Supplementary Figures

Legends

Figure 1. **Schematic illustrating the experimental strategy.** A four-way cross of diploid strains from different geographic origins (DBVPG6765, wine/European or WE; DBVPG6044, W. African or WA; YPS128, N. American or NA; Y12, sake/Asian or SA) generated a "synthetic population" that we used as the ancestral source for experimental populations. 12 replicate populations evolved in parallel and were sampled for sequencing at four timepoints: initially, and after 6, 12, and 18 rounds of forced outcrossing. Numbers in parentheses indicate the approximate number of mitotic divisions that have elapsed by each week.

Figure 2. **Evidence of allele frequency change across the genome. (**A) Sites that have changed the most over time in all replicate populations. Results of a genome scan for SNPs with significant variation in allele frequency between sampled timepoints (via an ANOVA on square root arcsin transformed allele frequencies treating "generation" as a continuous variable). y-axis values indicate transformed p-values from this ANOVA, such that higher values indicate higher levels of significance; the blue and red horizontal lines represent our empirically determined genome-wide alpha of 0.5 and 0.05, respectively. We find 5 peak regions that exceed our lower threshold, and label these A-E. (B) Haplotype change across the genome, represented as the average difference between founder haplotype frequency at week 18 from the ancestral founder haplotype frequency. We find three additional peaks and label these F-H. Note that absolute frequency differences are plotted for easier visualization; relative frequency differences are plotted in Figure S6 for comparison.

Figure 3. **Allele frequency trajectories at the most significant SNPs in the dataset.** Allele frequencies at each timepoint, averaged over all replicate populations for peaks A-H, The allele being modeled is the most significant SNP in each peak region.

Figure 4. **Effects of replication and sampling on signal.** ANOVA analysis used to generate Figure 2 was carried out on (A) the entire dataset consisting of all twelve replicate populations sampled at four timepoints (0, 180, 360 and 540 generations); (B) a dataset consisting of only five replicate populations sampled at the same four timepoints; (C) a dataset consisting of all twelve replicate populations sampled at two timepoints (zero and 360 generations) and (D) a dataset consisting of five replicate populations sampled at these two timepoints. Increasing both replication and sampling results in stronger, more localized signals of change.

Figure S1. Every week, experimental populations went through a "recombination protocol" to shuffle genotypes. Sporulation is induced on Fridays, random spores are recovered on Mondays, random mating and diploid recovery occurs during the next 48 hours. Recovered diploids are then passaged twice in rich media before sporulation is induced again. Thus, in this protocol diploid cells experience 2 days per week of noncompetitive growth on plates (~10 generations), and 2 days per week of competitive growth in rich liquid media (~20 generations).

Figure S2. Alternative models of change. (A) A linear model including generation as a factor was fit to the data; shown are results of ANOVA on lm(Y~generation (factor). (B) A linear model including a quadratic term was fit to the data; shown are results of ANOVA on lm(Y~generation+generation^2). In each case, Y represents square root arcsin transformed allele frequencies. y-axis values indicate transformed p-values from the ANOVA, such that higher values indicate higher levels of significance; the blue and red horizontal lines represent our empirically determined genome-wide alpha of 0.5 and 0.05, respectively. The localized peaks A-E observed in Figure 2 also emerge from these analyses, although not all peaks reach genome-wide significance.

Figure S3. ANCOVA for potentially heterogeneous sites. To detect positions at which alleles changed significantly in only one or a few populations, we fit the data to two linear models: lm(Y~generation+replicate) and lm(Y~generation+replicate+generation:replicate). In each case, Y represents square root arcsin transformed allele frequencies. Y-axis values indicate transformed p-values from an ANOVA comparing these two models, such that higher values would indicate heterogeneous allele frequency change across replicate populations (significance of the generation:replicate interaction term). The blue and red horizontal lines represent our empirically determined genome-wide alpha of 0.5 and 0.05, respectively.

Figure S4a-e. Close-up look at at regions corresponding to peaks A-E. Both SNP frequency change (top) and haplotype frequency change (bottom) is shown for markers within 50KB centered on the most significant marker per peak.

Figure S5. Close-up look at regions corresponding to peaks F-H. Both SNP frequency change (top) and haplotype frequency change (bottom) is shown for markers within 50KB centered on the most significant marker per peak. As in Figure 2B, absolute frequency differences from the ancestor are plotted.

Figure S6. Comparison of peak regions to previously identified "sporulation QTL". In each panel, points indicate mean founder haplotype frequency across all 12 experimental populations (relative to the ancestor, expressed as the difference between the mean founder haplotype frequency at generation 18 and the ancestral founder haplotype frequency), plotted for each founder. Vertical lines indicate loci at which large allele frequency differences were measured after the initial 12 intercross rounds (compare to Cubillos et al. 2013, Table S1). The color of the vertical line segment above zero indicates the founder that increased the most in frequency, and the color of the segment below zero indicates the founder that decreased the most in frequency. Peak regions described in this study (A-H) are labeled at the bottom of each panel for reference. For example, at the region corresponding to peak H, Cubillos et al., 2013 report evidence for a sporulation QTL; in their study the Y12/Asian allele increased and the YPS128/North American allele decreased after 12 rounds of sporulation/crossing. In our study we identified peak H because the Y12 haplotype frequency increased further over the 18 weeks of the experiment.

Figure S7a-h. SNP frequency trajectories for the most significant marker in each peak A-H, shown for all twelve replicate populations.

Figure S8a-h. Haploytpe frequency trajectories for the window that includes the most significant marker in each peak A-H, shown for all twelve replicate populations. Each of the four panels represents a different founder, from top to bottom: DBVPG6765, wine/European; DBVPG6044, W. African; YPS128, N. American; Y12, sake/Asian

Figure S9. (A) Q-Q plot of our expected (normal) versus observed quantiles on our statistic $\overline{\Delta}$, which is the mean absolute difference in square root arcsin transformed allele frequency between a haplotype's frequency in the ancestral population and it's frequency at the final timepoint over all 12 replicate populations and 4 haplotypes. It is visually apparent that values of $\overline{\Delta}$ bigger than 0.2 are very unlikely under the null hypothesis of no divergence. (B) $\overline{\Delta}$ plotted against genome position. Regions with $\overline{\Delta}$ values greater than 0.2 are colored in blue, and these correspond to our peak regions of high haplotype and allele frequency divergence (compare to Figure 2).

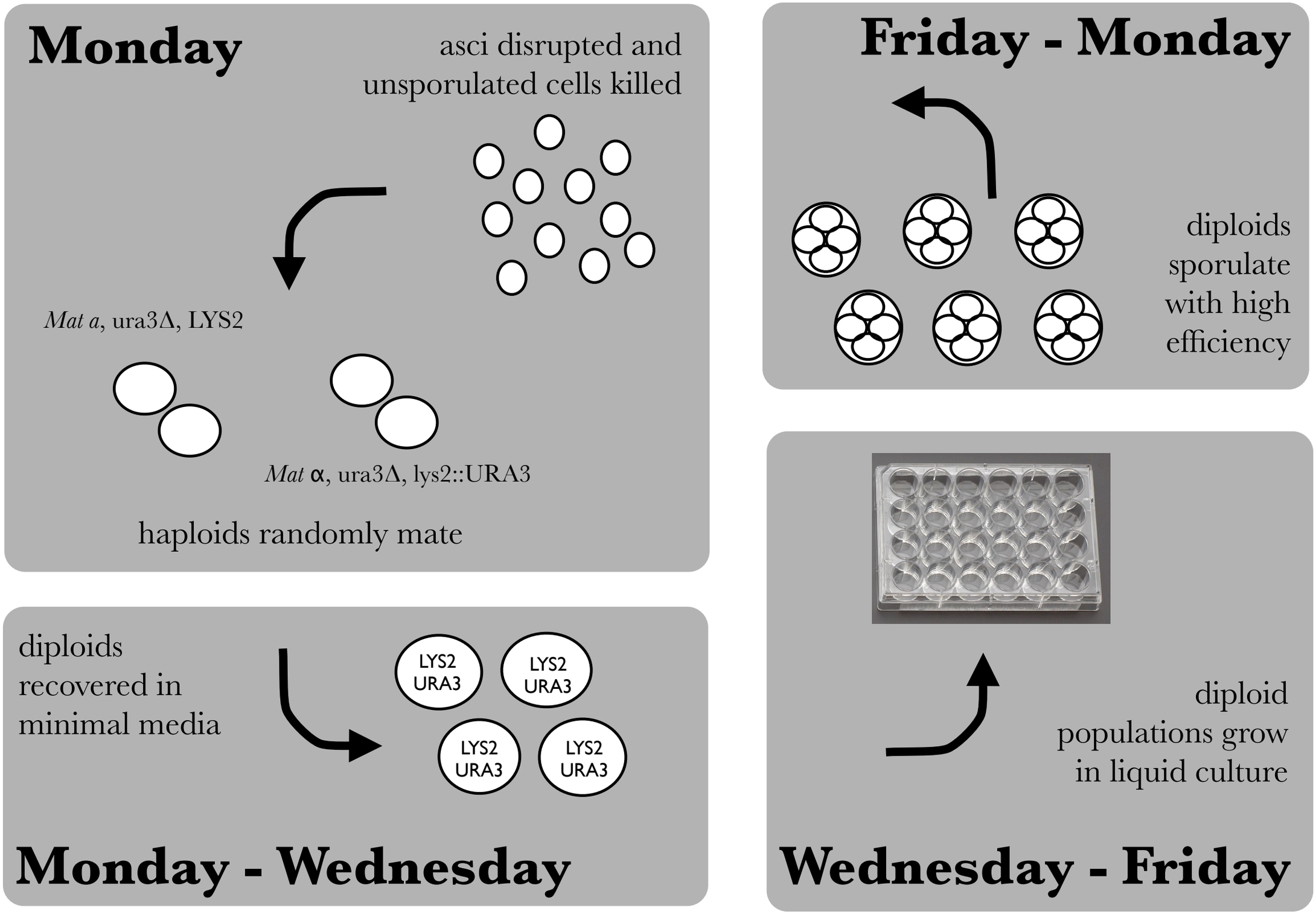

Monday
asci disrupted and
unsporulated cells killed
Mat a, ura3Δ, LYS2
Mat α, ura3Δ, lys2::URA3
haploids randomly mate
Friday - Monday
diploids
sporulate
with high
efficiency
diploids
recovered in
minimal media
LYS2
URA3
LYS2
URA3
LYS2
URA3
LYS2
URA3
Monday - Wednesday
diploid
populations grow
in liquid culture
Wednesday - Friday

Figure S1.

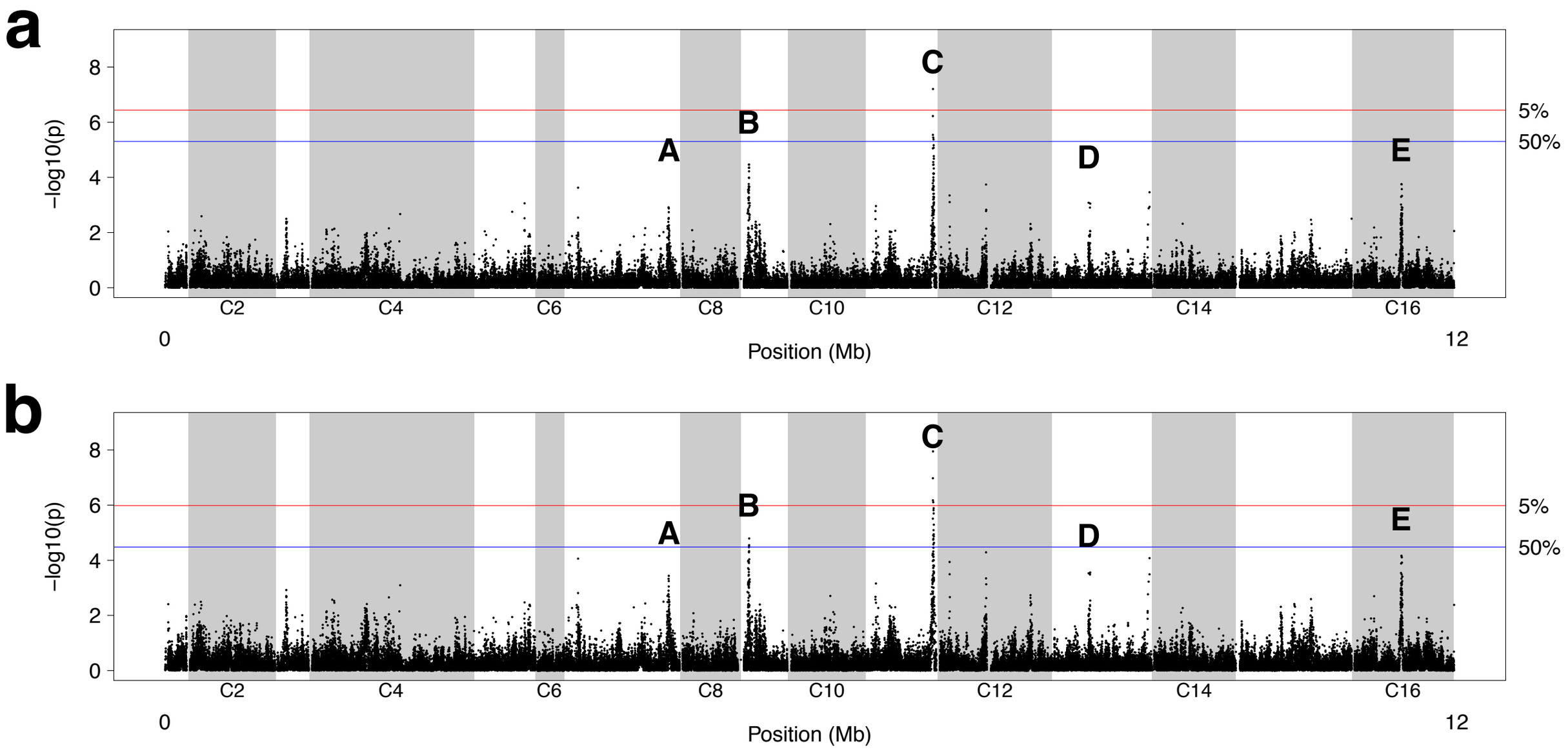

a
b
−log10(p)
0
2
4
6
8
A
B
C
D
E
5%
50%
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)

Figure S2.

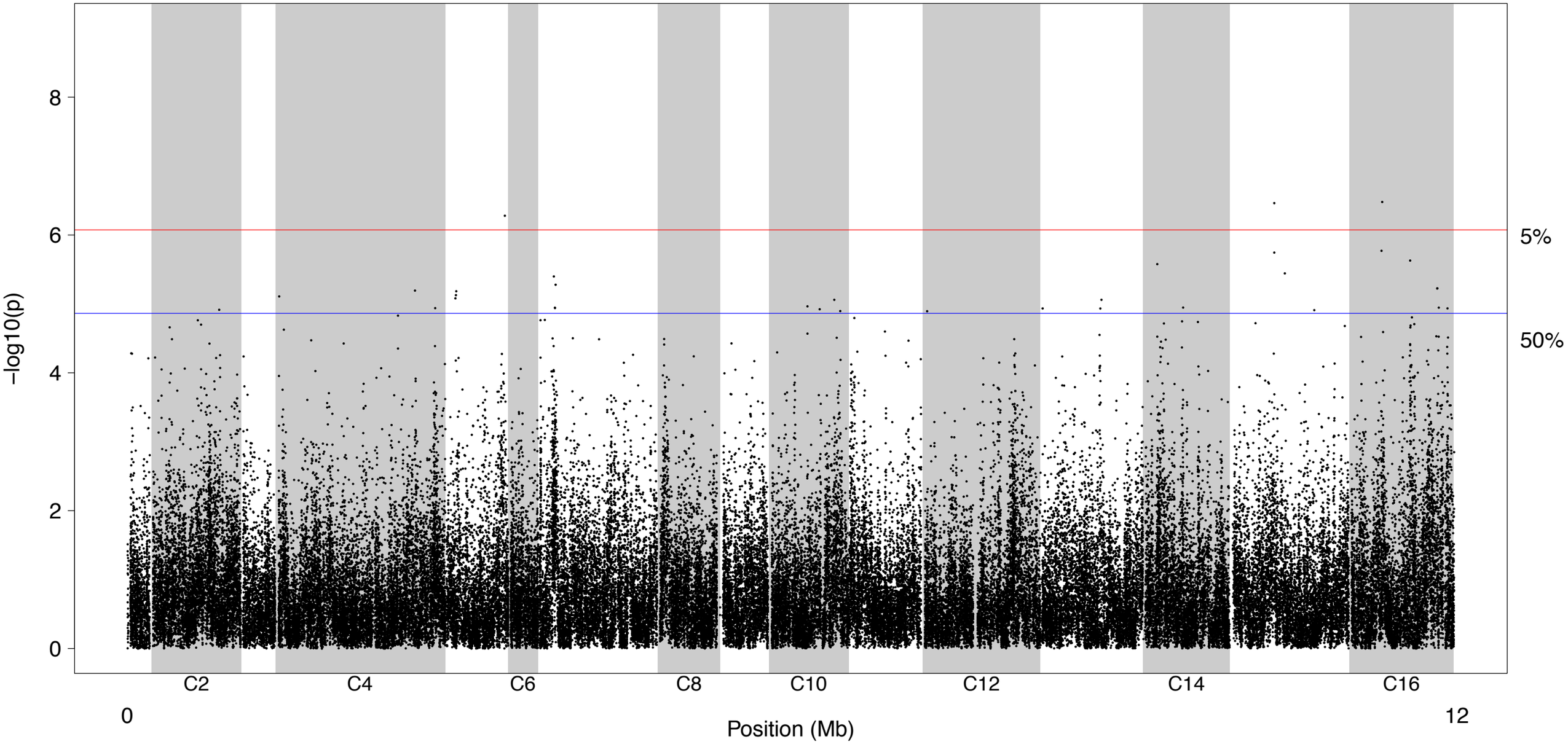


Figure S3.

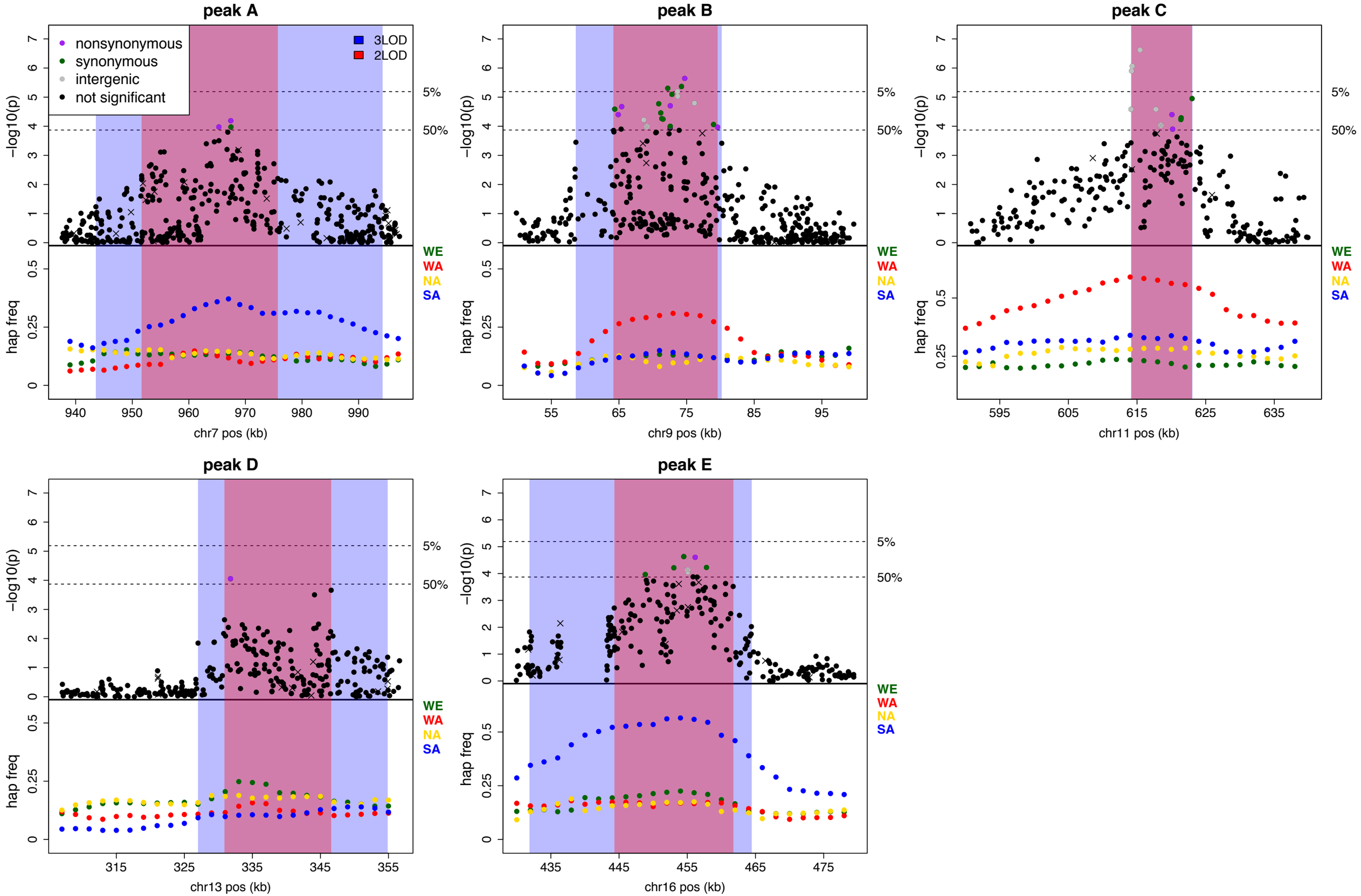

peak A
peak B
peak C
peak D
peak E
nonsynonymous
synonymous
intergenic
not significant
3LOD
2LOD
5%
50%
−log10(p)
hap freq
WE
WA
NA
SA
chr7 pos (kb)
chr9 pos (kb)
chr11 pos (kb)
chr13 pos (kb)
chr16 pos (kb)

Figure S4.

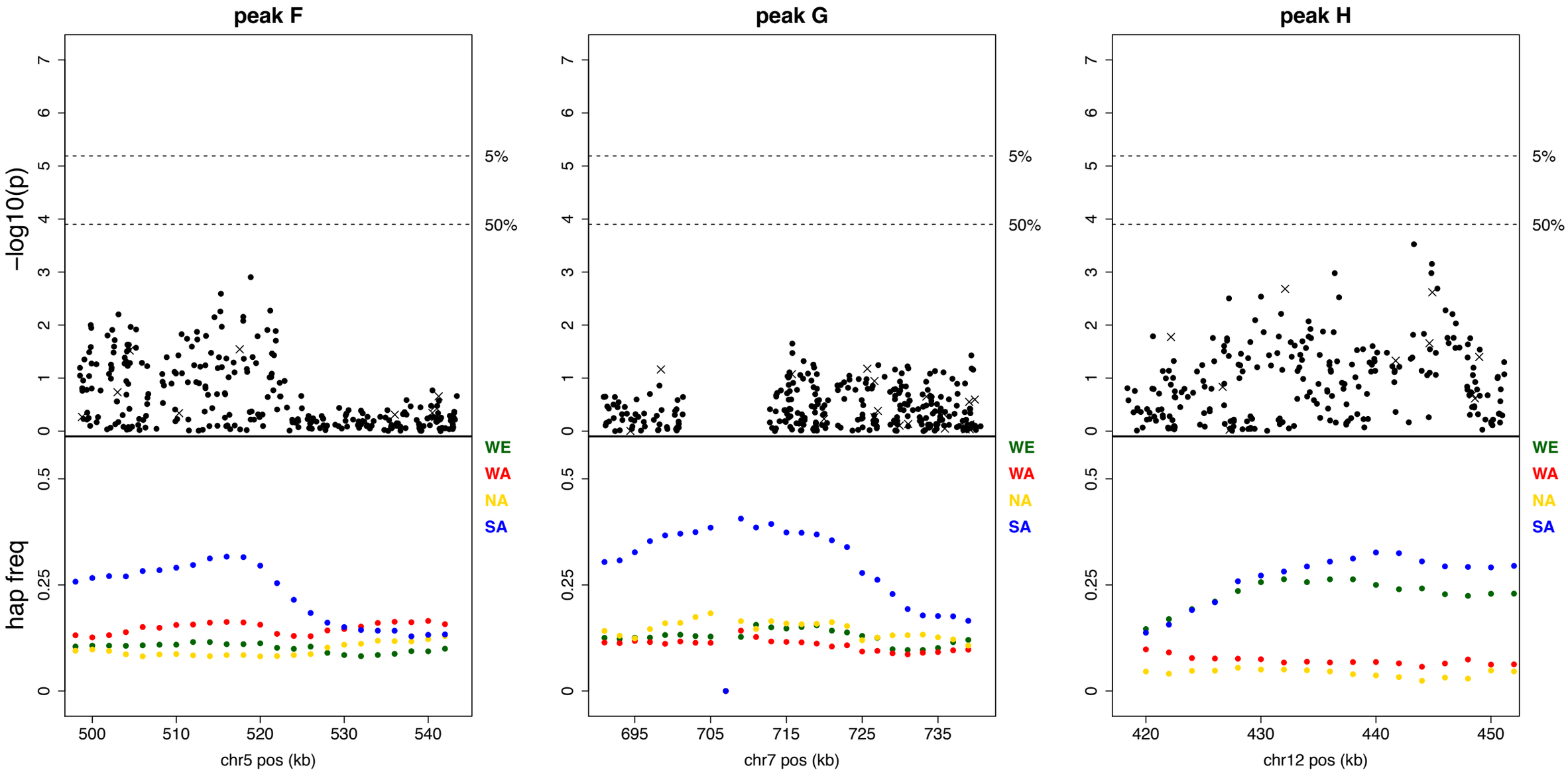
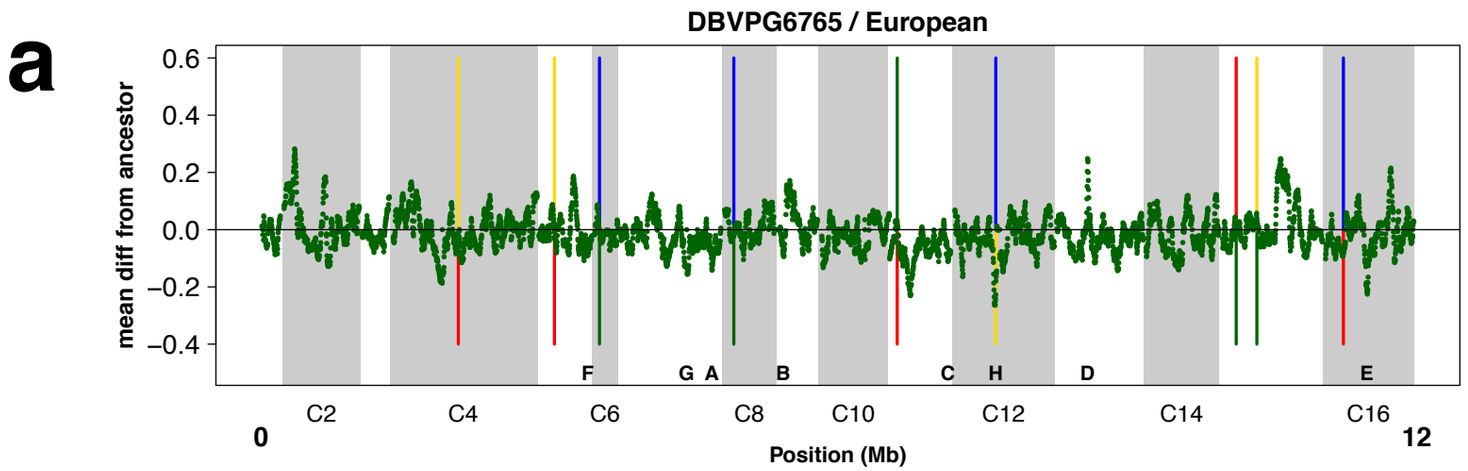
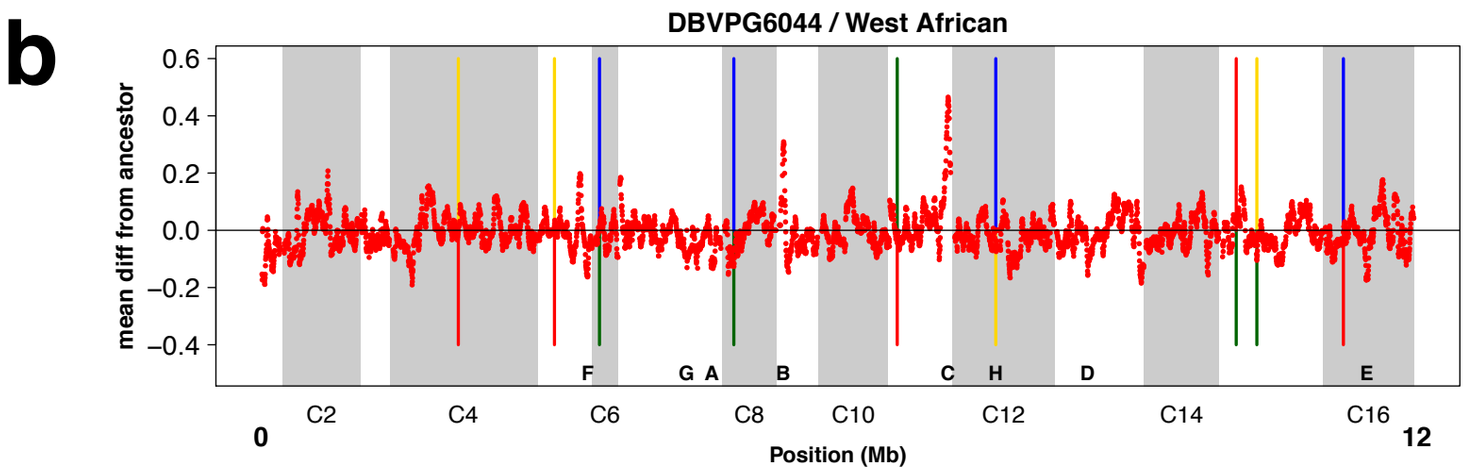
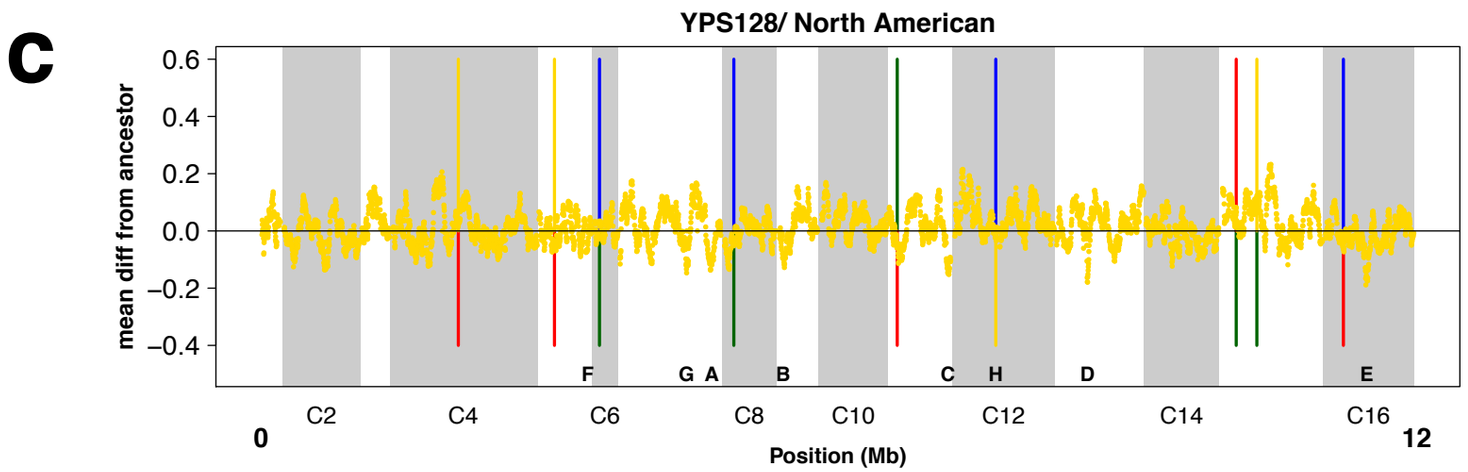

peak F
peak G
peak H
−log10(p)
hap freq
5%
50%
WE
WA
NA
SA
chr5 pos (kb)
chr7 pos (kb)
chr12 pos (kb)

Figure S5.


a
DBVPG6765 / European
mean diff from ancestor
0.6
0.4
0.2
0.0
−0.2
−0.4
F
G A
B
C
H
D
E
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)


b
DBVPG6044 / West African
mean diff from ancestor
0.6
0.4
0.2
0.0
−0.2
−0.4
F
G A
B
C
H
D
E
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)


c
YPS128/ North American
mean diff from ancestor
0.6
0.4
0.2
0.0
−0.2
−0.4
F
G A
B
C
H
D
E
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)

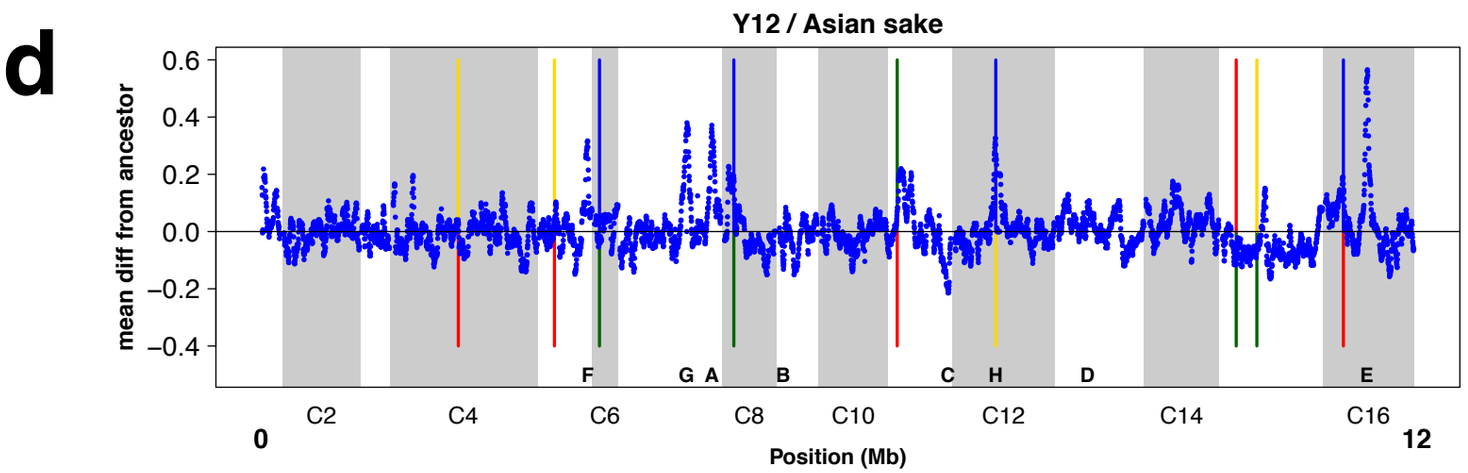

d
Y12 / Asian sake
mean diff from ancestor
0.6
0.4
0.2
0.0
−0.2
−0.4
F
G A
B
C
H
D
E
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)

Figure S6.

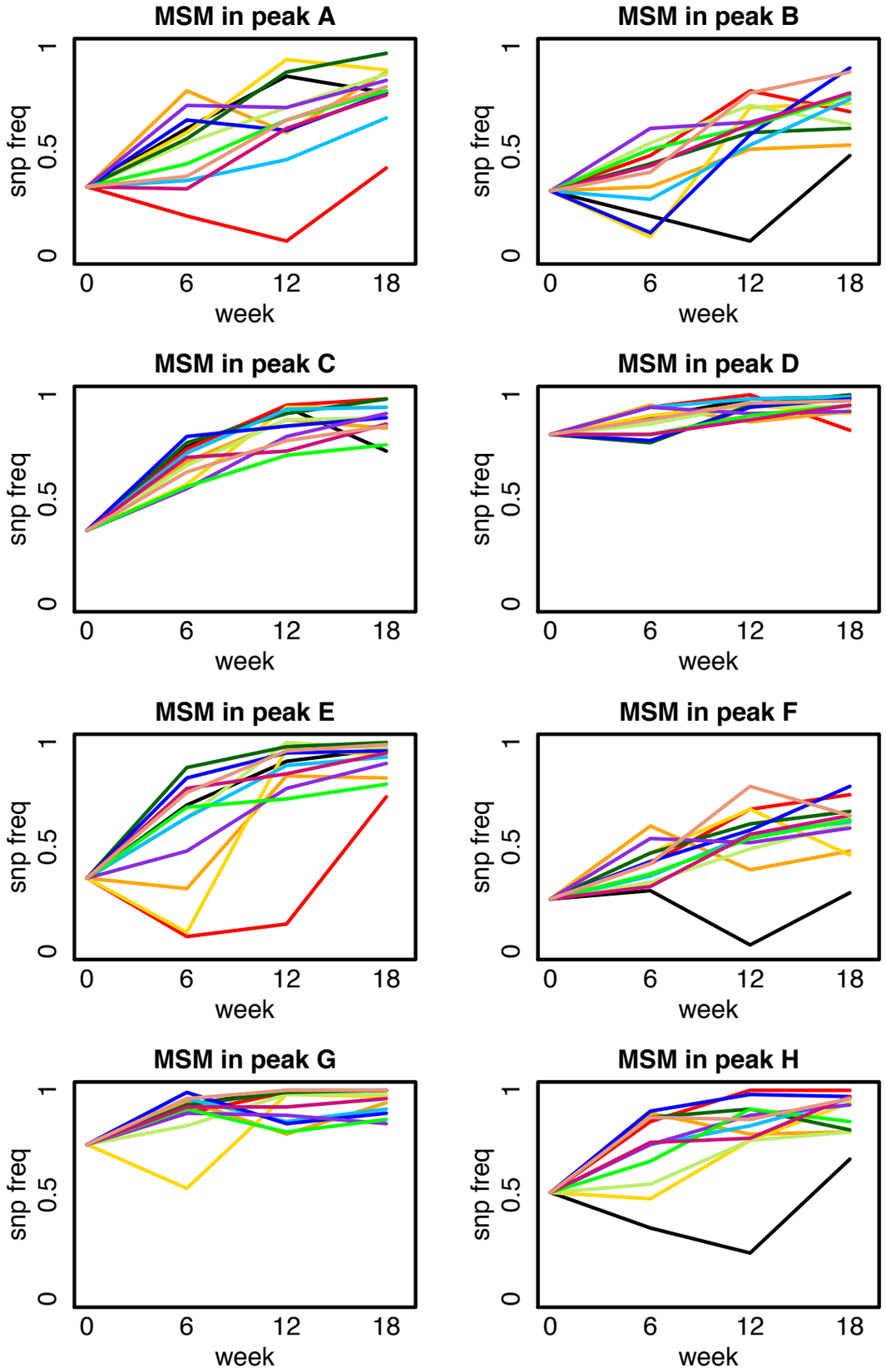

MSM in peak A
MSM in peak B
MSM in peak C
MSM in peak D
MSM in peak E
MSM in peak F
MSM in peak G
MSM in peak H
snp freq
0
0.5
1
0
6
12
18
week

Figure S7.

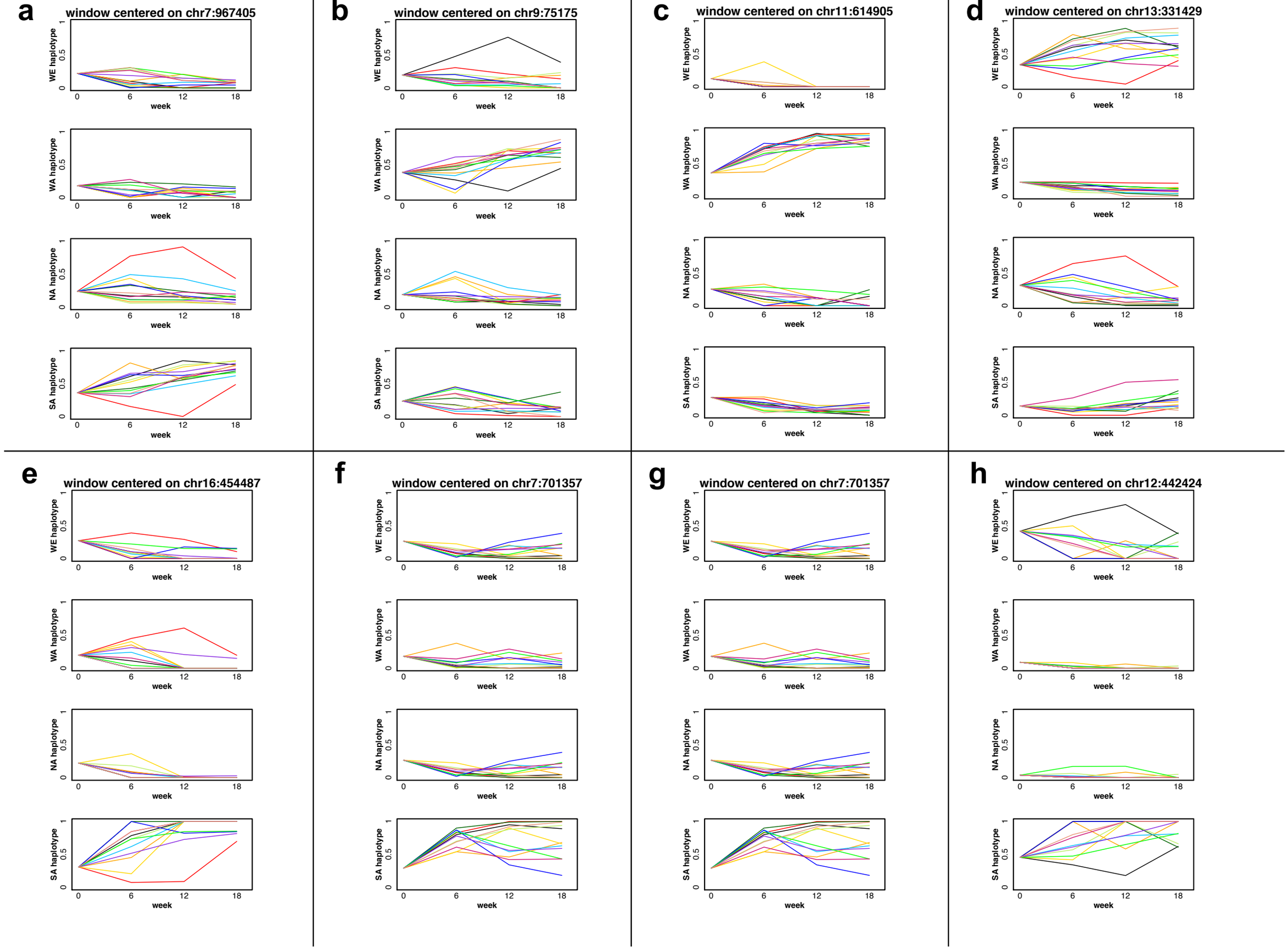
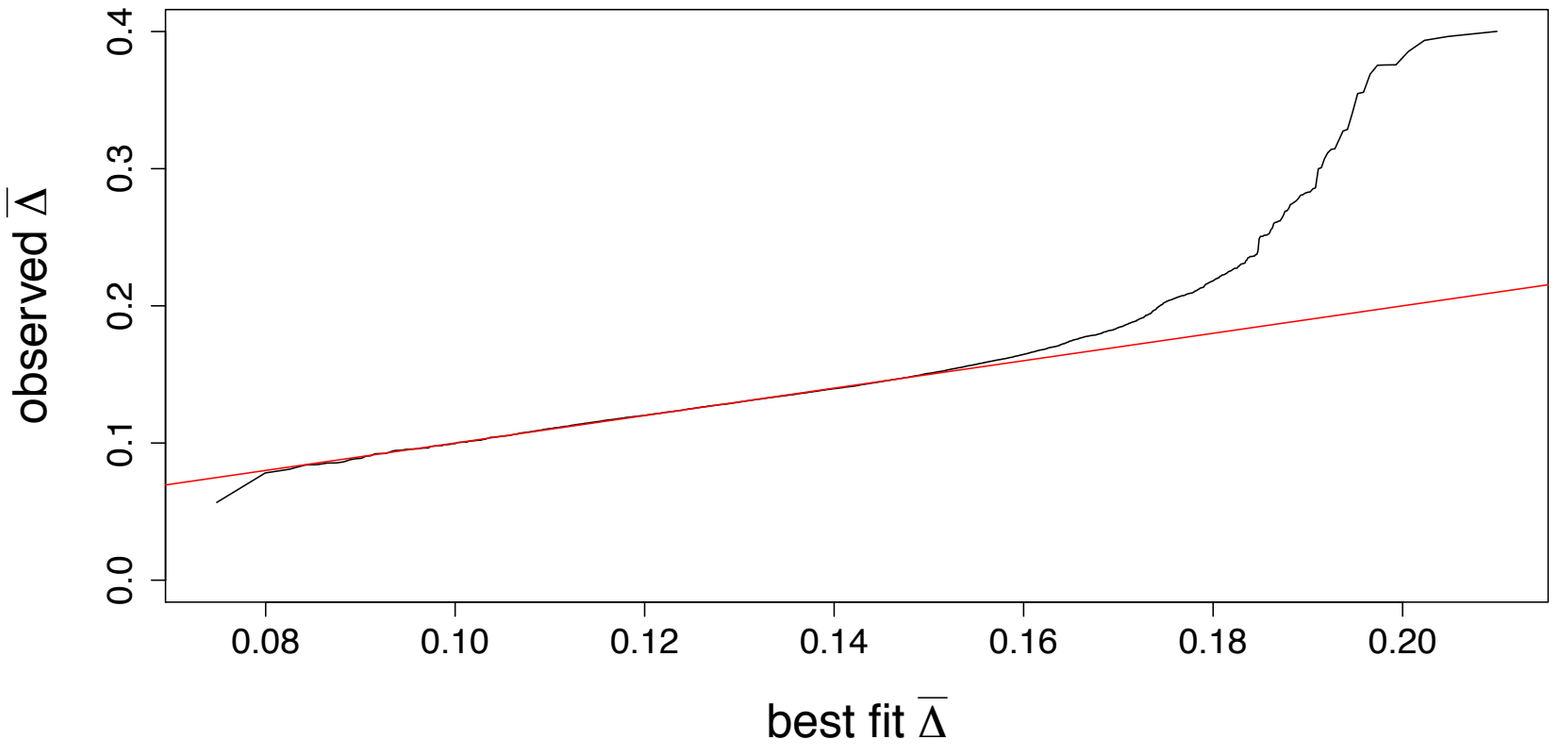

a
window centered on chr7:967405
b
window centered on chr9:75175
c
window centered on chr11:614905
d
window centered on chr13:331429
e
window centered on chr16:454487
f
window centered on chr7:701357
g
window centered on chr7:701357
h
window centered on chr12:442424
WE haplotype
WA haplotype
NA haplotype
SA haplotype
week
0
6
12
18
0.5
1

Figure S8.

a


observed $\overline{\Delta}$
0.0
0.1
0.2
0.3
0.4
0.08
0.10
0.12
0.14
0.16
0.18
0.20
best fit $\overline{\Delta}$

b

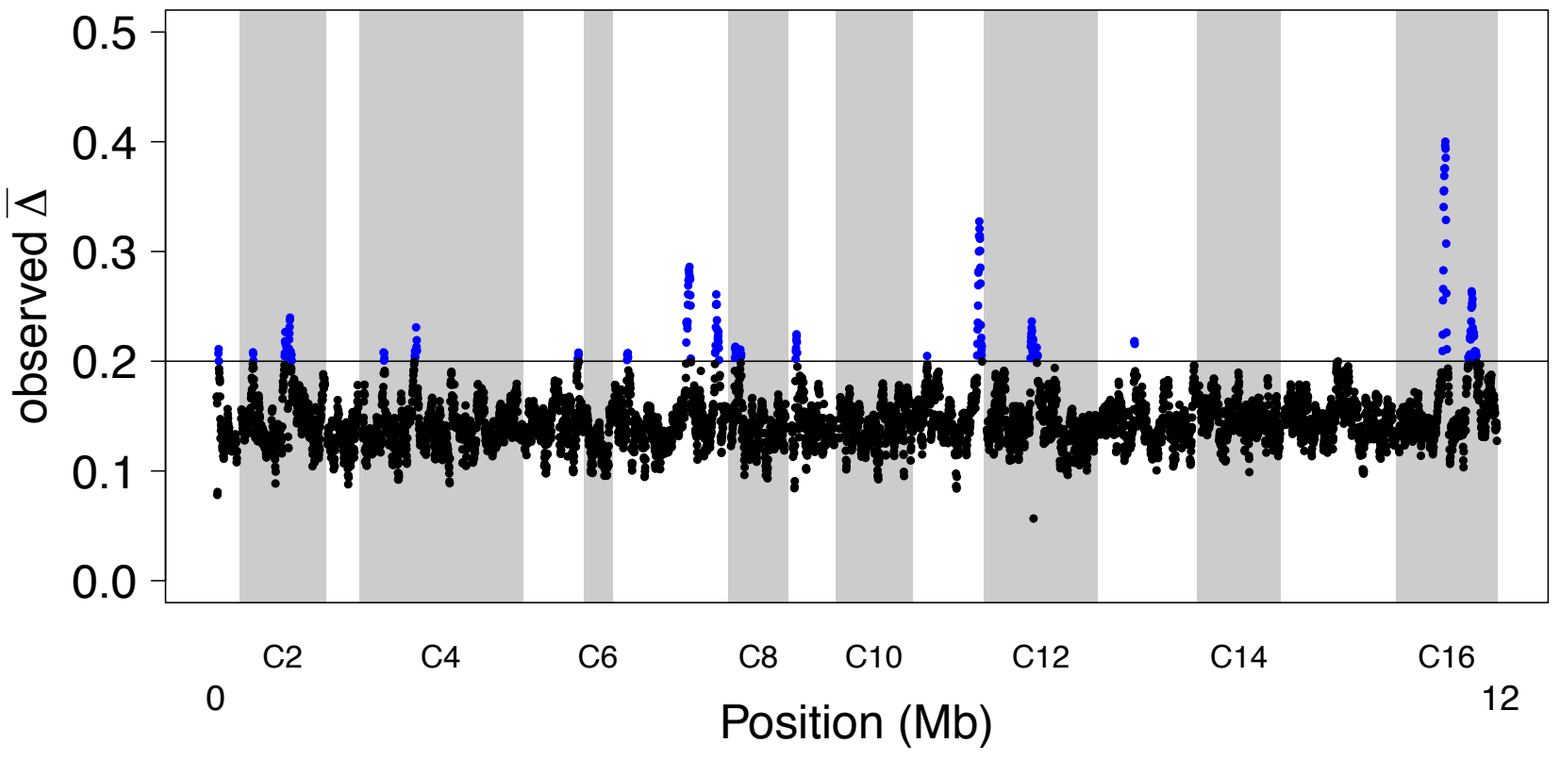

observed $\overline{\Delta}$
0.0
0.1
0.2
0.3
0.4
0.5
C2
C4
C6
C8
C10
C12
C14
C16
0
12
Position (Mb)

Figure S9.