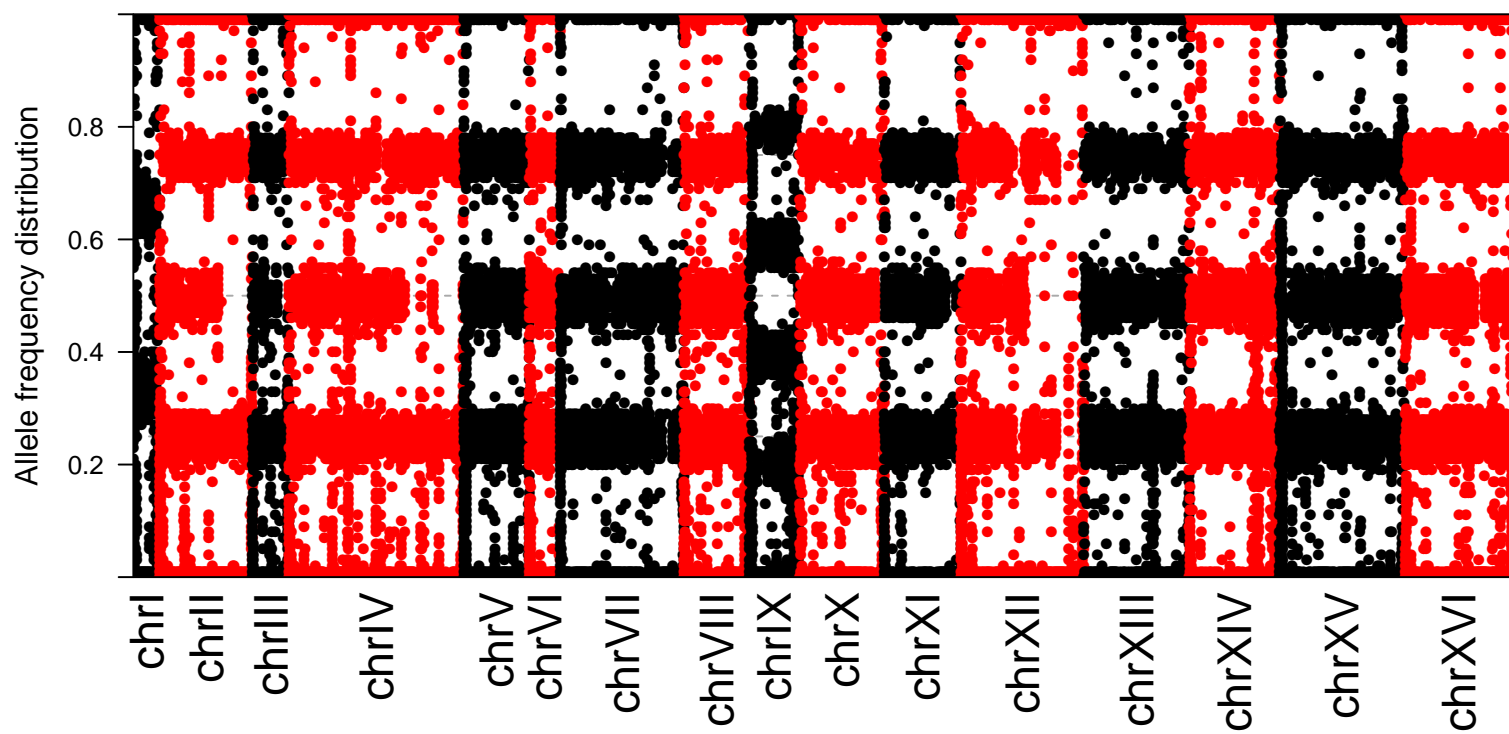
GH1

Supplementary Figure S3



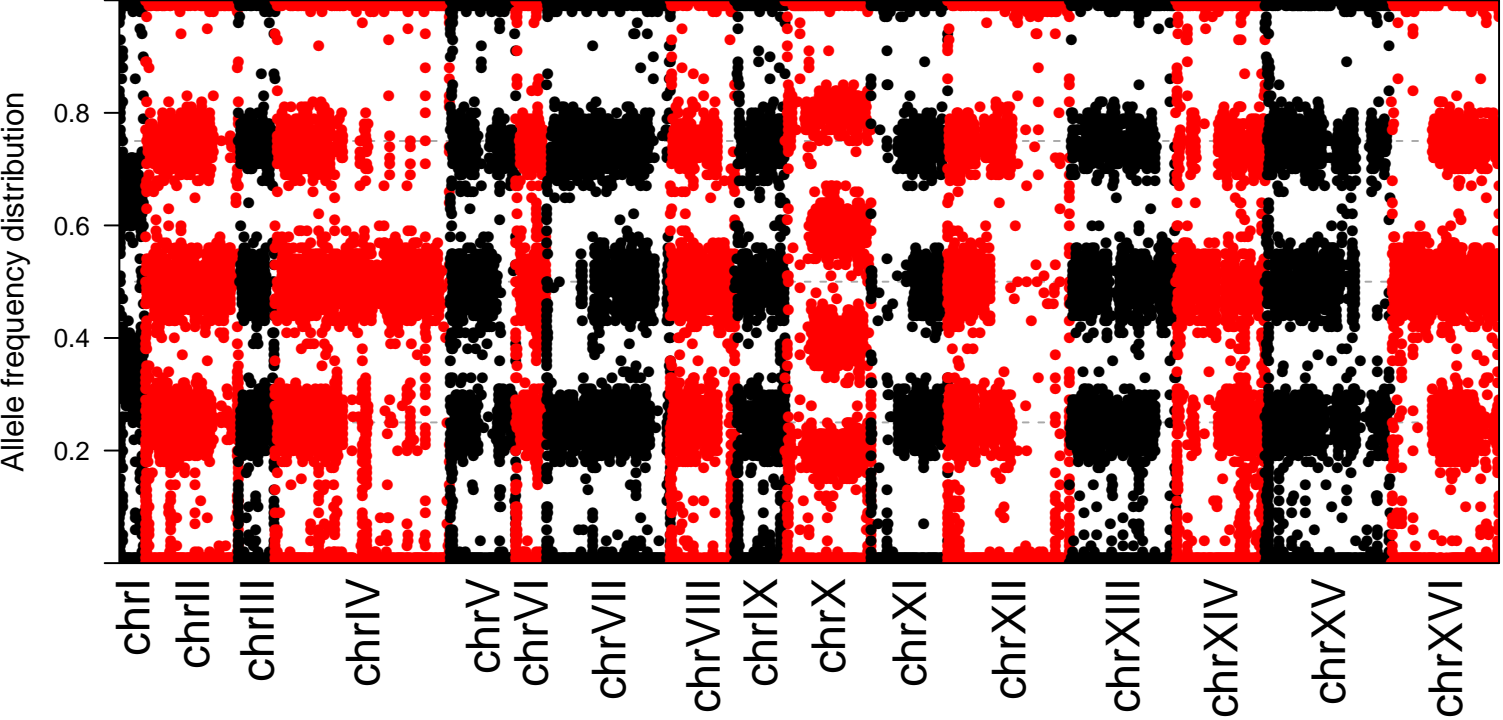
GH2

Supplementary Figure S3



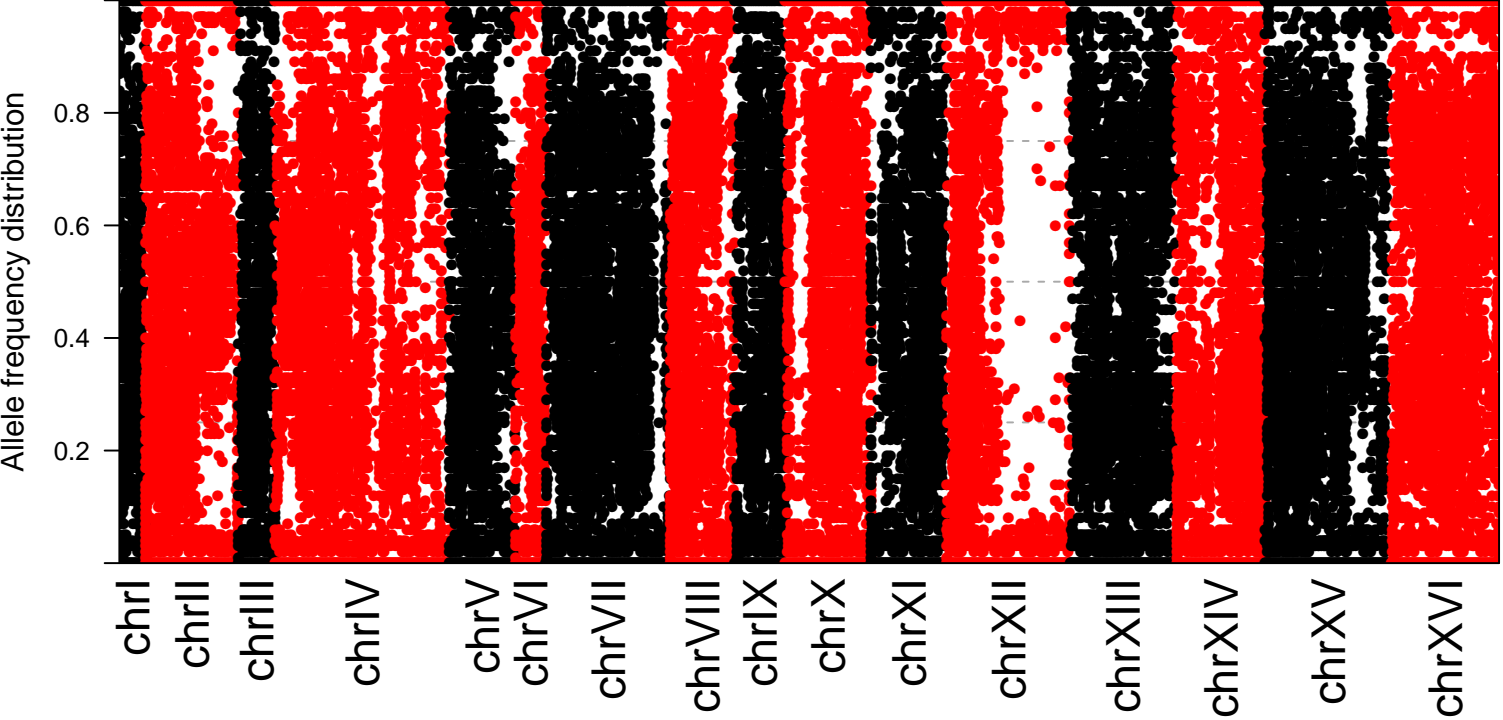
Granvin_1

Supplementary Figure S3



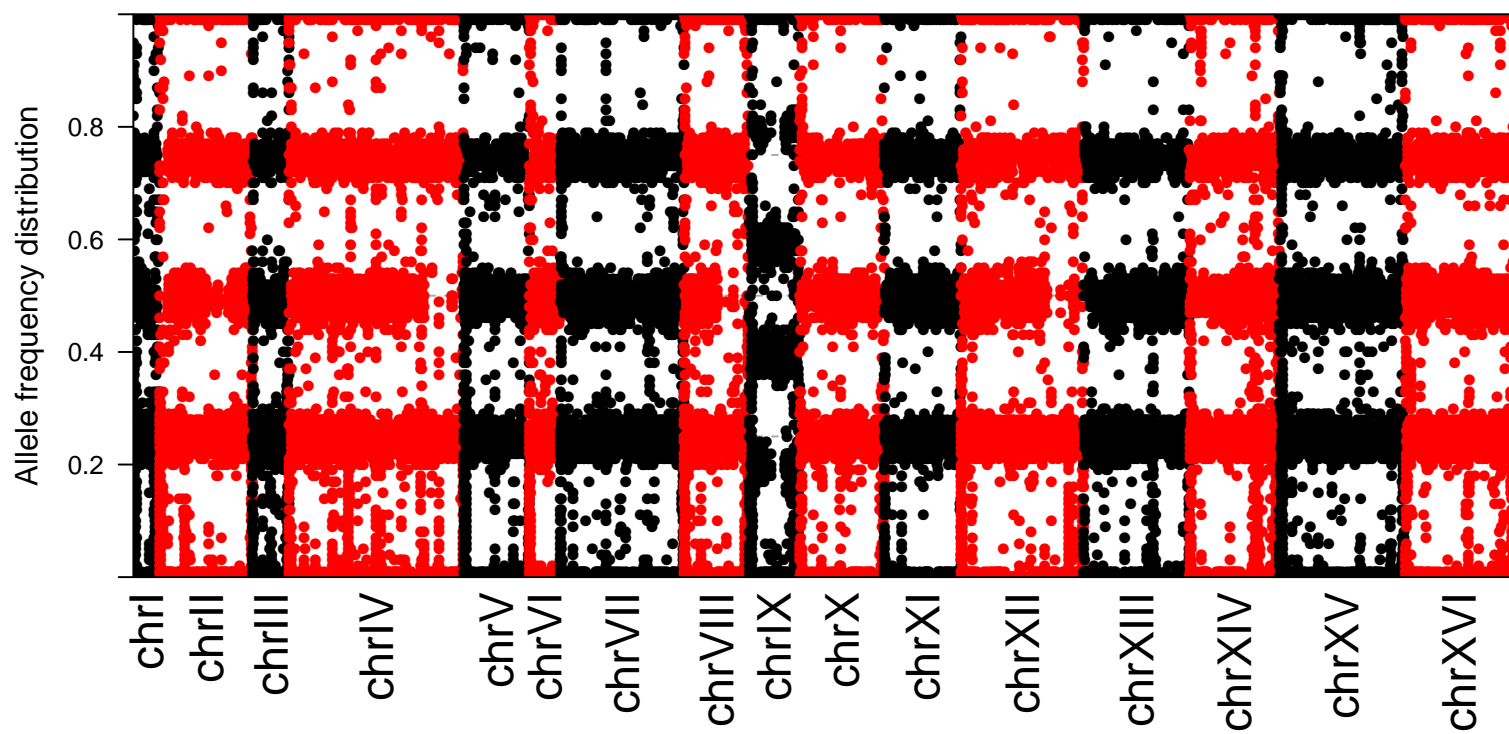
Halvorsgard_3

Supplementary Figure S3



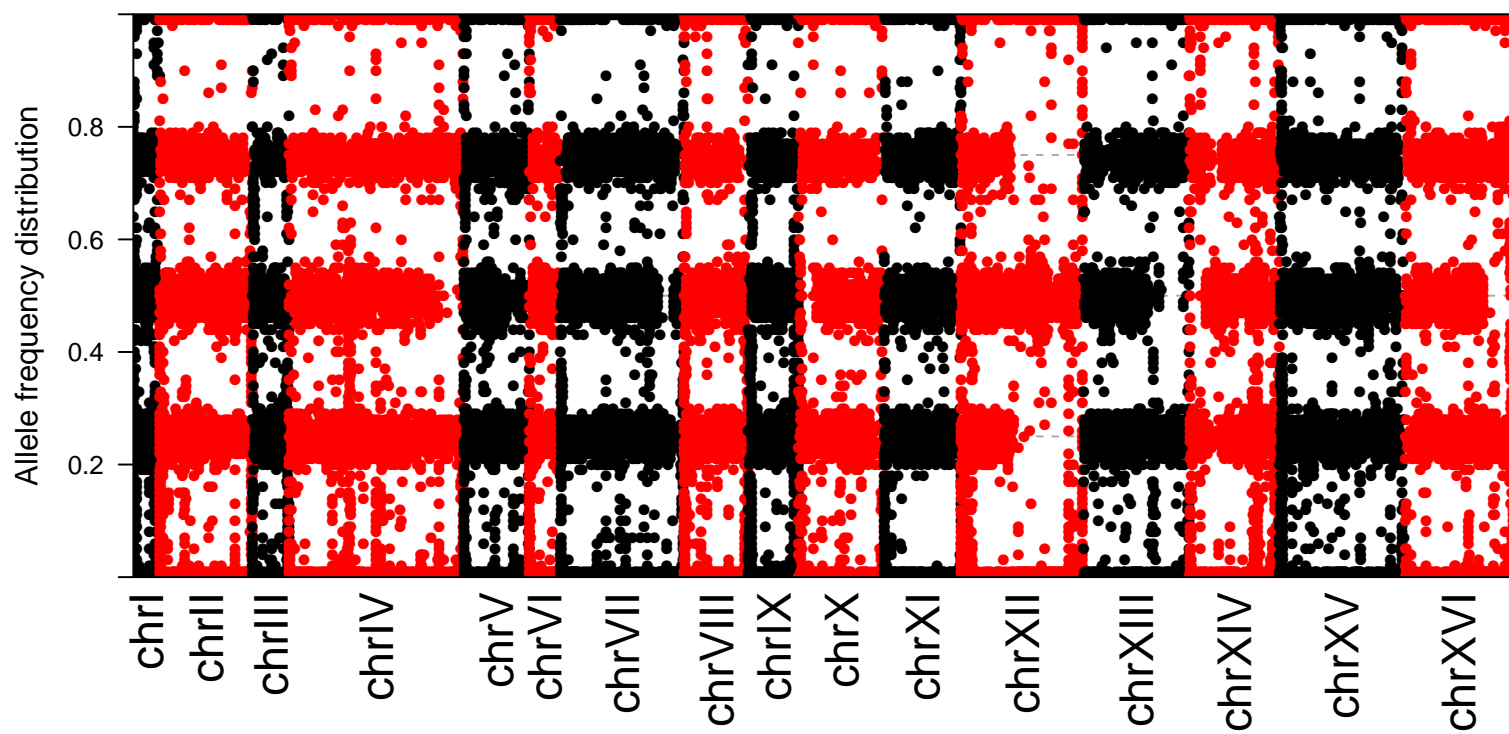
Halvorsgard_6

Supplementary Figure S3



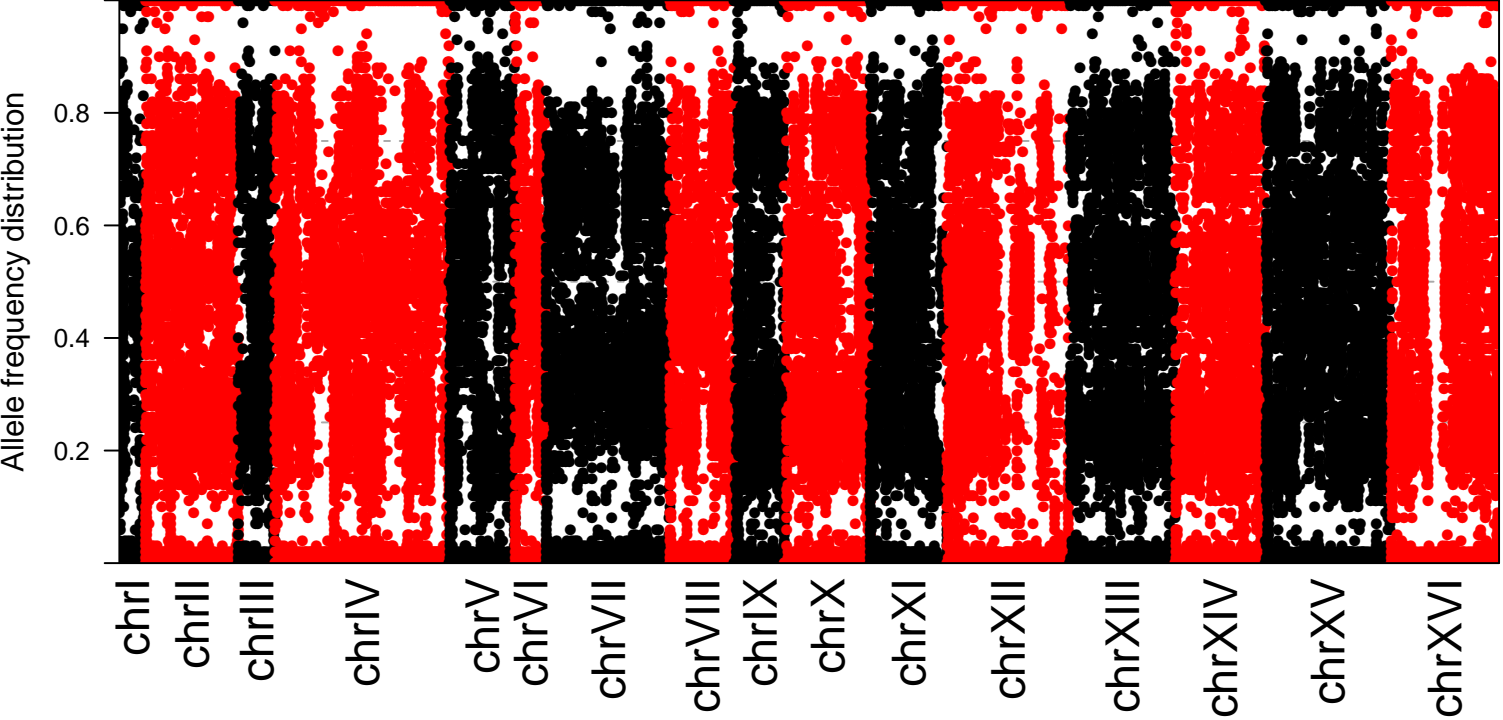
Hornindal_1

Supplementary Figure S3



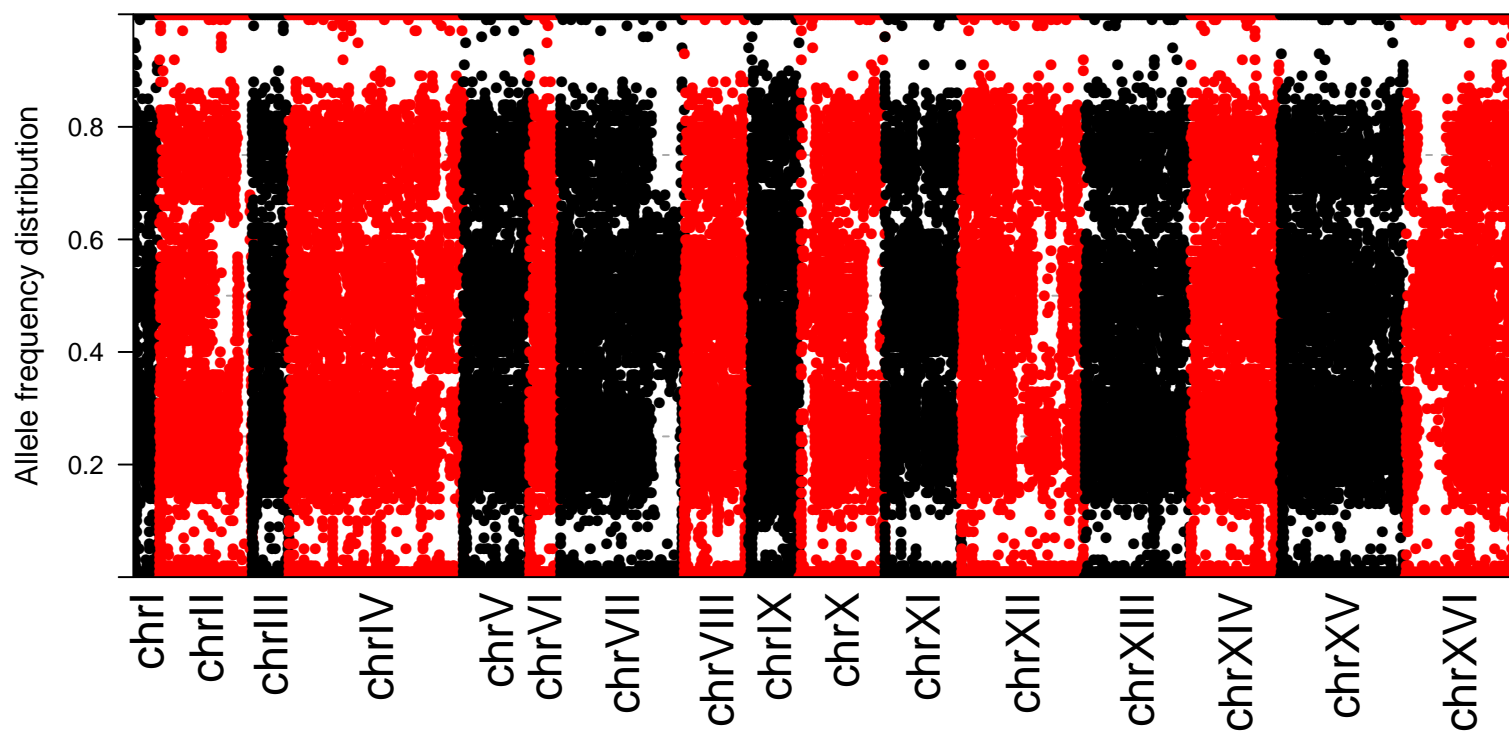
Hornindal_2

Supplementary Figure S3



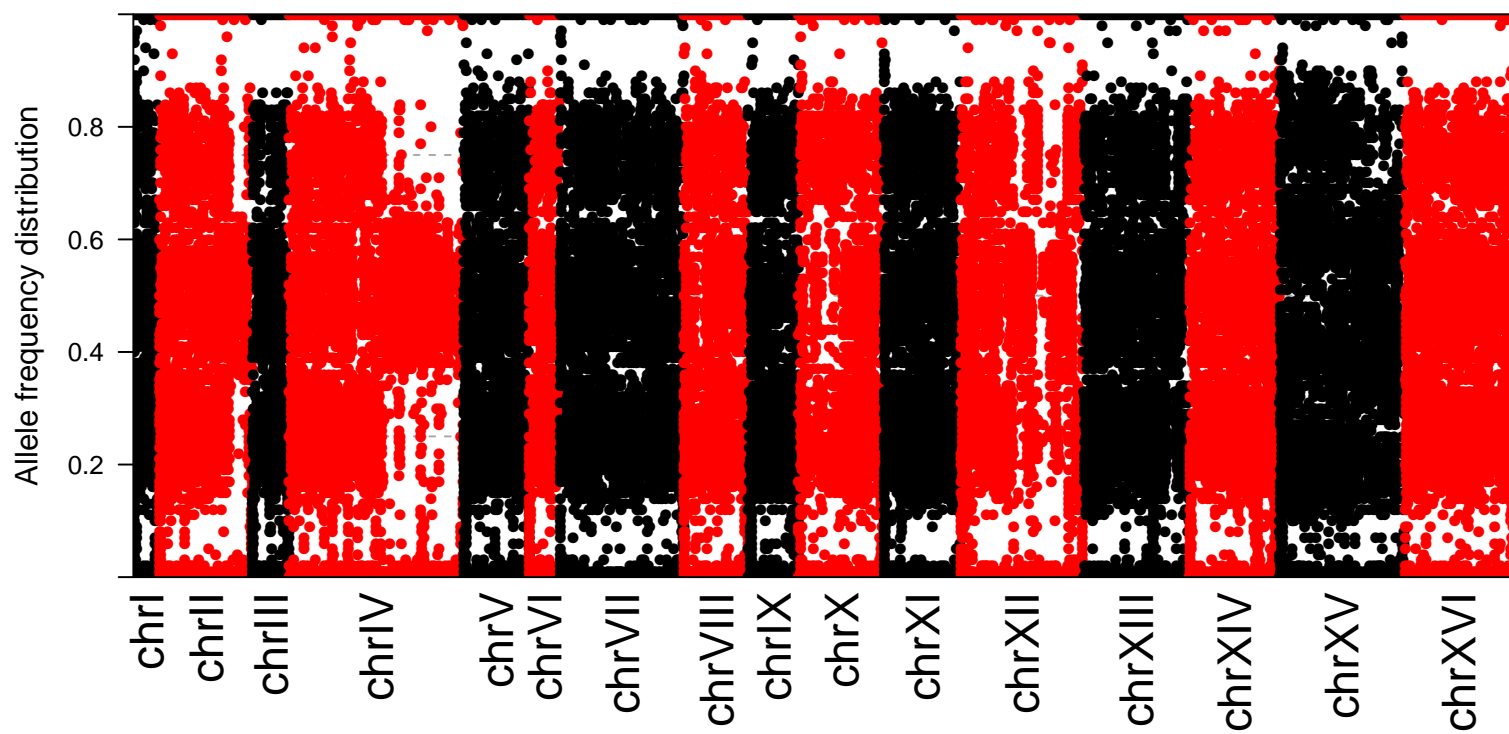
Ishlei1

Supplementary Figure S3



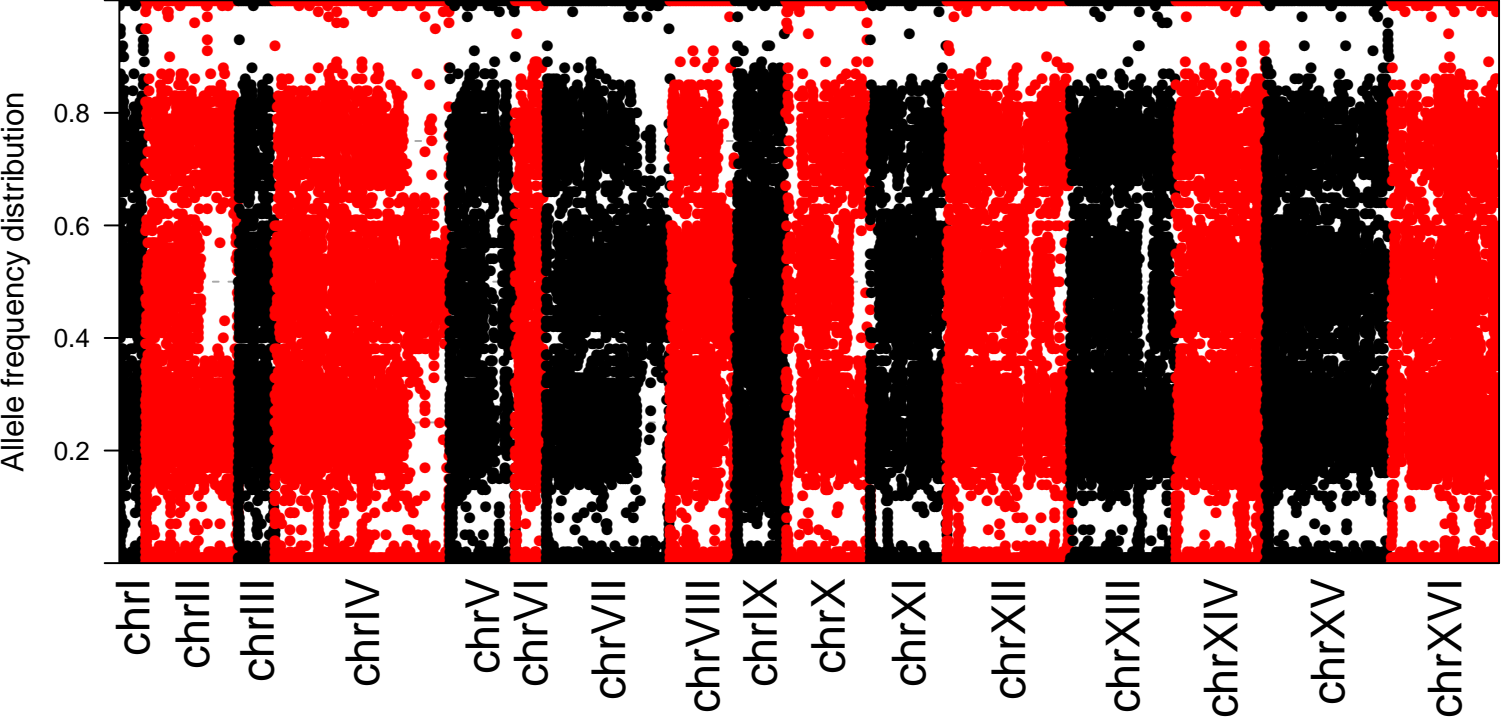
Ivar_Geithung_1

Supplementary Figure S3



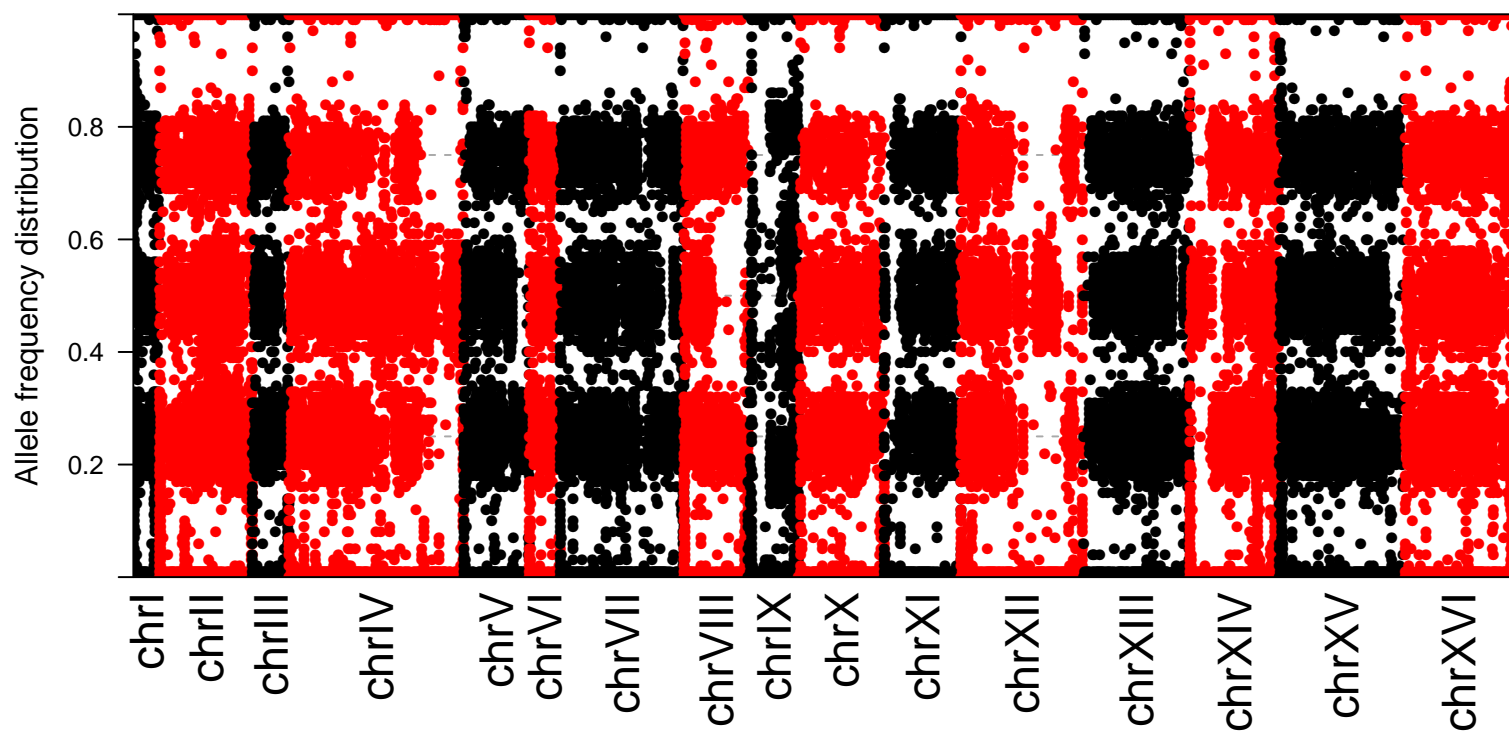
Ivar_Geithung_2

Supplementary Figure S3



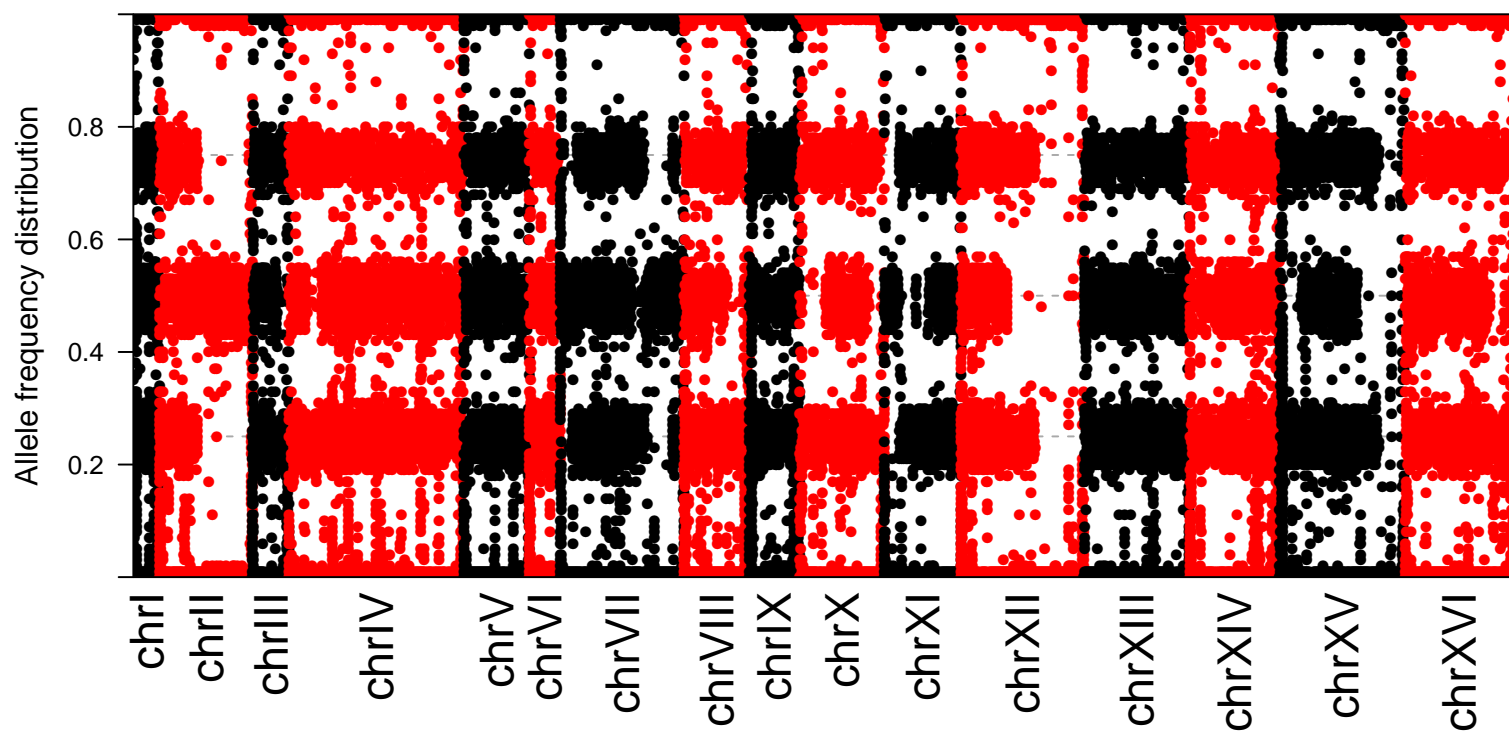
Ivar_Geithung_3

Supplementary Figure S3



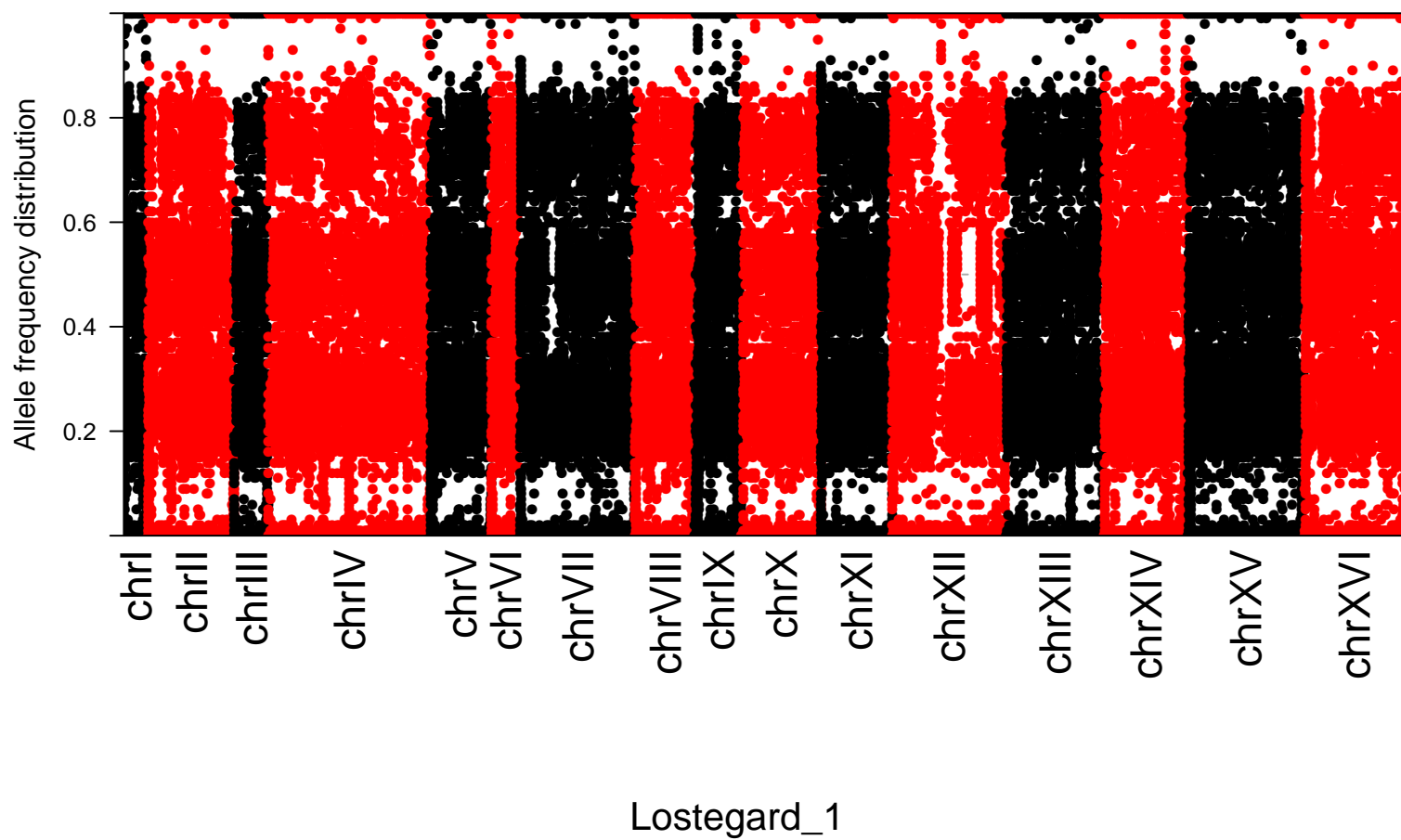
Jordal1

Supplementary Figure S3

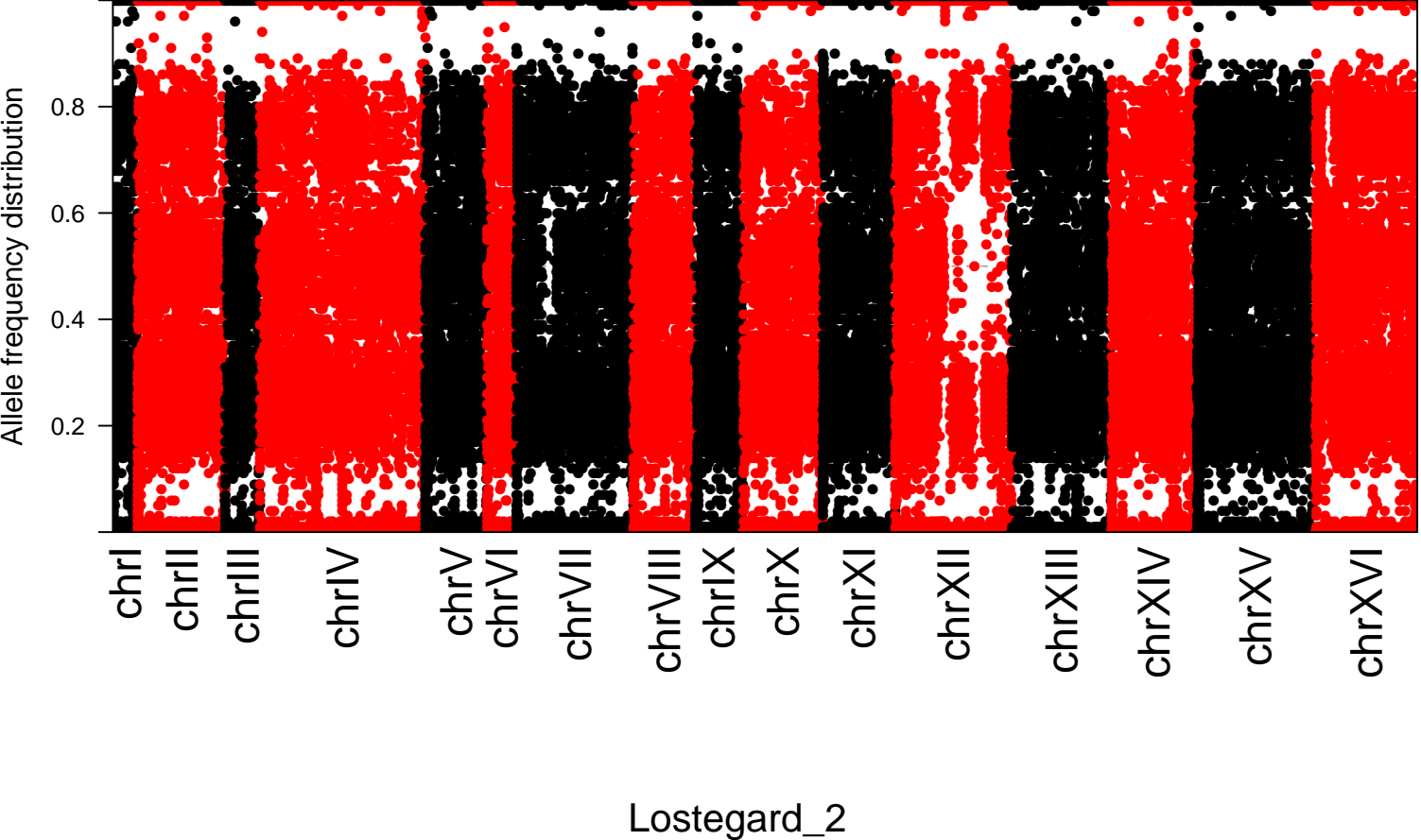


Laerdal_2

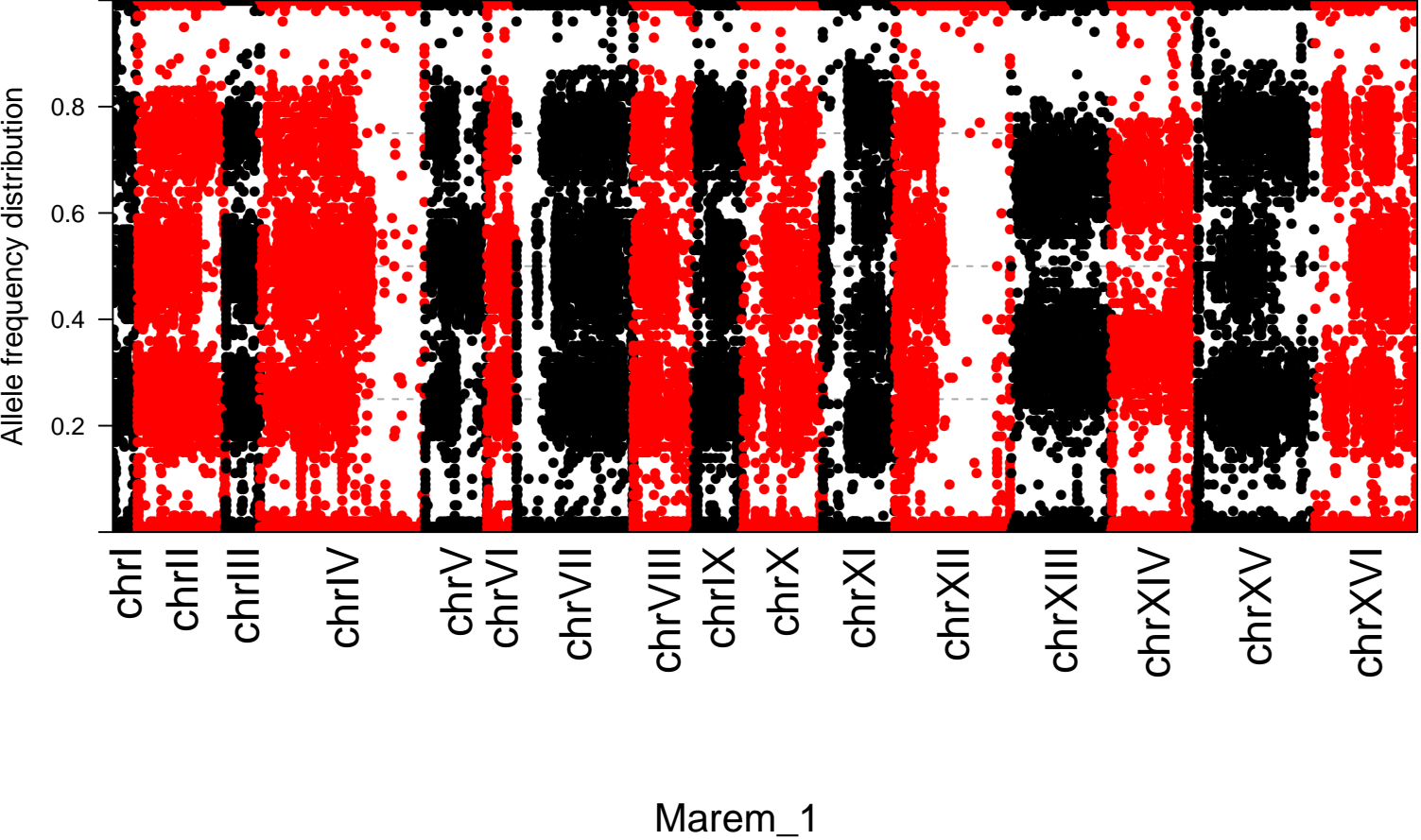
Supplementary Figure S3



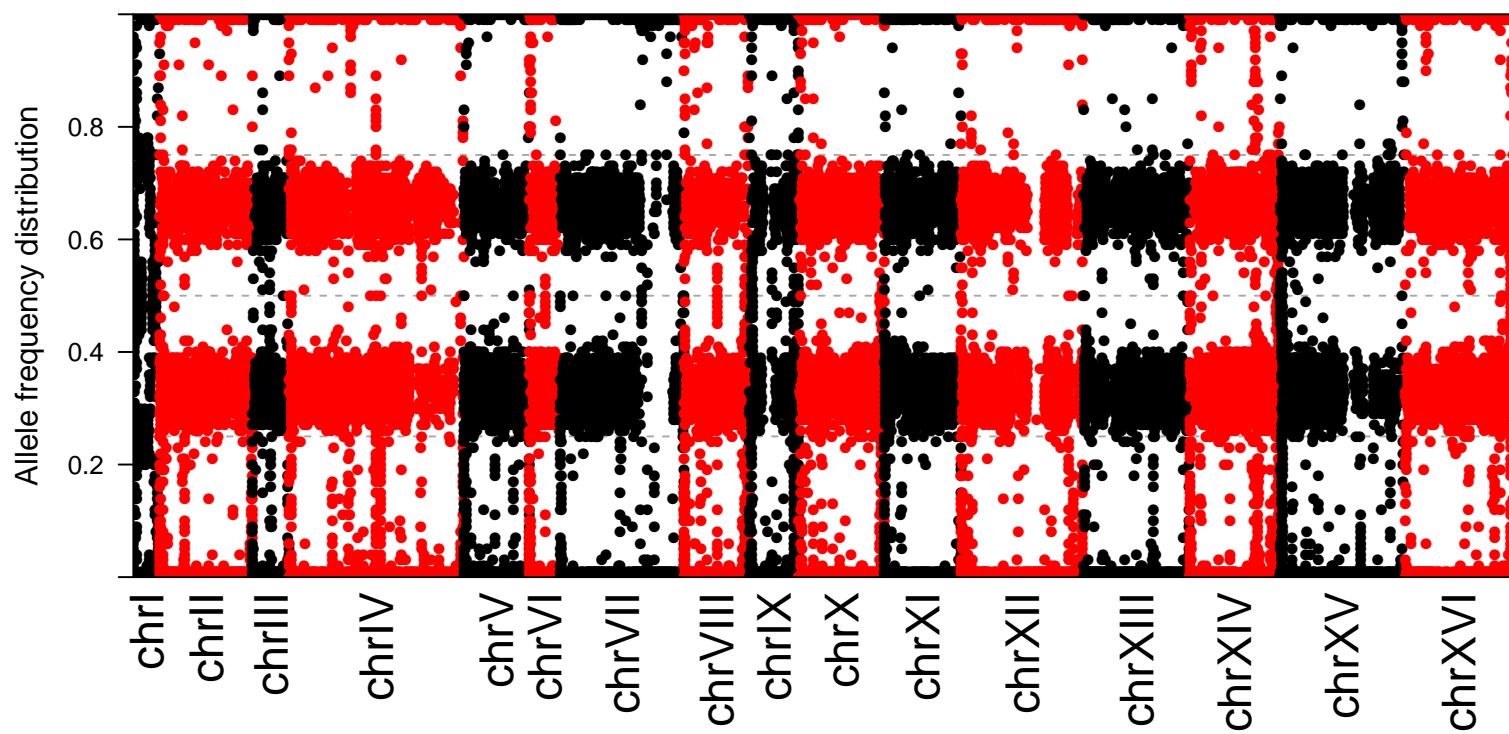
Supplementary Figure S3



Supplementary Figure S3

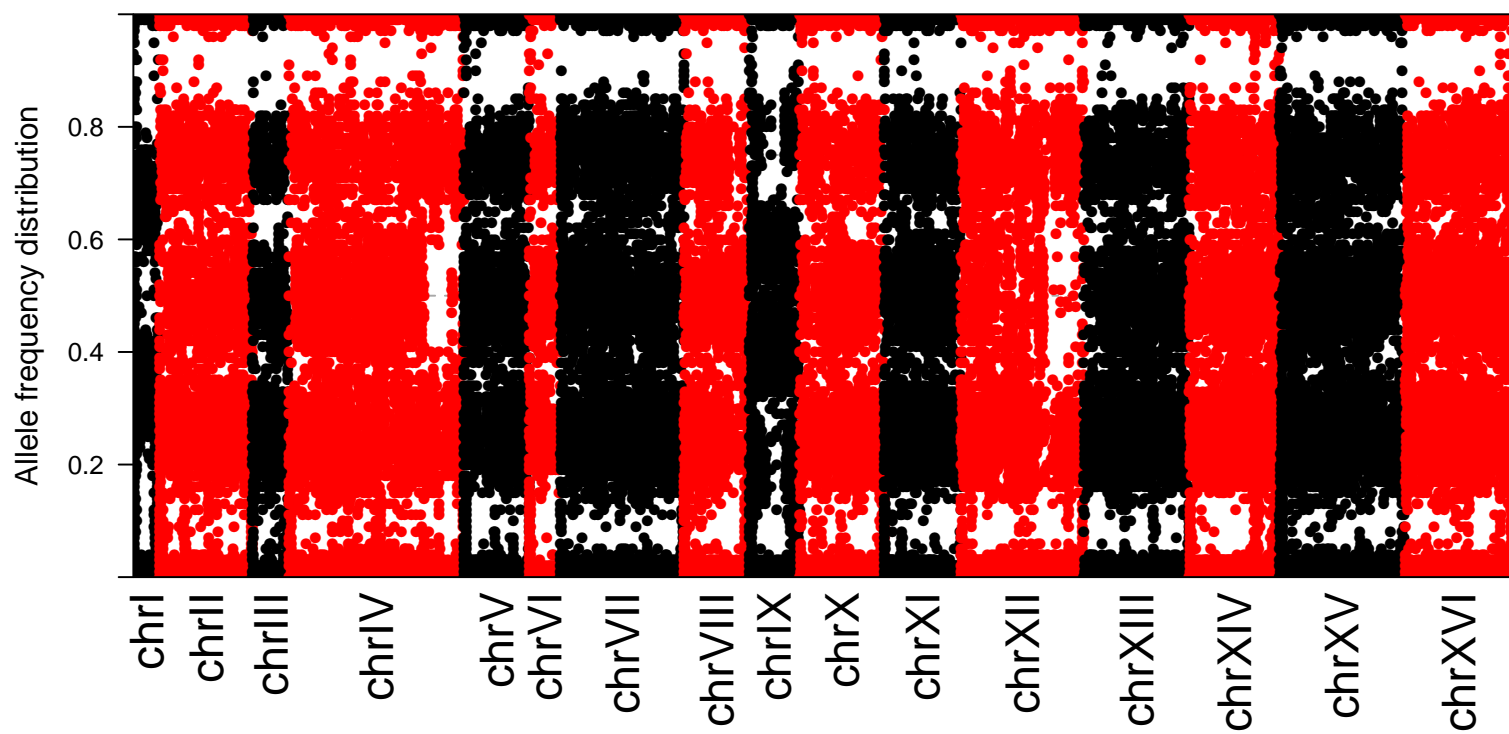


Supplementary Figure S3



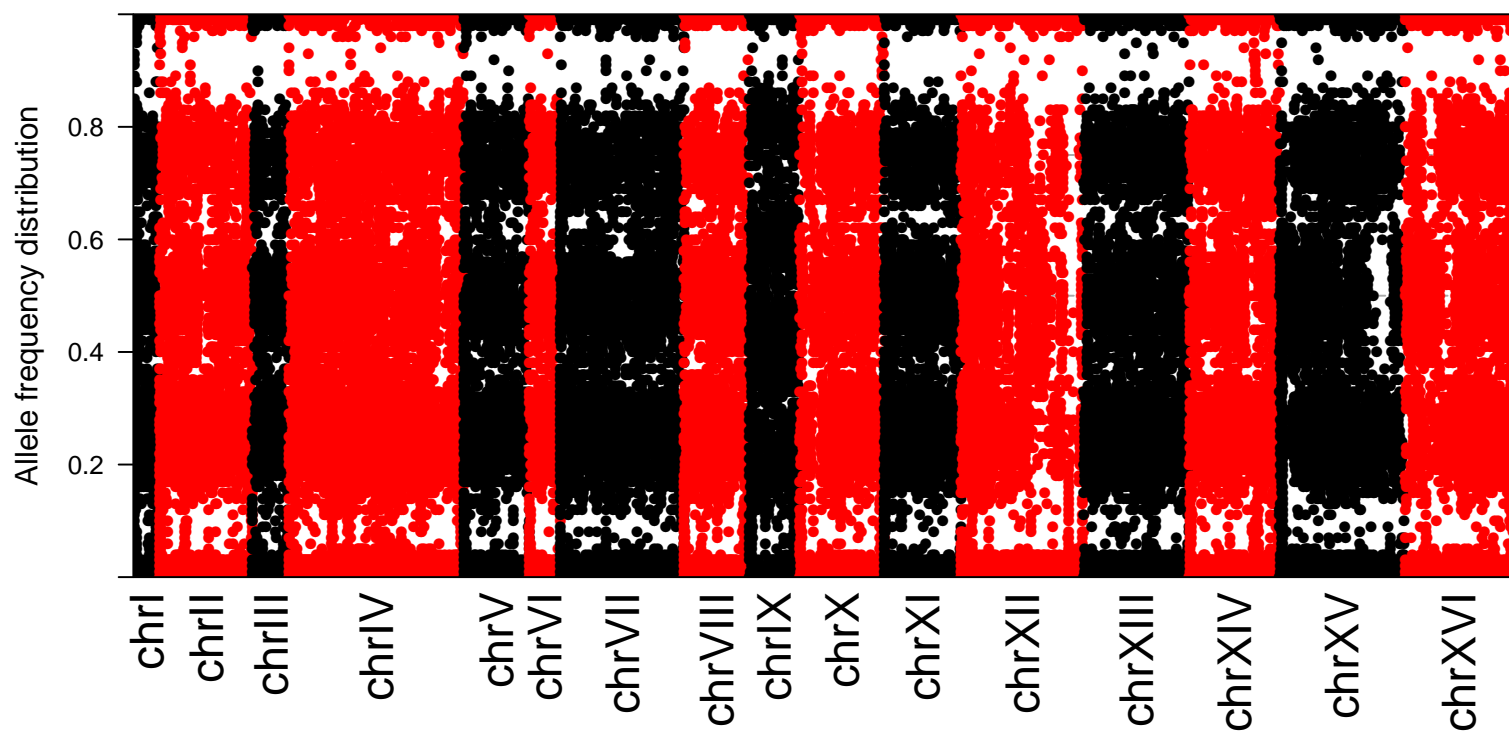
Marina_1

Supplementary Figure S3



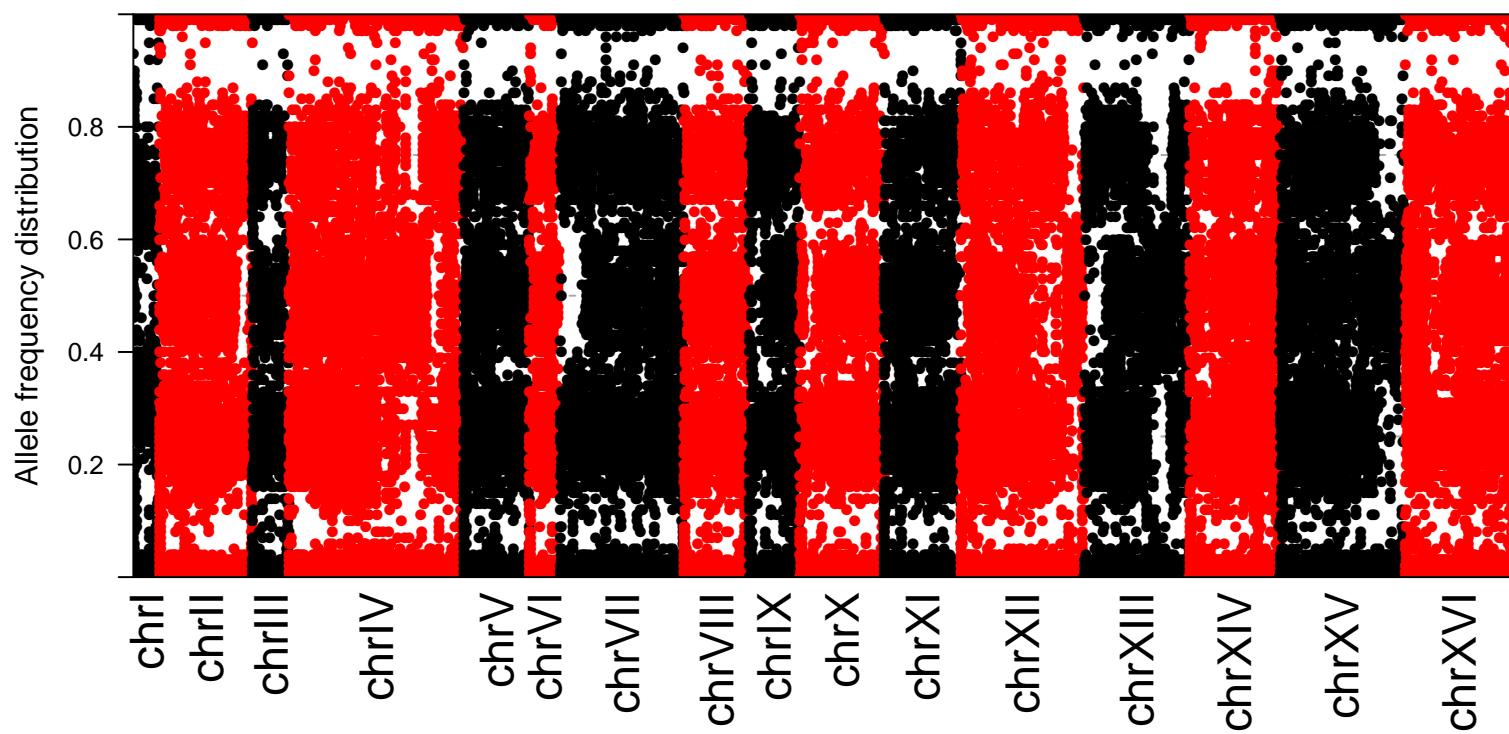
NCYC_Hornindal_1

Supplementary Figure S3



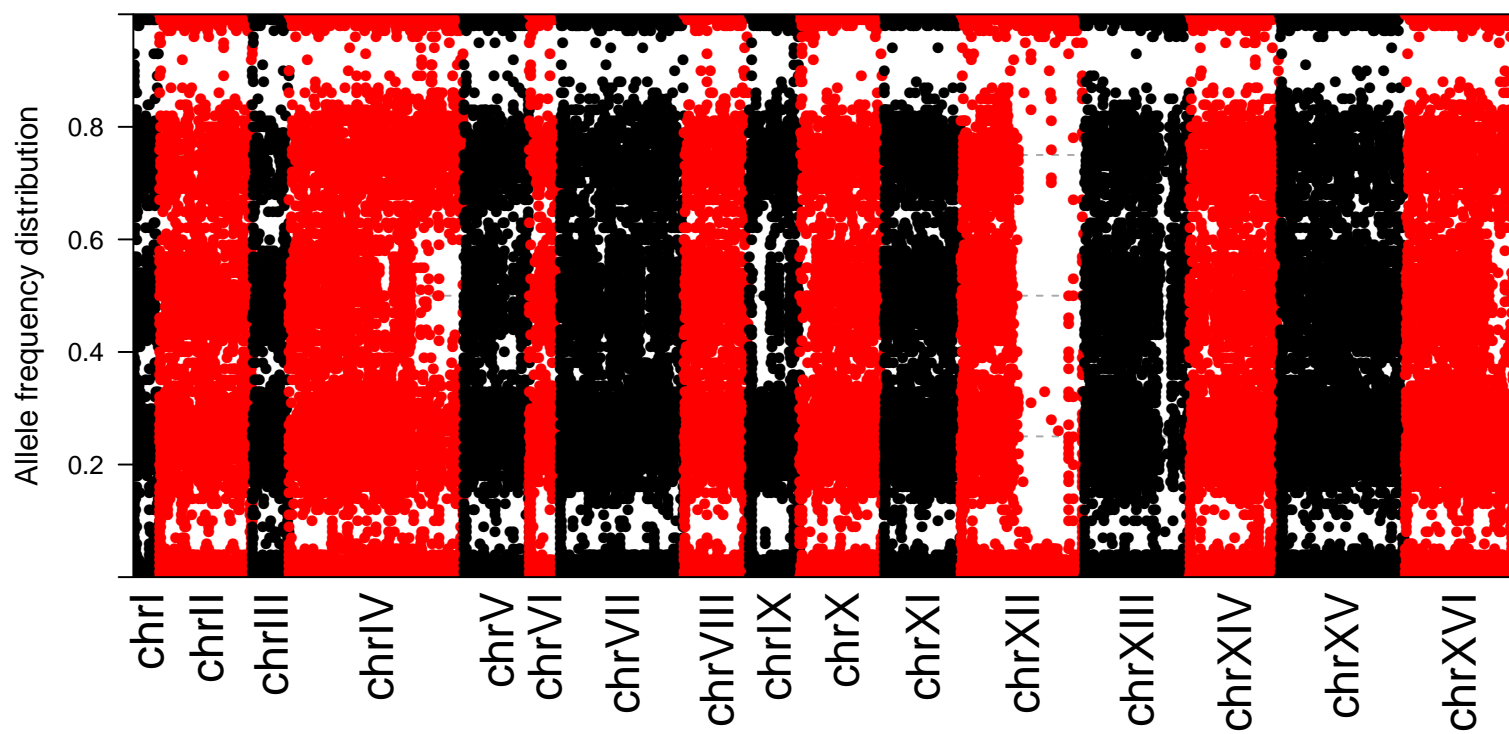
NCYC_Hornindal_2

Supplementary Figure S3



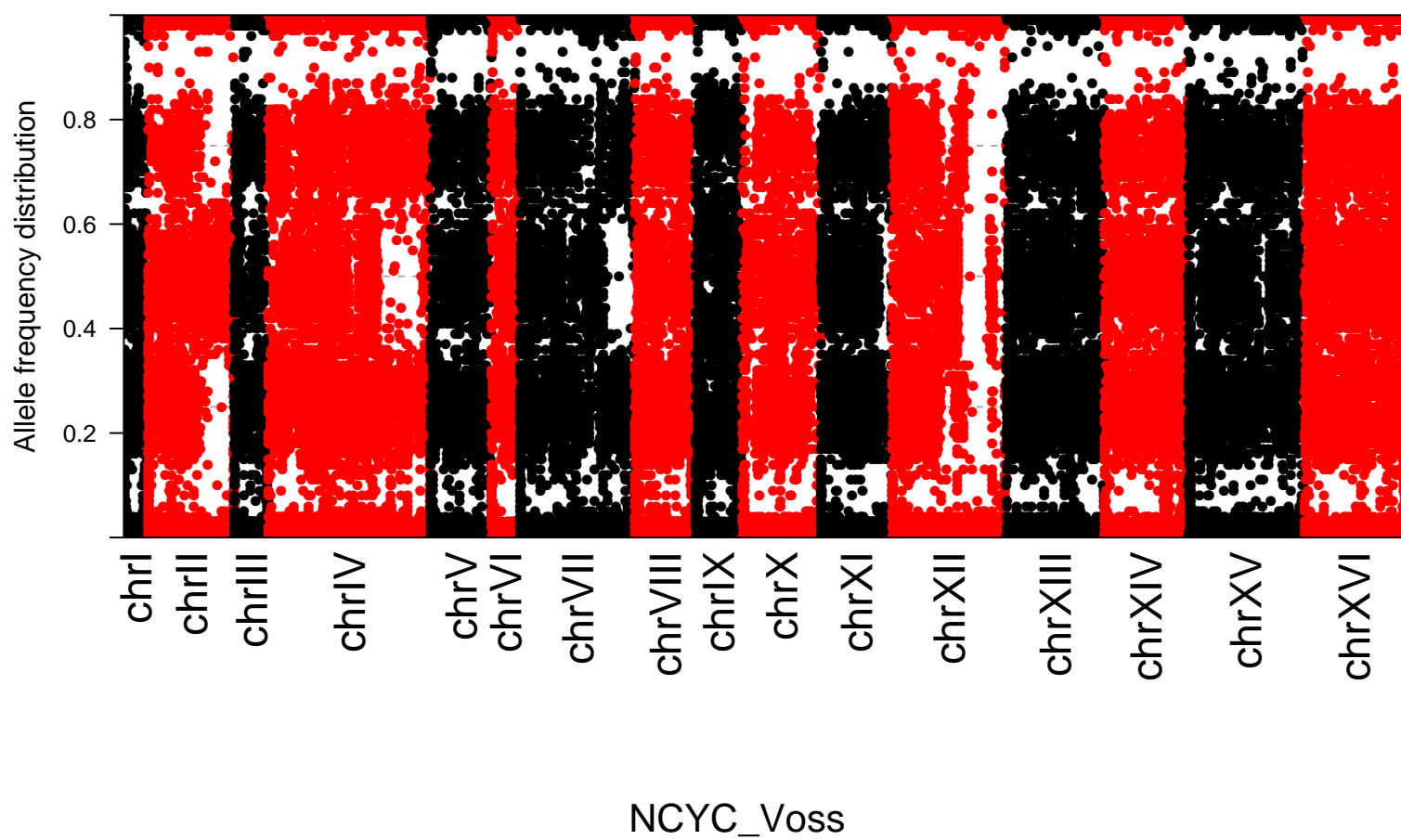
NCYC_Rivenes_1

Supplementary Figure S3

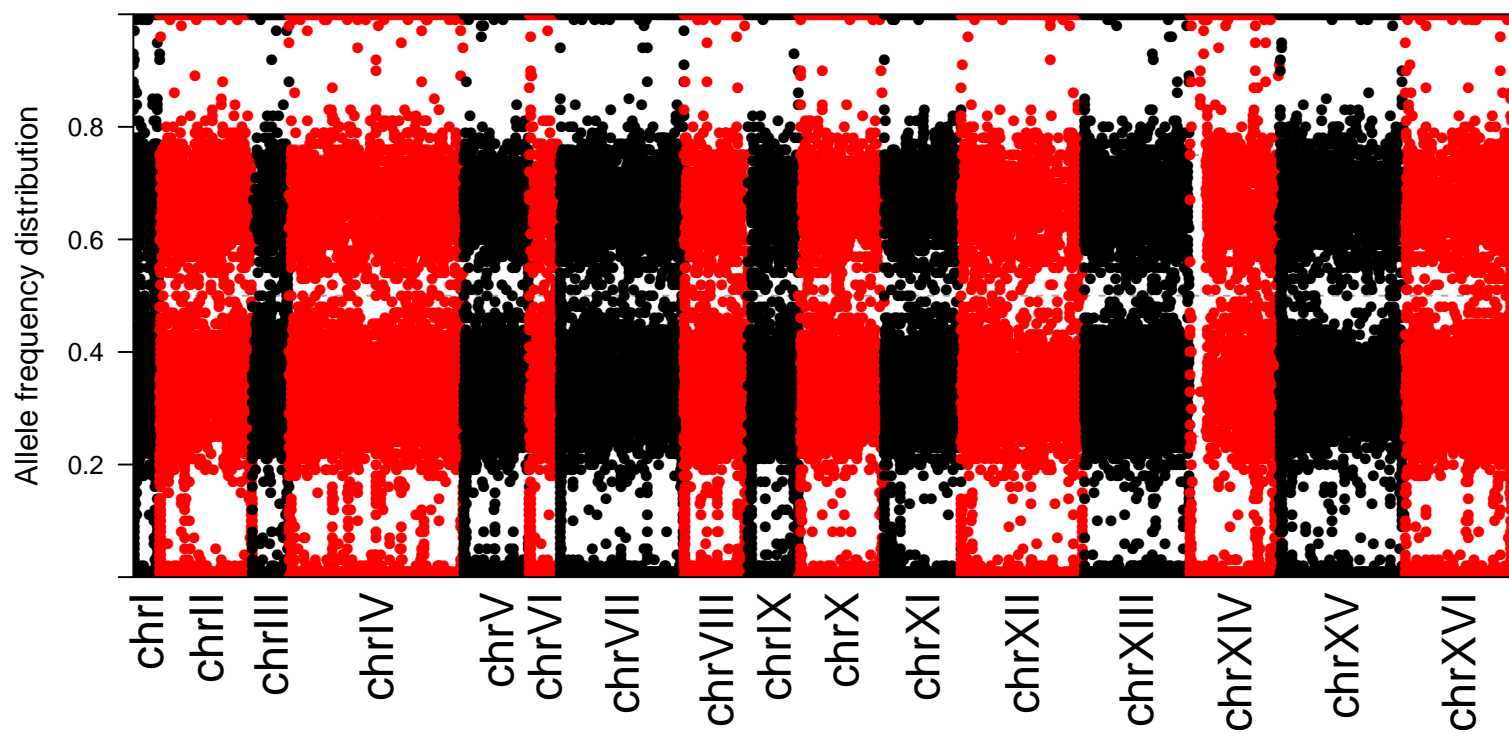


NCYC_Rivenes_2

Supplementary Figure S3

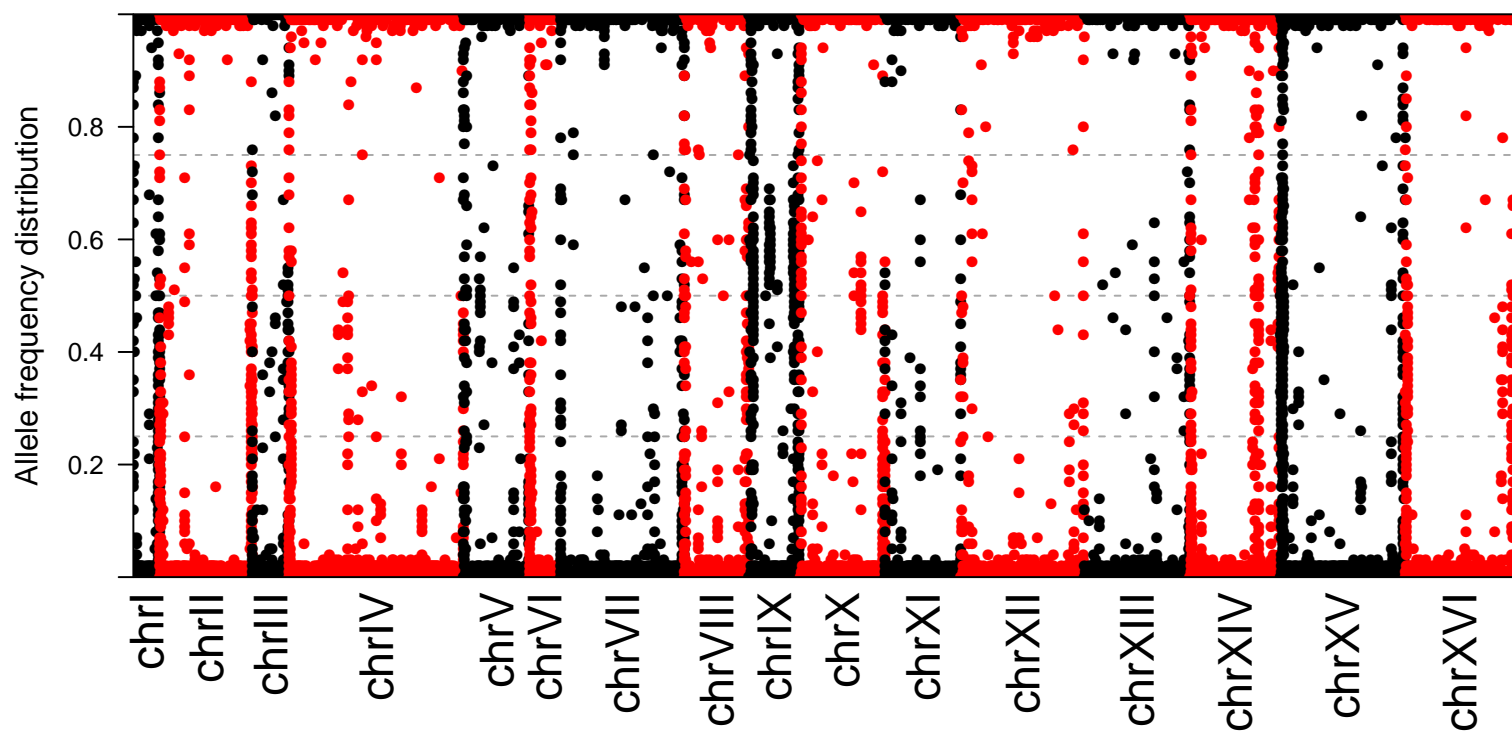


Supplementary Figure S3



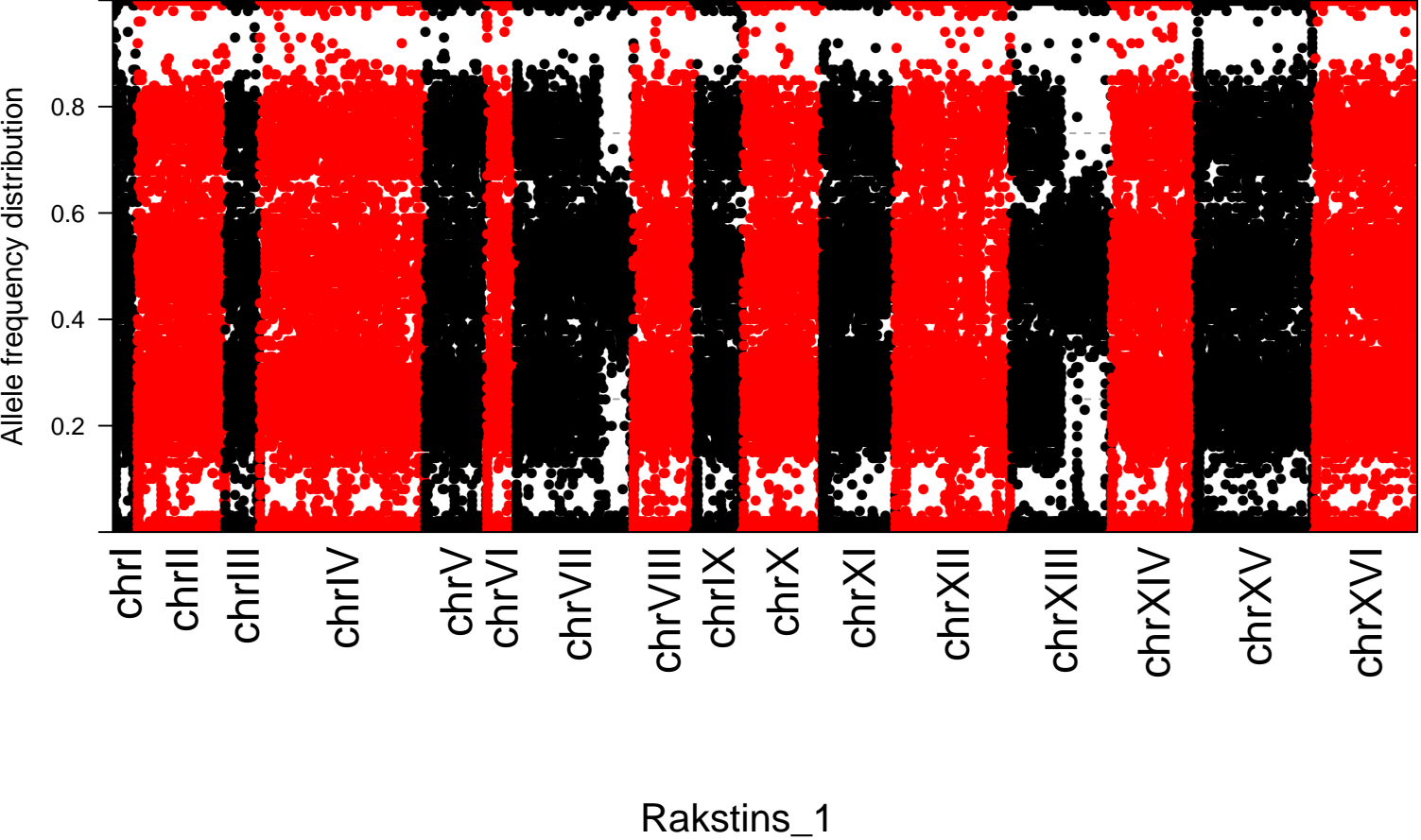
Pundurs_2

Supplementary Figure S3

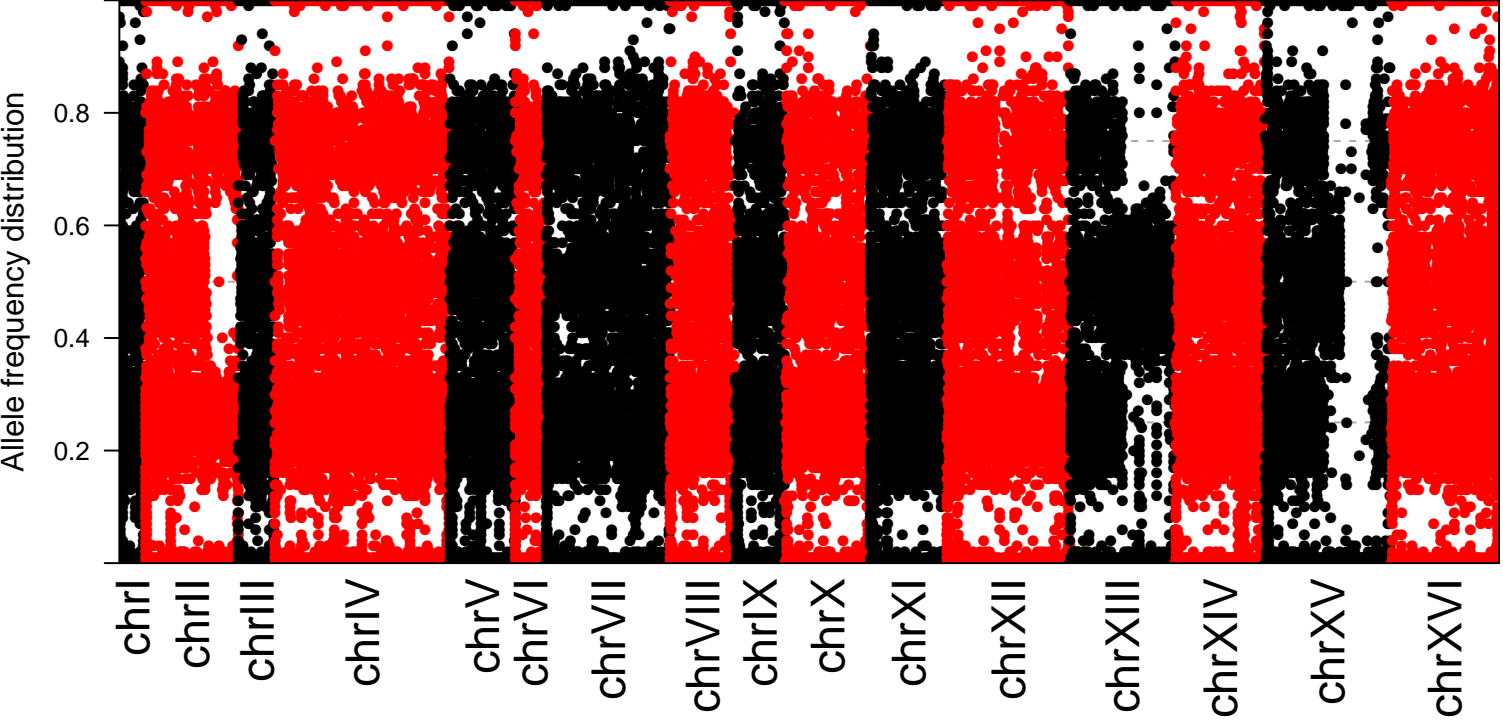


Pundurs1

Supplementary Figure S3

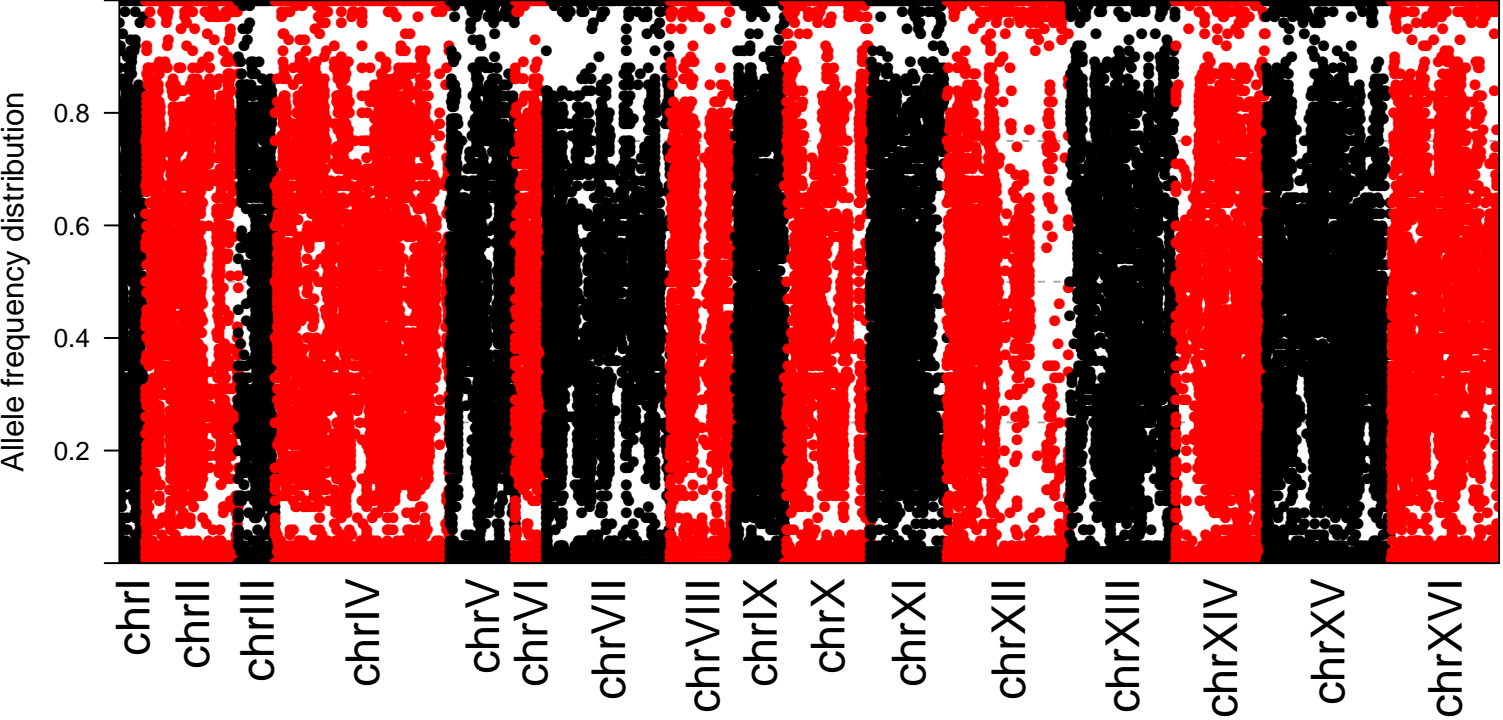


Supplementary Figure S3



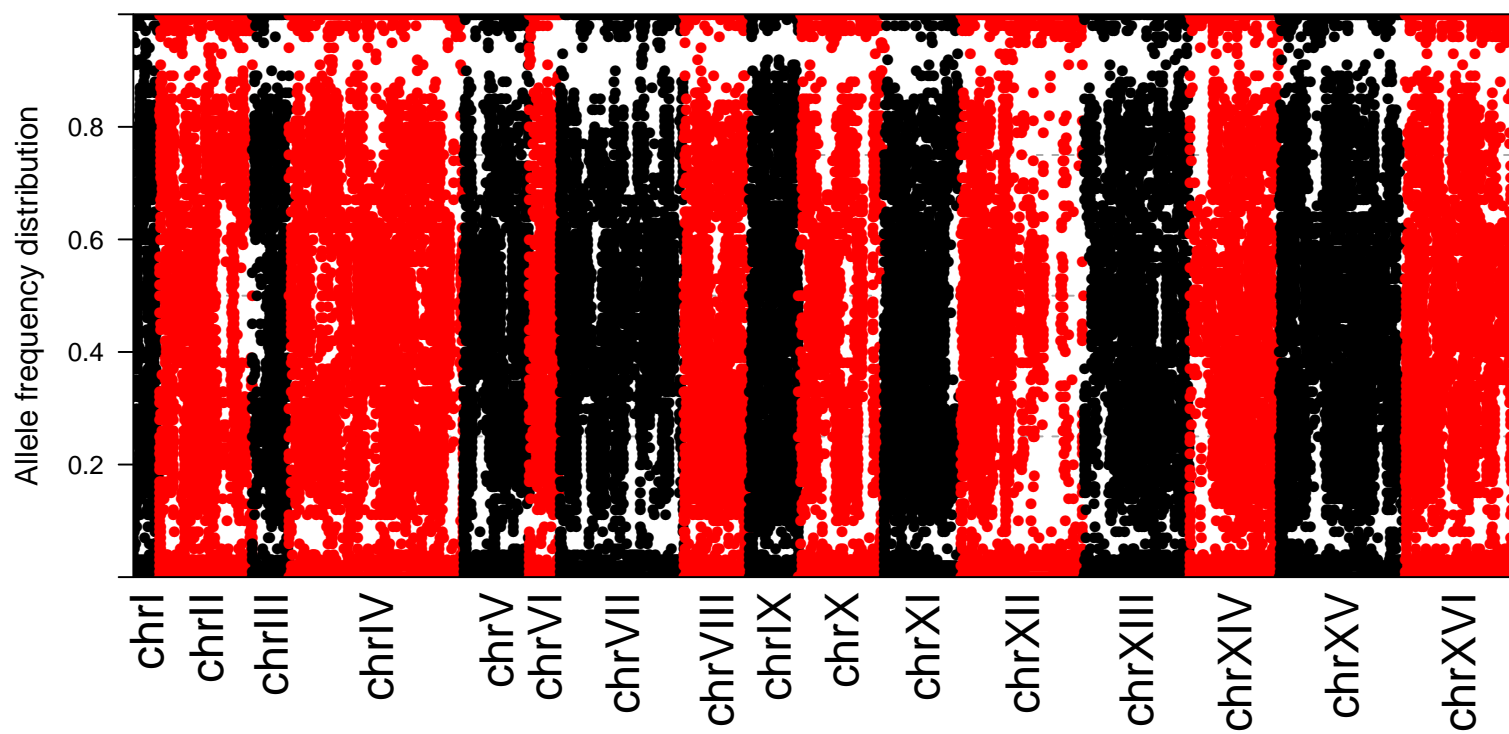
Rakstins_2

Supplementary Figure S3

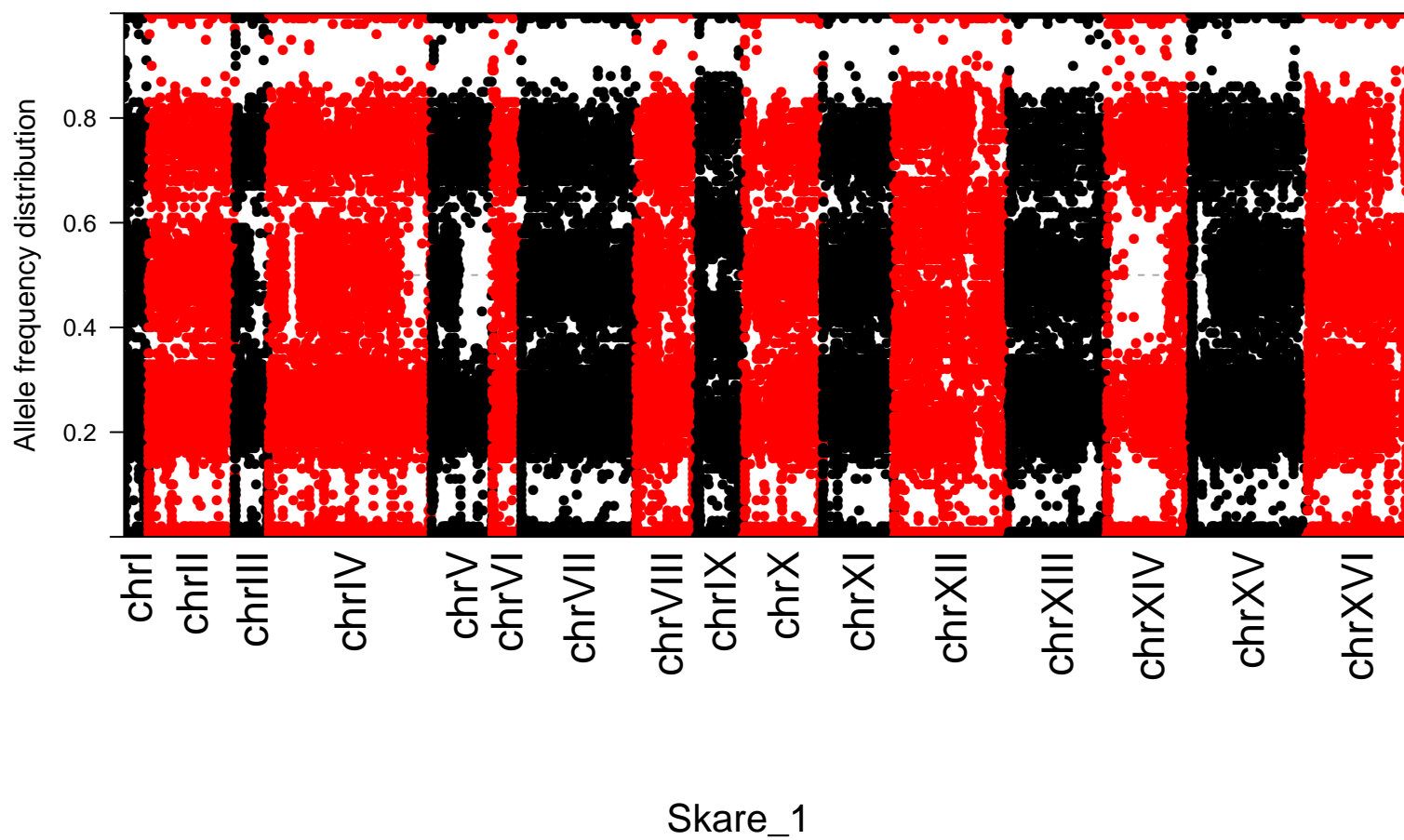


Rima1

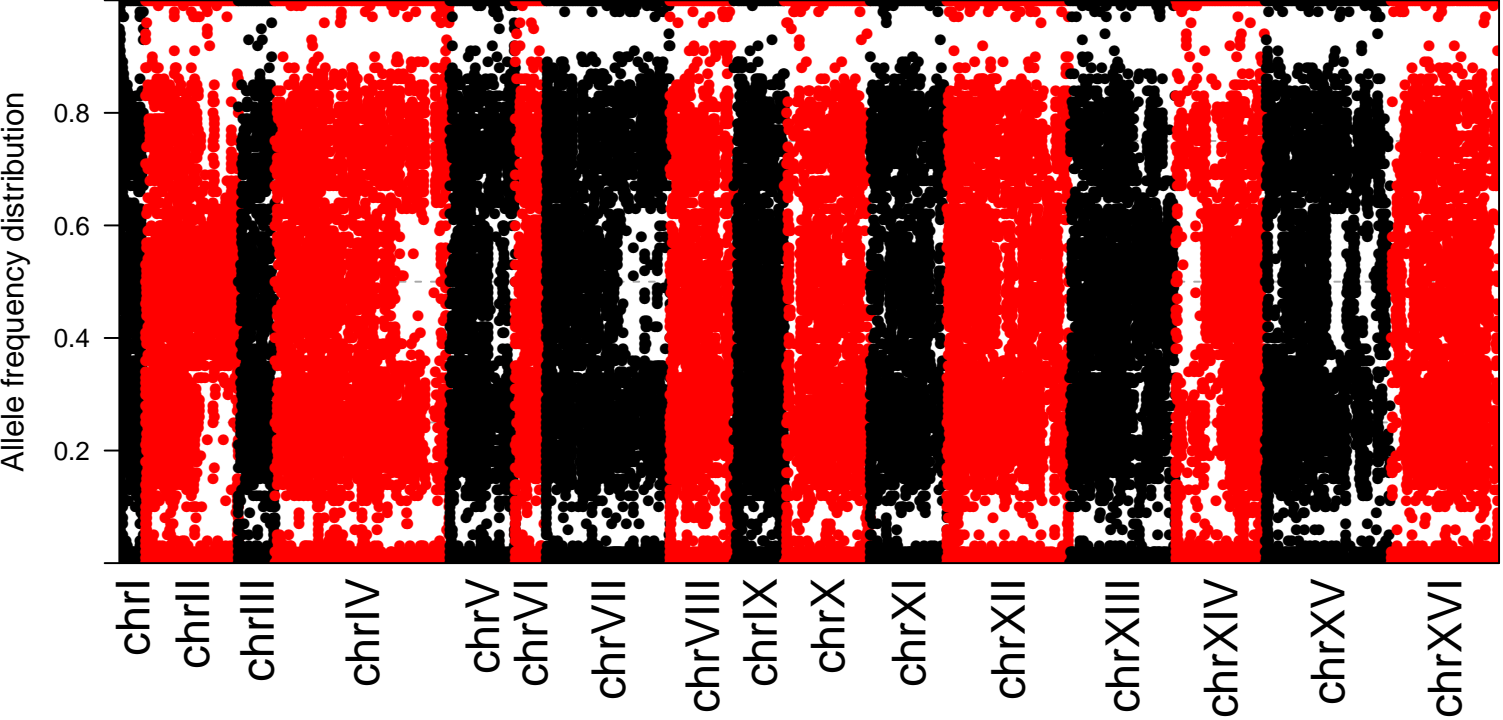
Supplementary Figure S3



Supplementary Figure S3

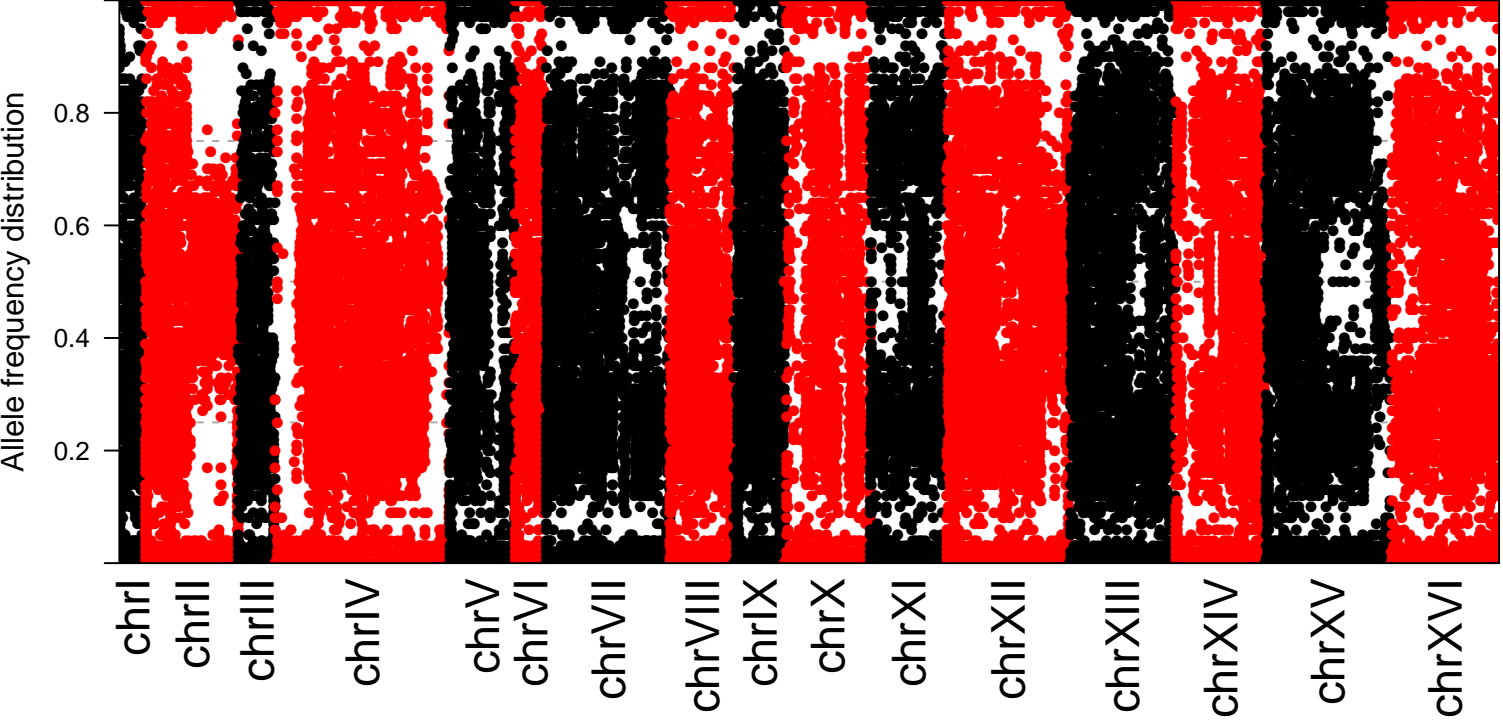


Supplementary Figure S3



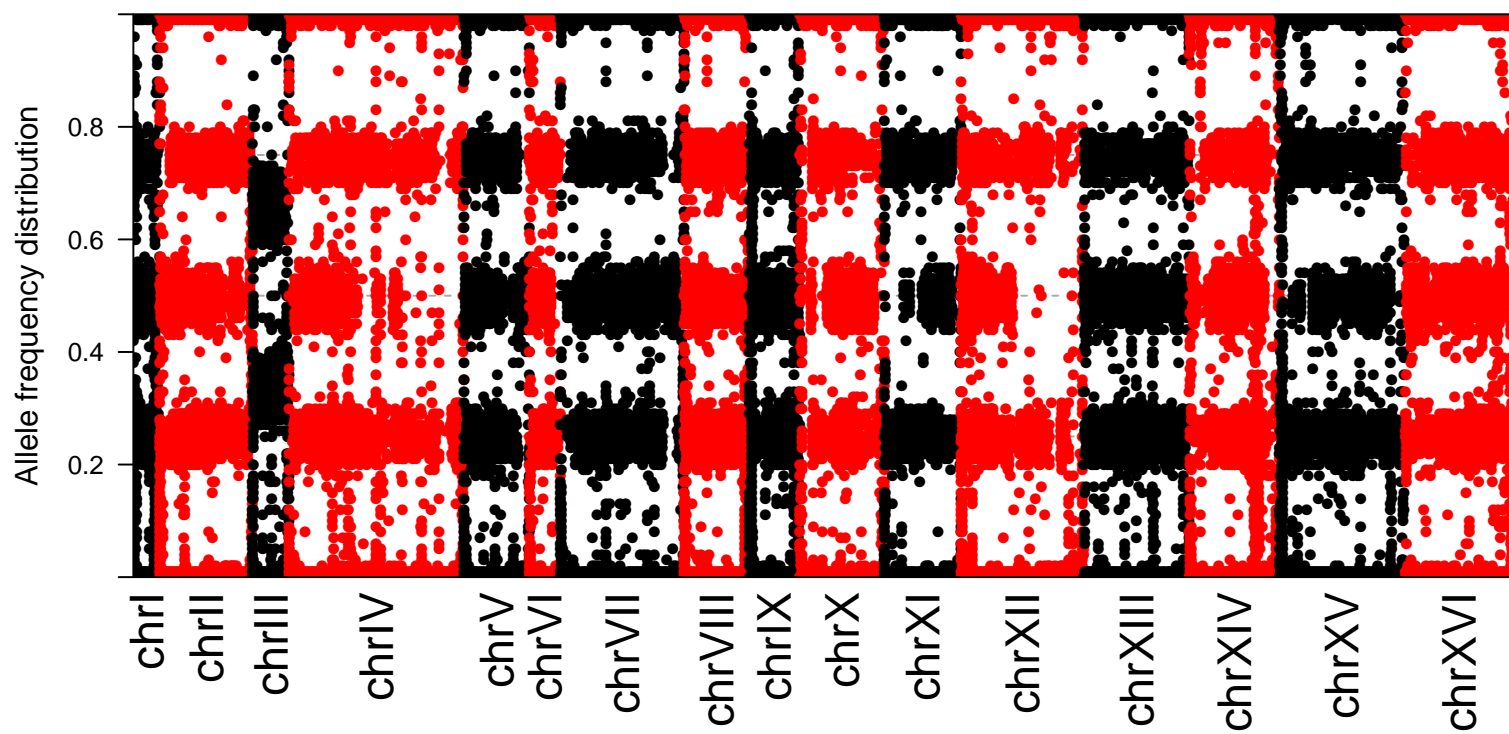
Skrindo2

Supplementary Figure S3



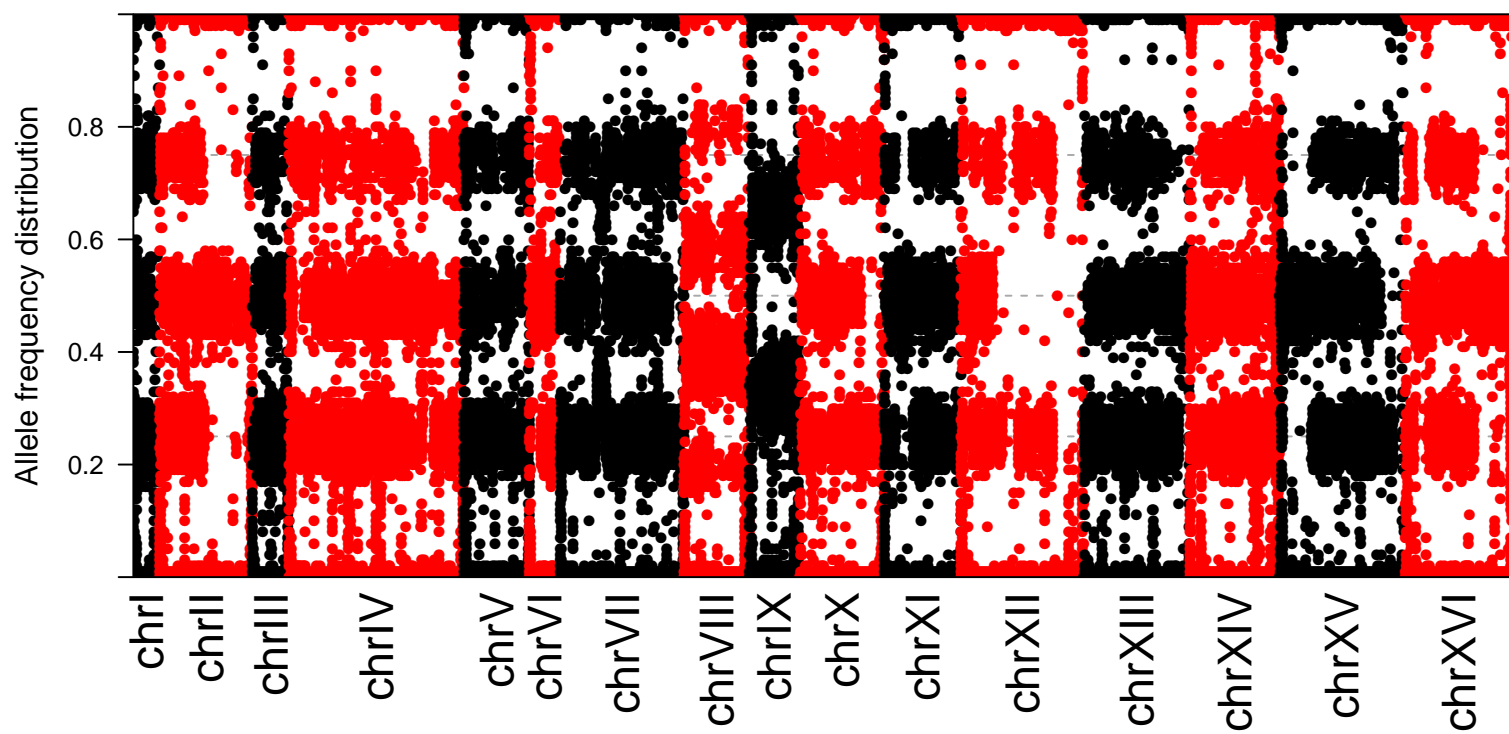
Skrindo5

Supplementary Figure S3



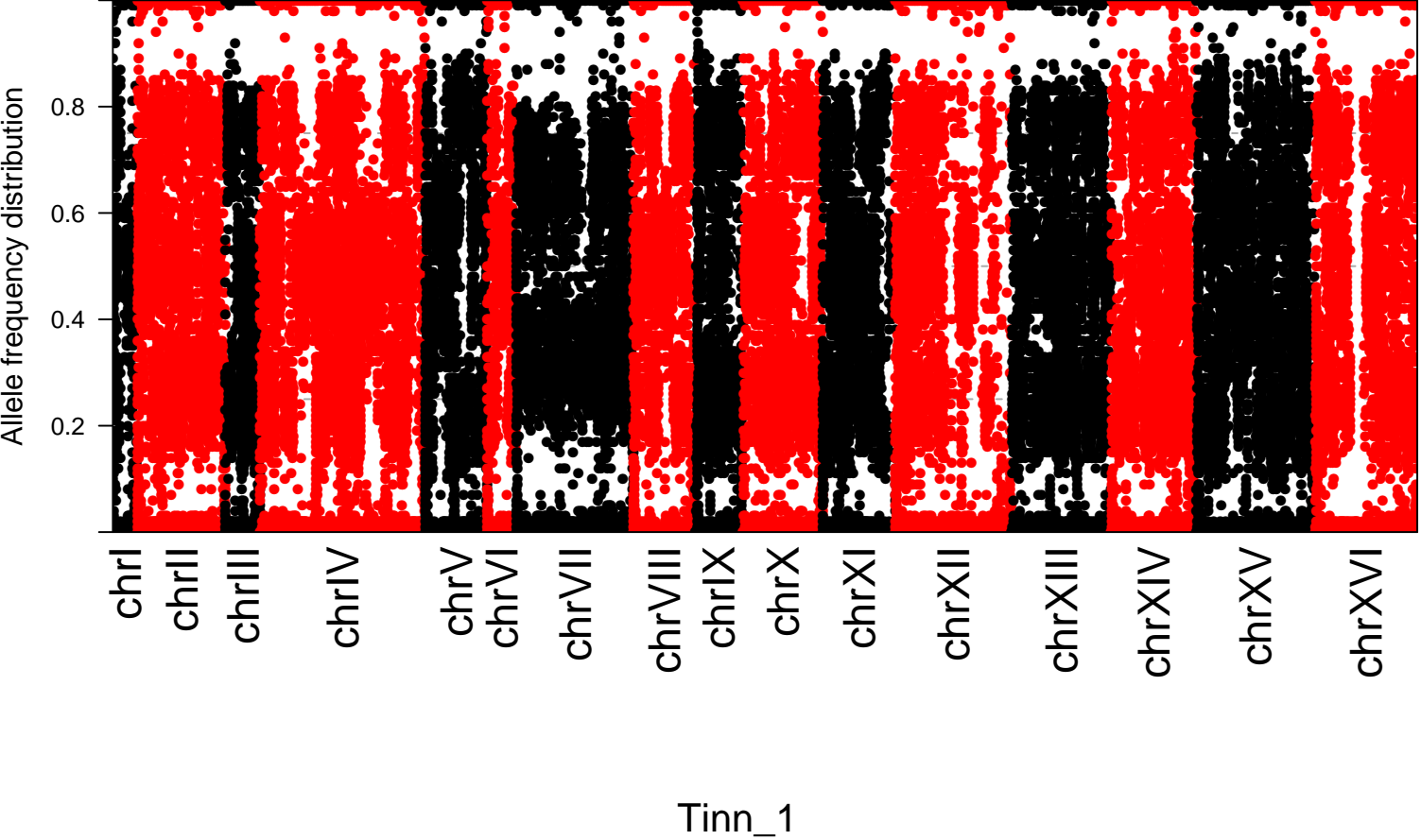
Stordal_Ebbegarden_1

Supplementary Figure S3

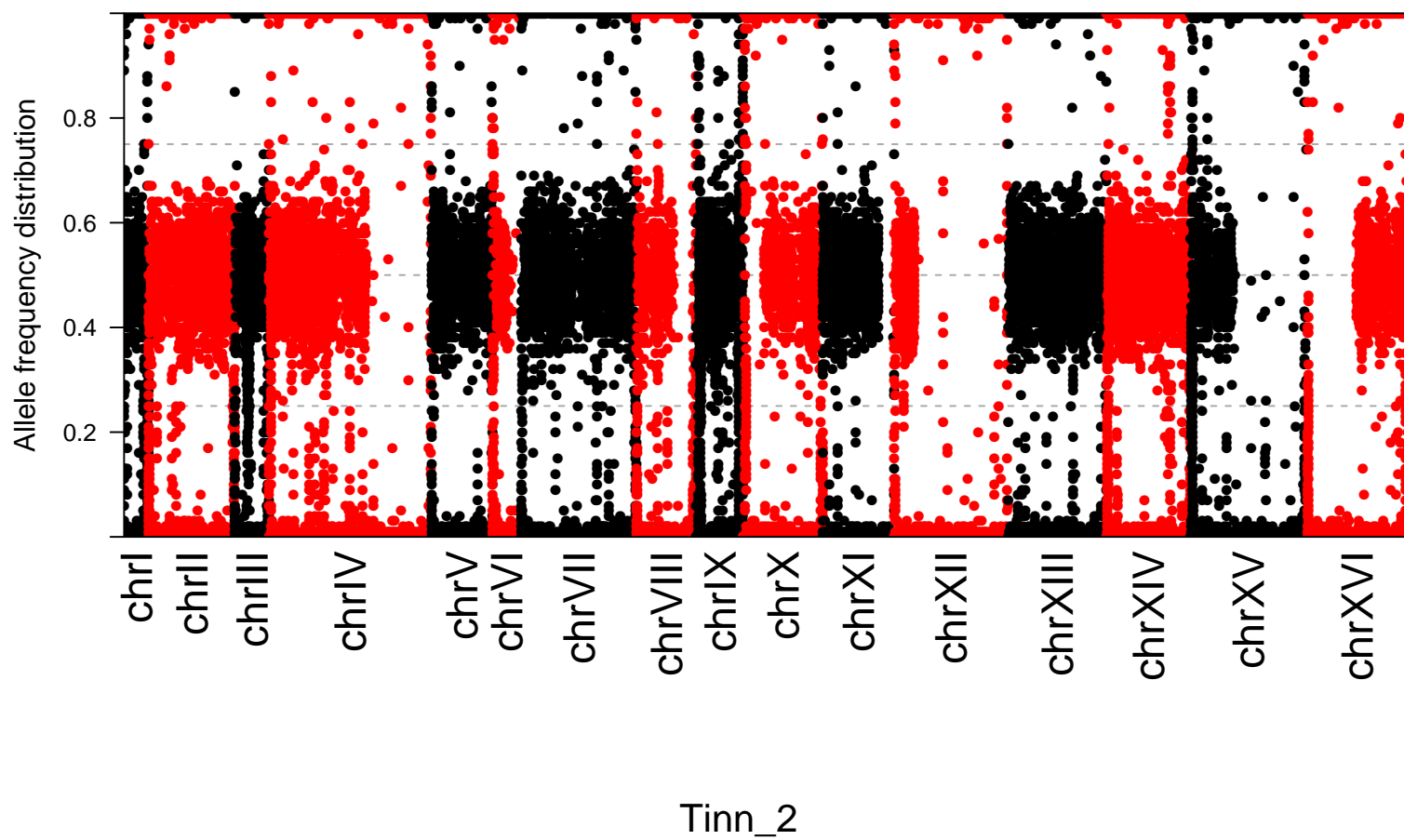


Suomen_Hiiva

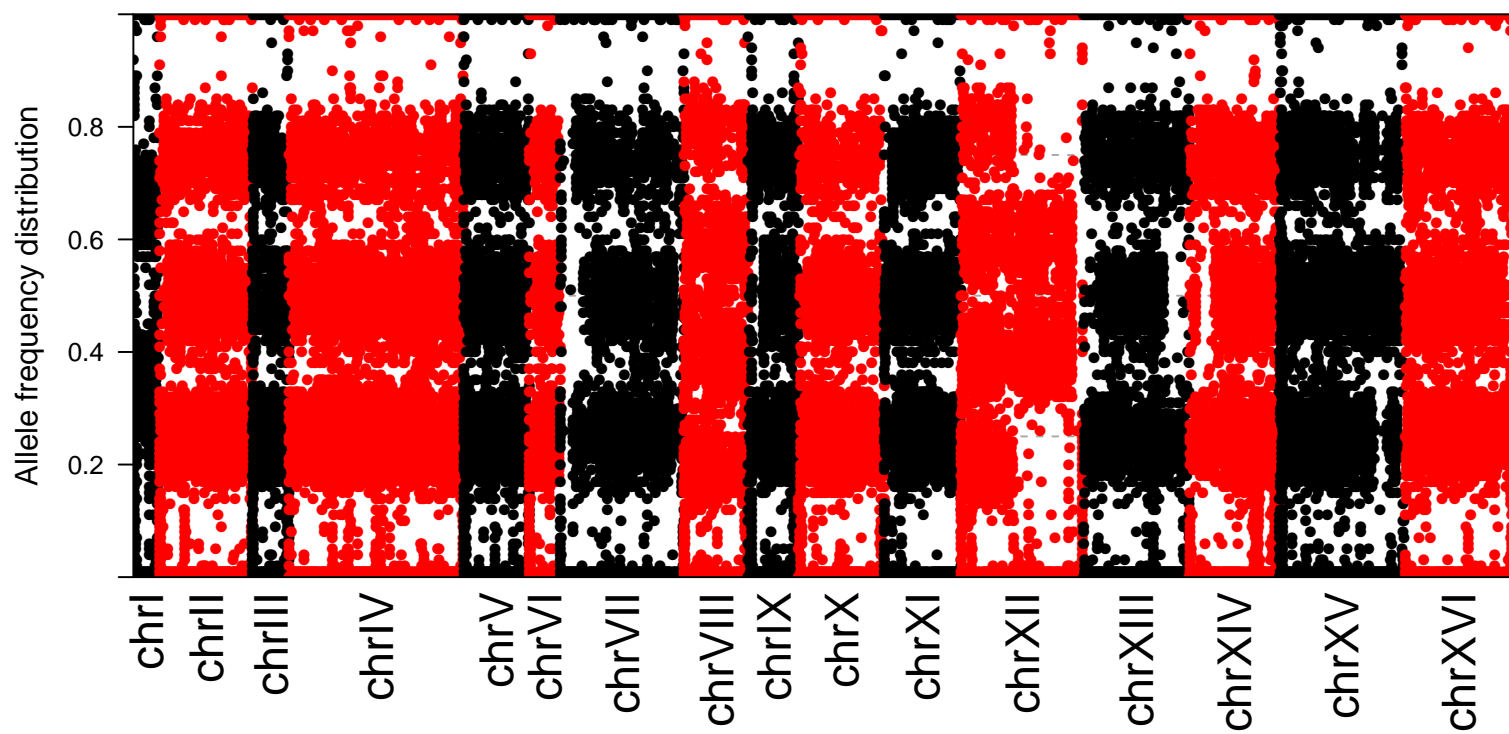
Supplementary Figure S3



Supplementary Figure S3

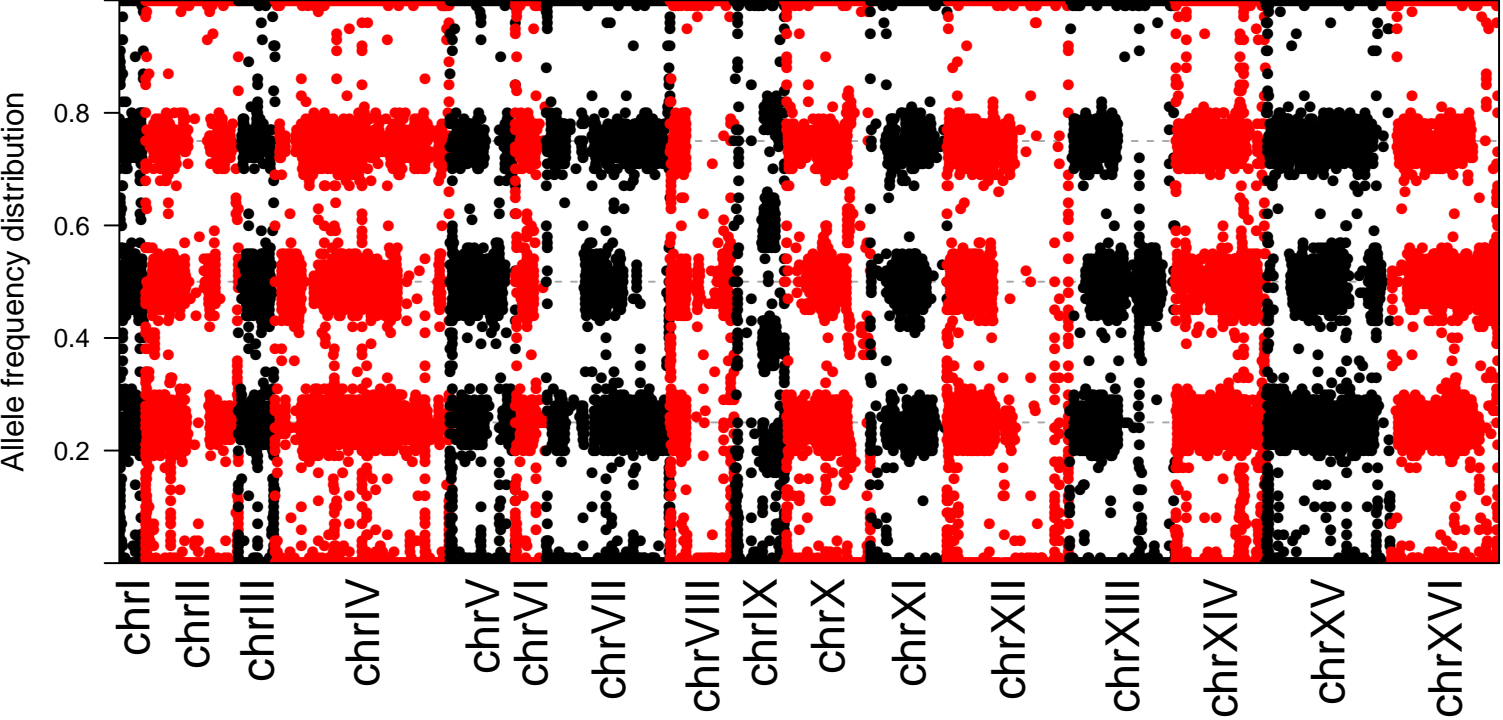


Supplementary Figure S3



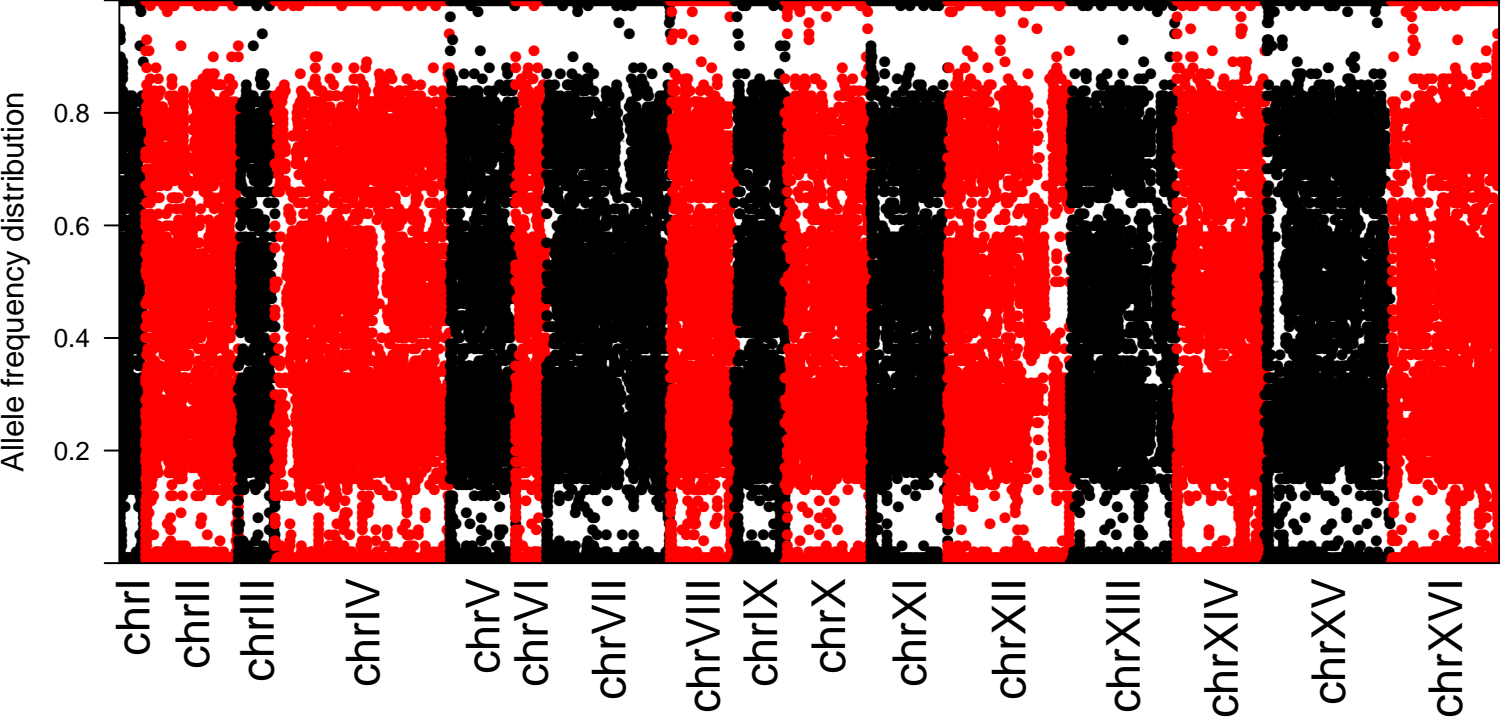
Tormodsgarden_1

Supplementary Figure S3



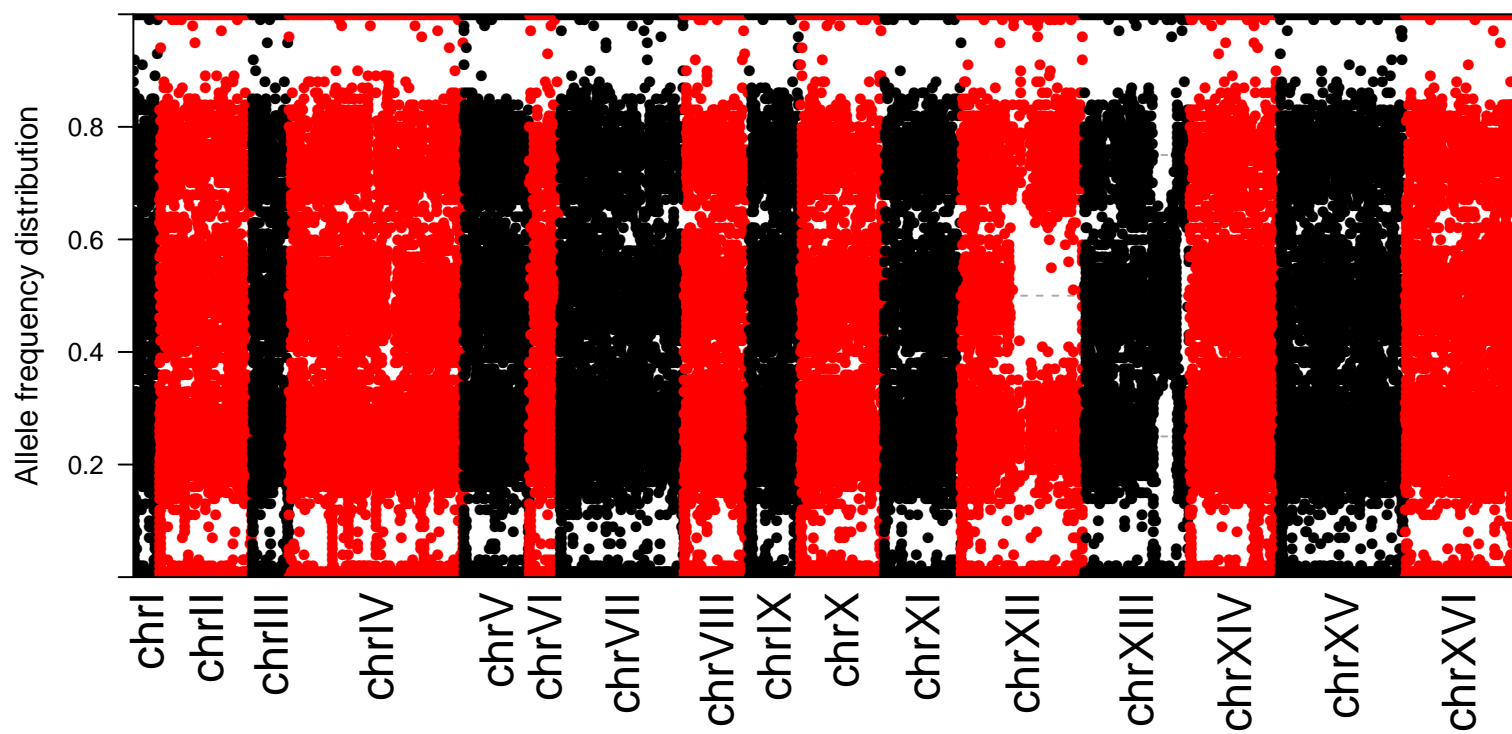
Überweizen

Supplementary Figure S3



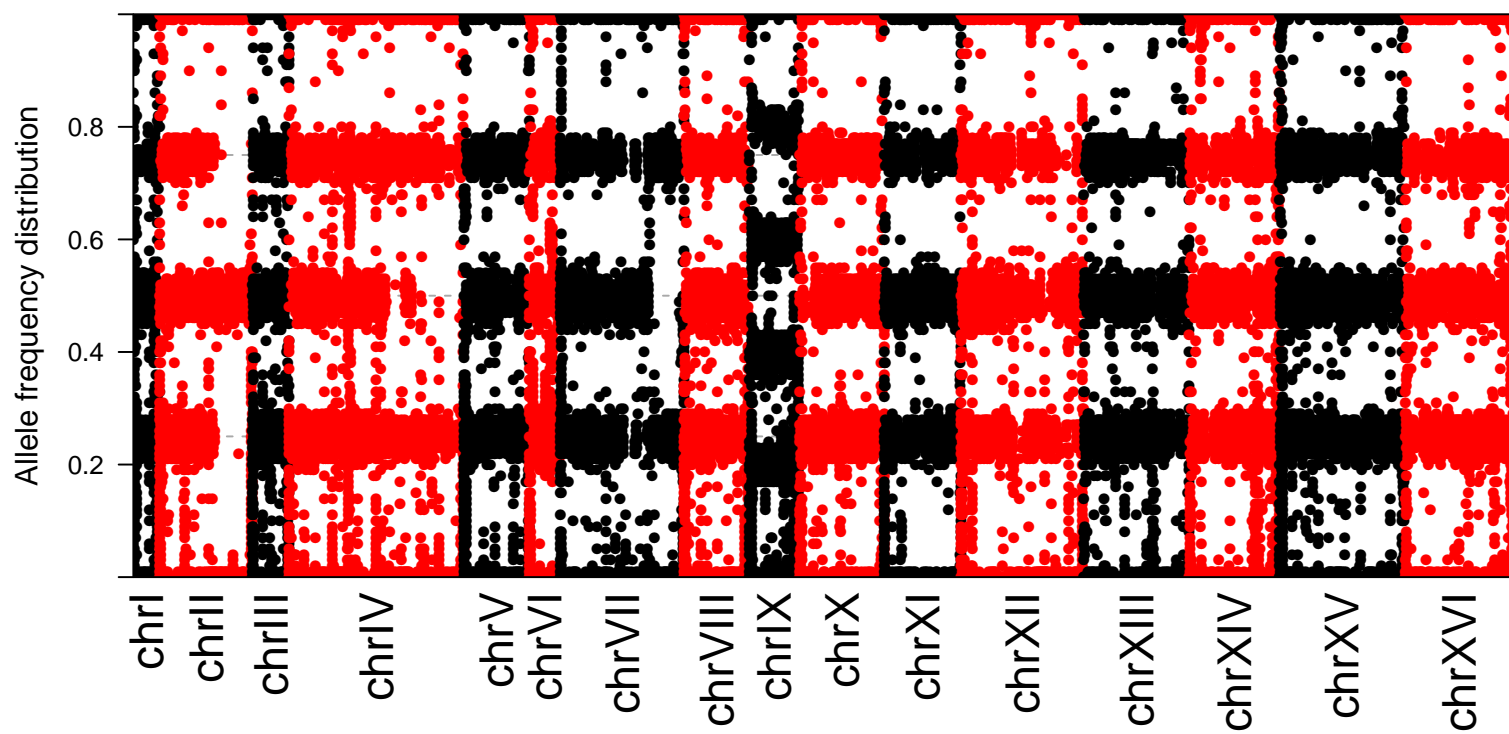
Vabalninkas_1

Supplementary Figure S3



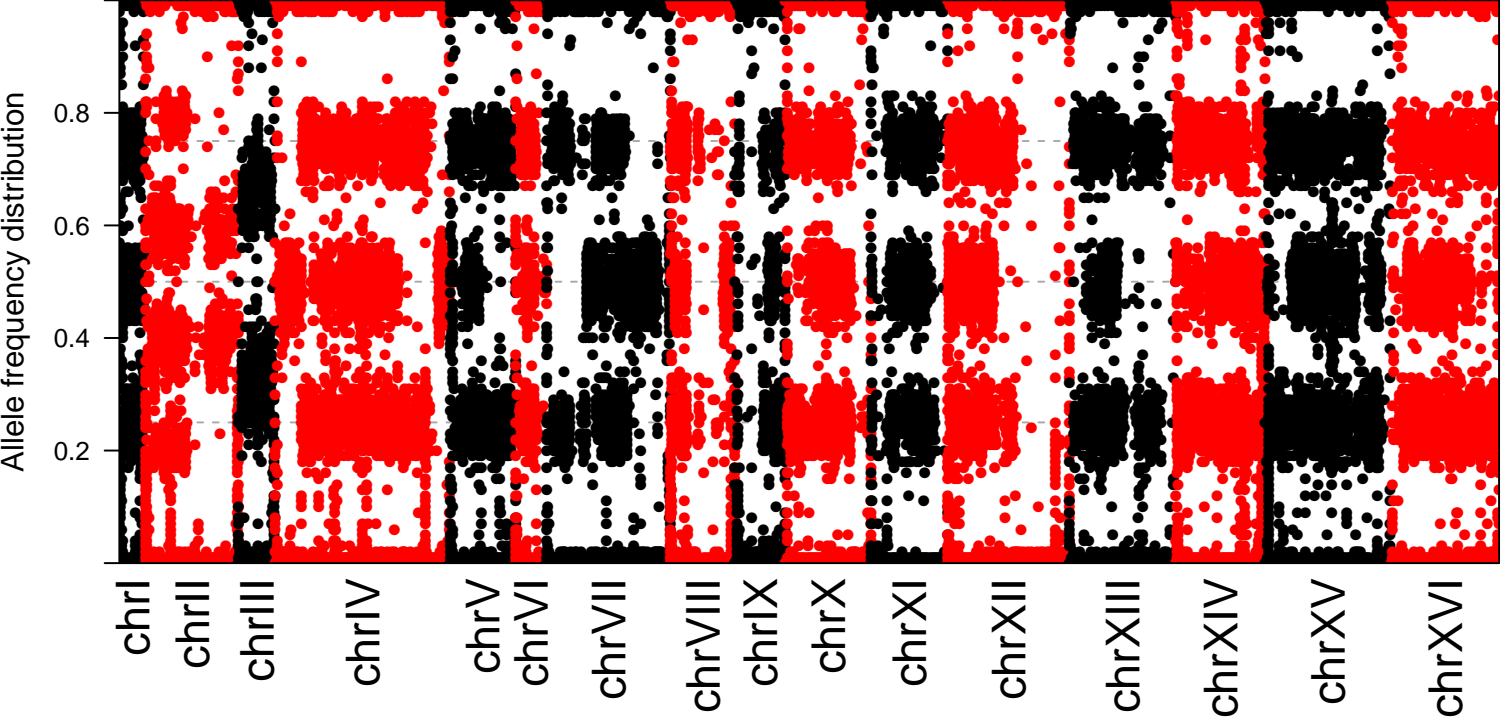
Vikintas

Supplementary Figure S3



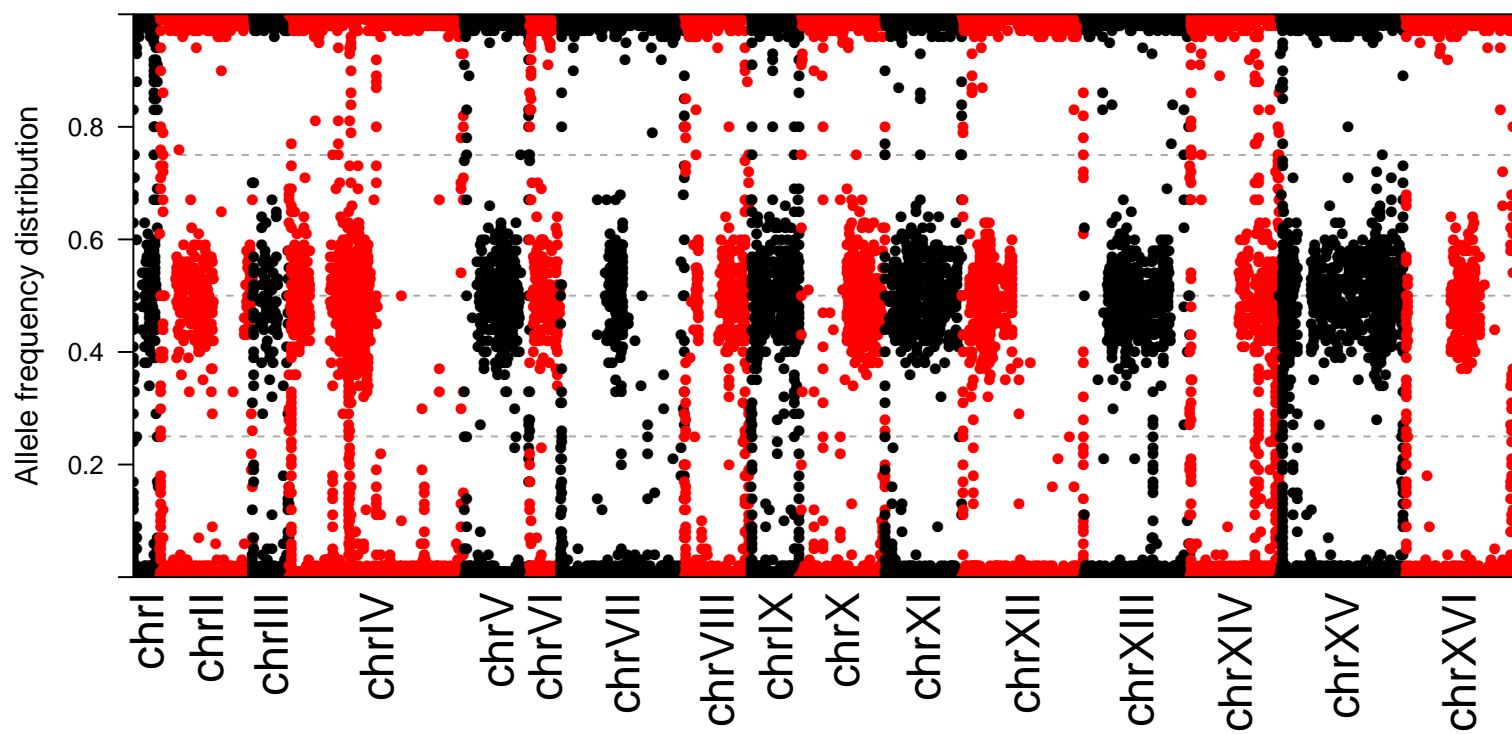
Voss_1

Supplementary Figure S3



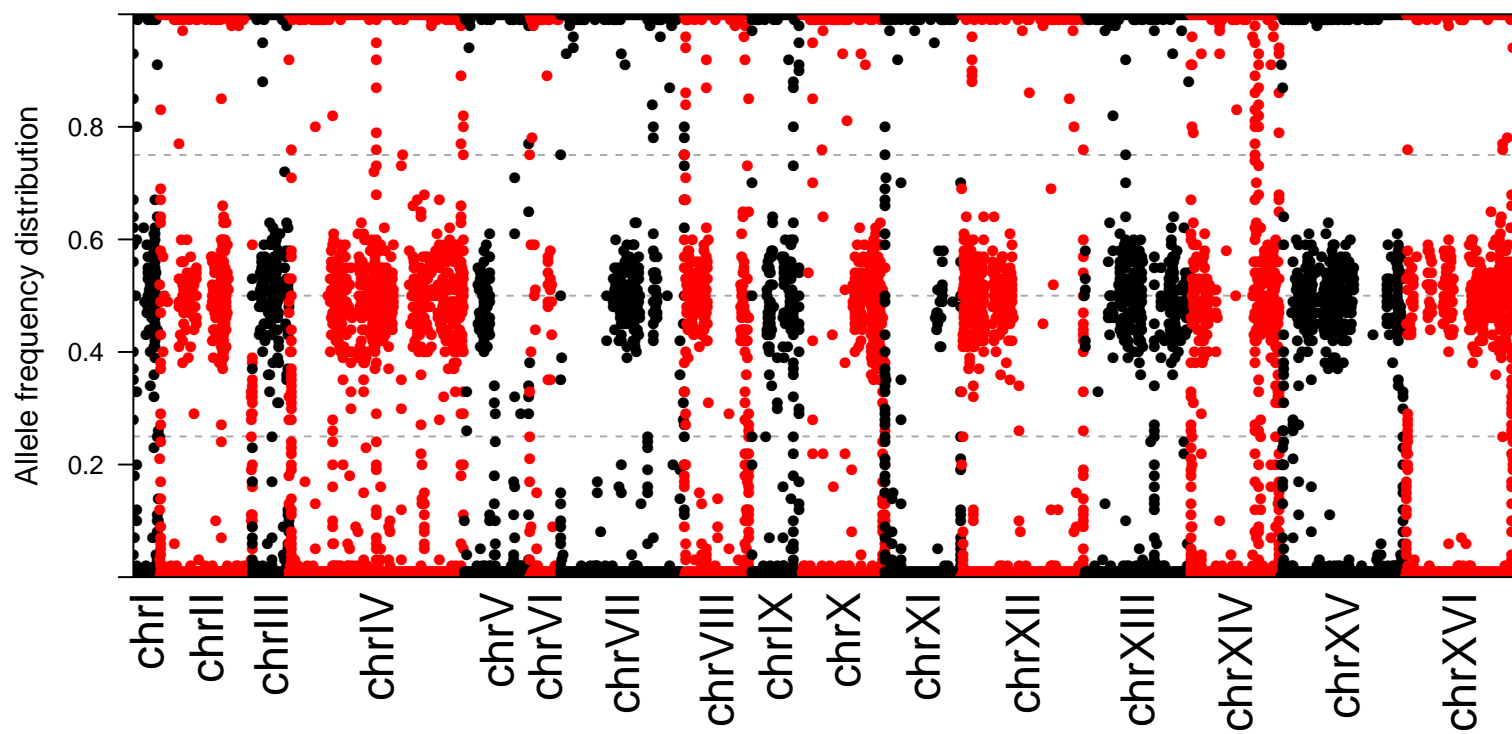
Weizen_1

Supplementary Figure S3



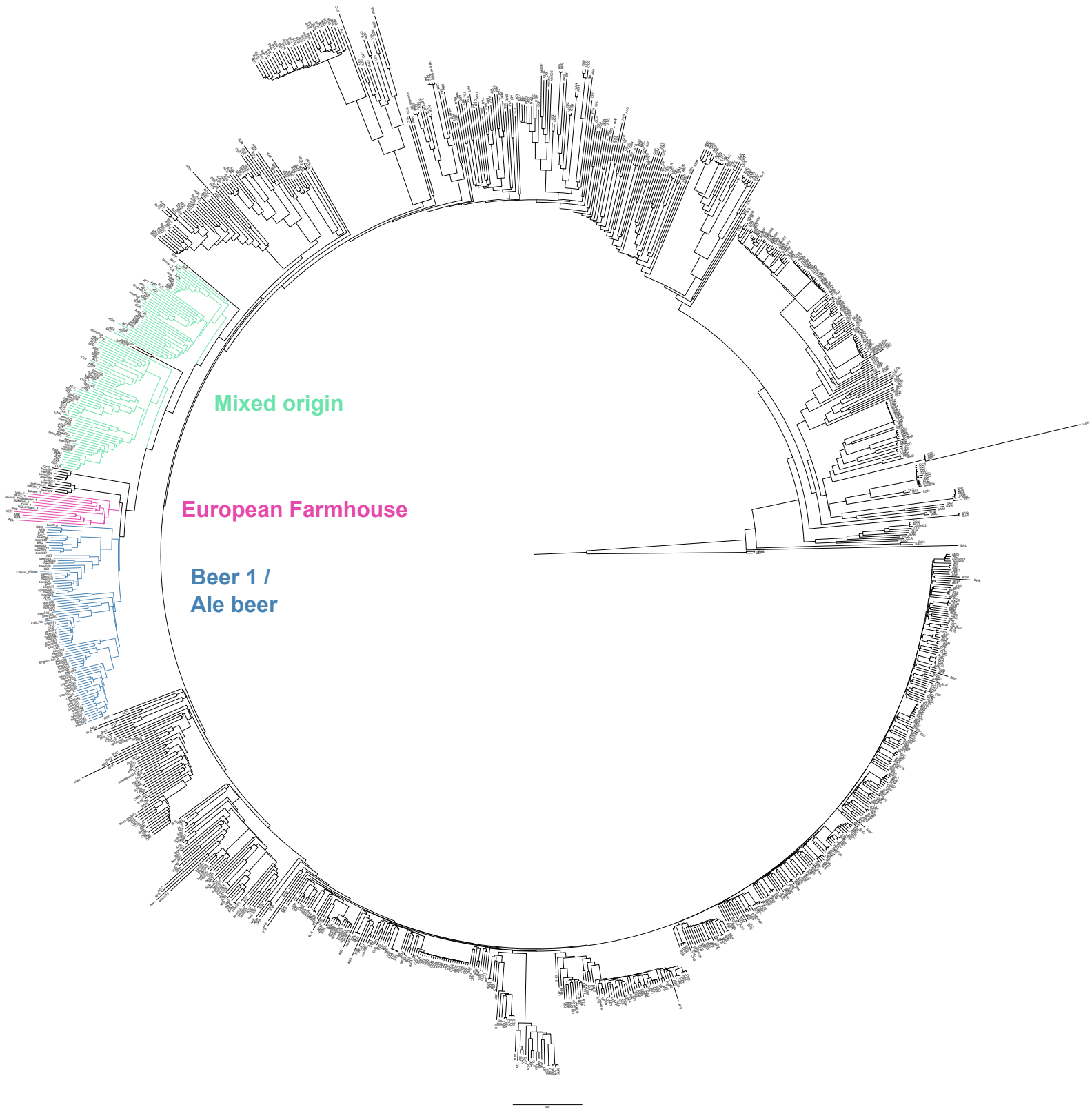
wine003

Supplementary Figure S3

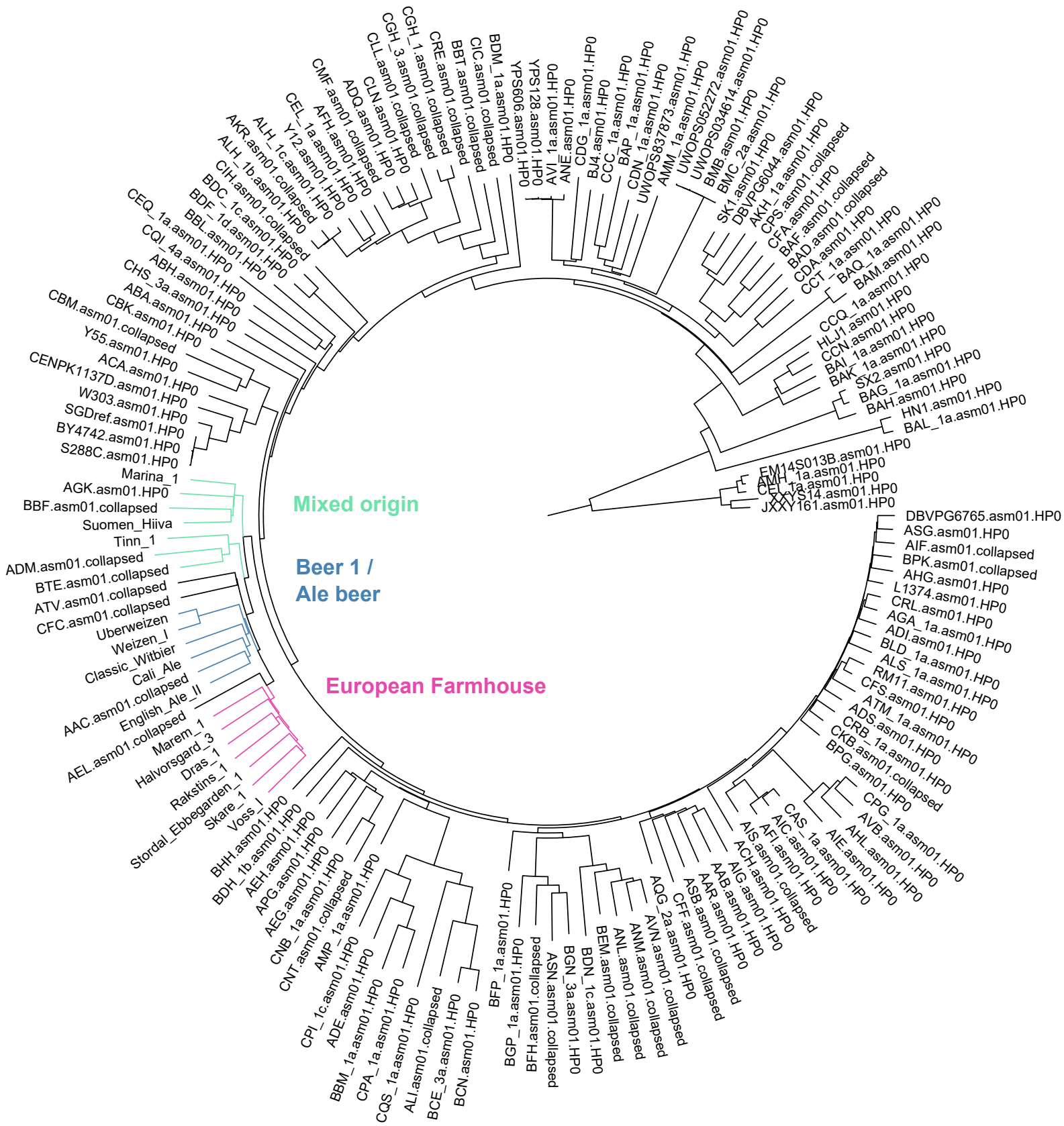


wine005

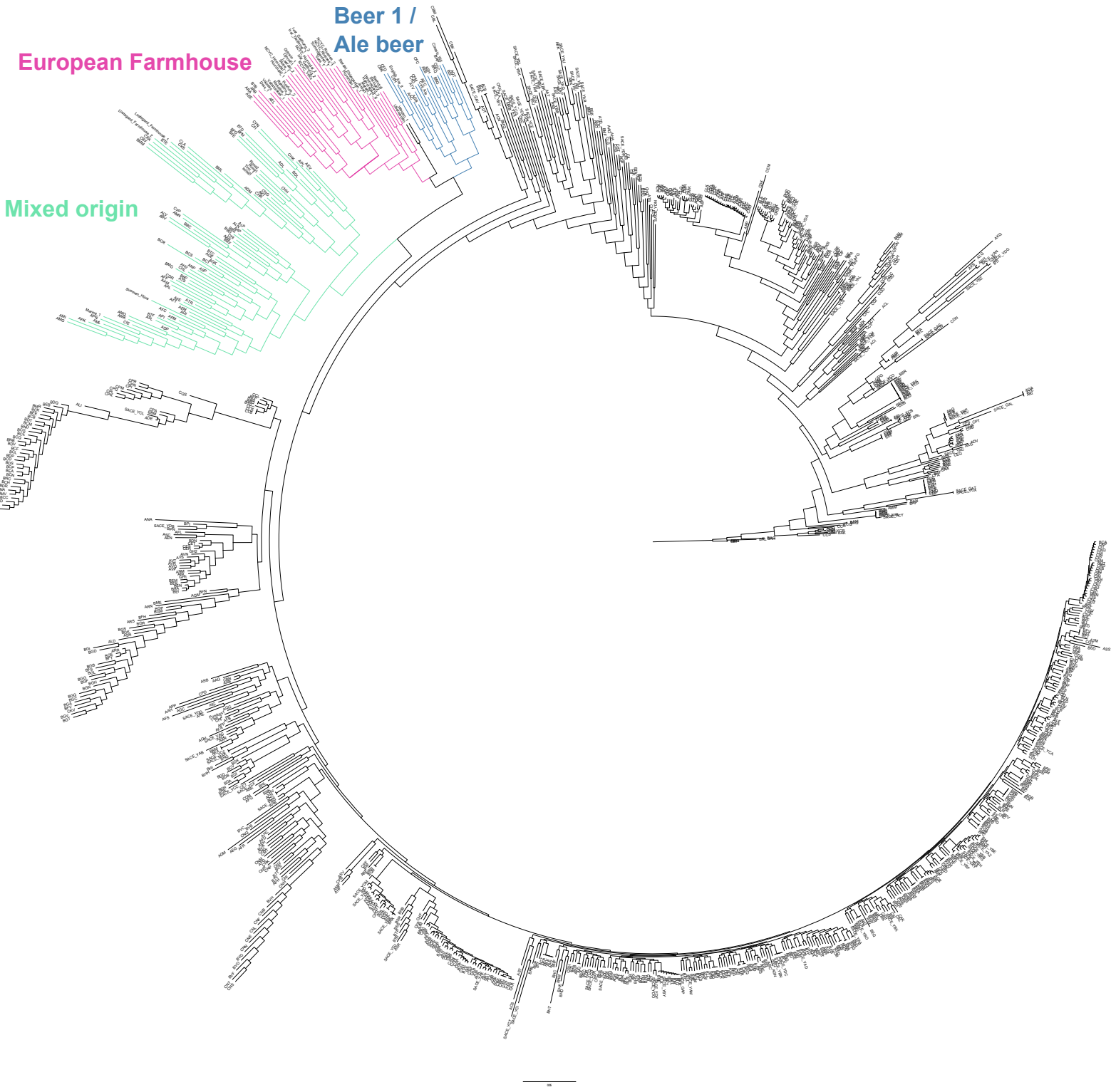
Supplementary Figure S4A



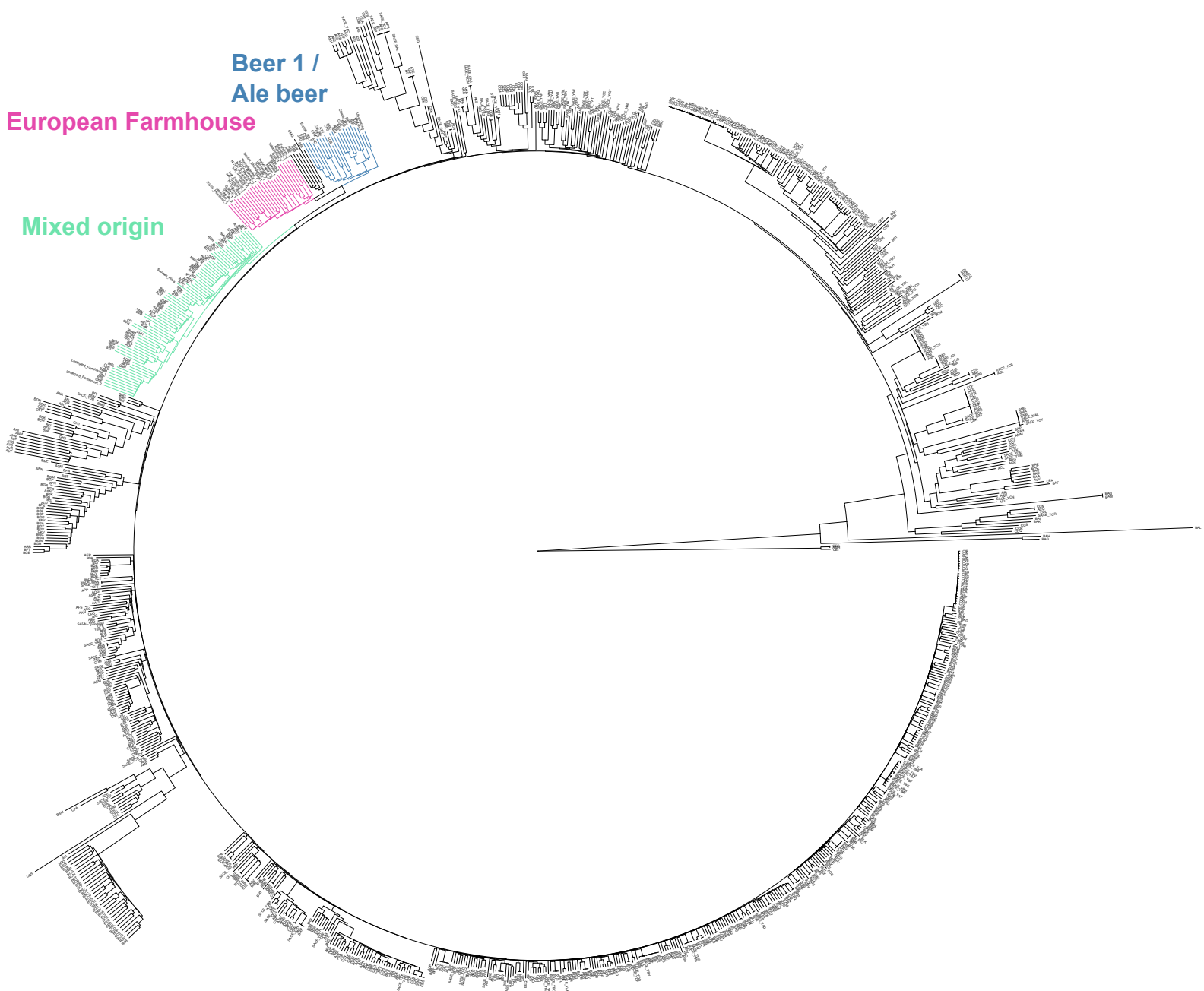
Supplementary Figure S4B



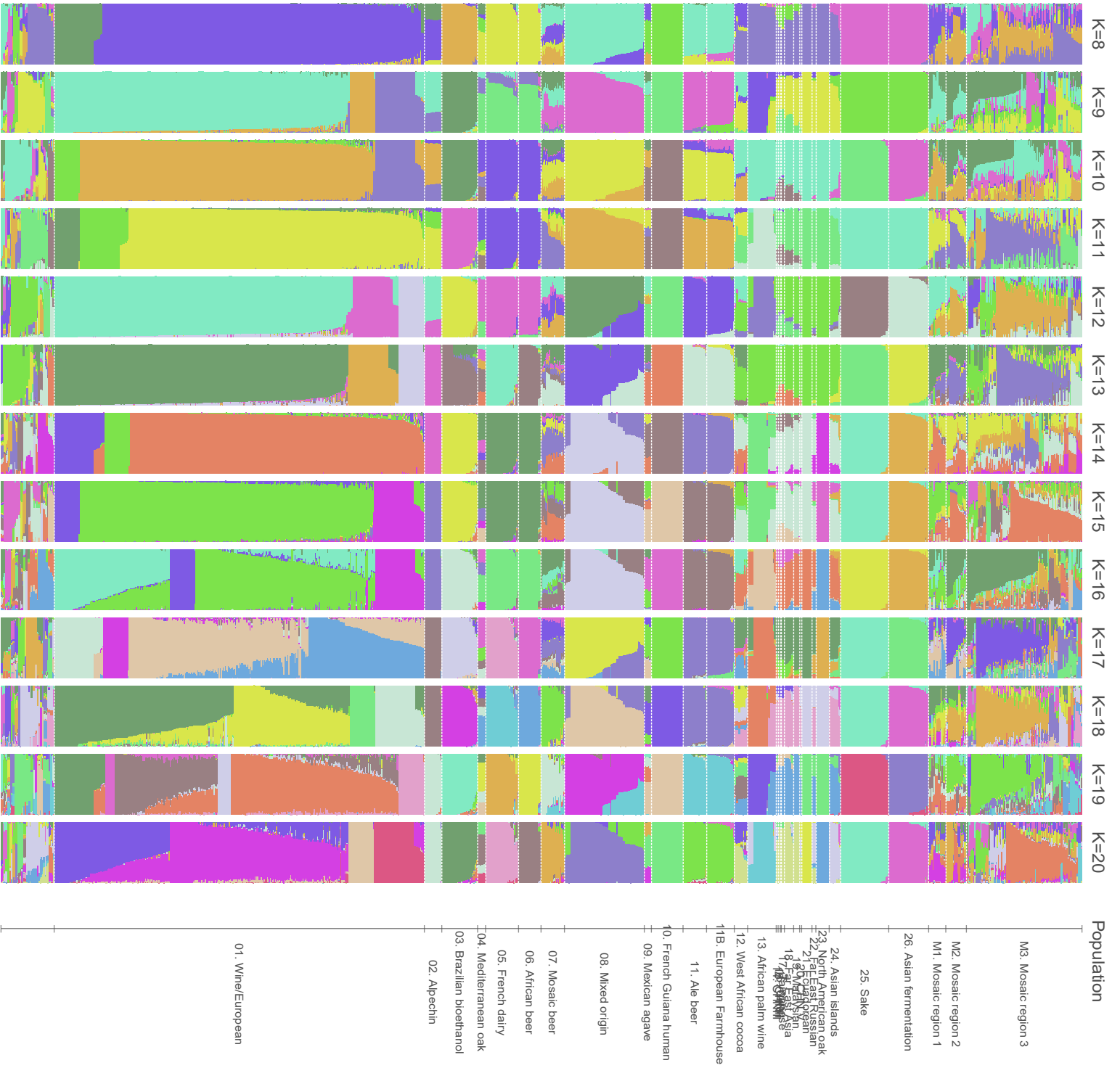
Supplementary Figure S5A



Supplementary Figure S5B

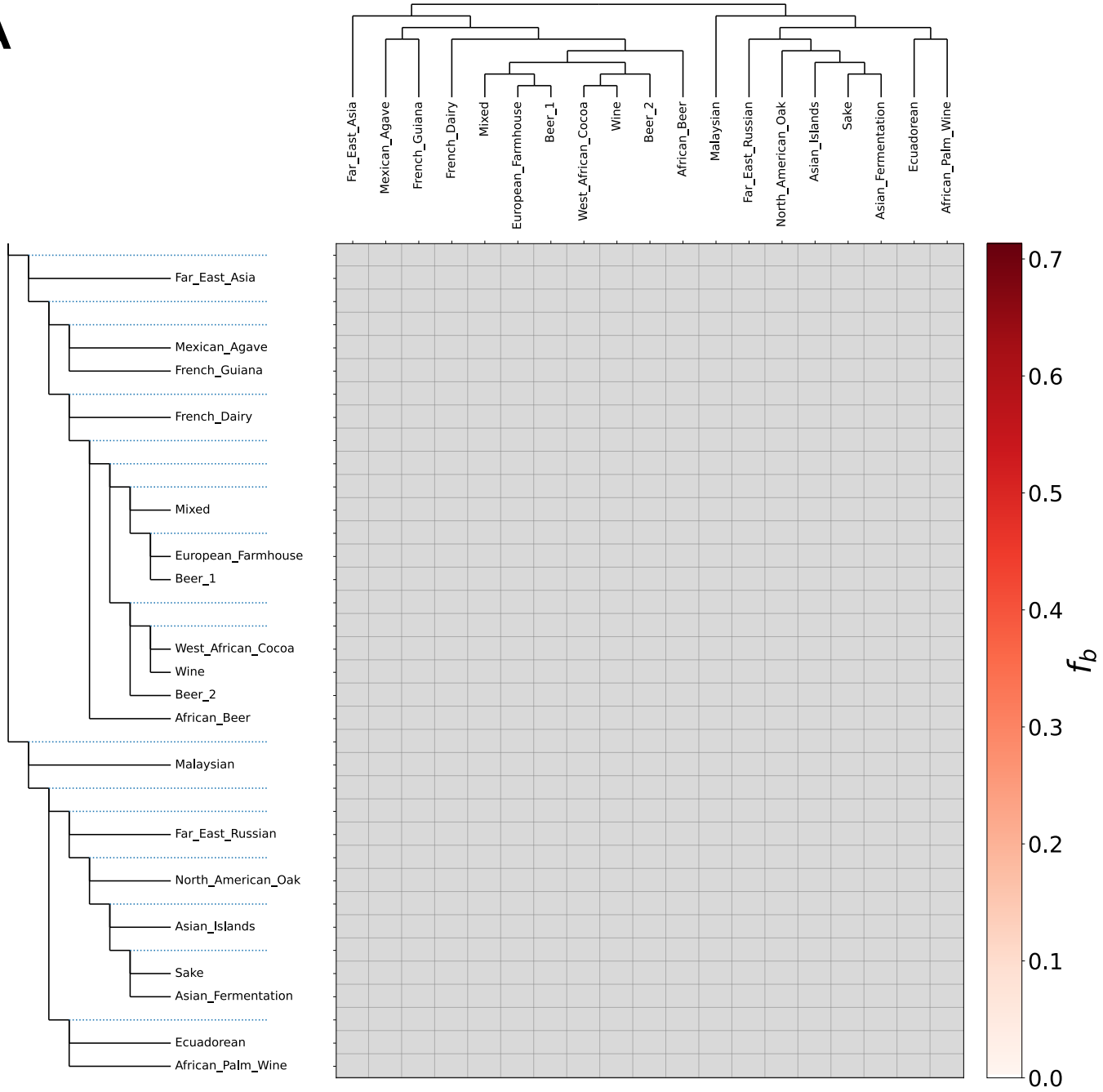


Supplementary Figure S6

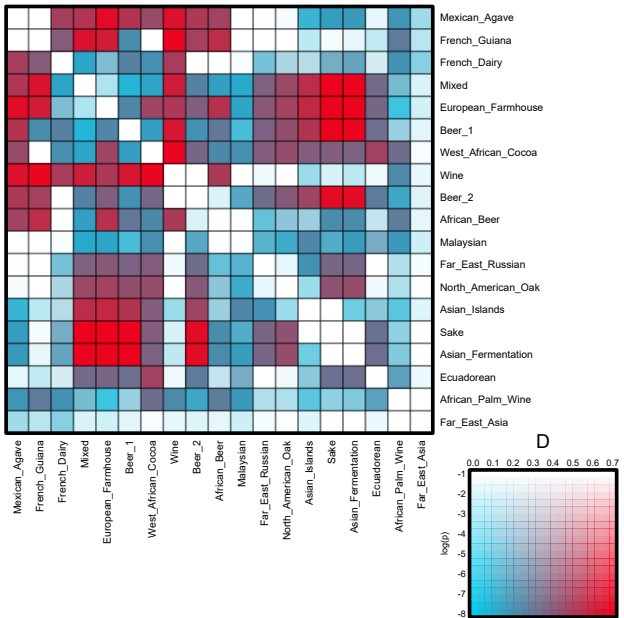


Supplementary Figure S7

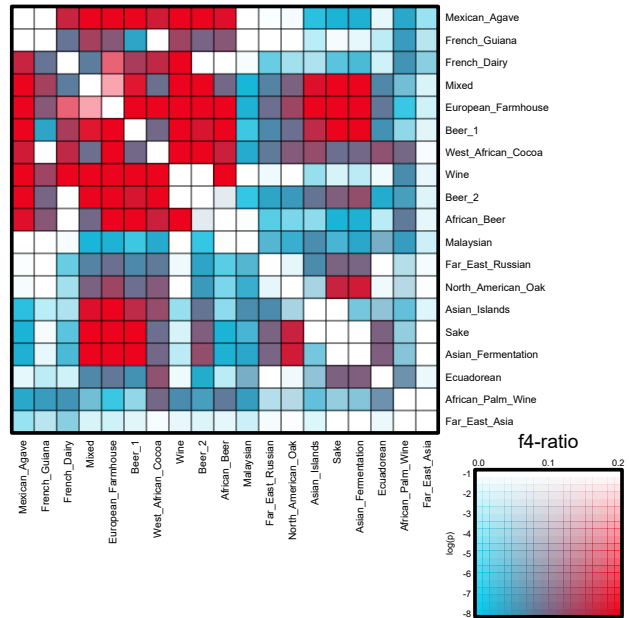
A



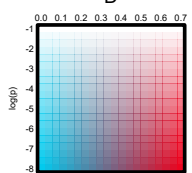
B



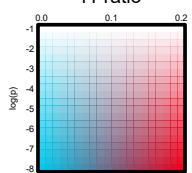
C



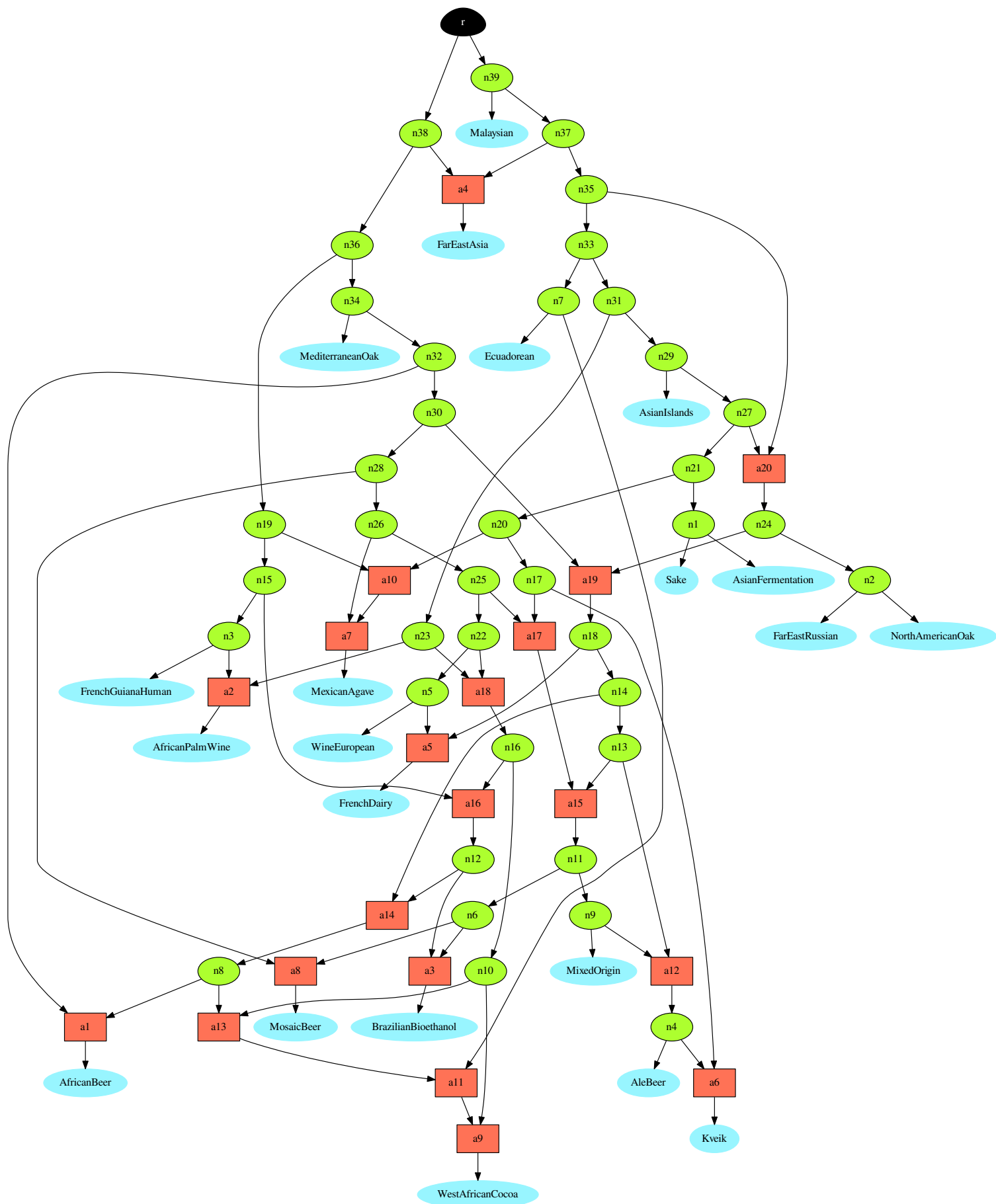
D



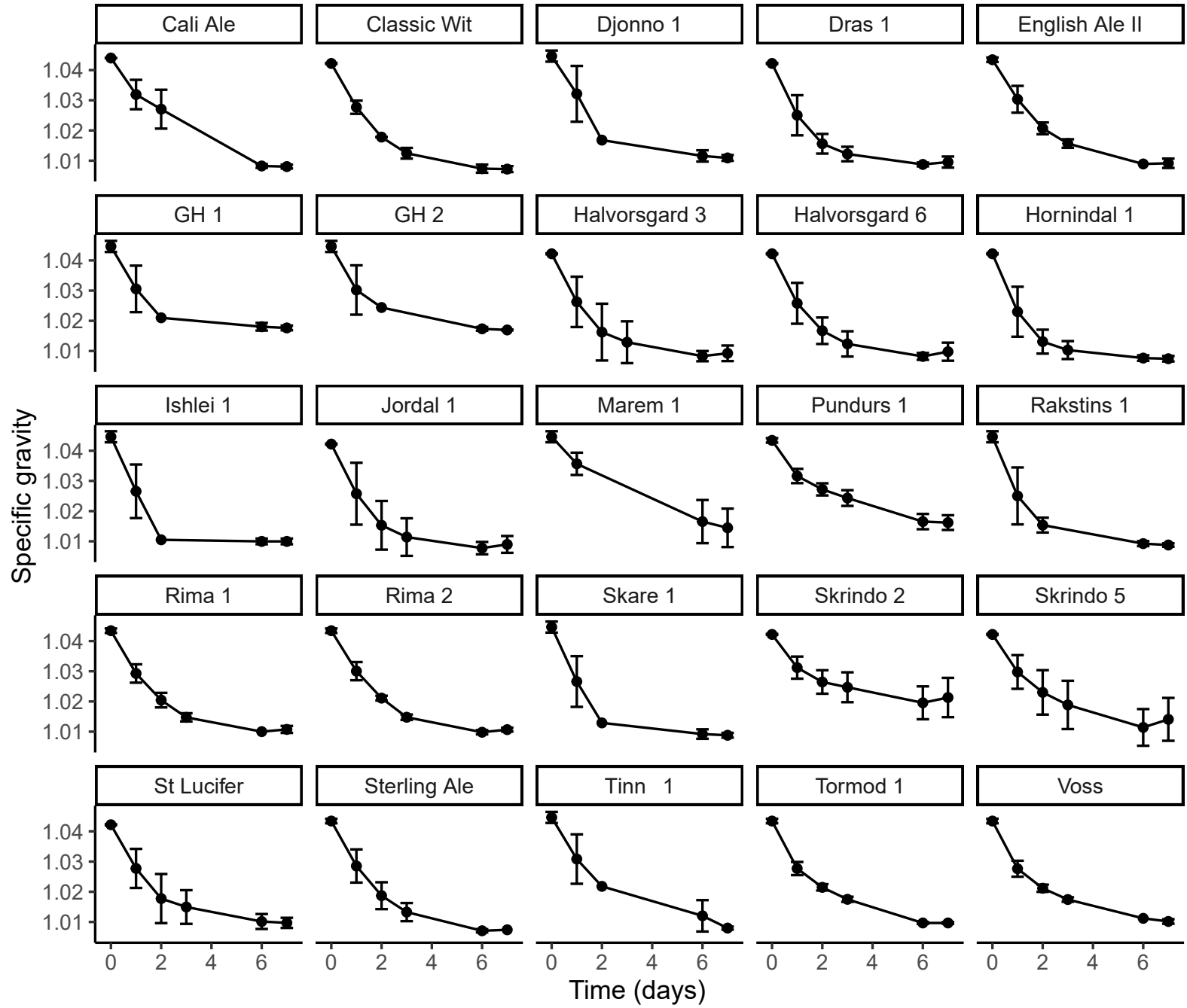
f4-ratio



Supplementary Figure S8

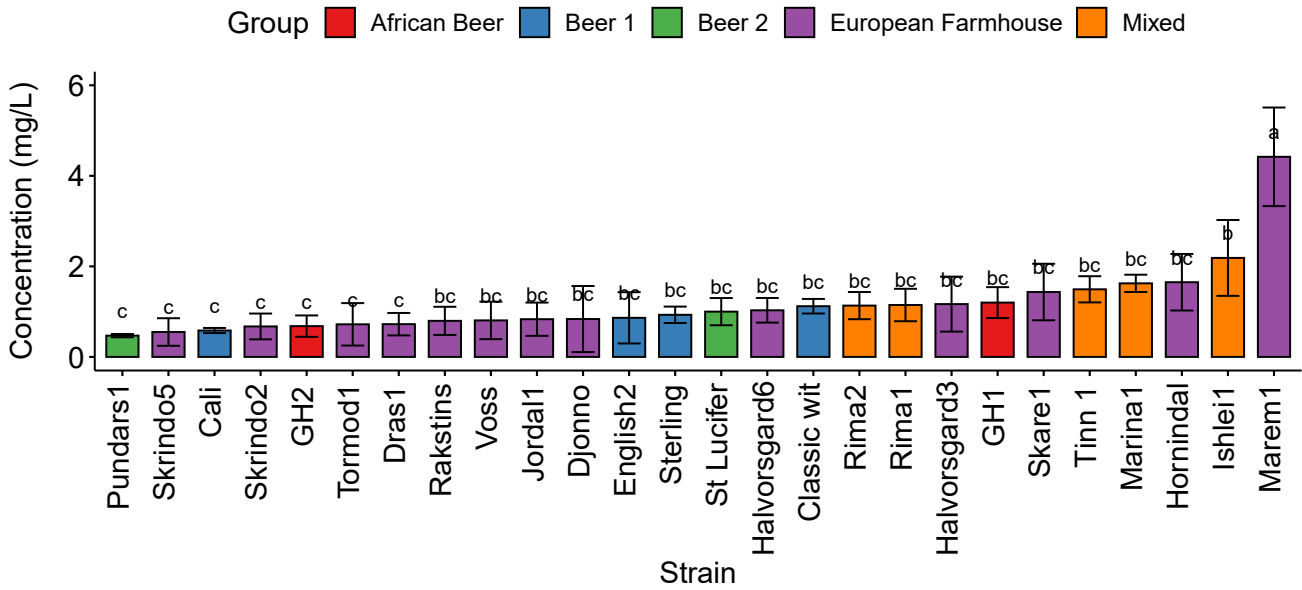


Supplementary Figure S9

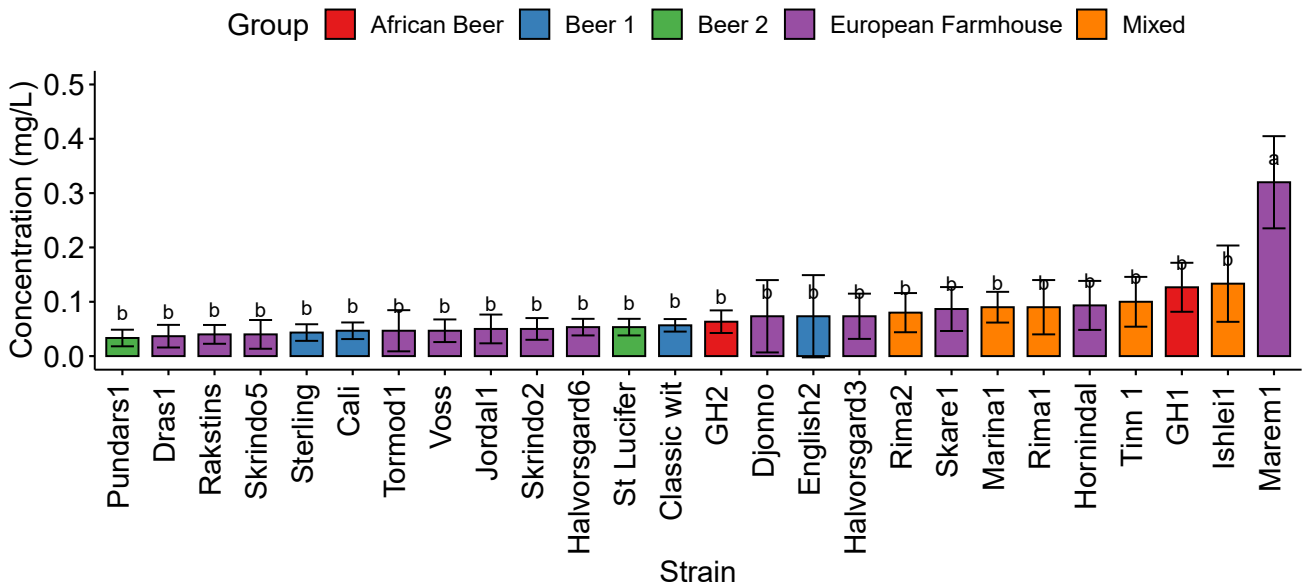


Supplementary Figure S10

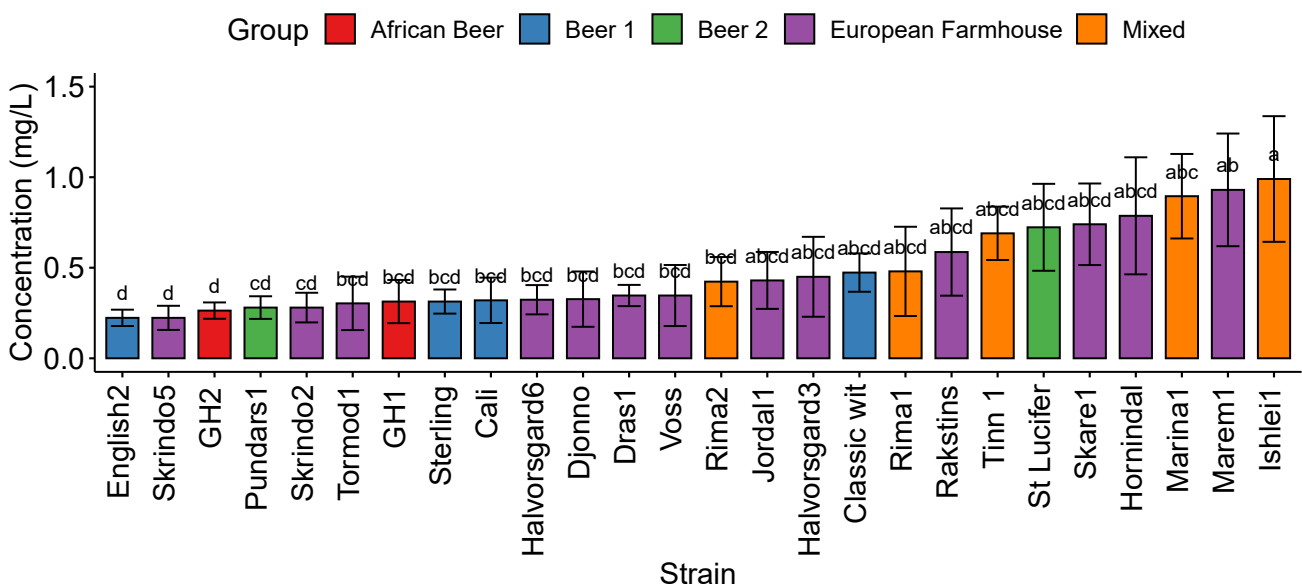
Isoamyl acetate



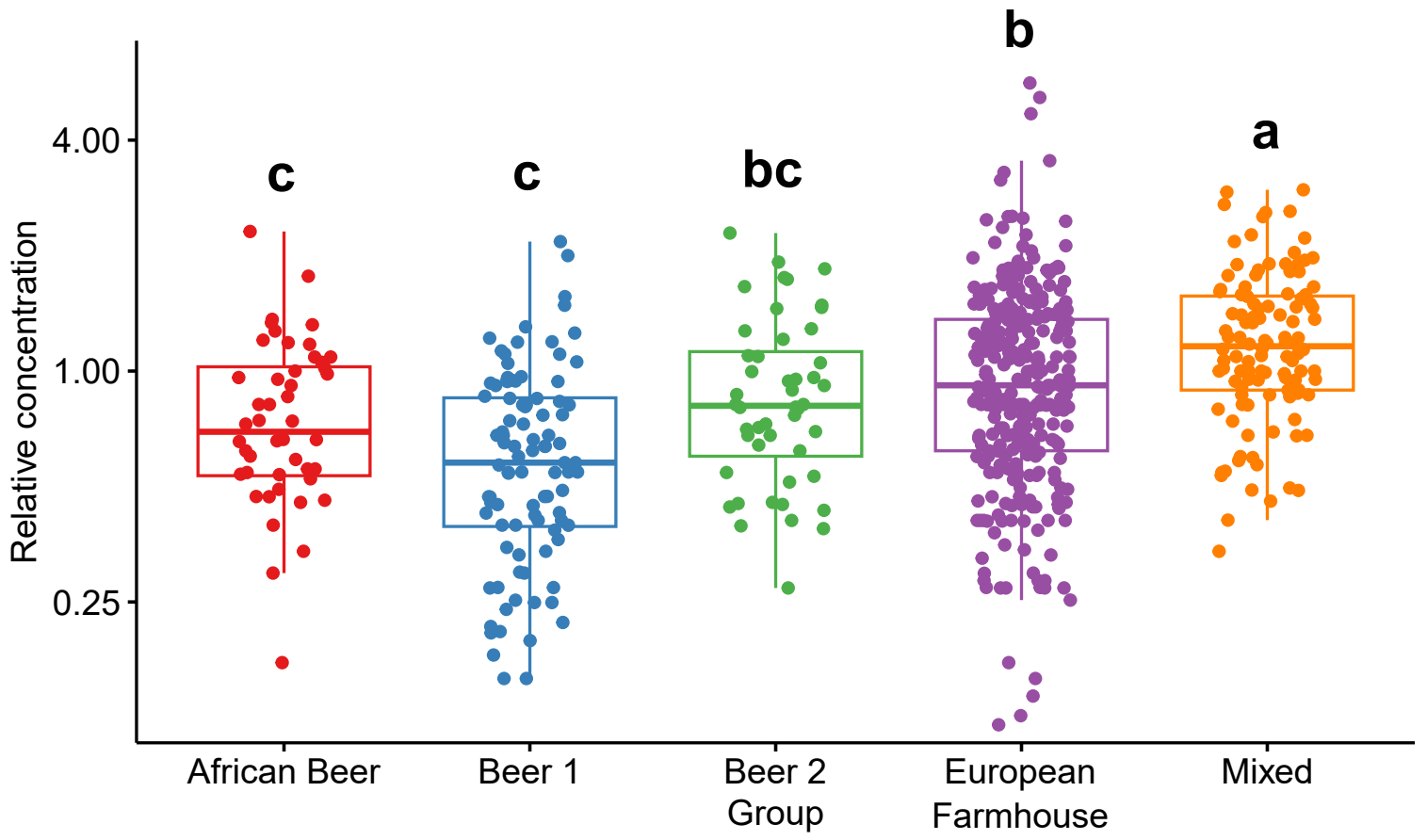
Isobutyl acetate



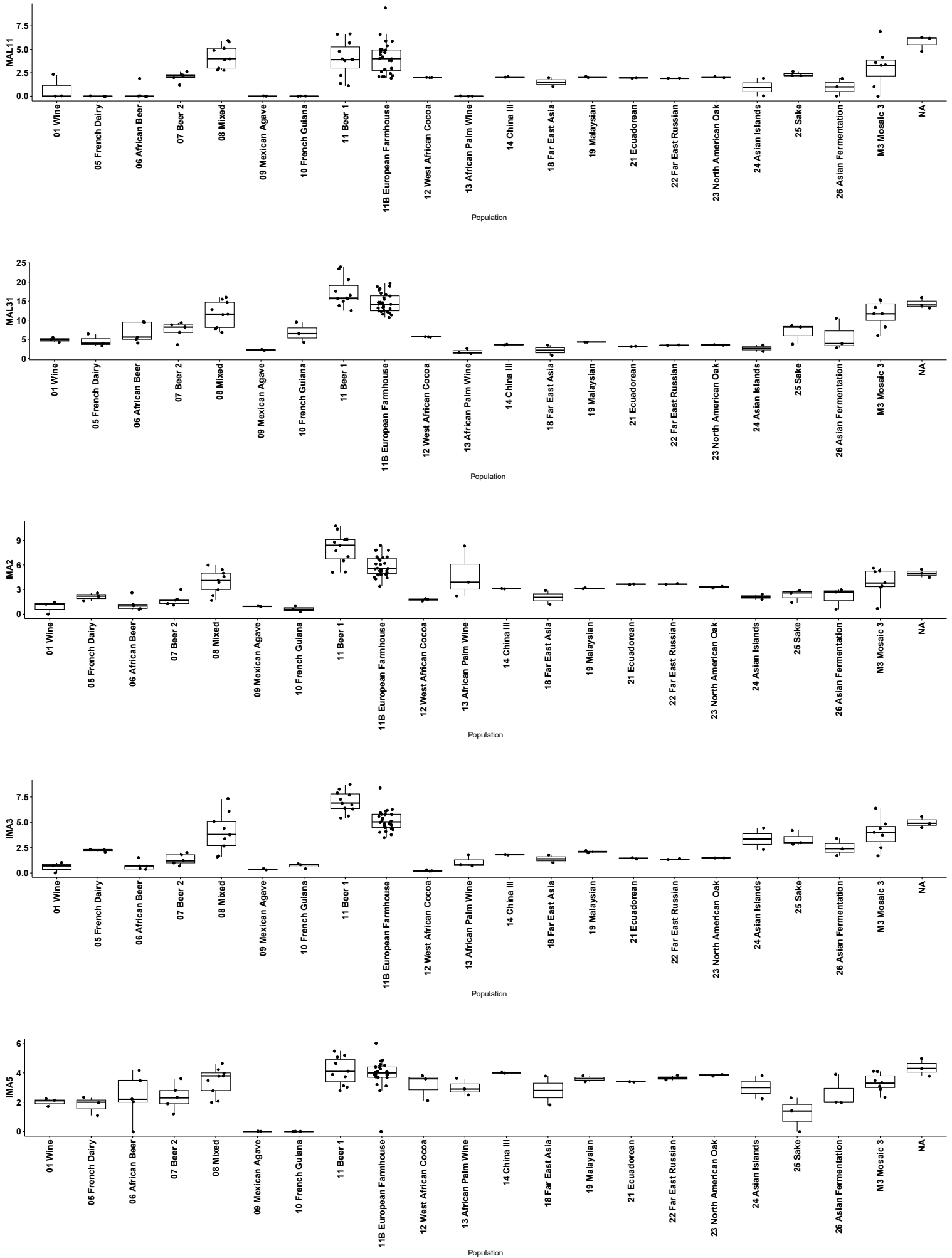
Phenylethyl acetate



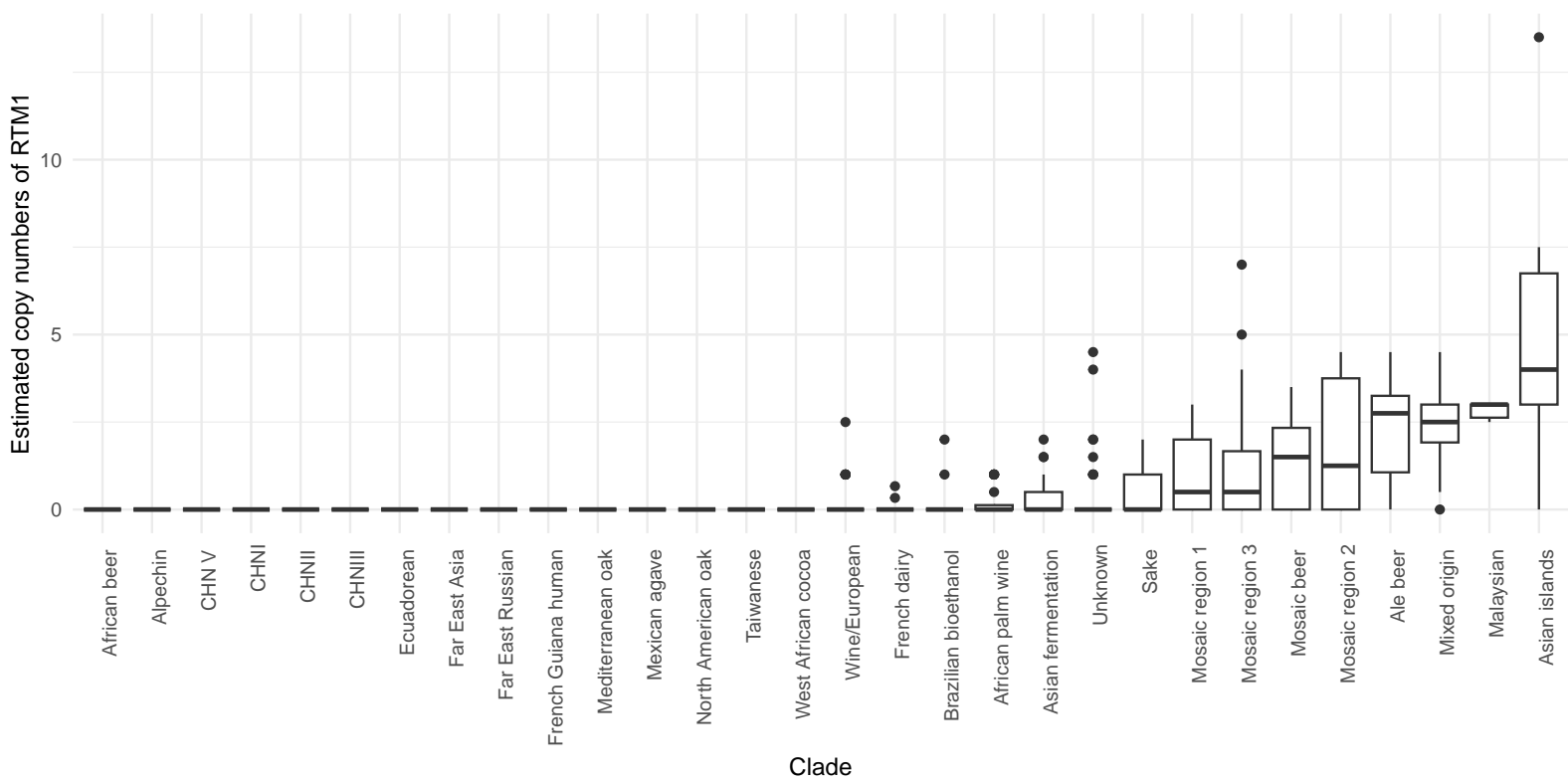
Supplementary Figure S11



Supplementary Figure S12



Supplementary Figure S13



Supplementary Figure S14

