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Supplemental Data

Multiethnic GWAS Reveals Polygenic Architecture of Earlobe Attachment

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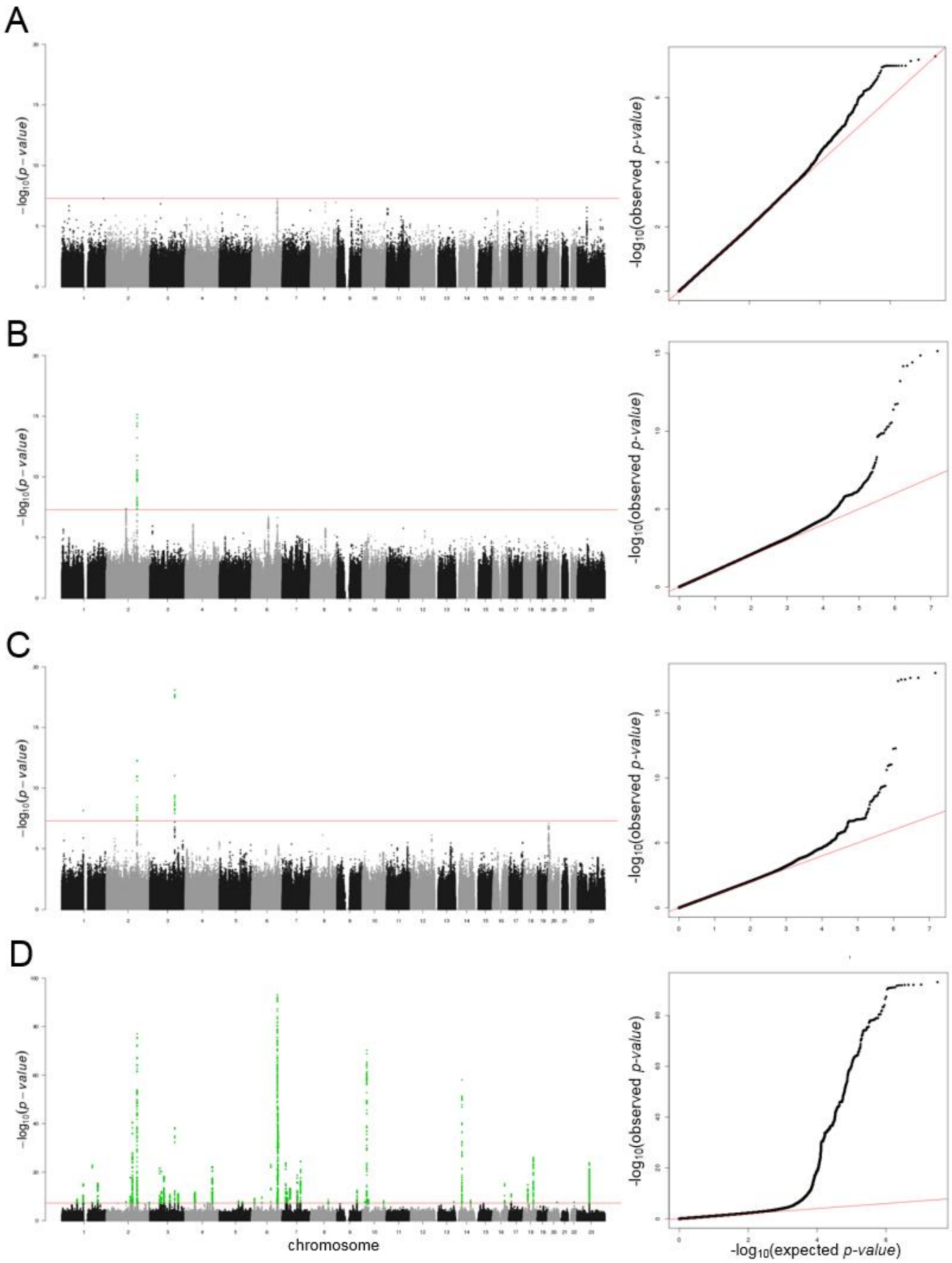
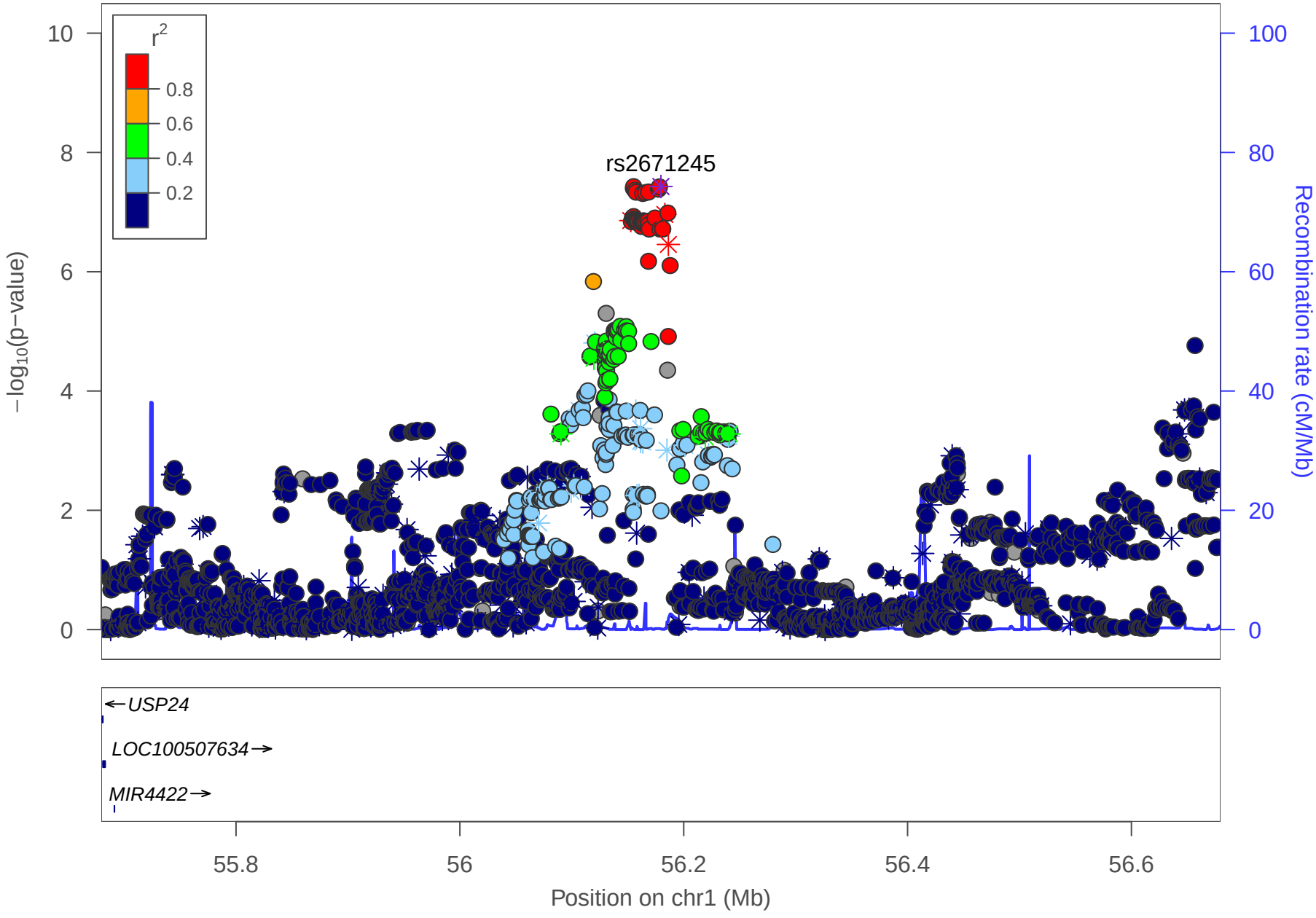
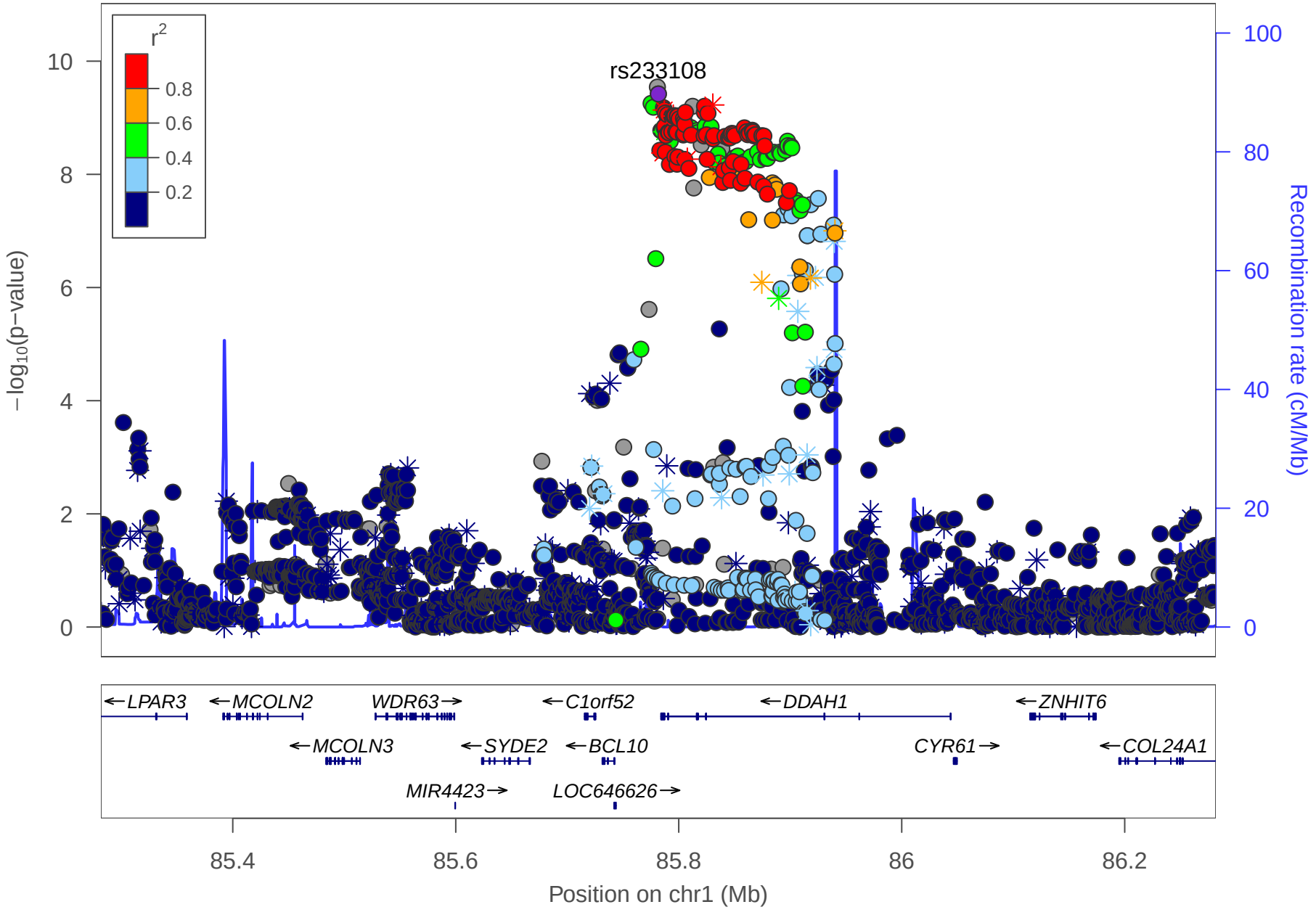
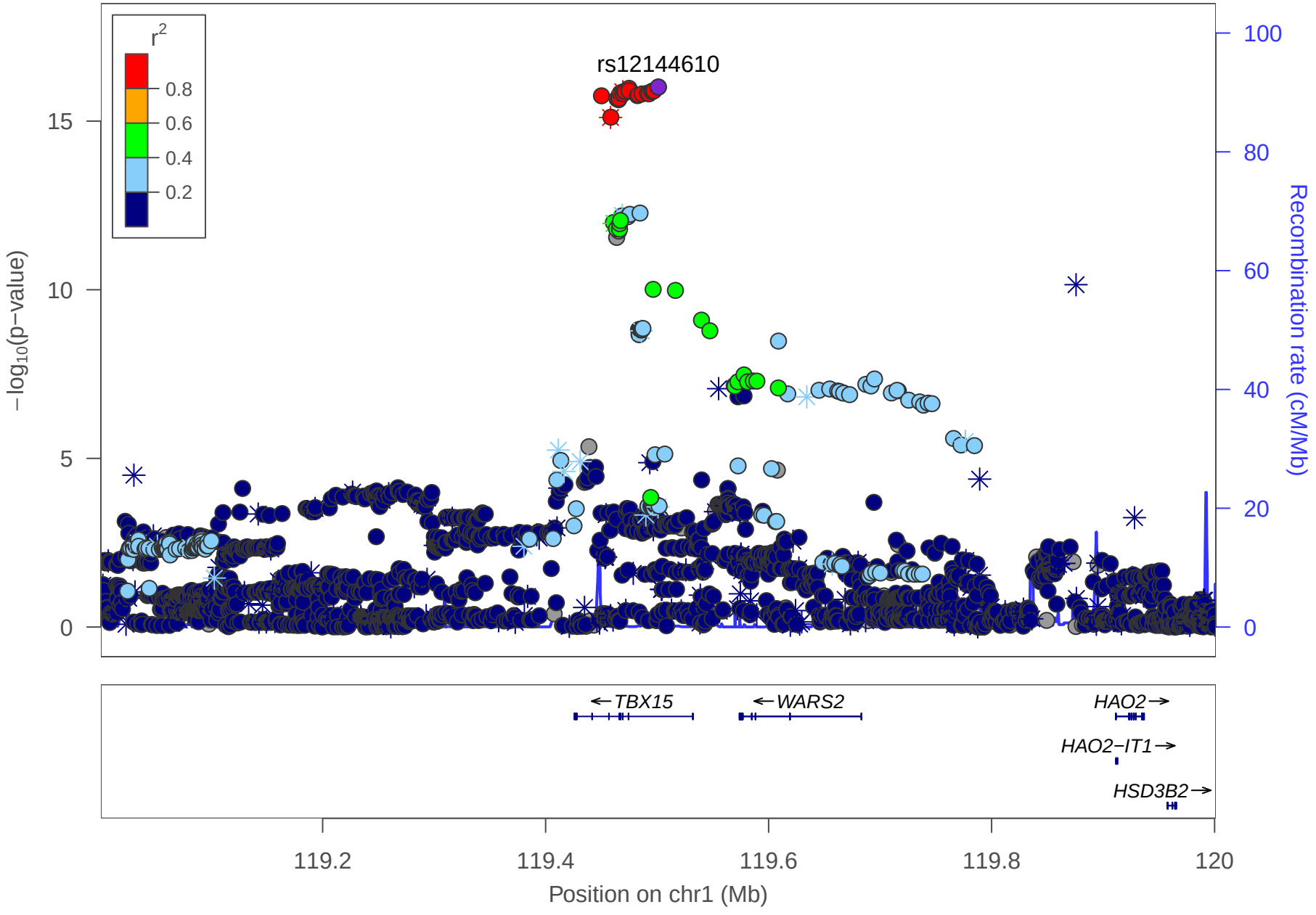
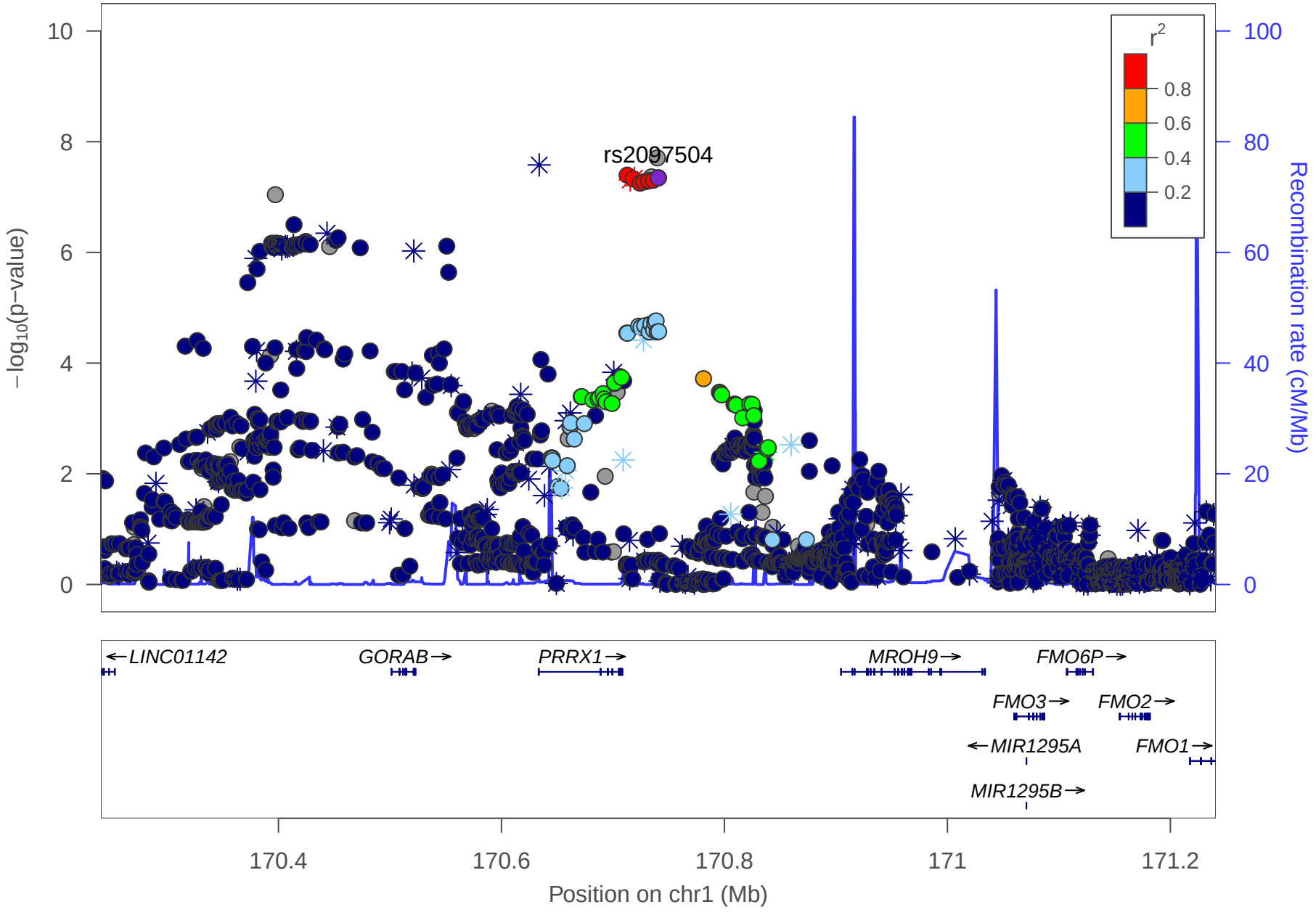


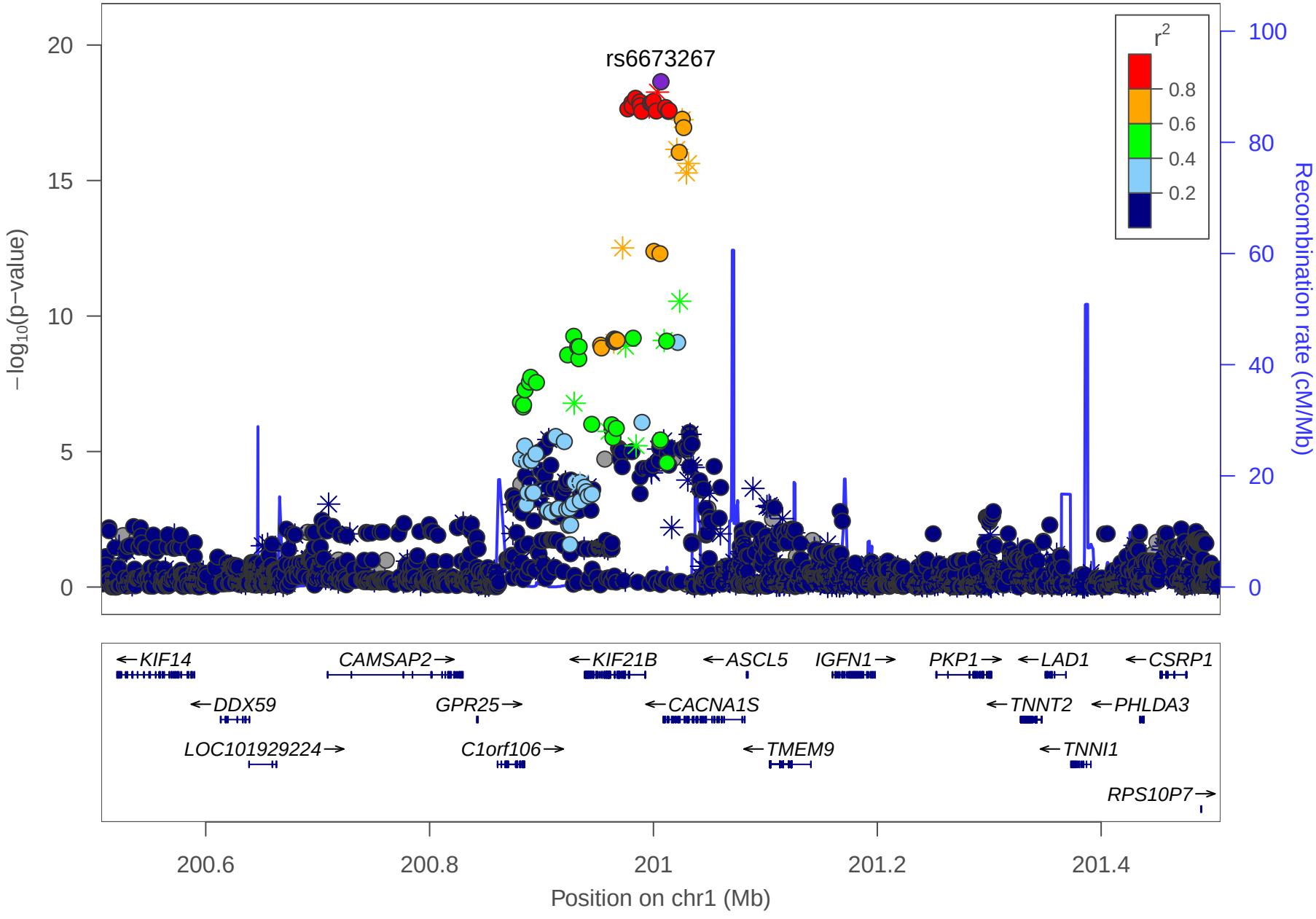
Figure S1: GWAS results shown as Manhattan (left) and quantile-quantile (right) plots for the (A) European American, (B) Latin American, (C) Chinese, and (D) 23andMe cohorts.

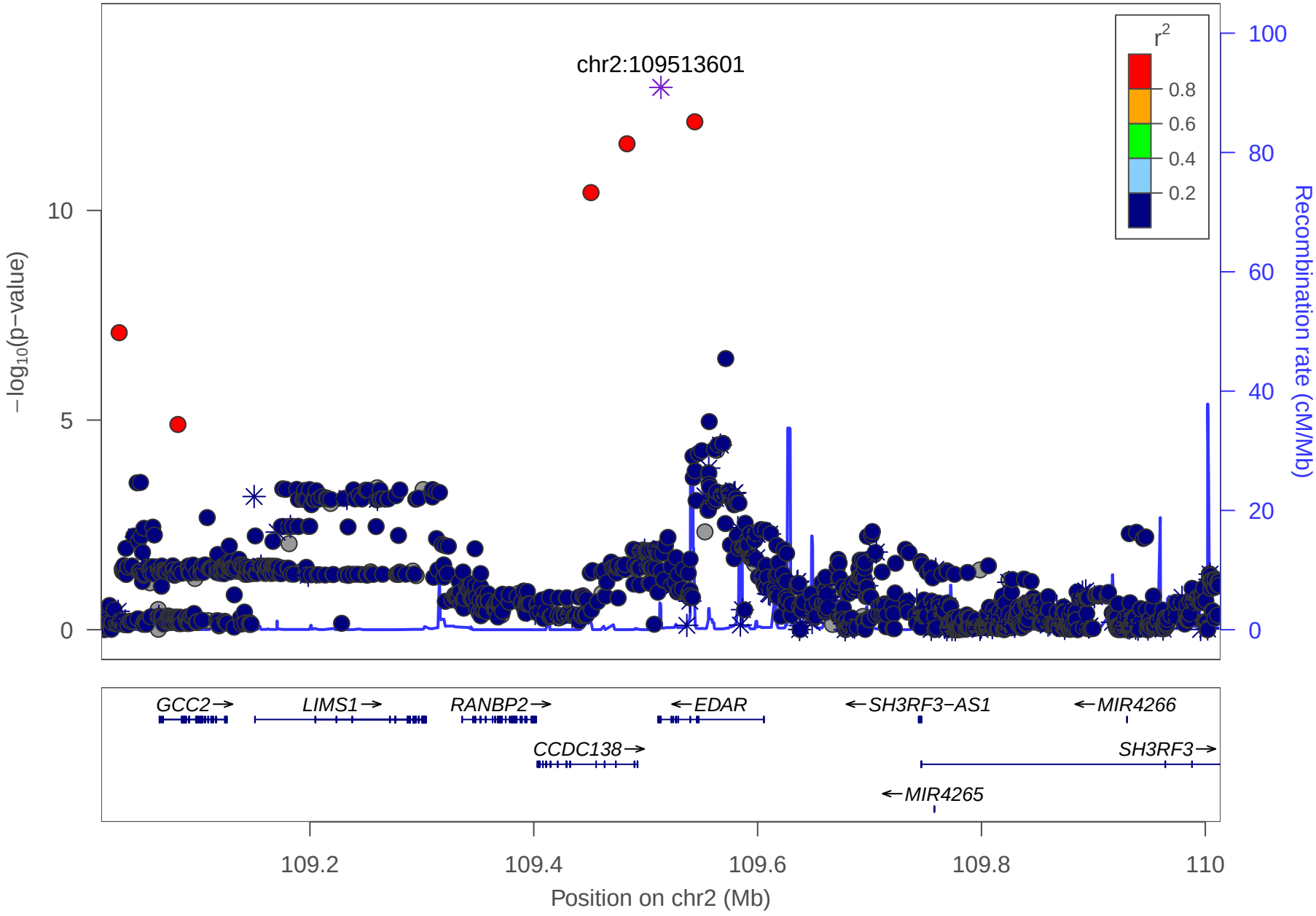

r2
0.8
0.6
0.4
0.2
rs2671245
10
8
6
4
2
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
←USP24
LOC100507634→
MIR4422→
55.8
56
56.2
56.4
56.6
Position on chr1 (Mb)

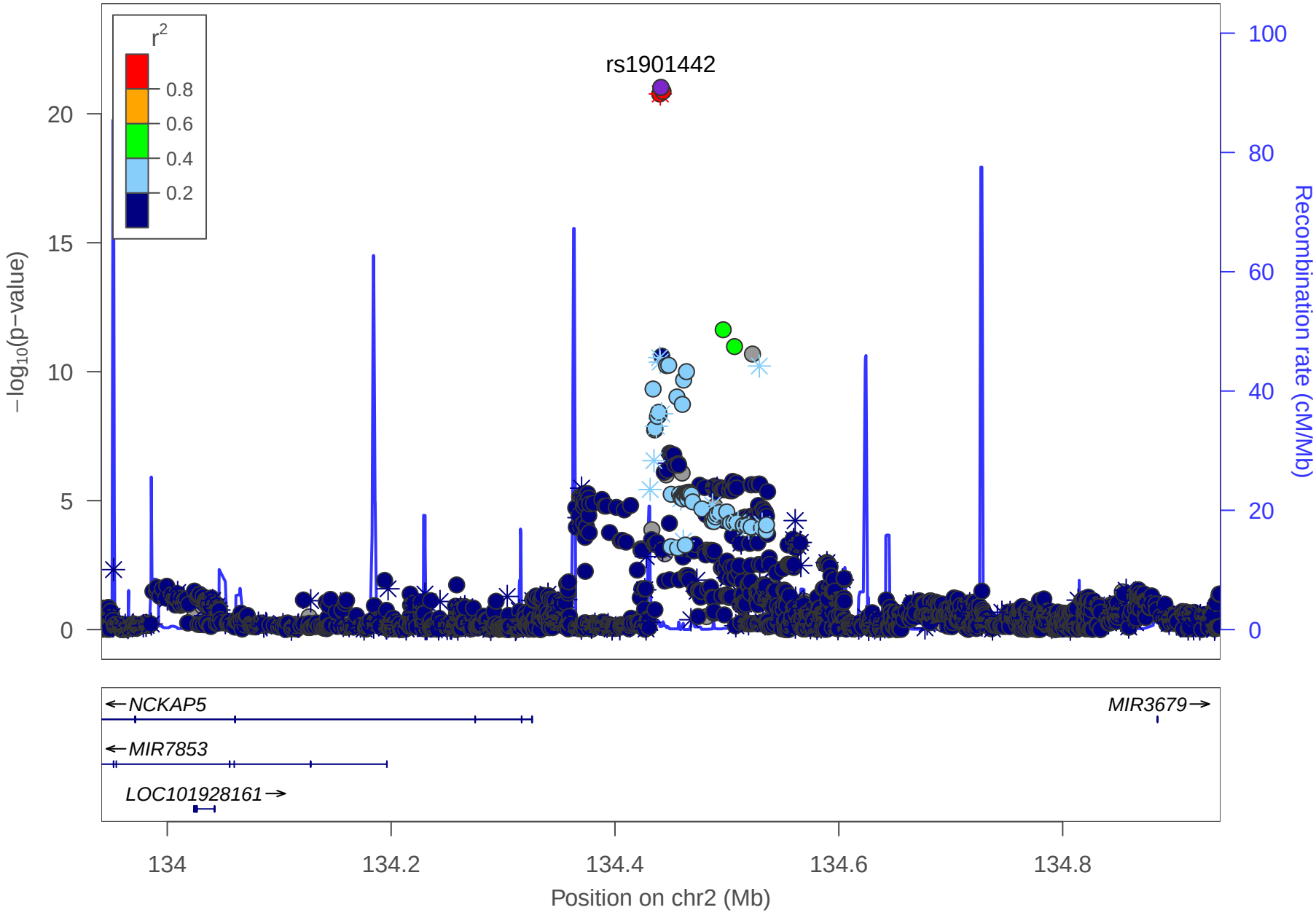

r2
0.8
0.6
0.4
0.2
rs233108
10
8
6
4
2
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←LPAR3
←MCOLN2
WDR63→
←C1orf52
←DDAH1
←ZNHIT6
←MCOLN3
←SYDE2
←BCL10
CYR61→
←COL24A1
MIR4423→
LOC646626→
85.4
85.6
85.8
86
86.2
Position on chr1 (Mb)

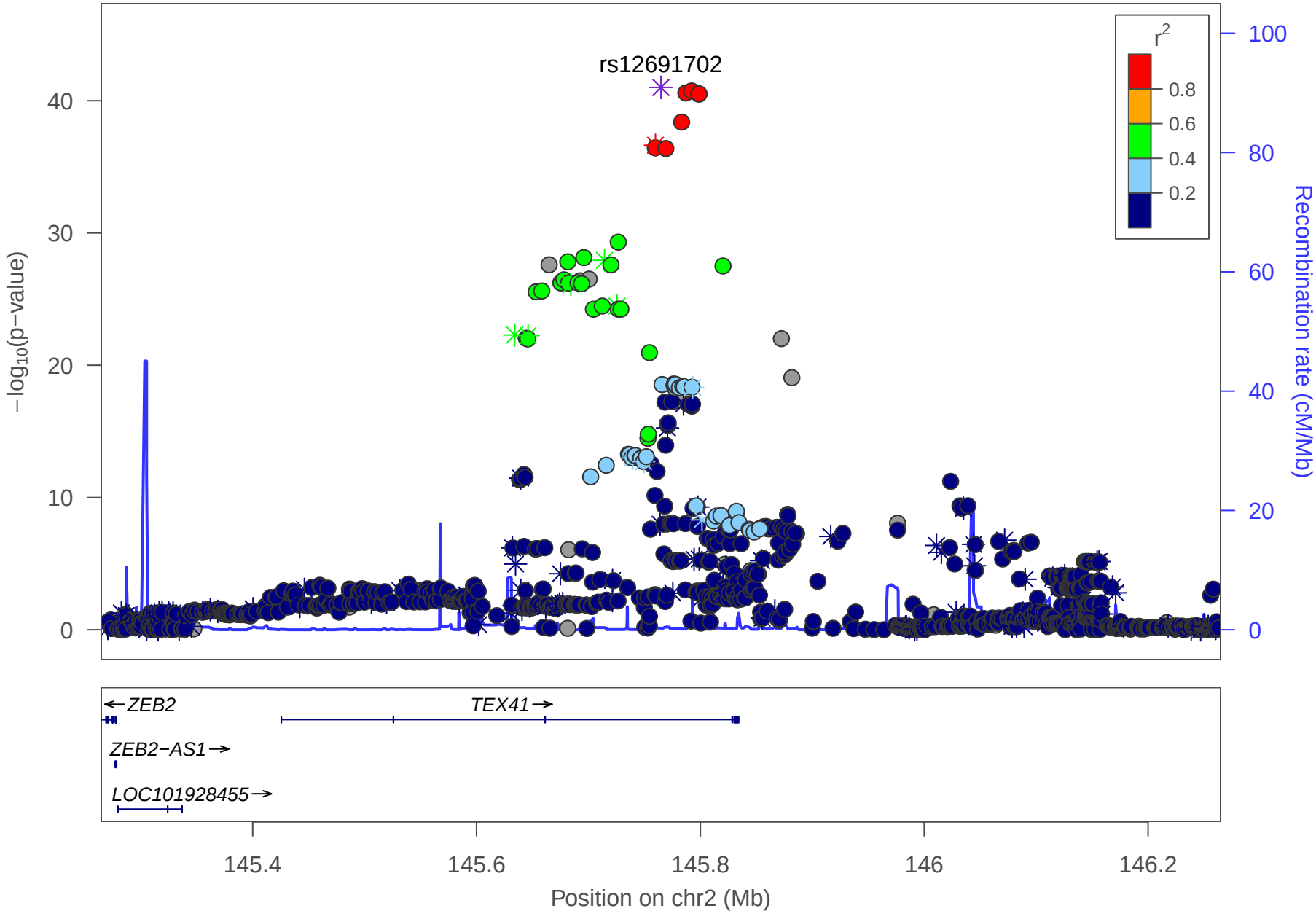

r2
0.8
0.6
0.4
0.2
rs12144610
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←TBX15
←WARS2
HAO2→
HAO2−IT1→
HSD3B2→
119.2
119.4
119.6
119.8
120
Position on chr1 (Mb)

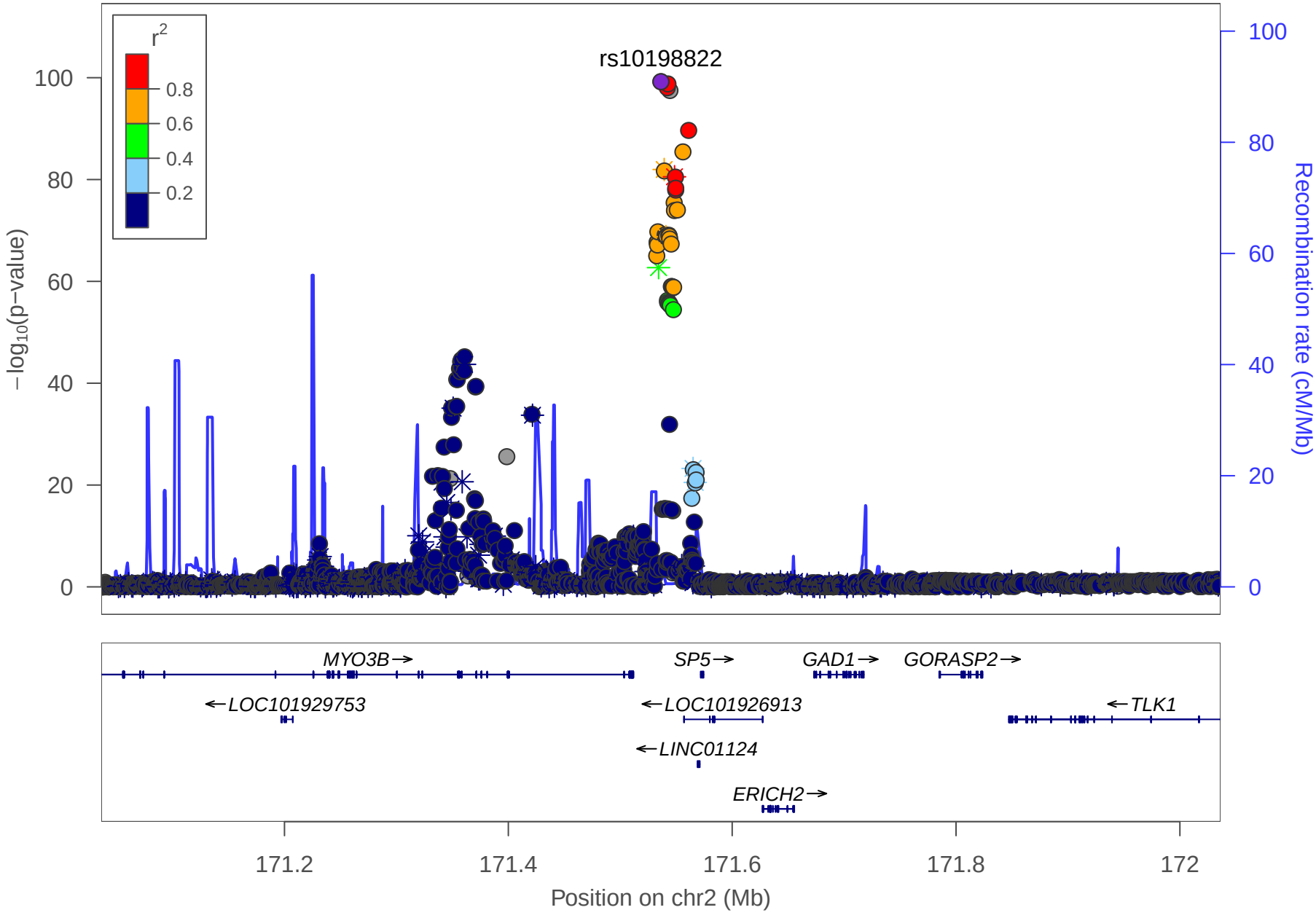

rs2097504
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
LINC01142
GORAB
PRRX1
MROH9
FMO6P
FMO3
FMO2
MIR1295A
FMO1
MIR1295B
170.4
170.6
170.8
171
171.2
Position on chr1 (Mb)

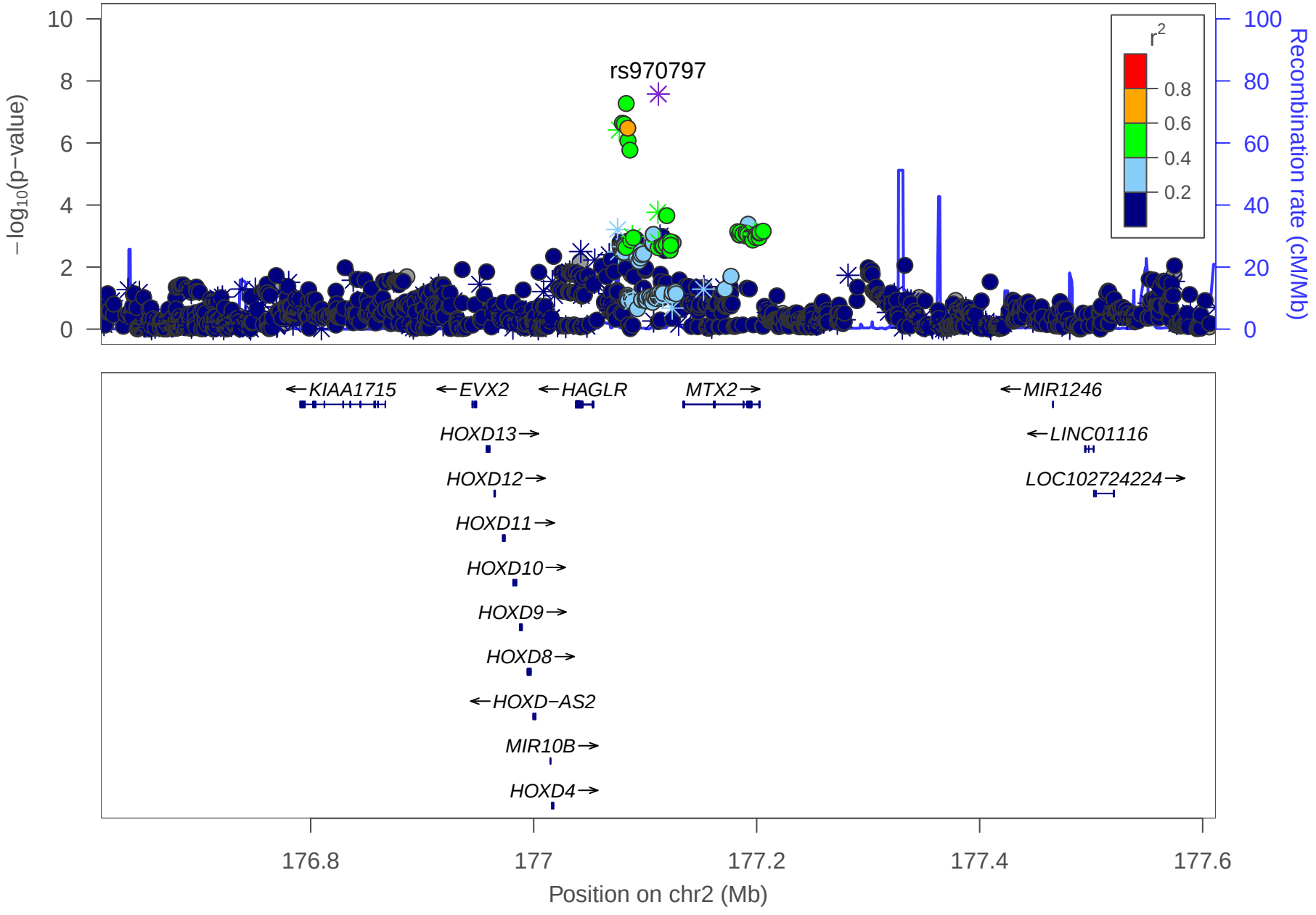

rs6673267
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
20
15
10
5
0
100
80
60
40
20
0
←KIF14
CAMSAP2→
←KIF21B
←ASCL5
IGFN1→
PKP1→
←LAD1
←CSRP1
←DDX59
GPR25→
←CACNA1S
←TNNT2
←PHLDA3
LOC101929224→
C1orf106→
←TMEM9
←TNNI1
RPS10P7→
200.6
200.8
201
201.2
201.4
Position on chr1 (Mb)

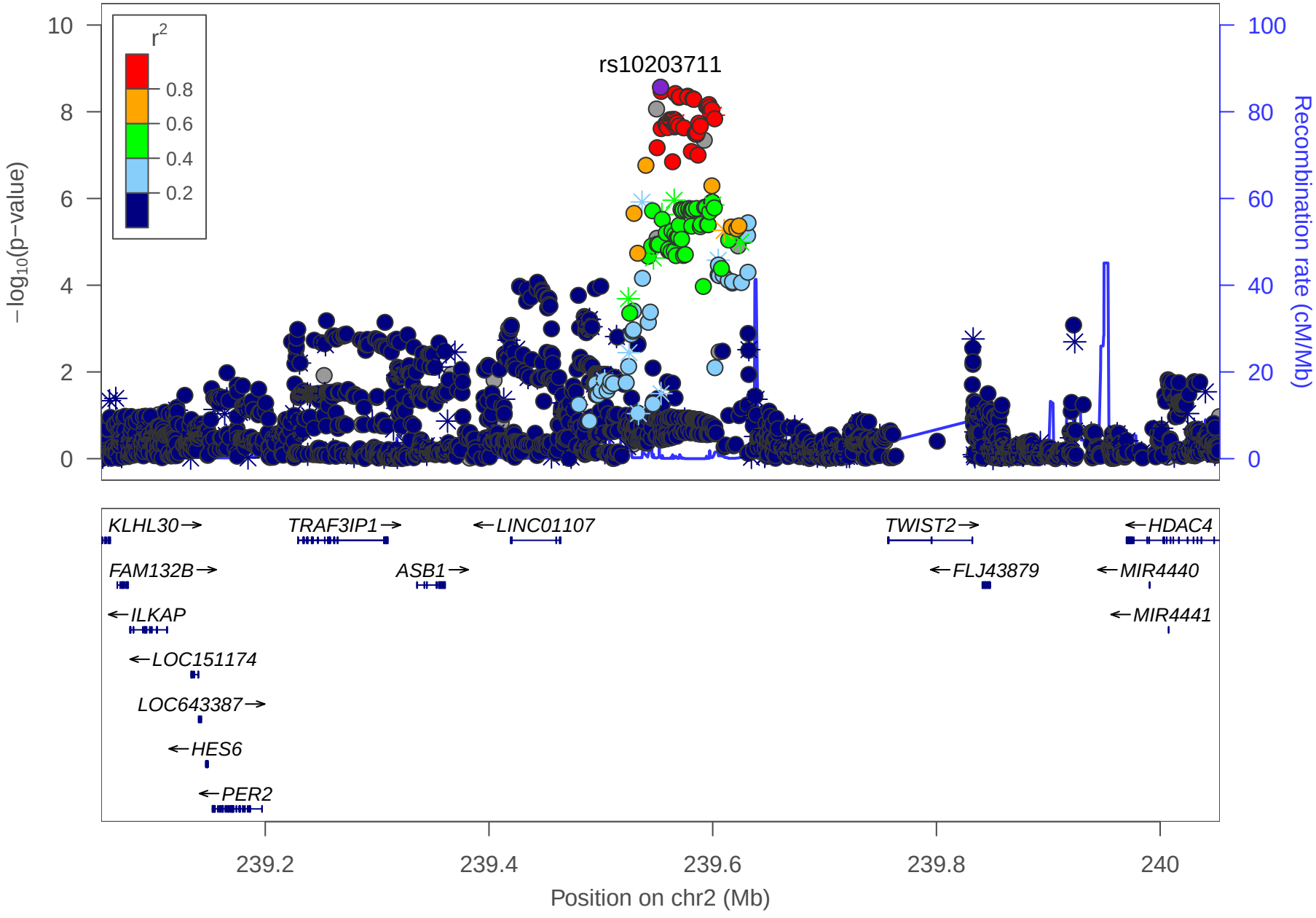

chr2:109513601
r2
0.8
0.6
0.4
0.2
−log10(p−value)
10
5
0
100
80
60
40
20
0
Recombination rate (cM/Mb)
GCC2→
LIMS1→
RANBP2→
←EDAR
←SH3RF3−AS1
←MIR4266
CCDC138→
SH3RF3→
←MIR4265
109.2
109.4
109.6
109.8
110
Position on chr2 (Mb)

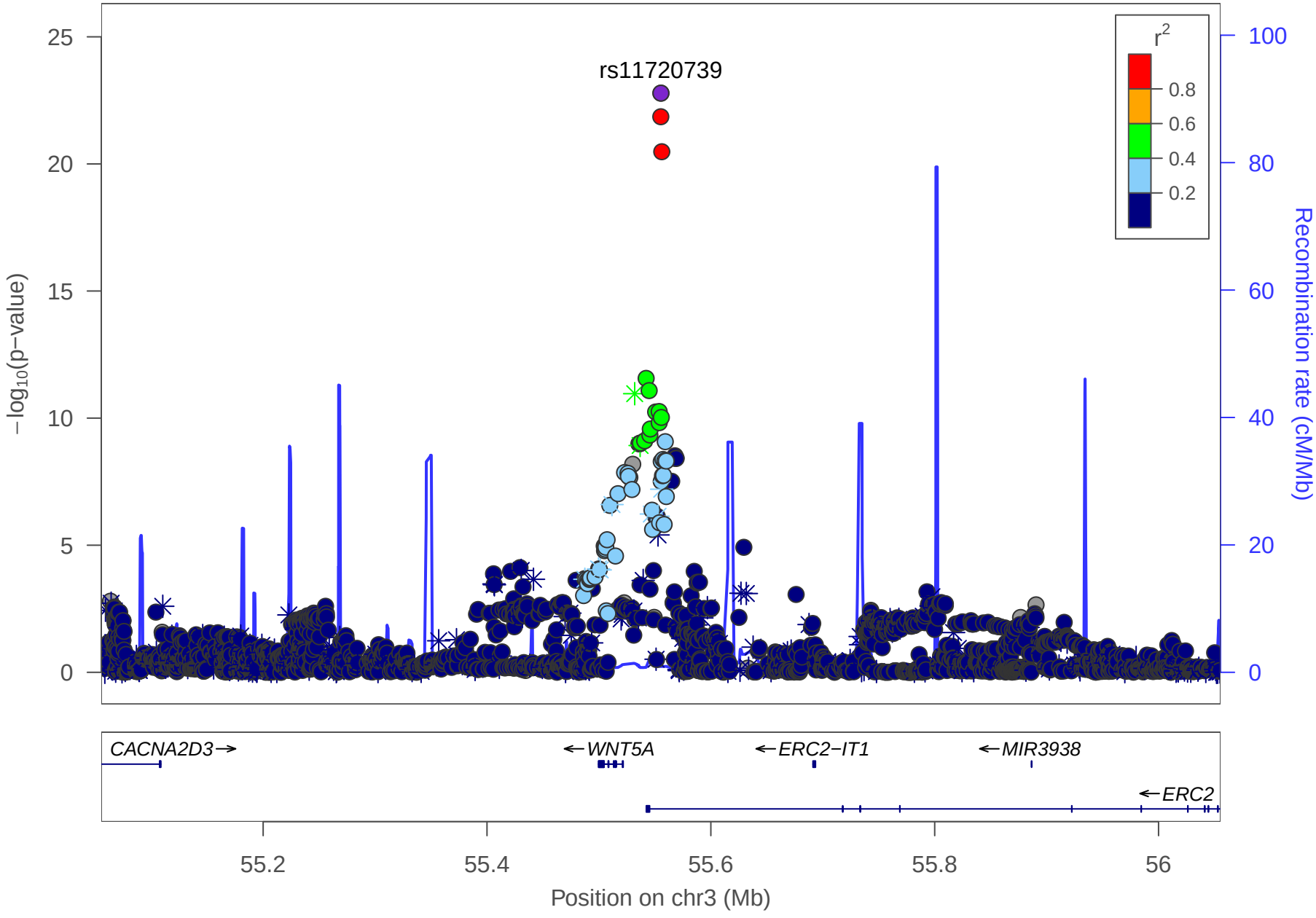

r2
0.8
0.6
0.4
0.2
rs1901442
20
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←NCKAP5
MIR3679→
←MIR7853
LOC101928161→
134
134.2
134.4
134.6
134.8
Position on chr2 (Mb)

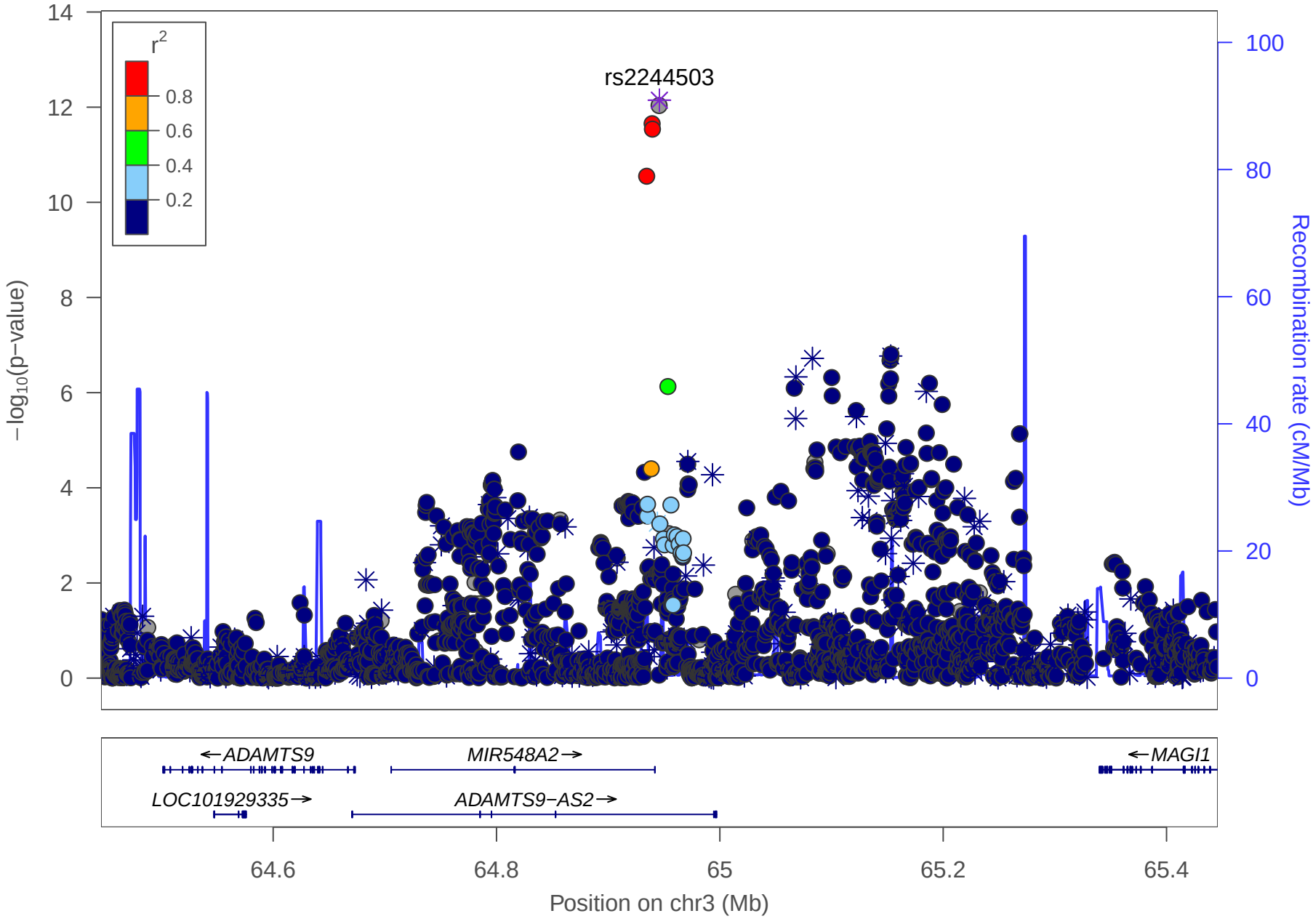

rs12691702
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
0
10
20
30
40
0
20
40
60
80
100
ZEB2
TEX41
ZEB2−AS1
LOC101928455
145.4
145.6
145.8
146
146.2
Position on chr2 (Mb)

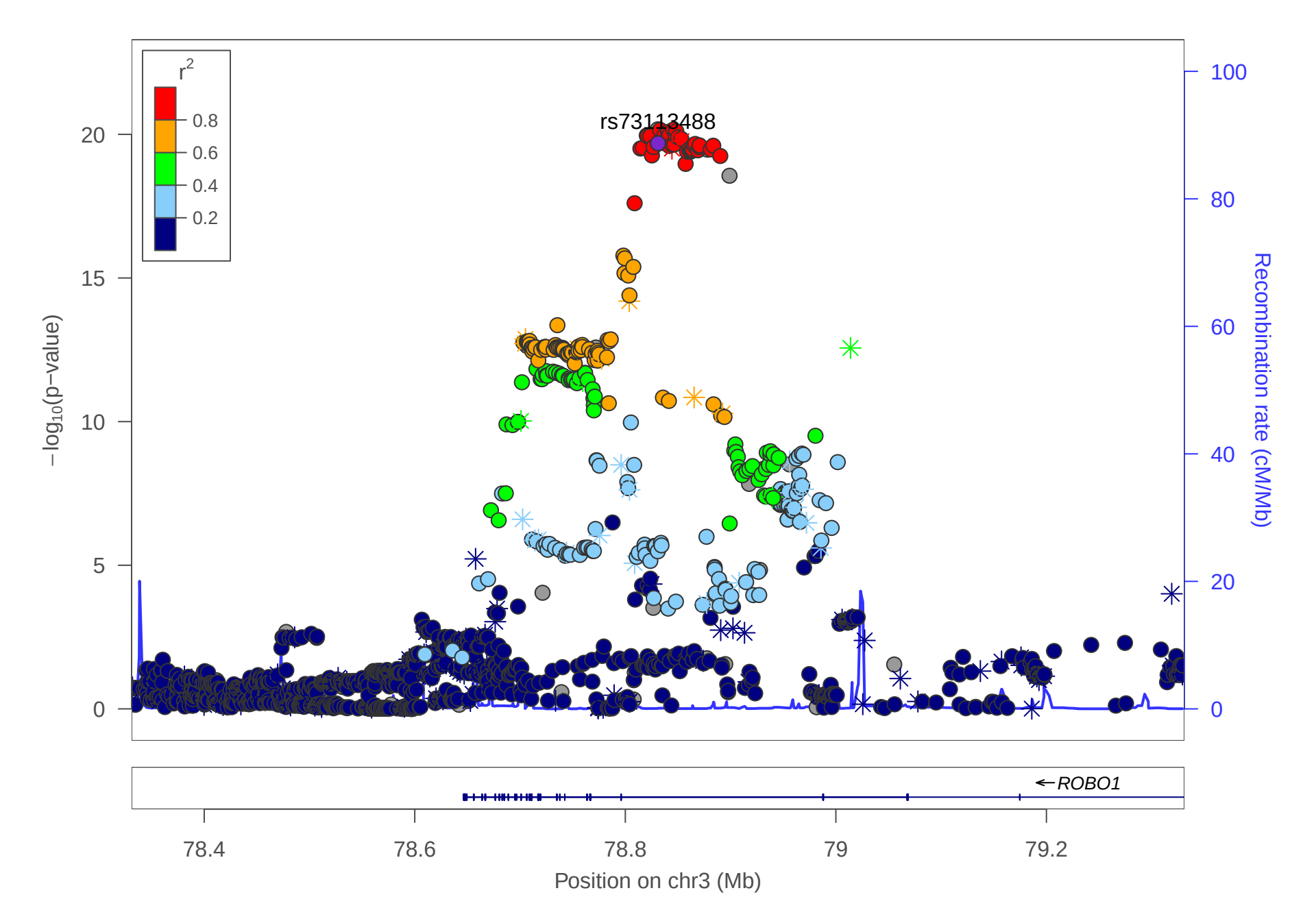

r2
0.8
0.6
0.4
0.2
rs10198822
100
80
60
40
20
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
MYO3B→
SP5→
GAD1→
GORASP2→
←LOC101929753
←LOC101926913
←TLK1
←LINC01124
ERICH2→
171.2
171.4
171.6
171.8
172
Position on chr2 (Mb)

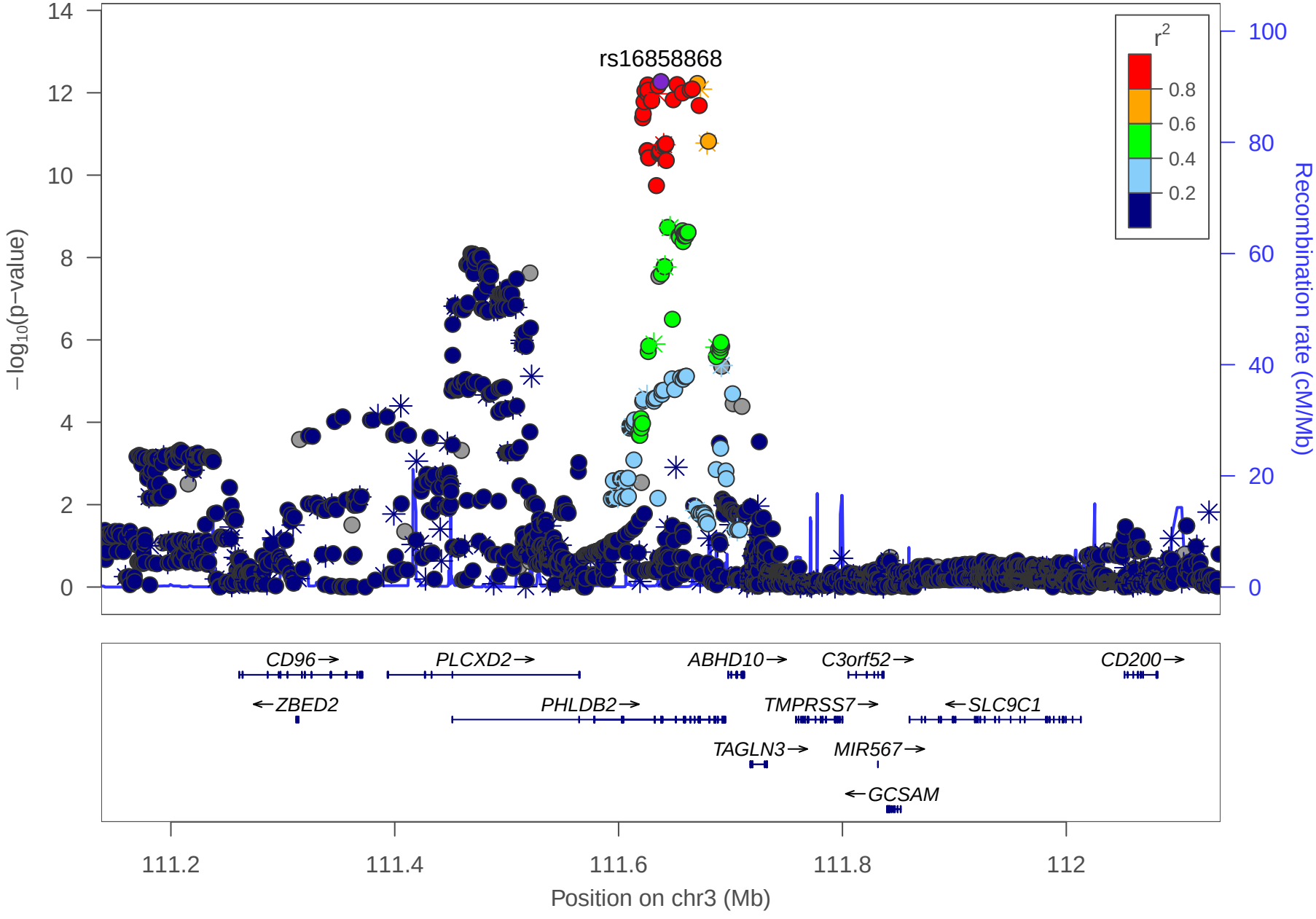

rs970797
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
←KIAA1715
←EVX2
←HAGLR
MTX2→
←MIR1246
HOXD13→
←LINC01116
HOXD12→
LOC102724224→
HOXD11→
HOXD10→
HOXD9→
HOXD8→
←HOXD−AS2
MIR10B→
HOXD4→
176.8
177
177.2
177.4
177.6
Position on chr2 (Mb)

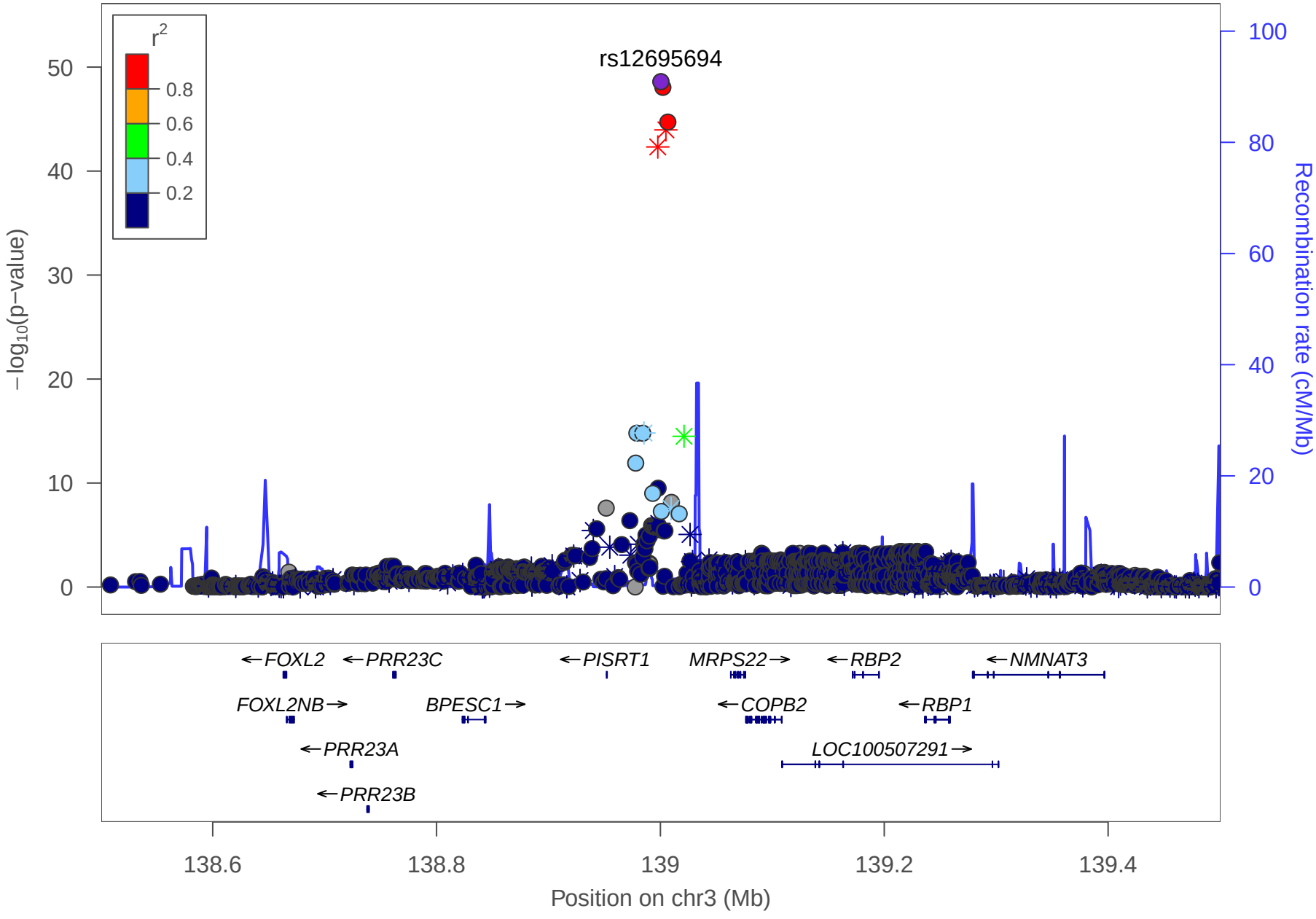

rs10203711
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
KLHL30
TRAF3IP1
LINC01107
TWIST2
HDAC4
FAM132B
ASB1
FLJ43879
MIR4440
ILKAP
MIR4441
LOC151174
LOC643387
HES6
PER2
239.2
239.4
239.6
239.8
240
Position on chr2 (Mb)

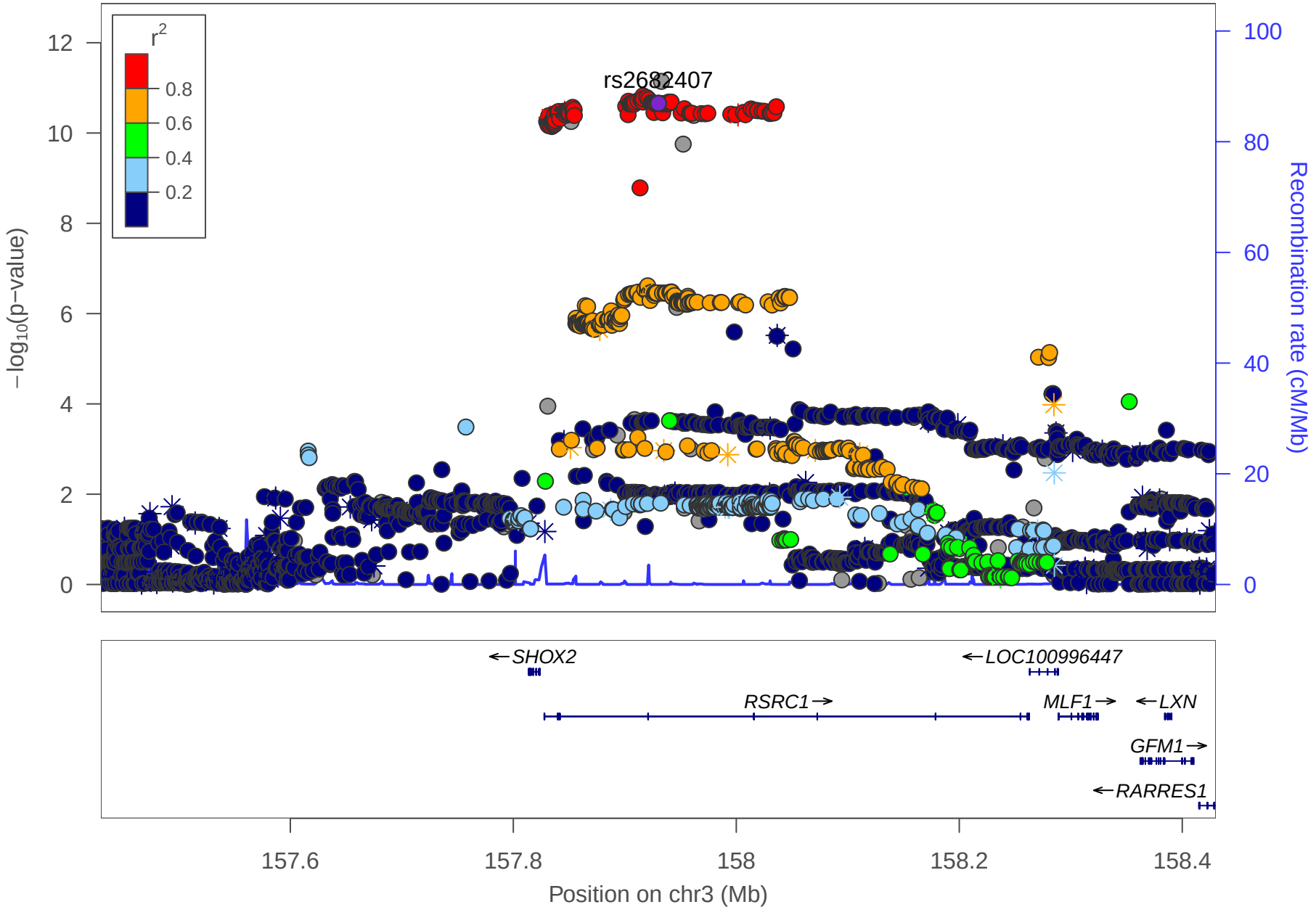

rs11720739
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
25
20
15
10
5
0
100
80
60
40
20
0
CACNA2D3→
←WNT5A
←ERC2−IT1
←MIR3938
←ERC2
55.2
55.4
55.6
55.8
56
Position on chr3 (Mb)

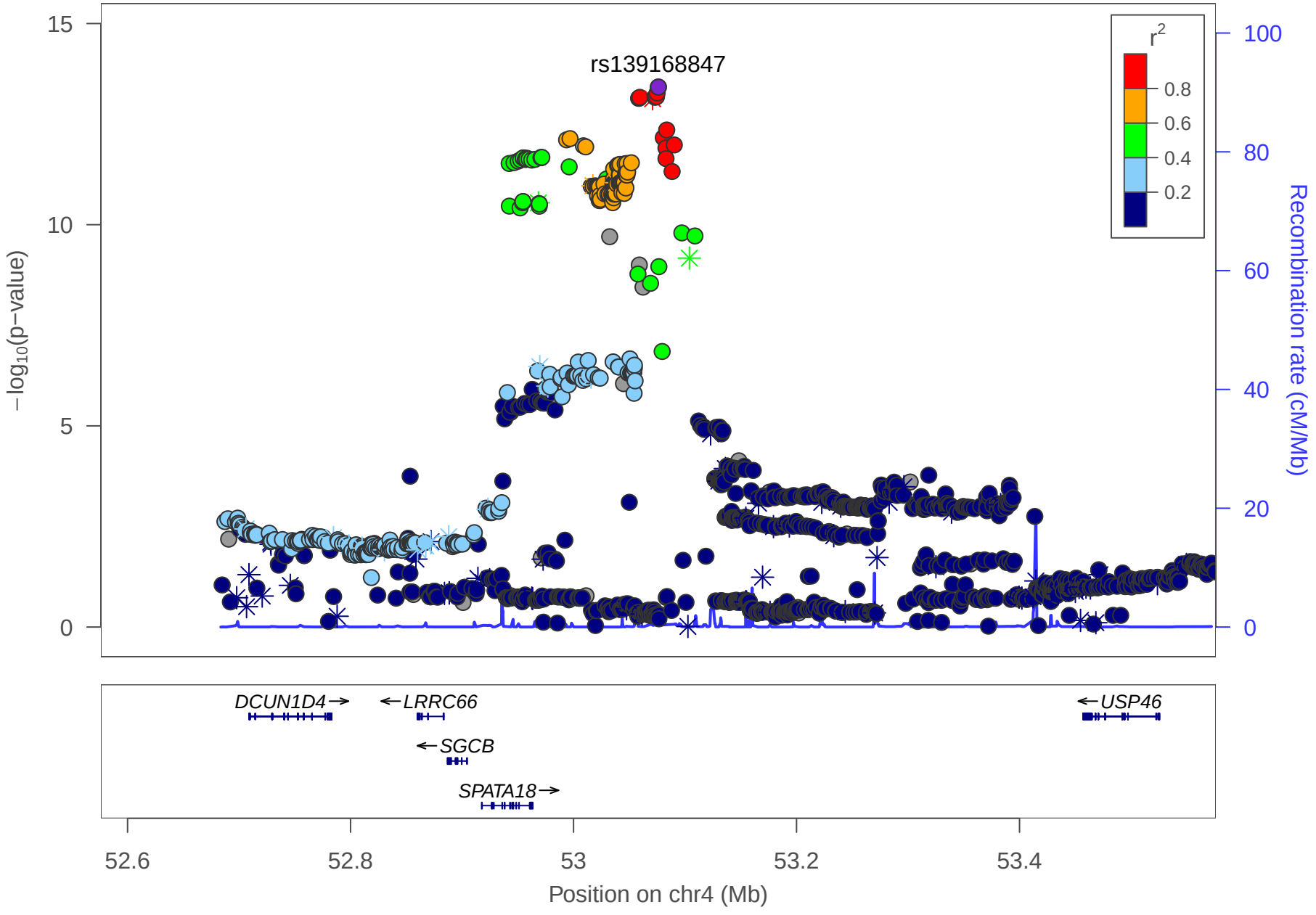

rs2244503
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
ADAMTS9
MIR548A2
MAGI1
LOC101929335
ADAMTS9−AS2
Position on chr3 (Mb)

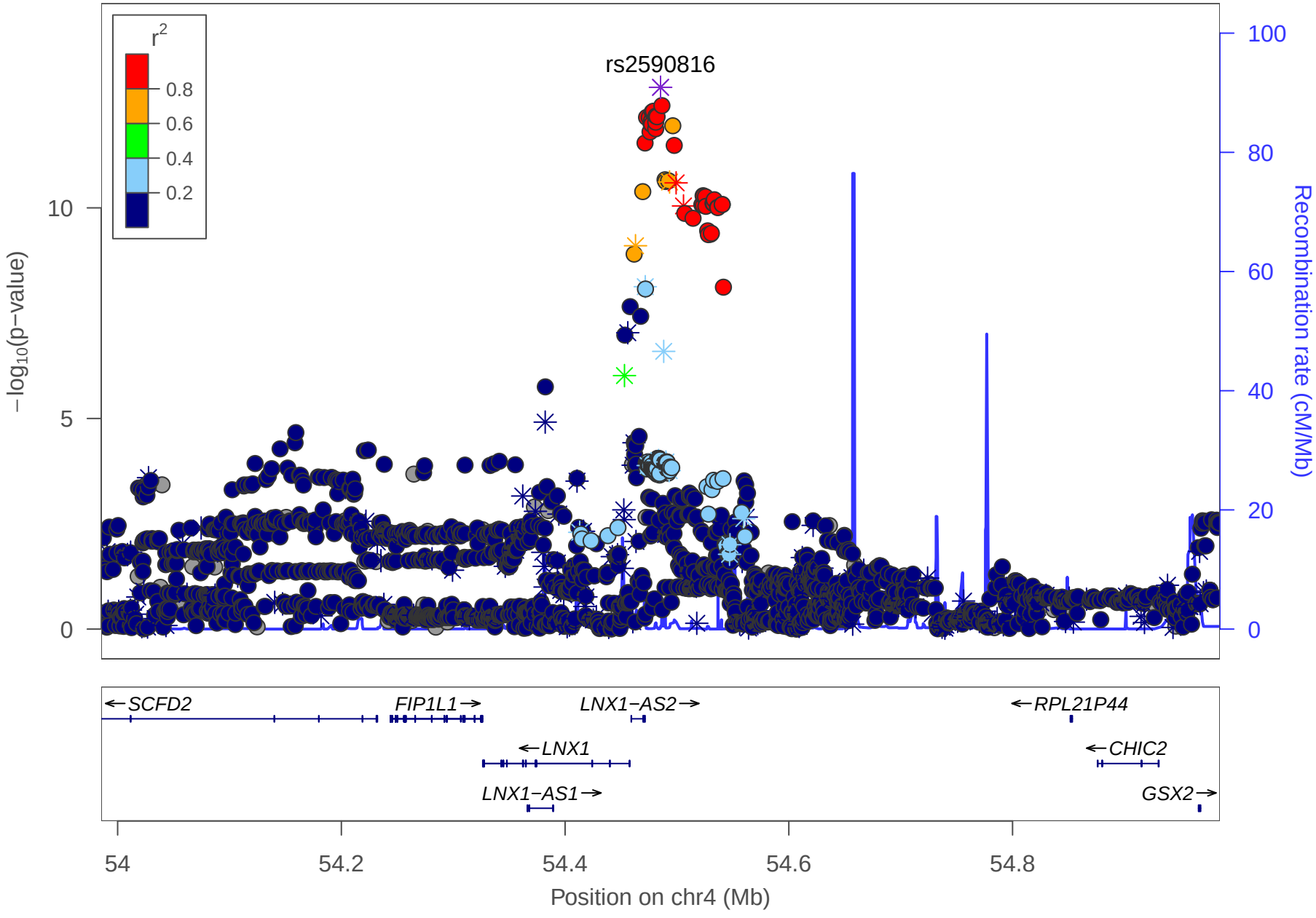

r2
0.8
0.6
0.4
0.2
rs73113488
20
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←ROBO1
78.4
78.6
78.8
79
79.2
Position on chr3 (Mb)

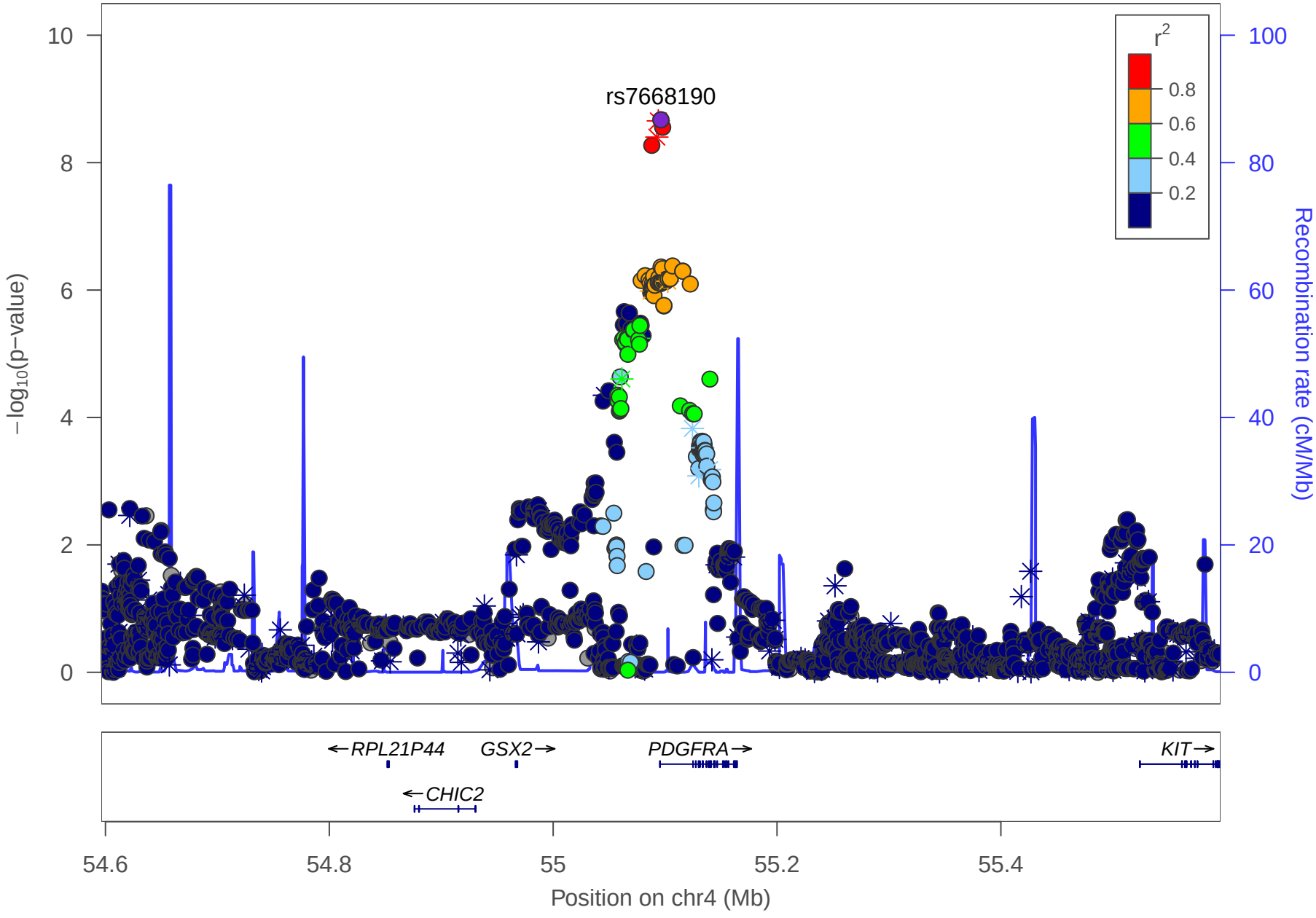

rs16858868
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
14
12
10
8
6
4
2
0
100
80
60
40
20
0
CD96→
PLCXD2→
ABHD10→
C3orf52→
CD200→
←ZBED2
PHLDB2→
TMPRSS7→
←SLC9C1
TAGLN3→
MIR567→
←GCSAM
111.2
111.4
111.6
111.8
112
Position on chr3 (Mb)

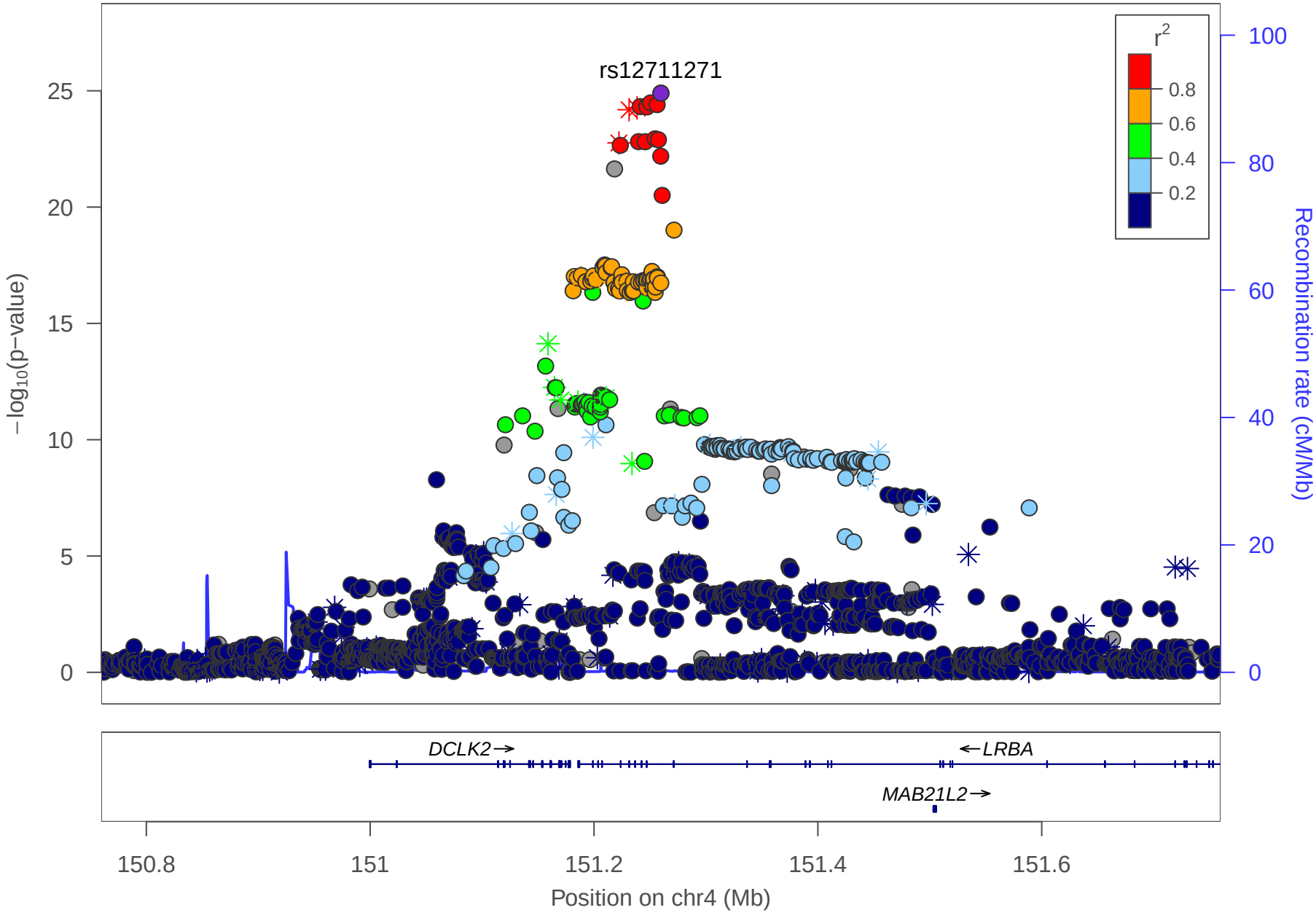

rs12695694
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
FOXL2
PRR23C
PISRT1
MRPS22
RBP2
NMNAT3
FOXL2NB
BPESC1
COPB2
RBP1
PRR23A
LOC100507291
PRR23B
Position on chr3 (Mb)

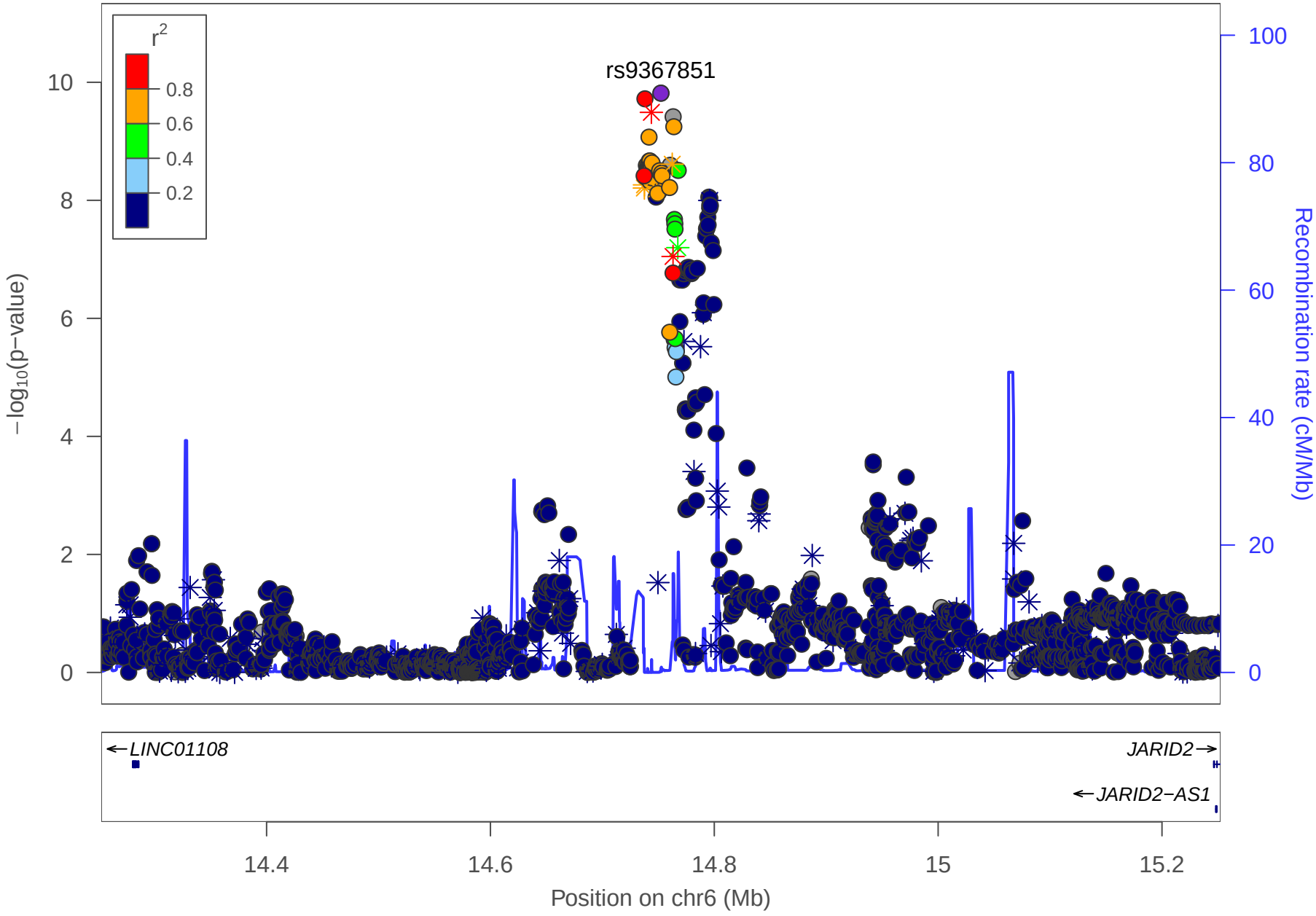

r2
0.8
0.6
0.4
0.2
rs2682407
−log10(p−value)
Recombination rate (cM/Mb)
SHOX2
LOC100996447
RSRC1
MLF1
LXN
GFM1
RARRES1
Position on chr3 (Mb)

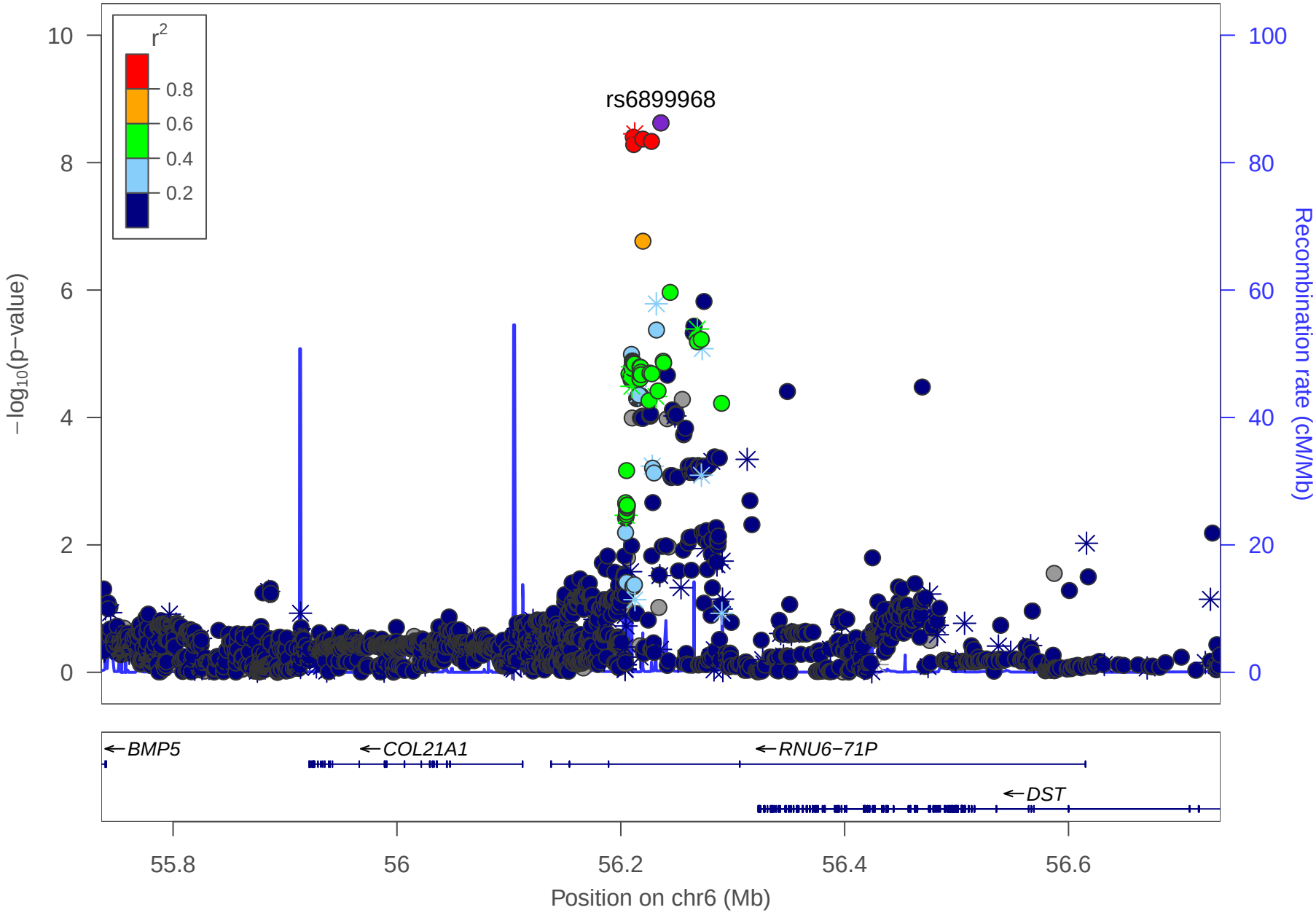

rs139168847
r2
0.8
0.6
0.4
0.2
15
10
5
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
DCUN1D4→
←LRRC66
←USP46
←SGCB
SPATA18→
52.6
52.8
53
53.2
53.4
Position on chr4 (Mb)

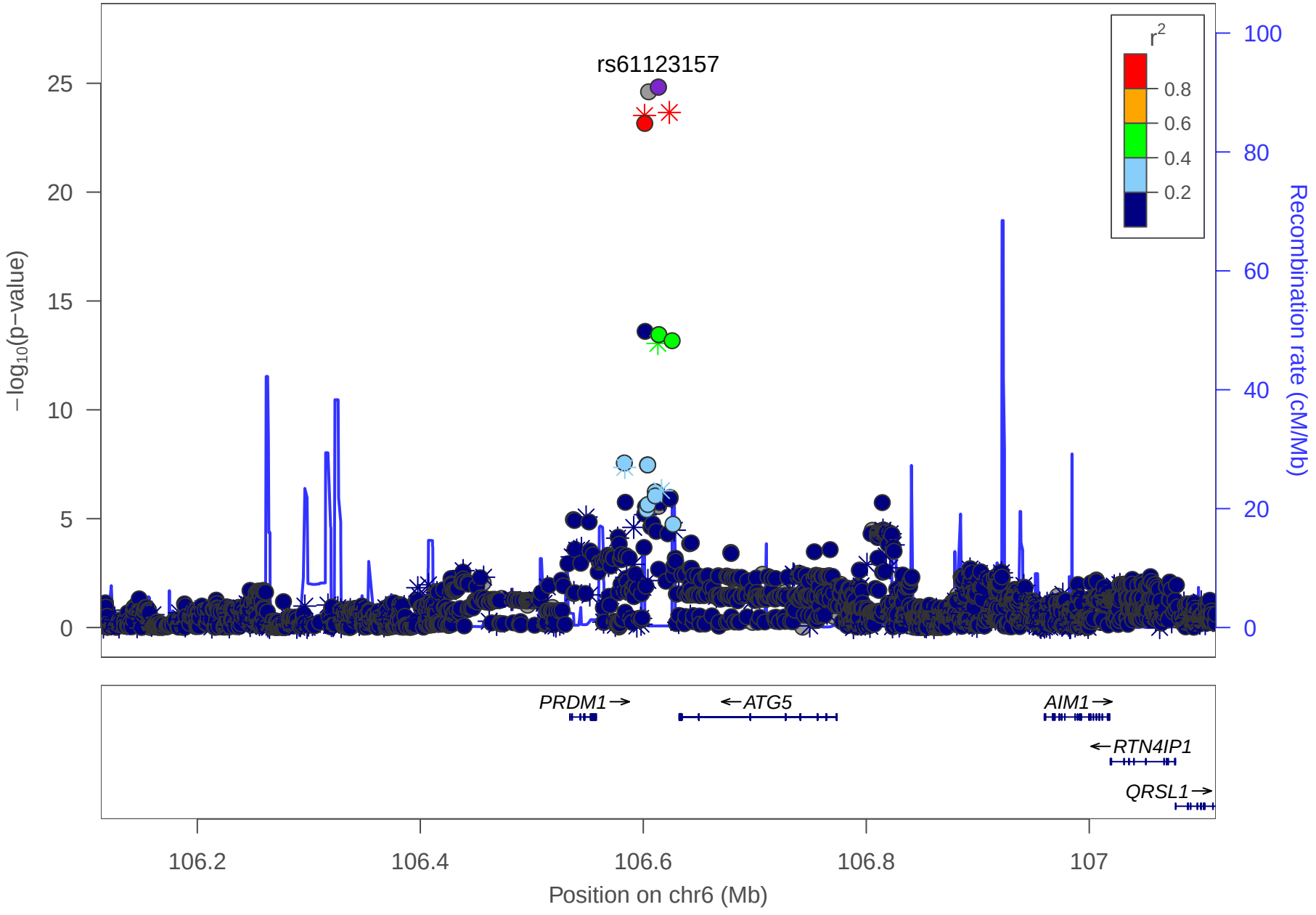

r2
0.8
0.6
0.4
0.2
rs2590816
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←SCFD2
FIP1L1→
LNX1−AS2→
←RPL21P44
←LNX1
←CHIC2
LNX1−AS1→
GSX2→
54
54.2
54.4
54.6
54.8
Position on chr4 (Mb)

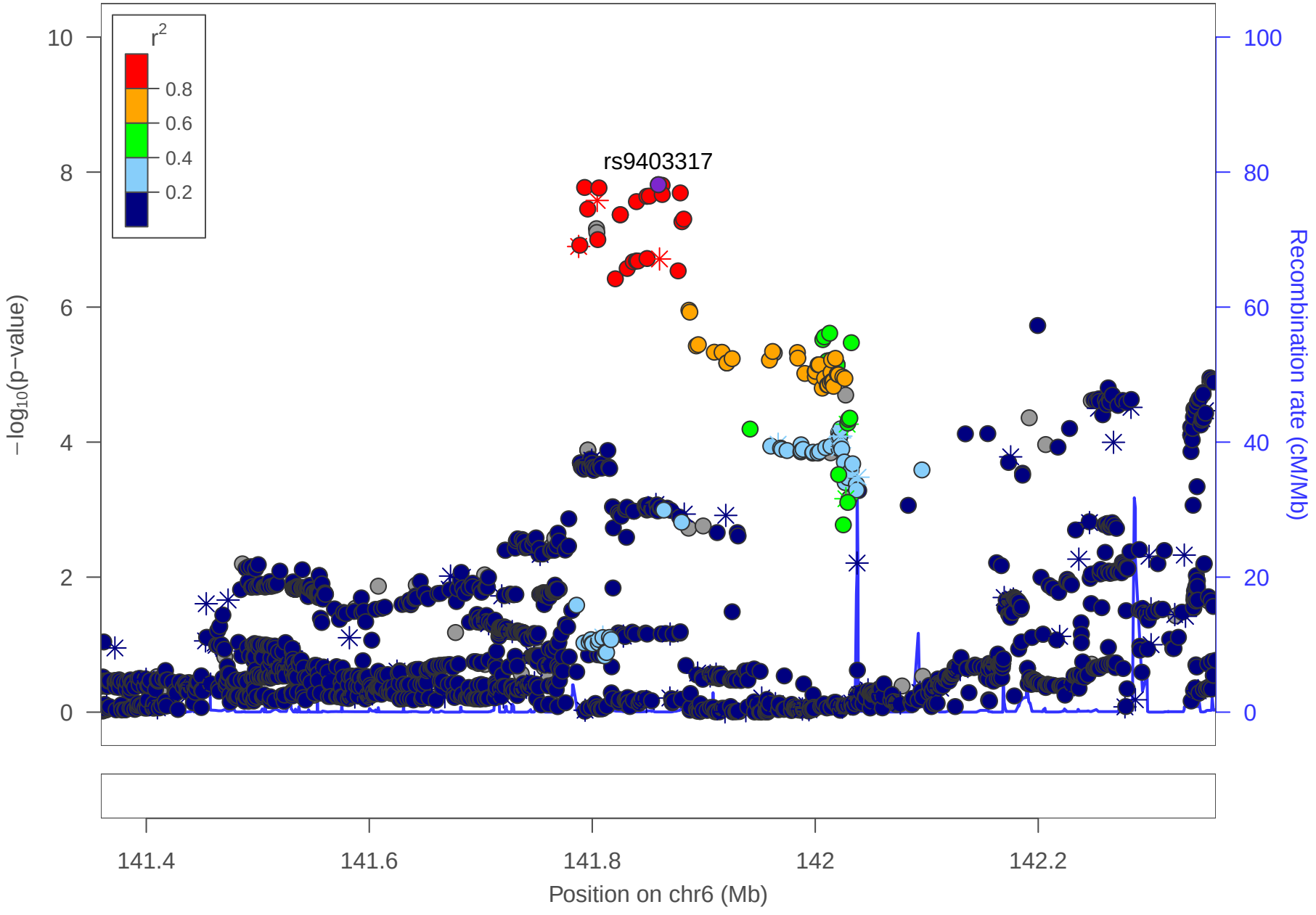

rs7668190
r2
0.8
0.6
0.4
0.2
10
8
6
4
2
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
←RPL21P44
GSX2→
PDGFRA→
KIT→
←CHIC2
54.6
54.8
55
55.2
55.4
Position on chr4 (Mb)

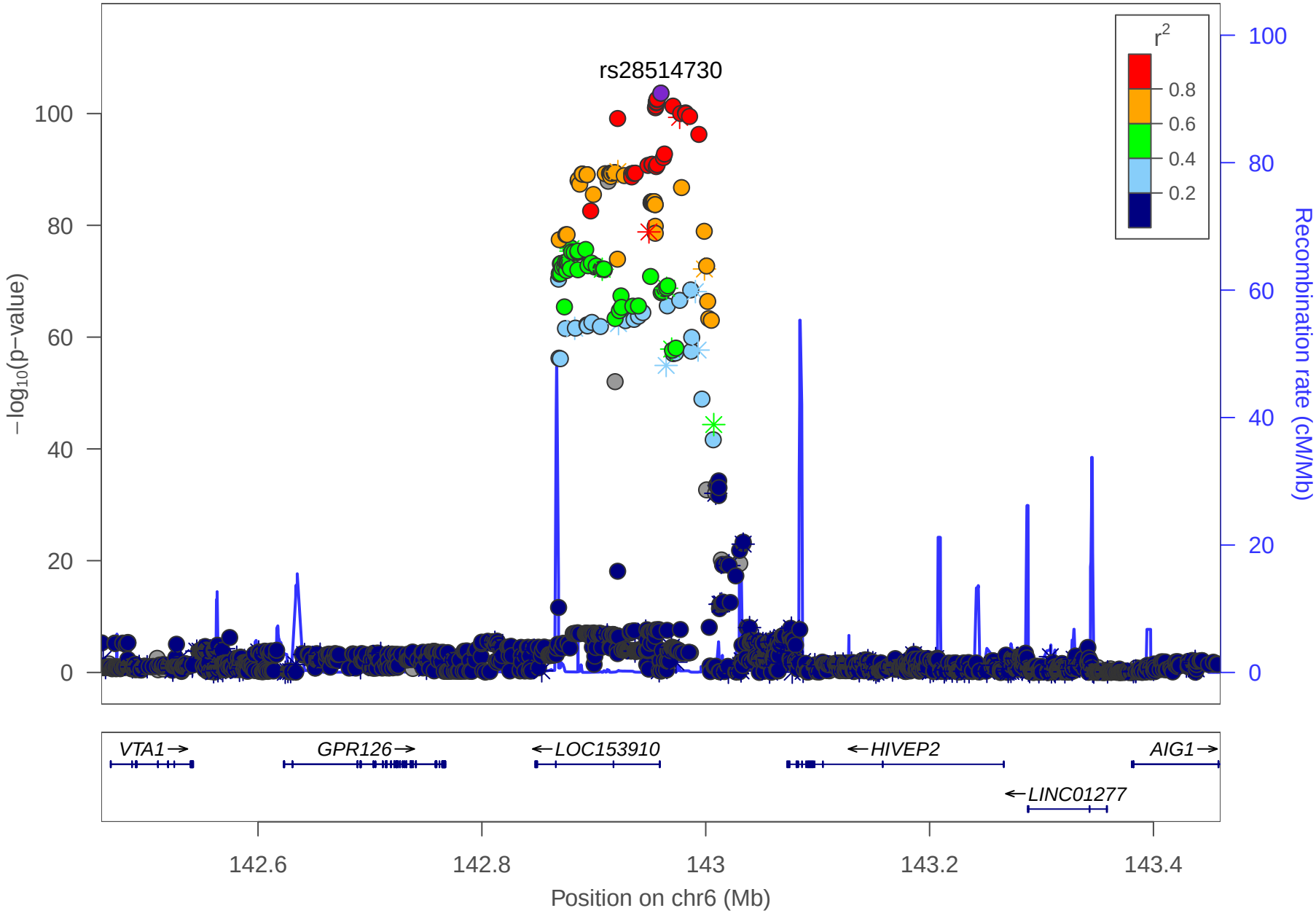

rs12711271
r2
0.8
0.6
0.4
0.2
25
20
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
DCLK2→
←LRBA
MAB21L2→
150.8
151
151.2
151.4
151.6
Position on chr4 (Mb)

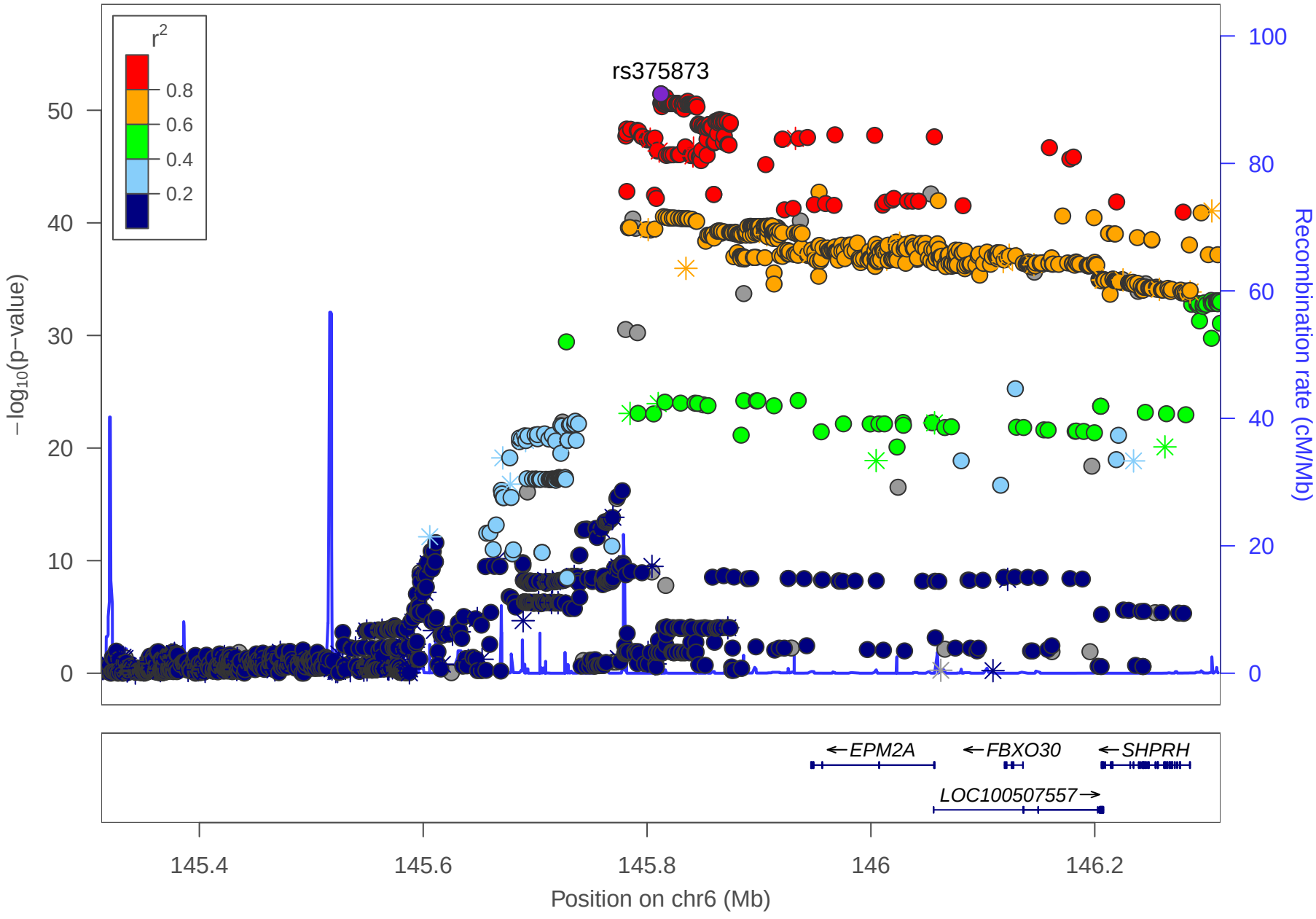

r2
0.8
0.6
0.4
0.2
rs9367851
10
8
6
4
2
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←LINC01108
JARID2→
←JARID2−AS1
14.4
14.6
14.8
15
15.2
Position on chr6 (Mb)

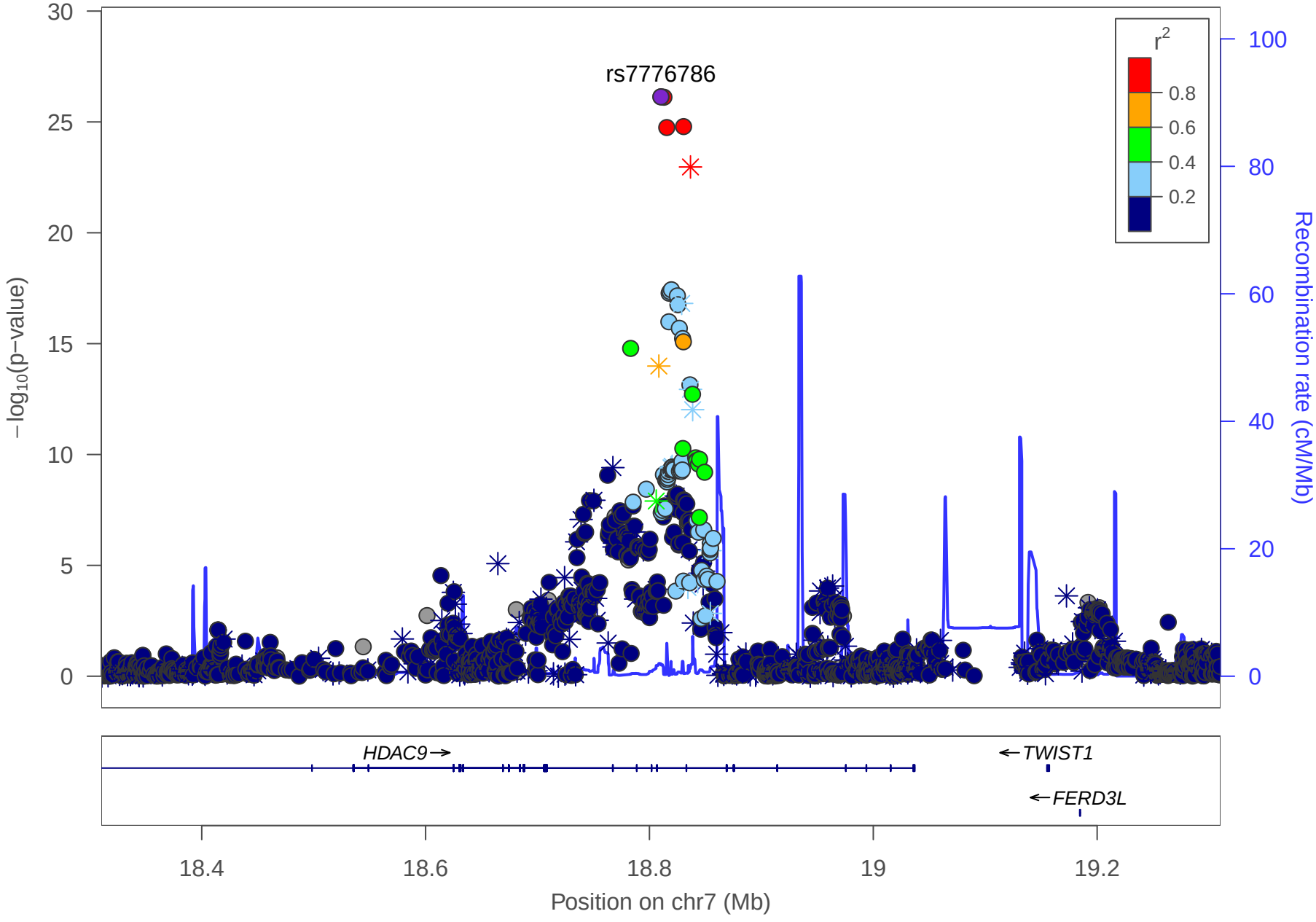

r2
0.8
0.6
0.4
0.2
rs6899968
10
8
6
4
2
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
←BMP5
←COL21A1
←RNU6−71P
←DST
55.8
56
56.2
56.4
56.6
Position on chr6 (Mb)

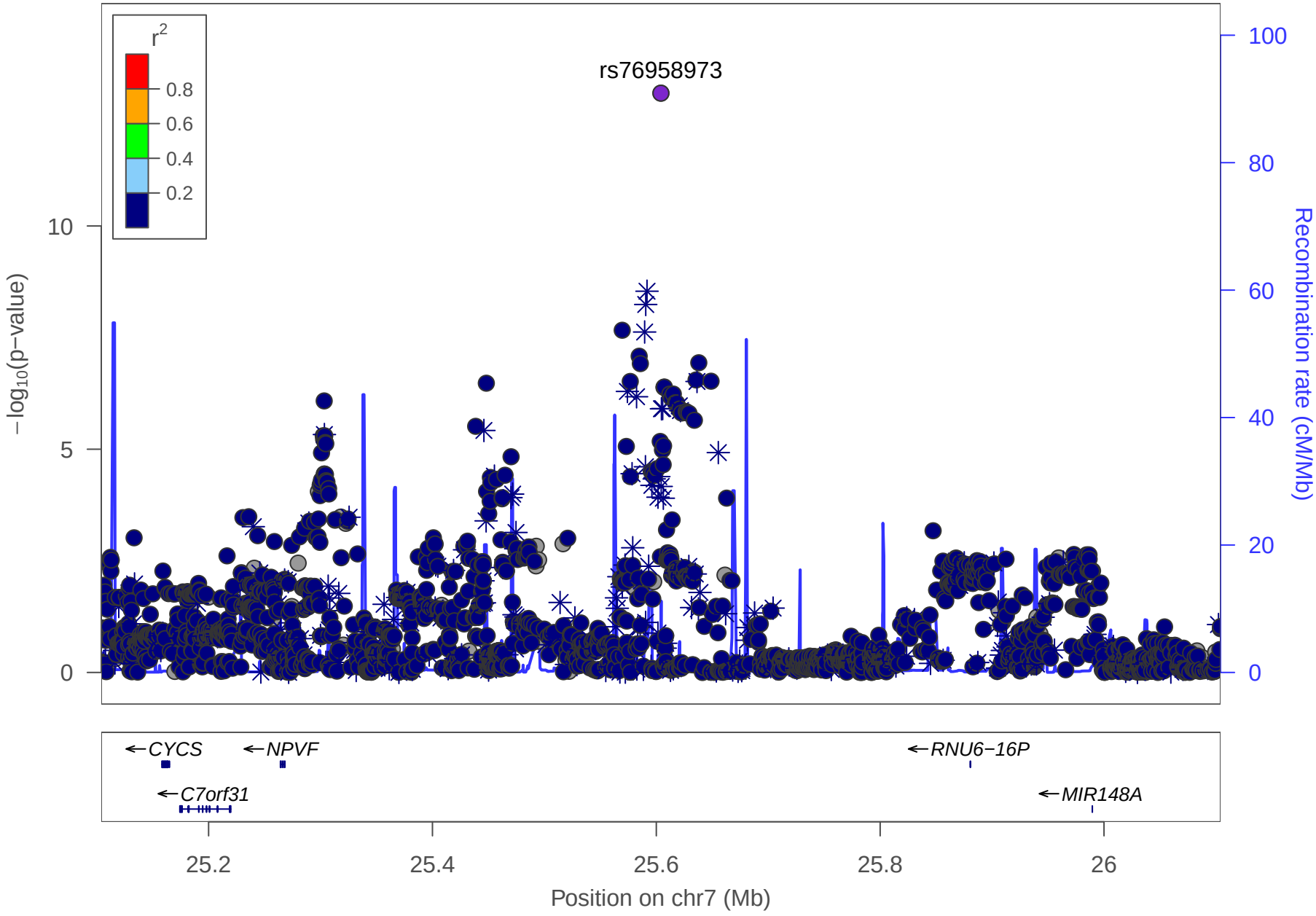

rs61123157
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
25
20
15
10
5
0
100
80
60
40
20
0
PRDM1
ATG5
AIM1
RTN4IP1
QRSL1
106.2
106.4
106.6
106.8
107
Position on chr6 (Mb)

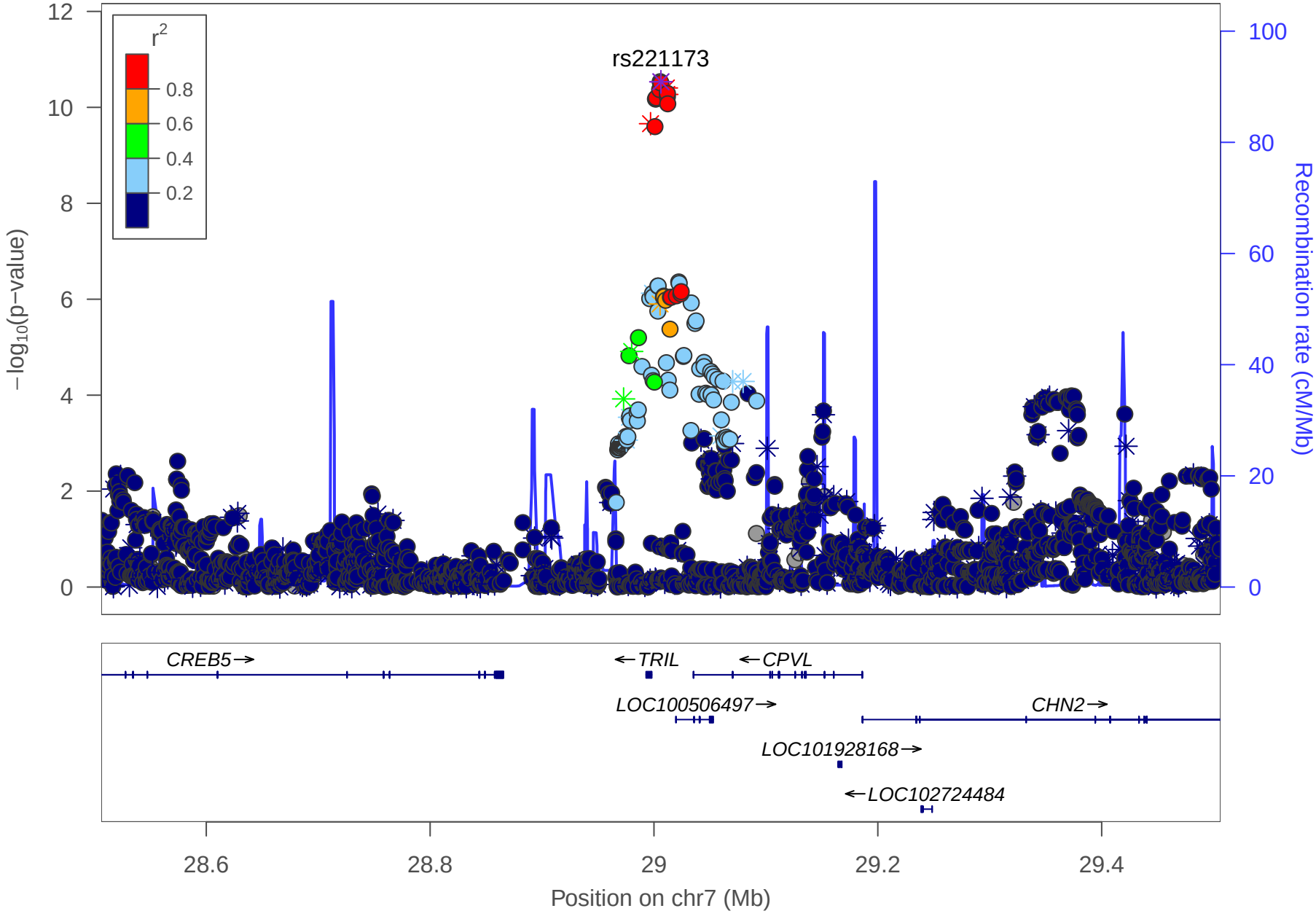

r2
0.8
0.6
0.4
0.2
rs9403317
10
8
6
4
2
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
141.4
141.6
141.8
142
142.2
Position on chr6 (Mb)

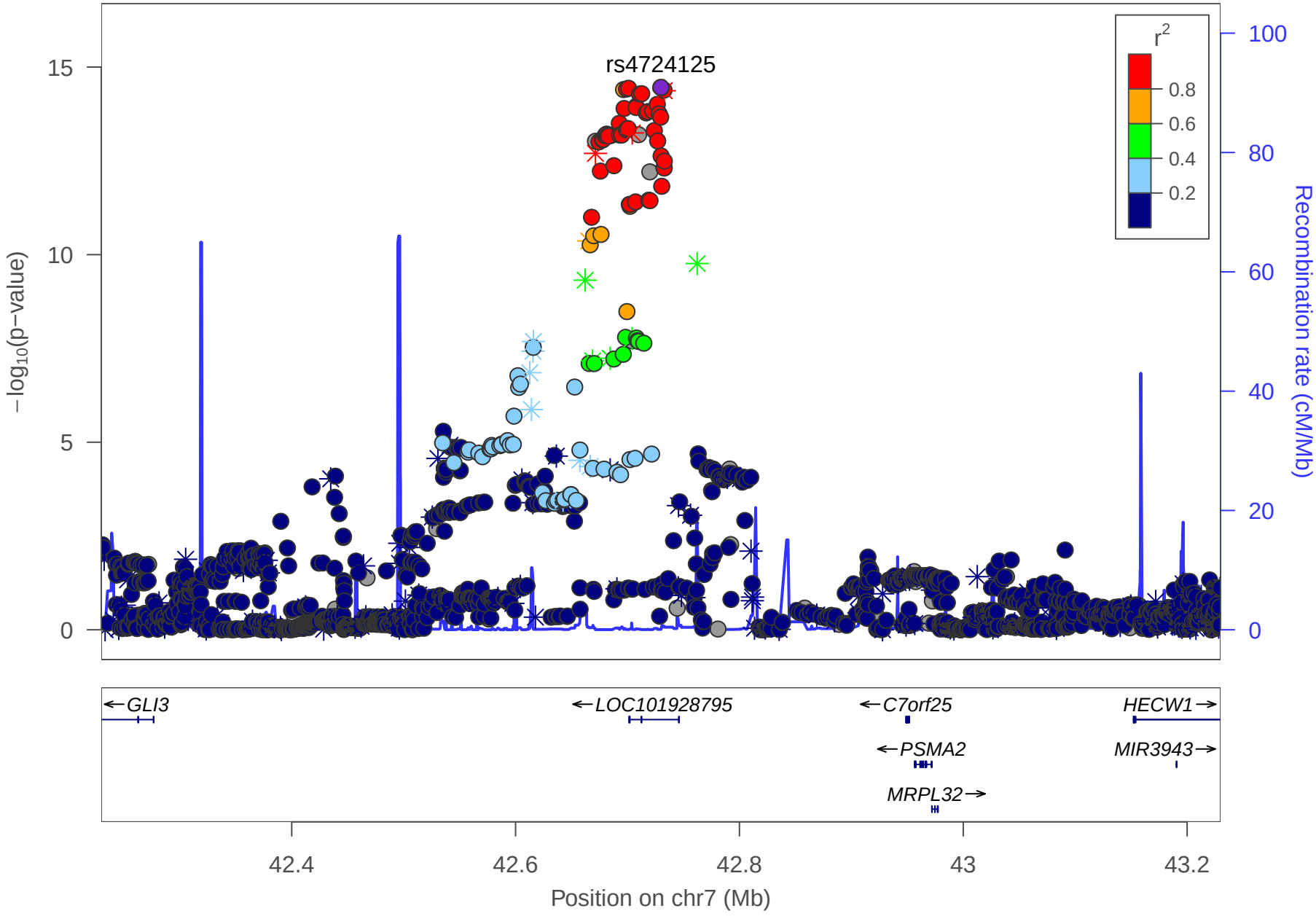

rs28514730
r2
0.8
0.6
0.4
0.2
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
VTA1
GPR126
LOC153910
HIVEP2
AIG1
LINC01277
142.6
142.8
143
143.2
143.4
Position on chr6 (Mb)

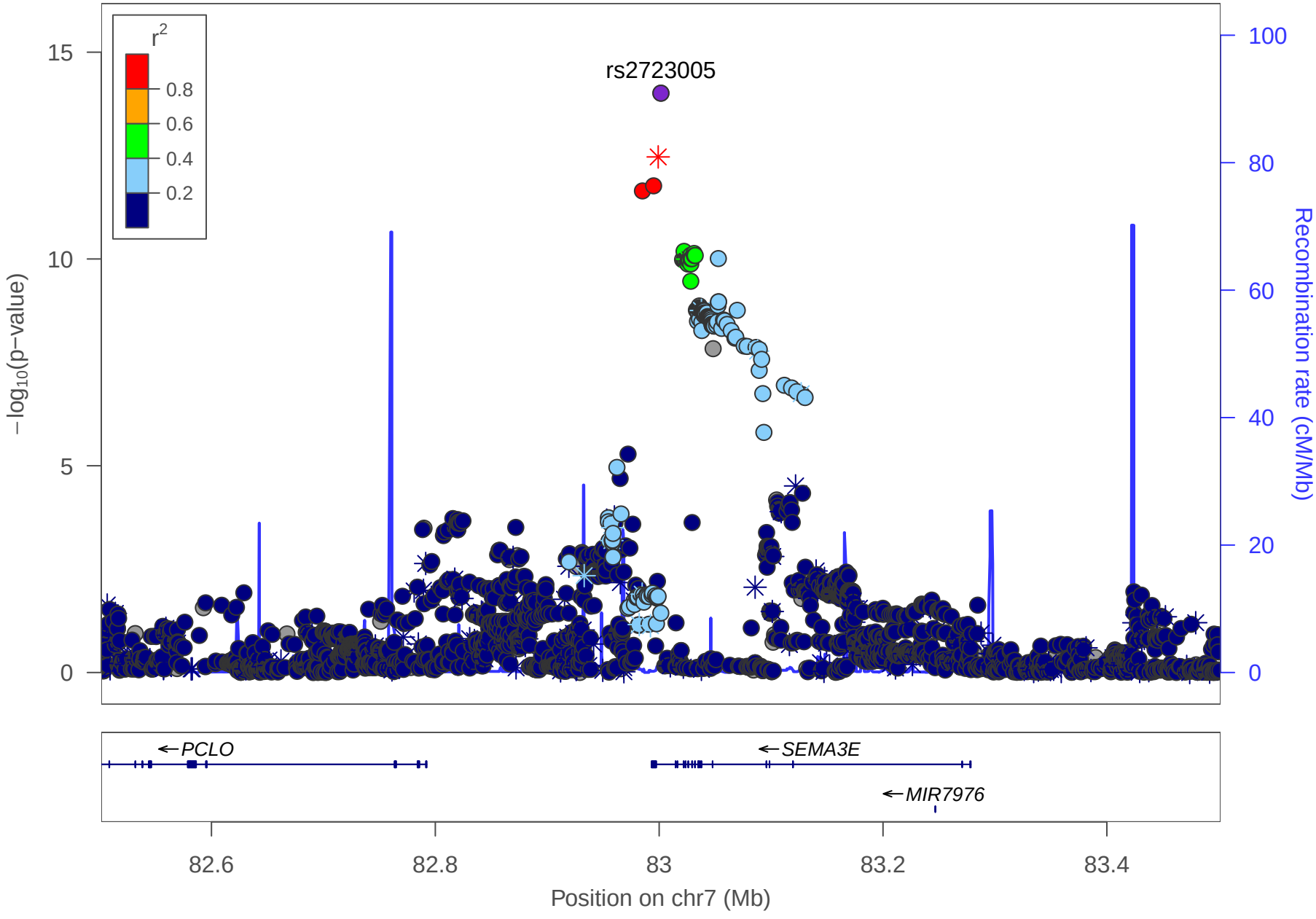

r2
0.8
0.6
0.4
0.2
rs375873
−log10(p−value)
Recombination rate (cM/Mb)
0
10
20
30
40
50
0
20
40
60
80
100
EPM2A
FBXO30
SHPRH
LOC100507557
145.4
145.6
145.8
146
146.2
Position on chr6 (Mb)

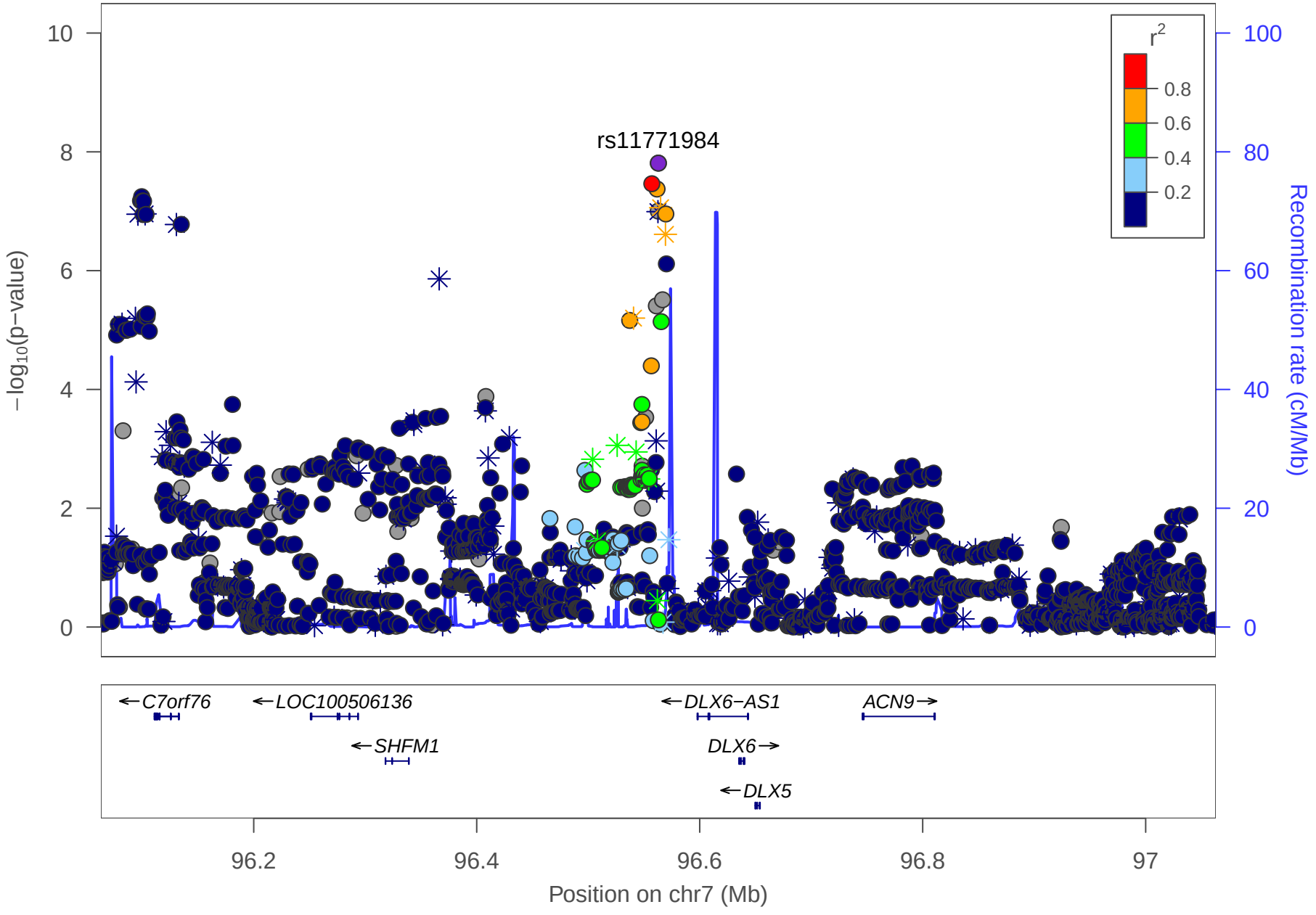

rs7776786
r2
0.8
0.6
0.4
0.2
30
25
20
15
10
5
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
HDAC9
TWIST1
FERD3L
18.4
18.6
18.8
19
19.2
Position on chr7 (Mb)

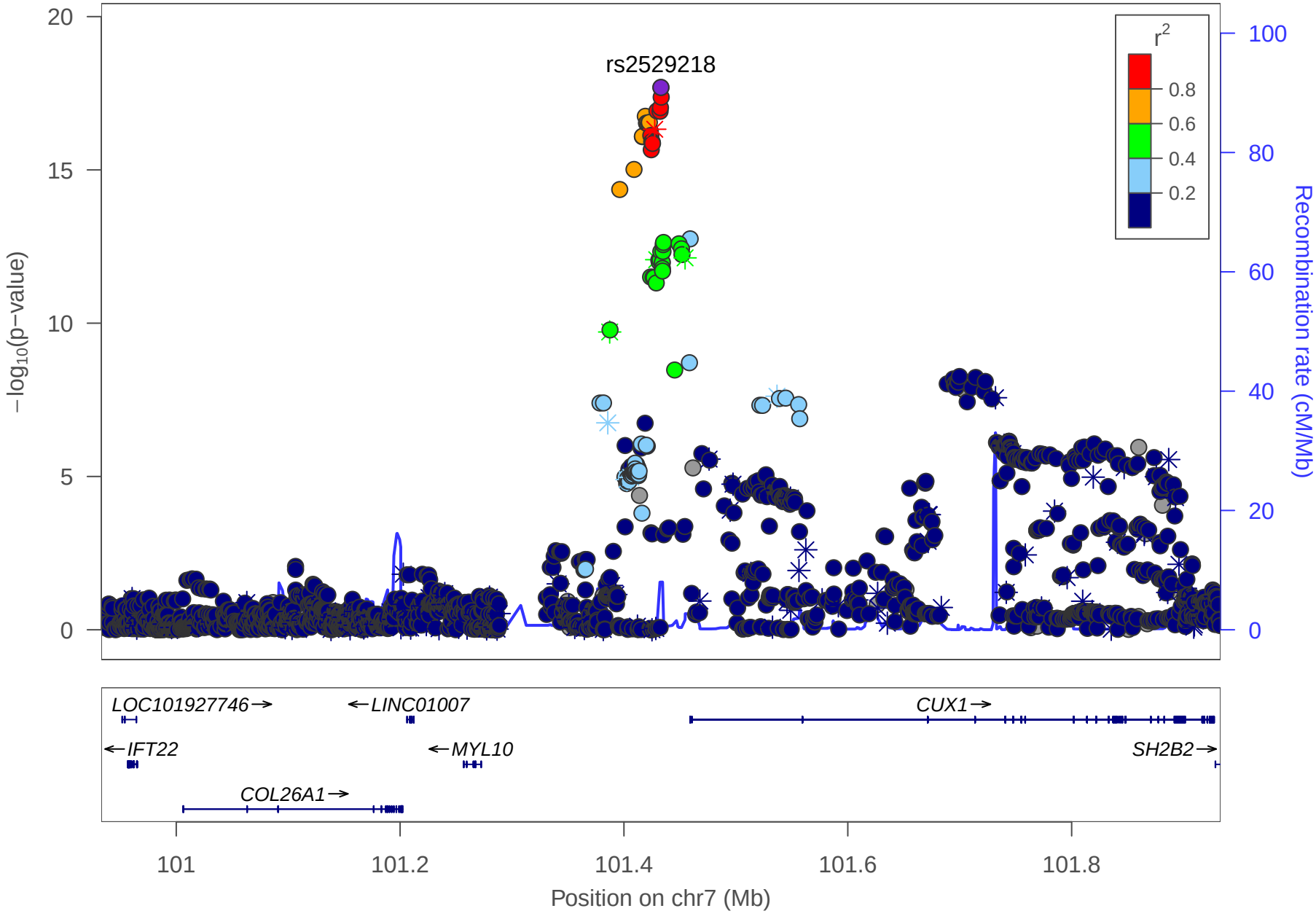

r2
0.8
0.6
0.4
0.2
rs76958973
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←CYCS
←NPVF
←RNU6−16P
←C7orf31
←MIR148A
25.2
25.4
25.6
25.8
26
Position on chr7 (Mb)

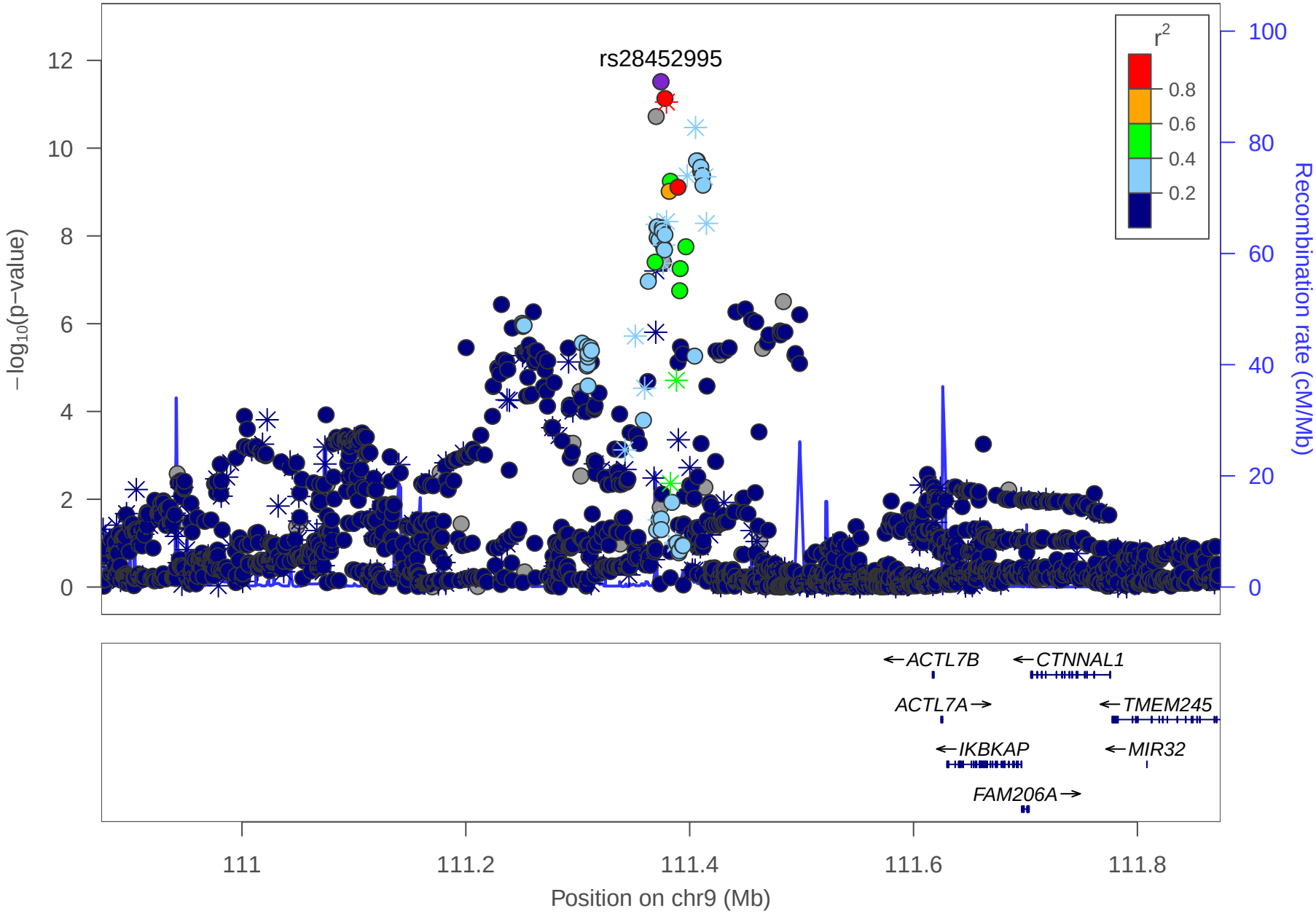

rs221173
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
CREB5→
←TRIL
←CPVL
LOC100506497→
CHN2→
LOC101928168→
←LOC102724484
Position on chr7 (Mb)

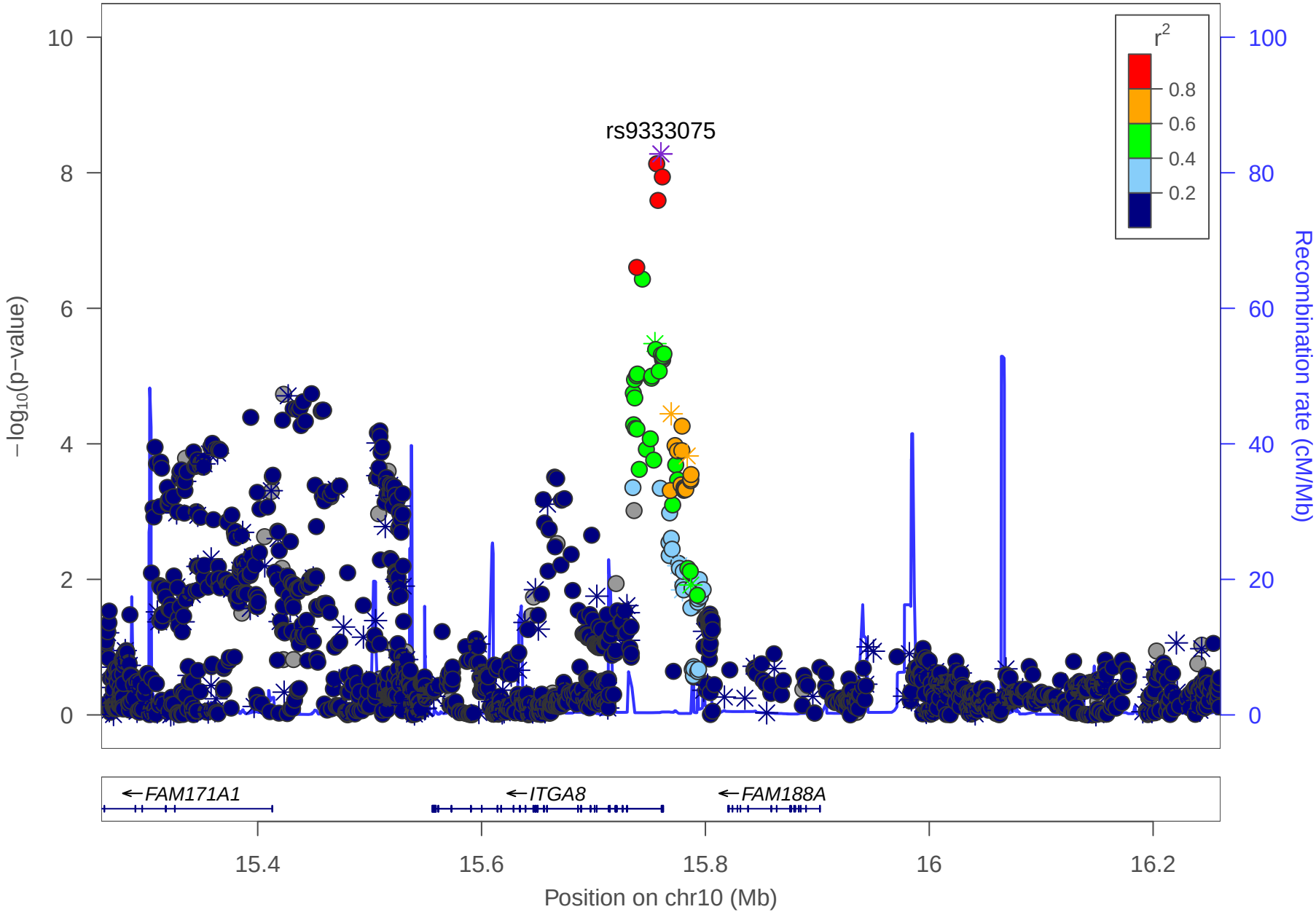

rs4724125
r2
0.8
0.6
0.4
0.2
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←GLI3
←LOC101928795
←C7orf25
HECW1→
←PSMA2
MIR3943→
MRPL32→
42.4
42.6
42.8
43
43.2
Position on chr7 (Mb)

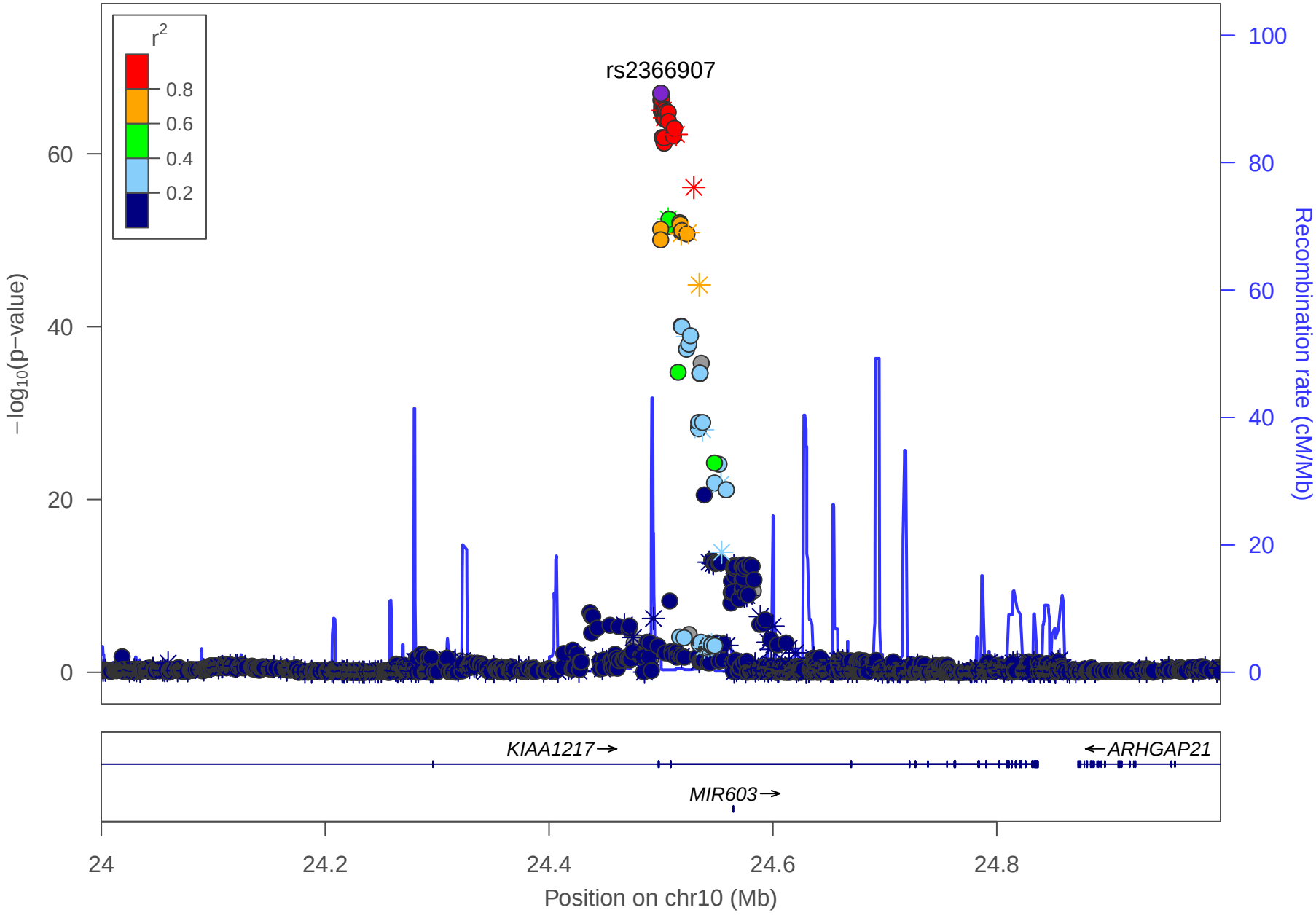

r2
0.8
0.6
0.4
0.2
rs2723005
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←PCLO
←SEMA3E
←MIR7976
82.6
82.8
83
83.2
83.4
Position on chr7 (Mb)

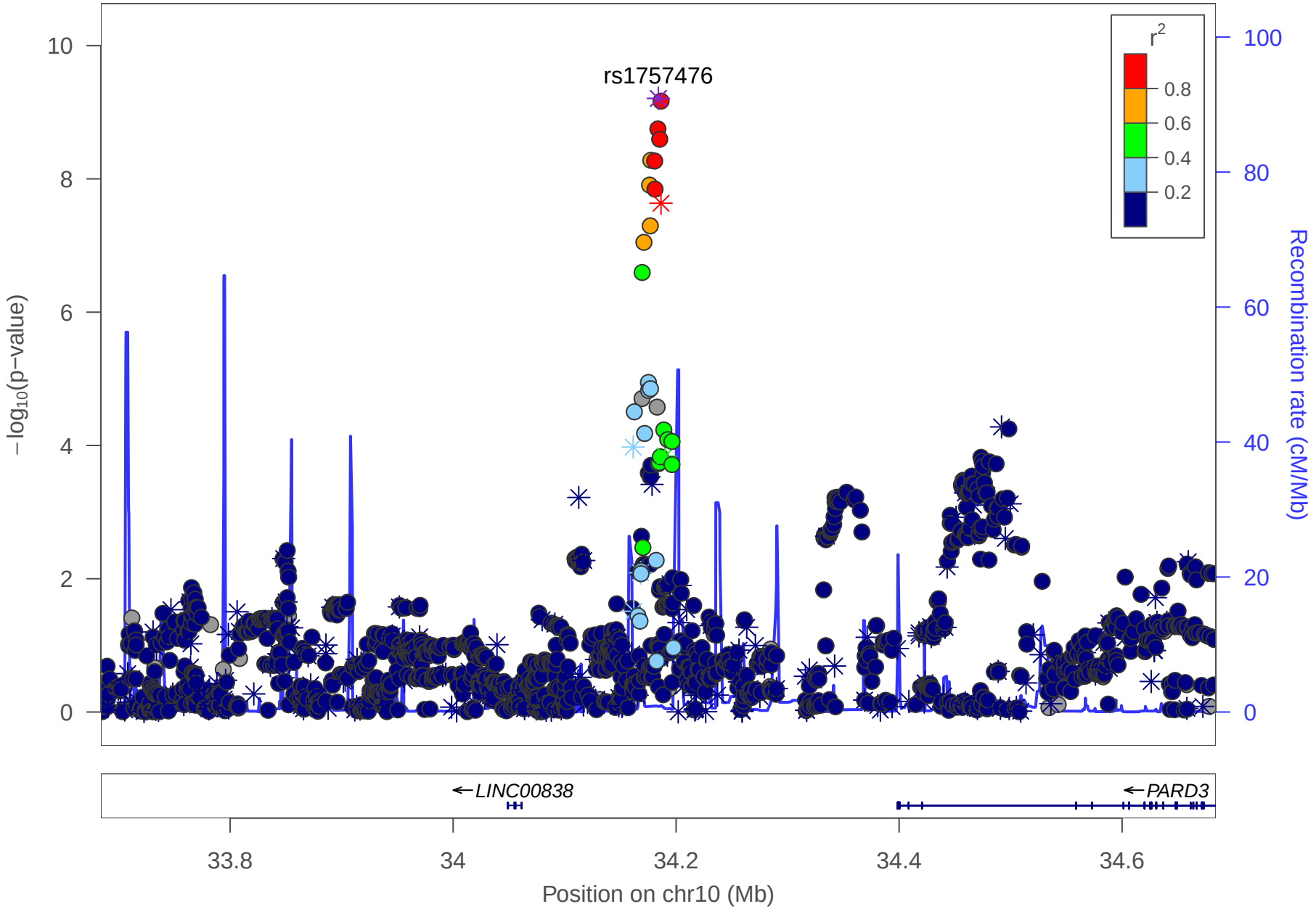

rs11771984
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
←C7orf76
←LOC100506136
←SHFM1
←DLX6−AS1
ACN9→
DLX6→
←DLX5
96.2
96.4
96.6
96.8
97
Position on chr7 (Mb)

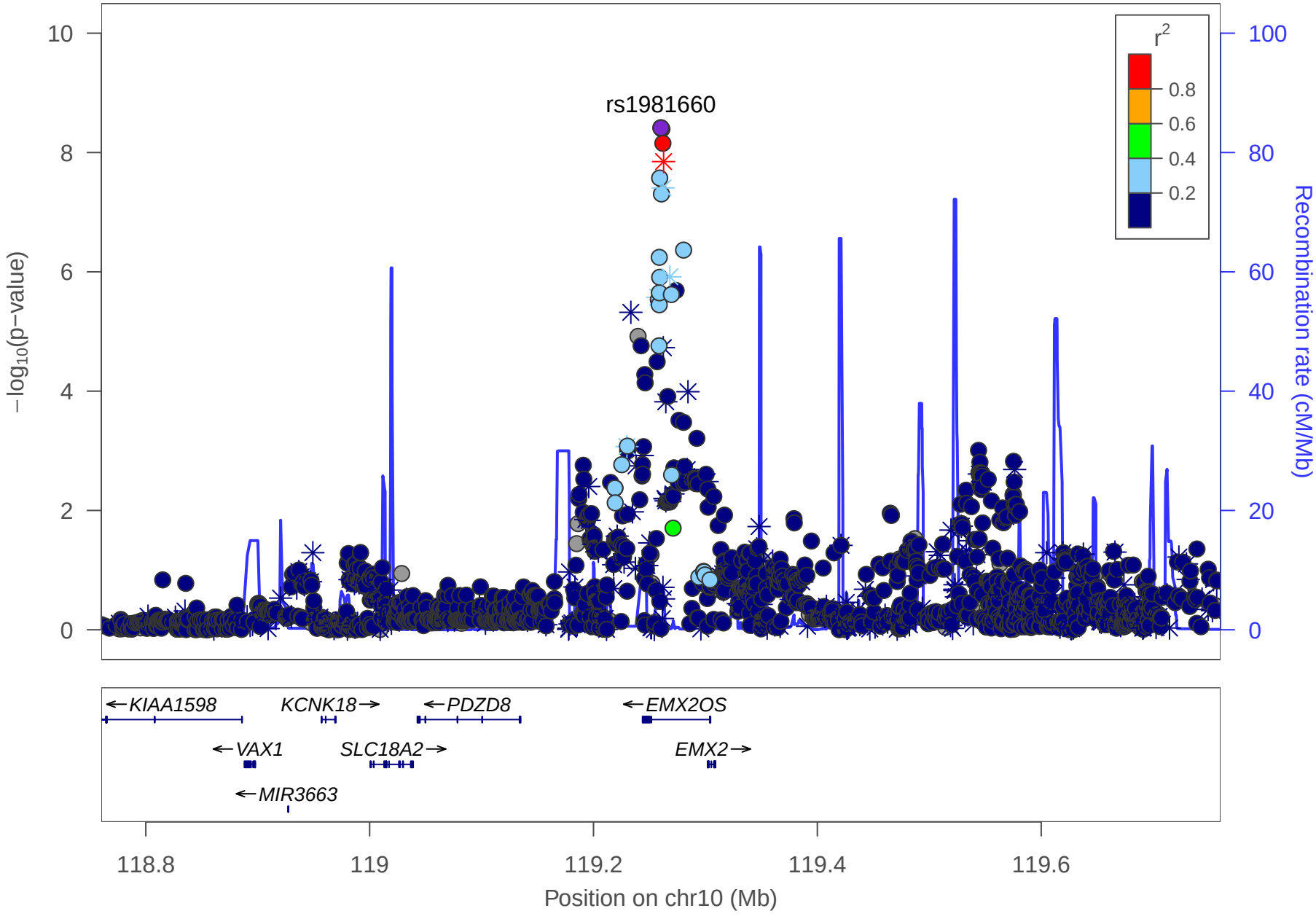

rs2529218
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
20
15
10
5
0
100
80
60
40
20
0
LOC101927746
LINC01007
CUX1
IFT22
MYL10
SH2B2
COL26A1
101
101.2
101.4
101.6
101.8
Position on chr7 (Mb)

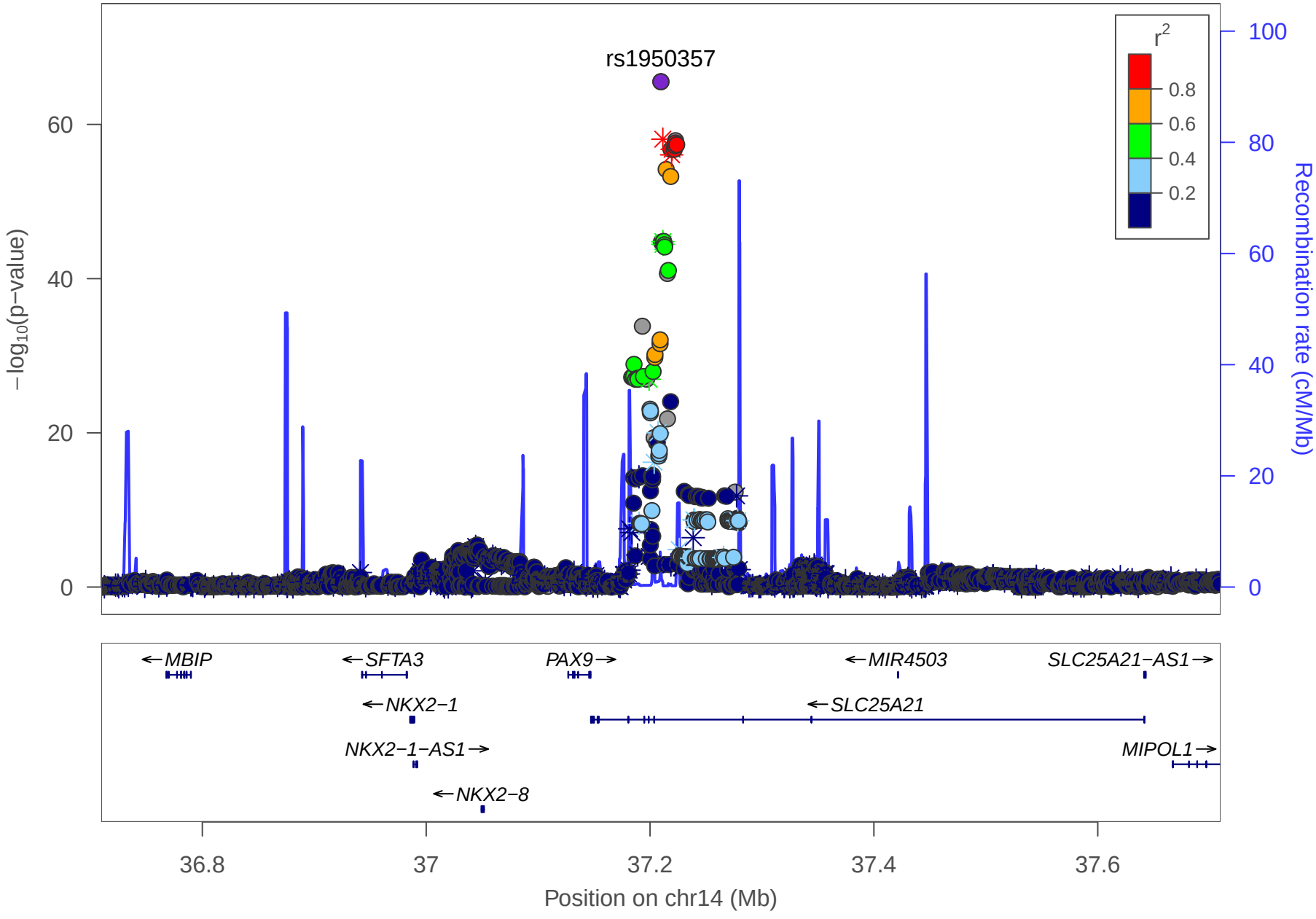

rs28452995
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
0
2
4
6
8
10
12
0
20
40
60
80
100
ACTL7B
CTNNAL1
ACTL7A
TMEM245
IKBKAP
MIR32
FAM206A
111
111.2
111.4
111.6
111.8
Position on chr9 (Mb)

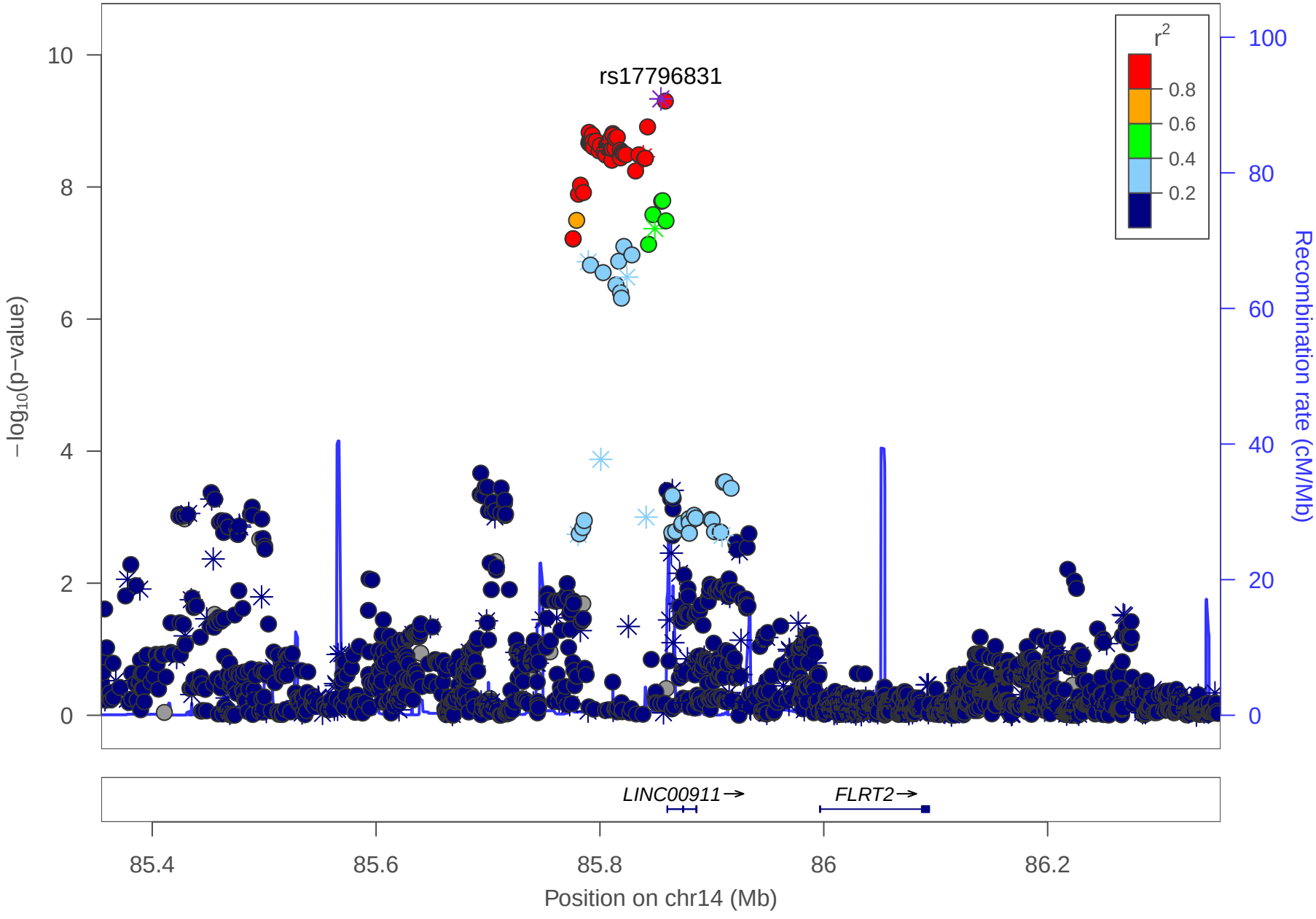

rs9333075
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
FAM171A1
ITGA8
FAM188A
15.4
15.6
15.8
16
16.2
Position on chr10 (Mb)

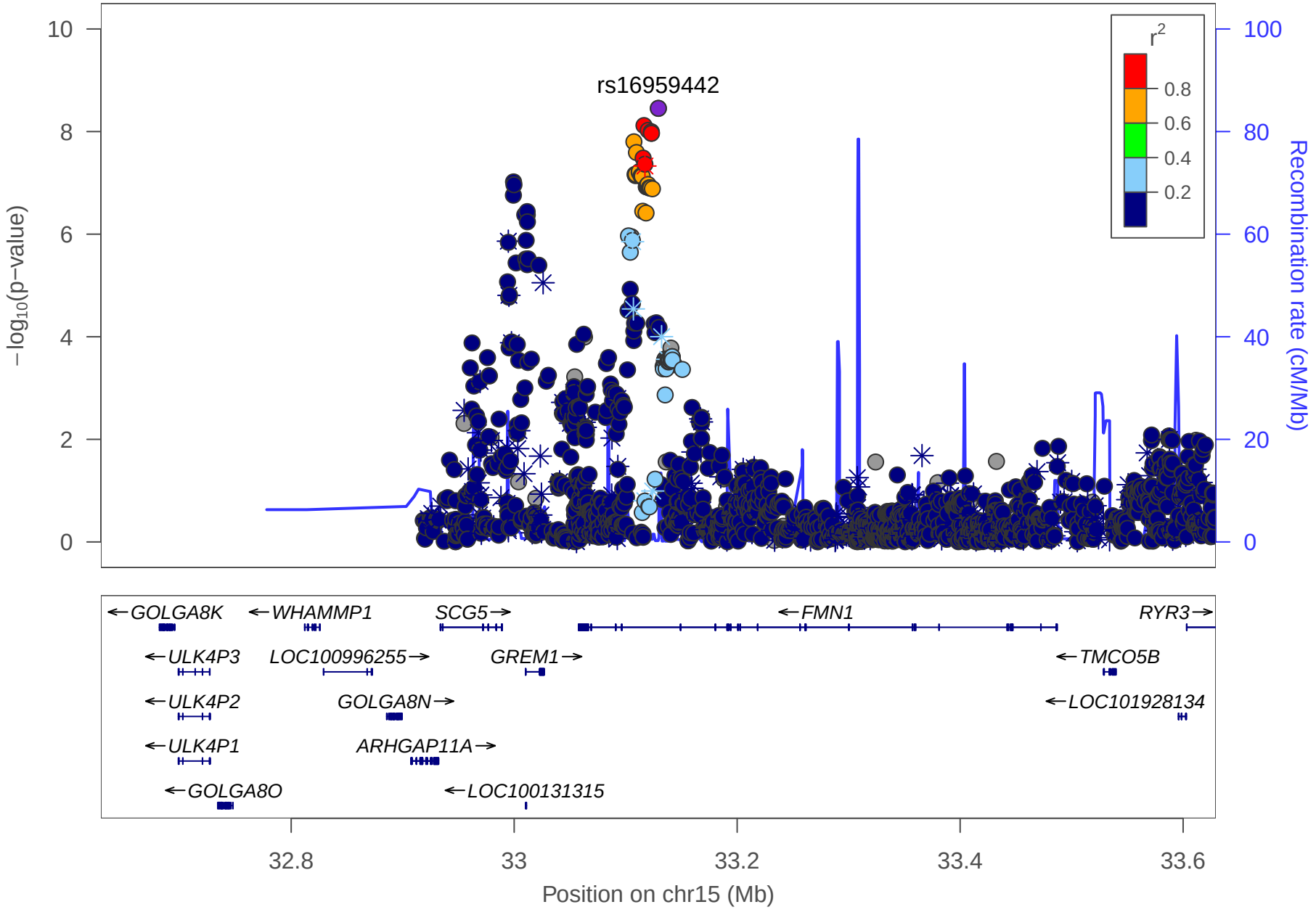

r2
0.8
0.6
0.4
0.2
rs2366907
60
40
20
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
KIAA1217→
←ARHGAP21
MIR603→
24
24.2
24.4
24.6
24.8
Position on chr10 (Mb)

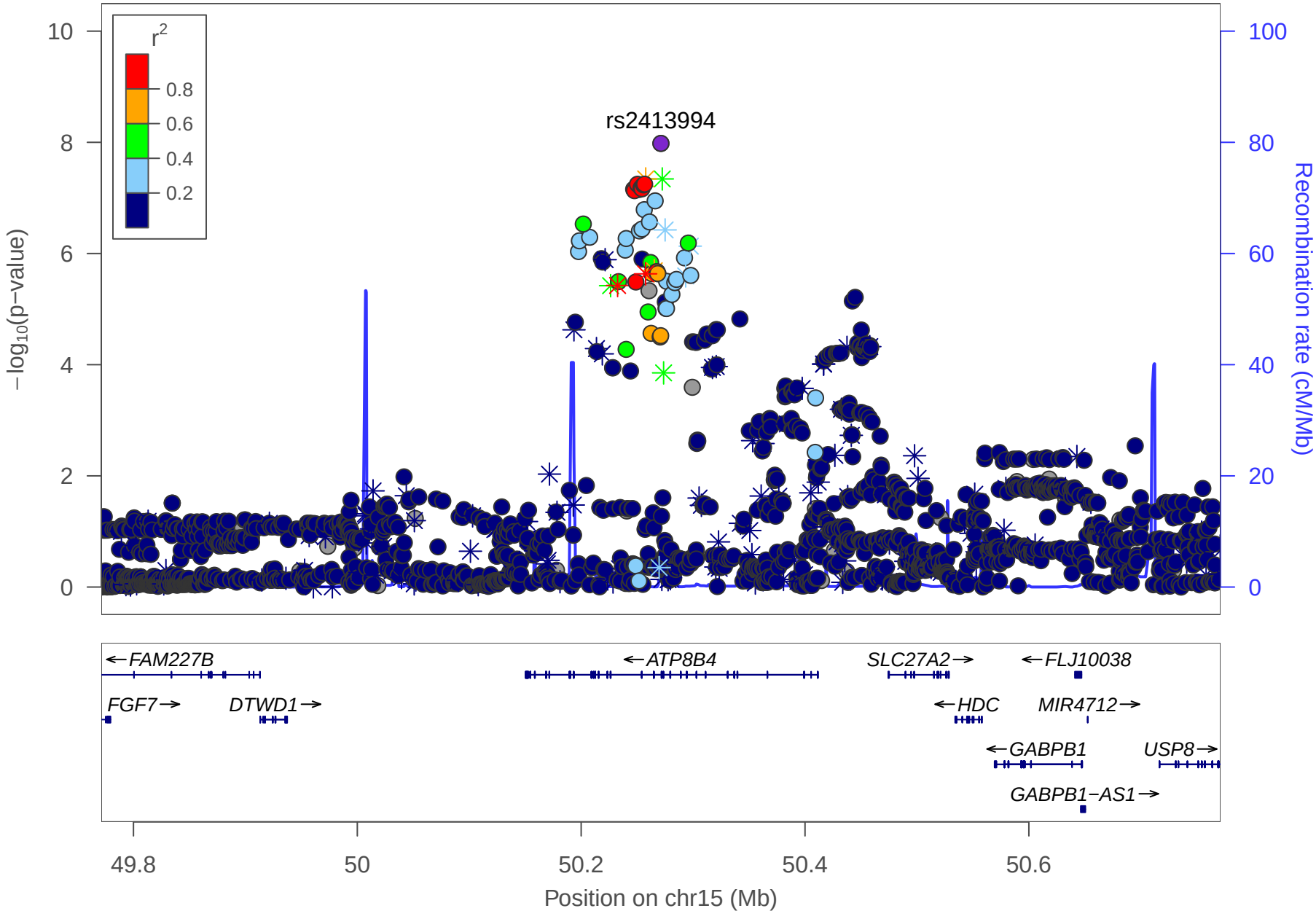

rs1757476
r²
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
←LINC00838
←PARD3
33.8
34
34.2
34.4
34.6
Position on chr10 (Mb)

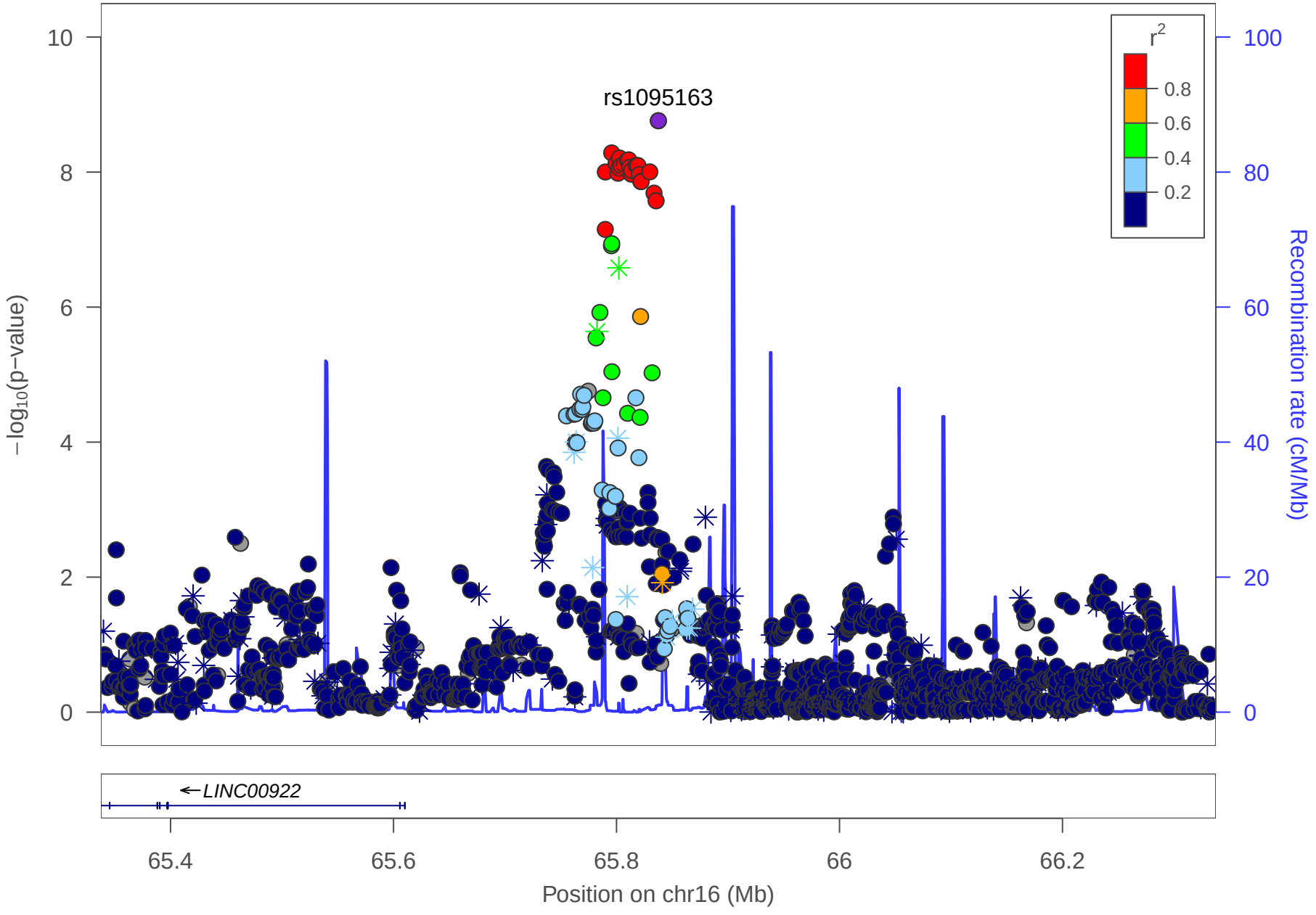

rs1981660
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
KIAA1598
KCNK18
PDZD8
EMX2OS
VAX1
SLC18A2
EMX2
MIR3663
118.8
119
119.2
119.4
119.6
Position on chr10 (Mb)

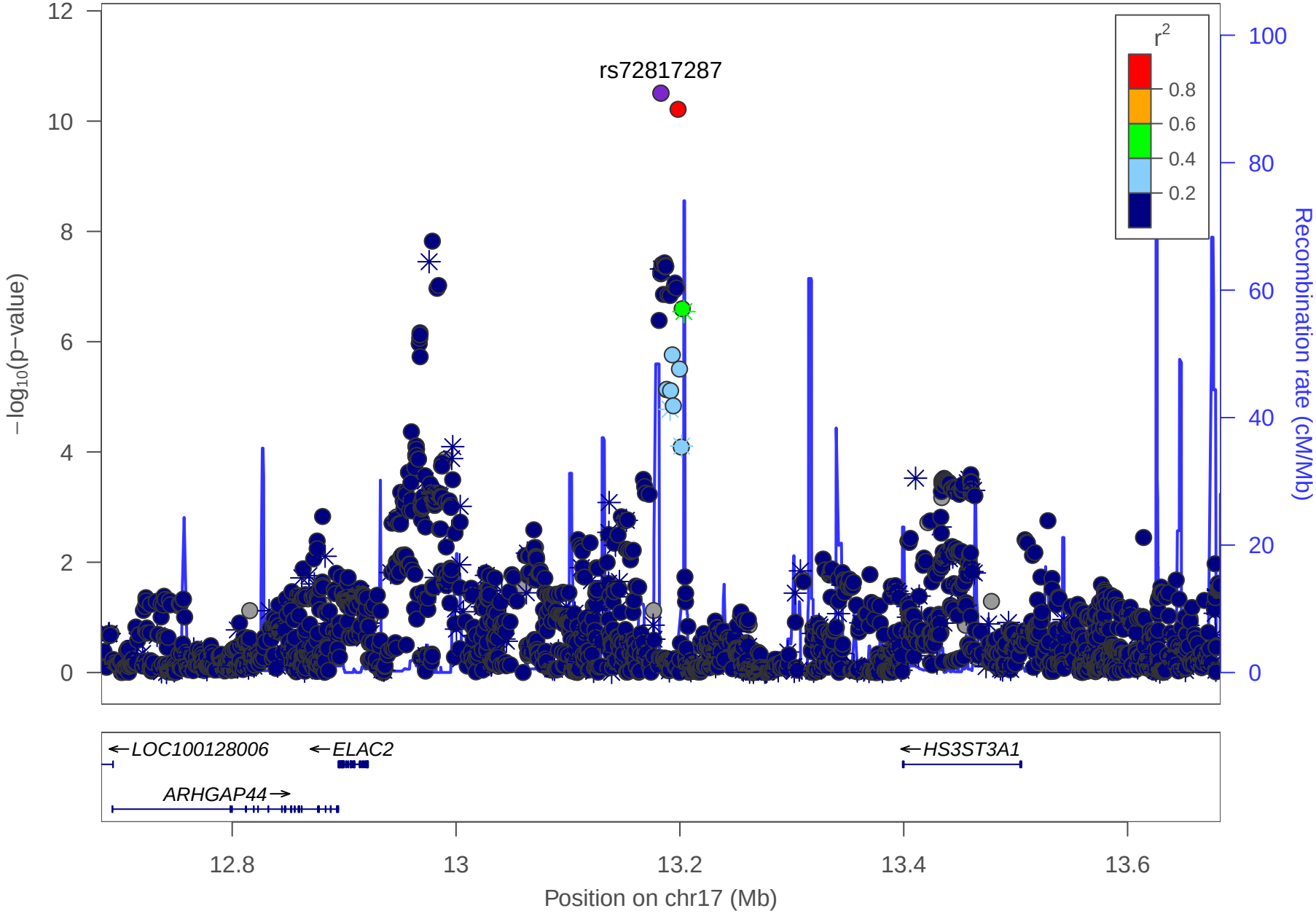

rs1950357
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
0
20
40
60
0
20
40
60
80
100
←MBIP
←SFTA3
PAX9→
←MIR4503
SLC25A21−AS1→
←NKX2−1
←SLC25A21
NKX2−1−AS1→
MIPOL1→
←NKX2−8
36.8
37
37.2
37.4
37.6
Position on chr14 (Mb)

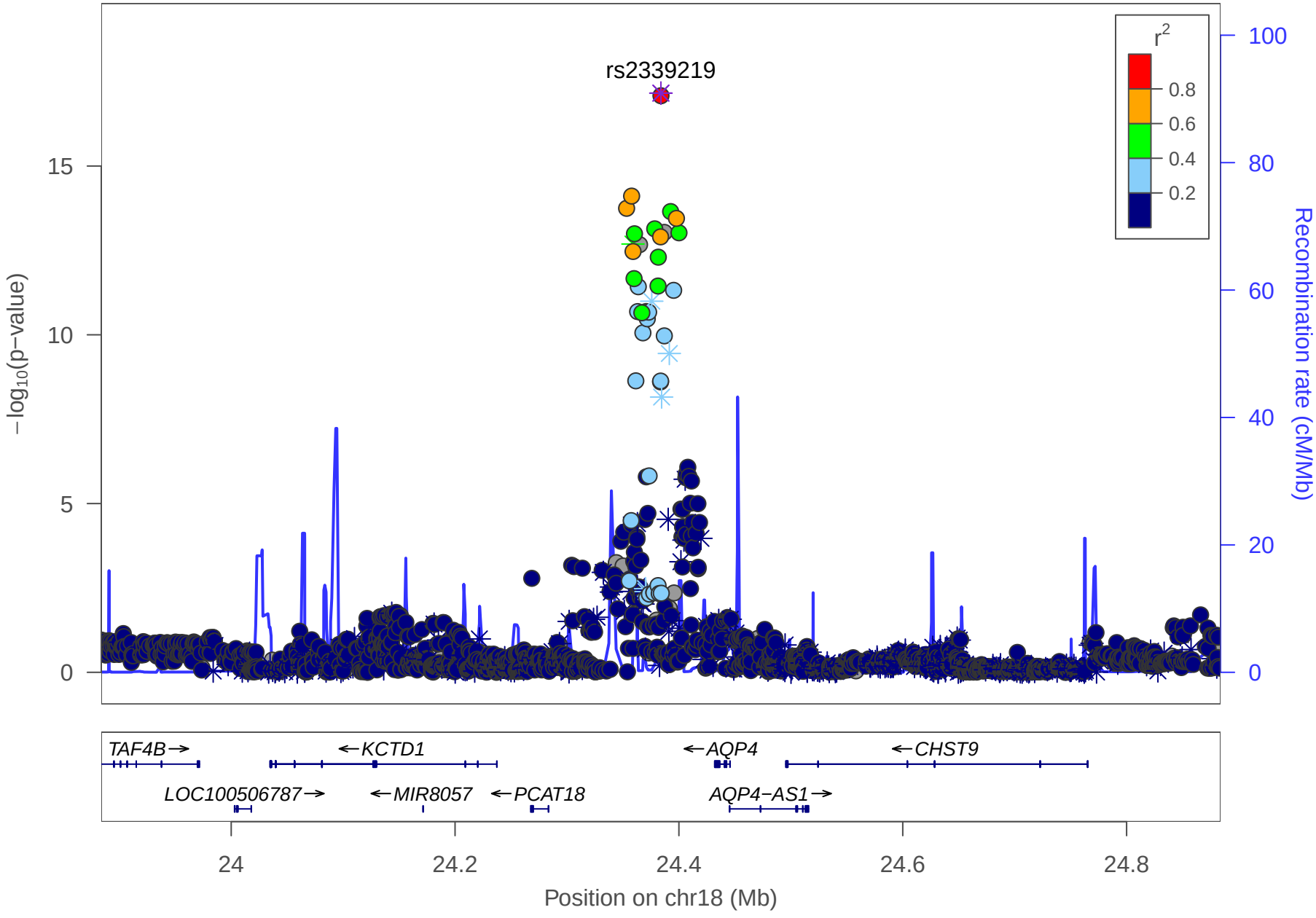

rs17796831
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
LINC00911
FLRT2
Position on chr14 (Mb)
85.4
85.6
85.8
86
86.2

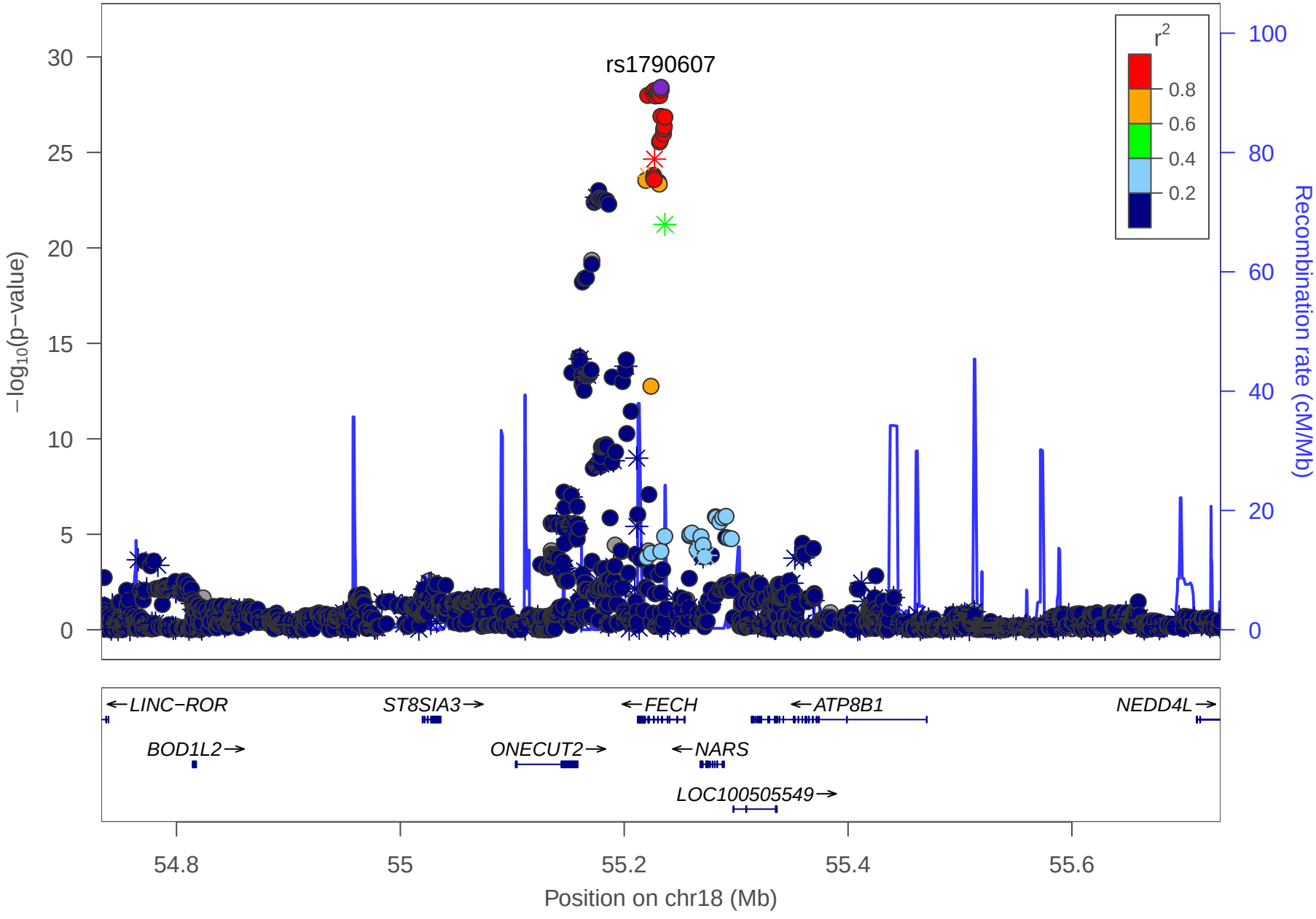

rs16959442
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
GOLGA8K
WHAMMP1
SCG5
FMN1
RYR3
ULK4P3
LOC100996255
GREM1
TMCO5B
ULK4P2
GOLGA8N
LOC101928134
ULK4P1
ARHGAP11A
GOLGA8O
LOC100131315
32.8
33
33.2
33.4
33.6
Position on chr15 (Mb)

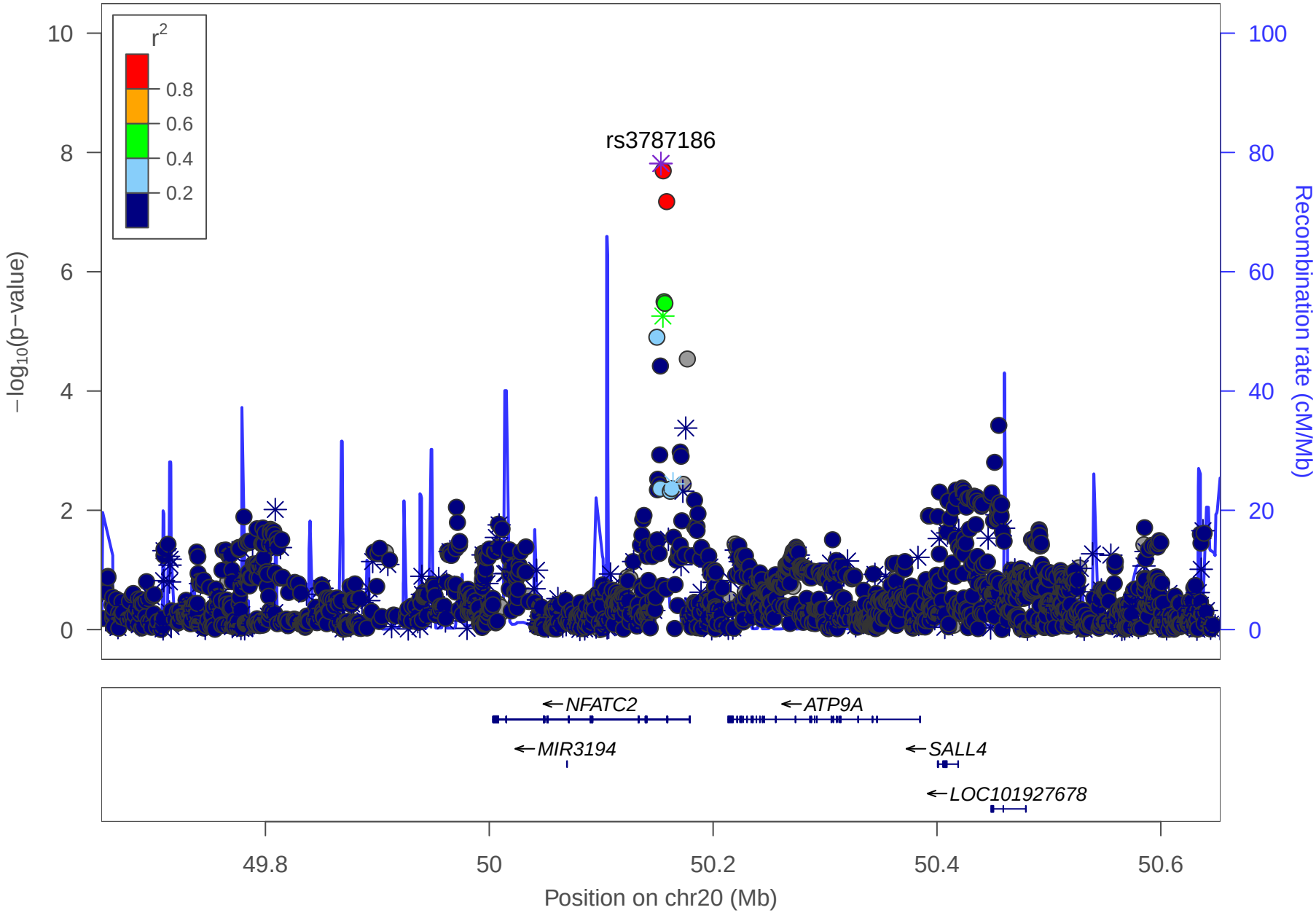

r2
0.8
0.6
0.4
0.2
rs2413994
−log10(p−value)
Recombination rate (cM/Mb)
FAM227B
ATP8B4
SLC27A2
FLJ10038
FGF7
DTWD1
HDC
MIR4712
GABPB1
USP8
GABPB1−AS1
Position on chr15 (Mb)


rs1095163
r2
0.8
0.6
0.4
0.2
10
8
6
4
2
0
100
80
60
40
20
0
−log10(p−value)
Recombination rate (cM/Mb)
←LINC00922
65.4
65.6
65.8
66
66.2
Position on chr16 (Mb)


rs72817287
r2
0.8
0.6
0.4
0.2
12
10
8
6
4
2
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
←LOC100128006
←ELAC2
←HS3ST3A1
ARHGAP44→
12.8
13
13.2
13.4
13.6
Position on chr17 (Mb)


rs2339219
r2
0.8
0.6
0.4
0.2
15
10
5
0
−log10(p−value)
100
80
60
40
20
0
Recombination rate (cM/Mb)
TAF4B
KCTD1
AQP4
CHST9
LOC100506787
MIR8057
PCAT18
AQP4−AS1
24
24.2
24.4
24.6
24.8
Position on chr18 (Mb)


rs1790607
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
30
25
20
15
10
5
0
100
80
60
40
20
0
←LINC−ROR
ST8SIA3→
←FECH
←ATP8B1
NEDD4L→
BOD1L2→
ONECUT2→
←NARS
LOC100505549→
54.8
55
55.2
55.4
55.6
Position on chr18 (Mb)


r2
0.8
0.6
0.4
0.2
rs3787186
−log10(p−value)
Recombination rate (cM/Mb)
10
8
6
4
2
0
100
80
60
40
20
0
NFATC2
ATP9A
MIR3194
SALL4
LOC101927678
49.8
50
50.2
50.4
50.6
Position on chr20 (Mb)

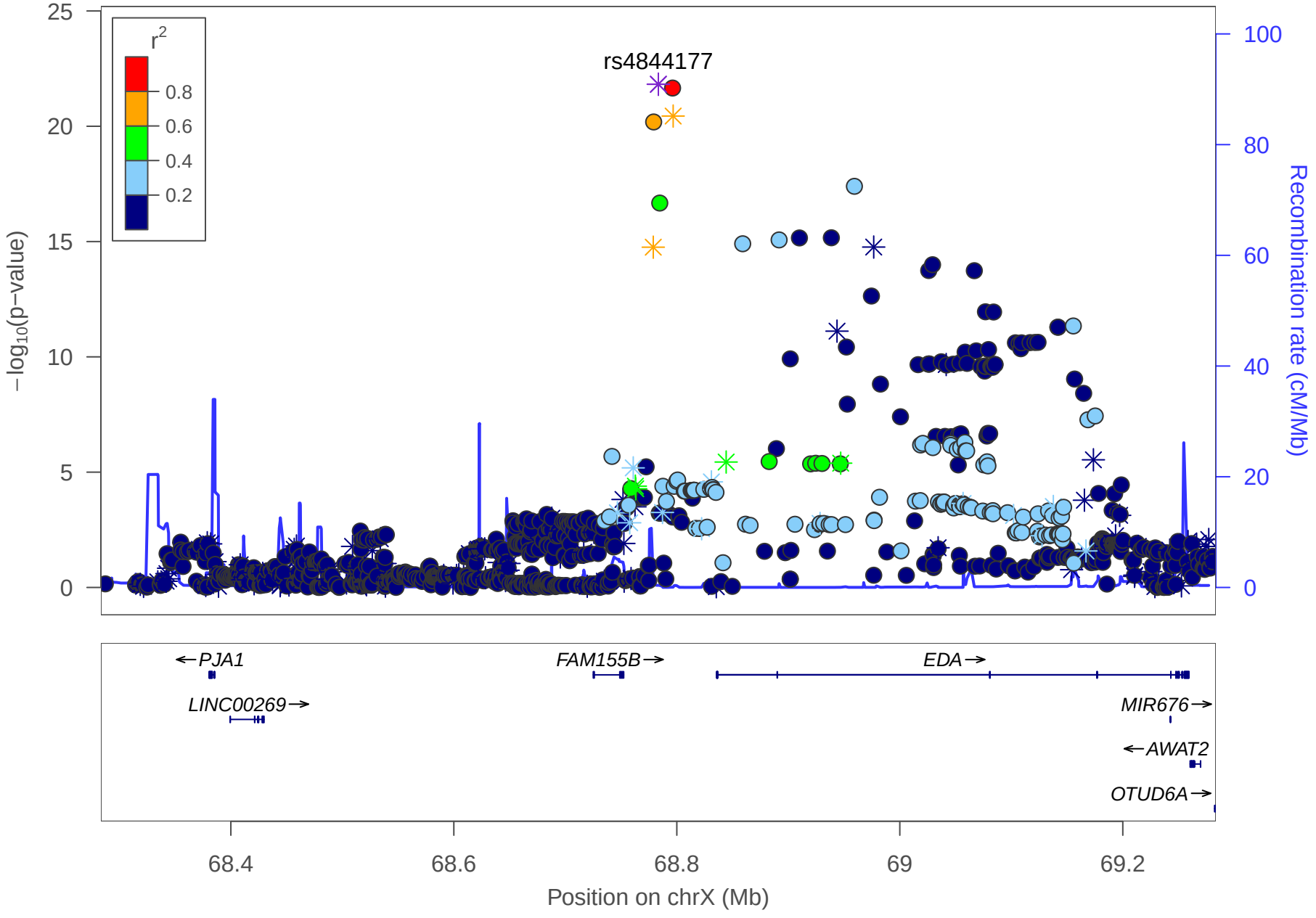

rs4844177
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
25
20
15
10
5
0
100
80
60
40
20
0
PJA1
FAM155B
EDA
LINC00269
MIR676
AWAT2
OTUD6A
68.4
68.6
68.8
69
69.2
Position on chrX (Mb)

Figure S2: Regional association plots showing significant associations observed in the meta-analysis across all four cohorts.

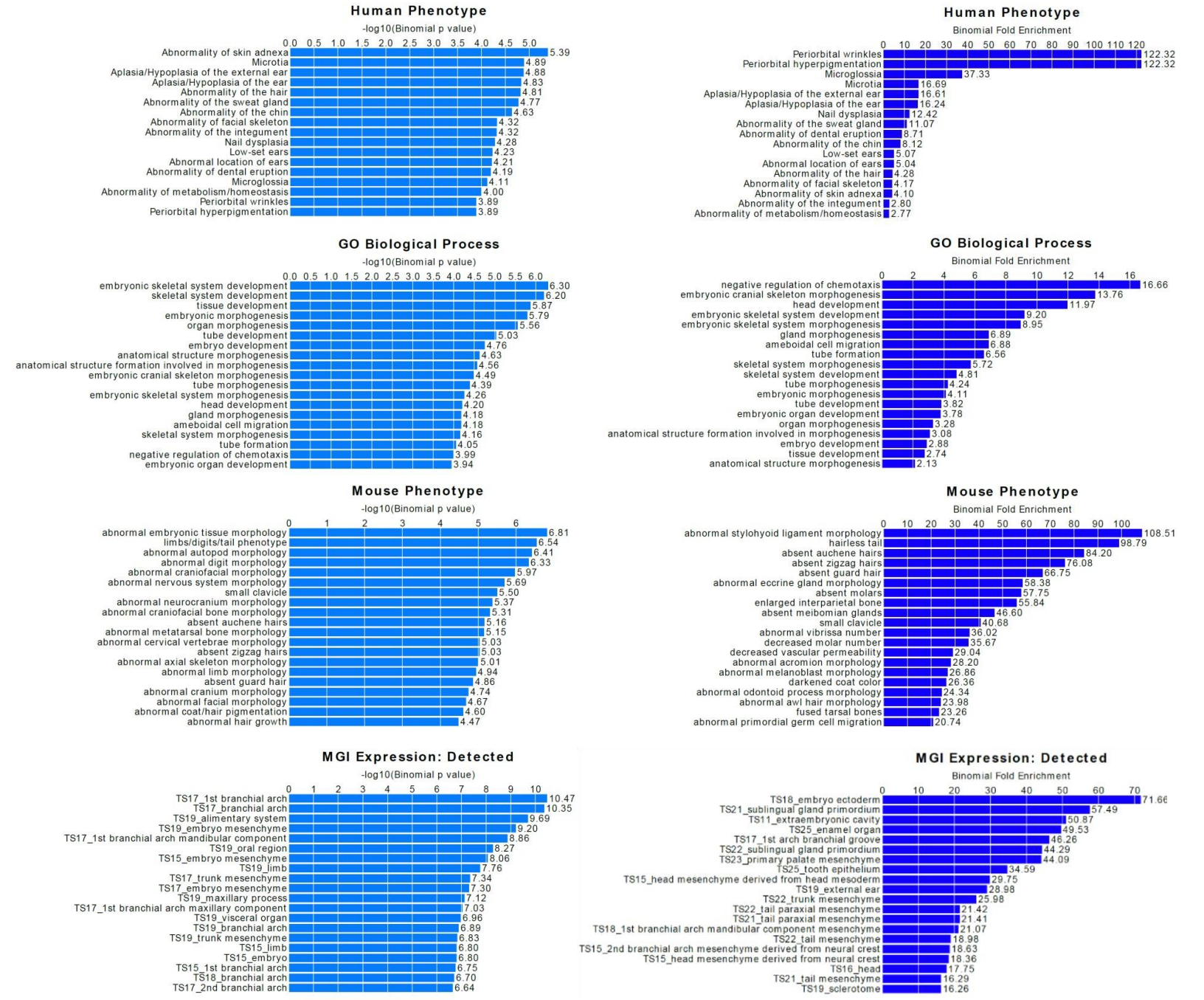


Figure S3: Enrichment for annotation across ontologies for 91 genes (i.e., nearest two genes up to 1000kb from the lead SNP) across 49 loci identified in the meta-analysis across all four cohorts. From top to bottom: human phenotype, Gene Ontology (GO) biological processes, mouse phenotype, and MGI expression ontologies. Left: significant annotations ordered by -log10-transformed p-values (up to 20 shown). Right: significant annotations order by fold enrichment (up to 20 shown).

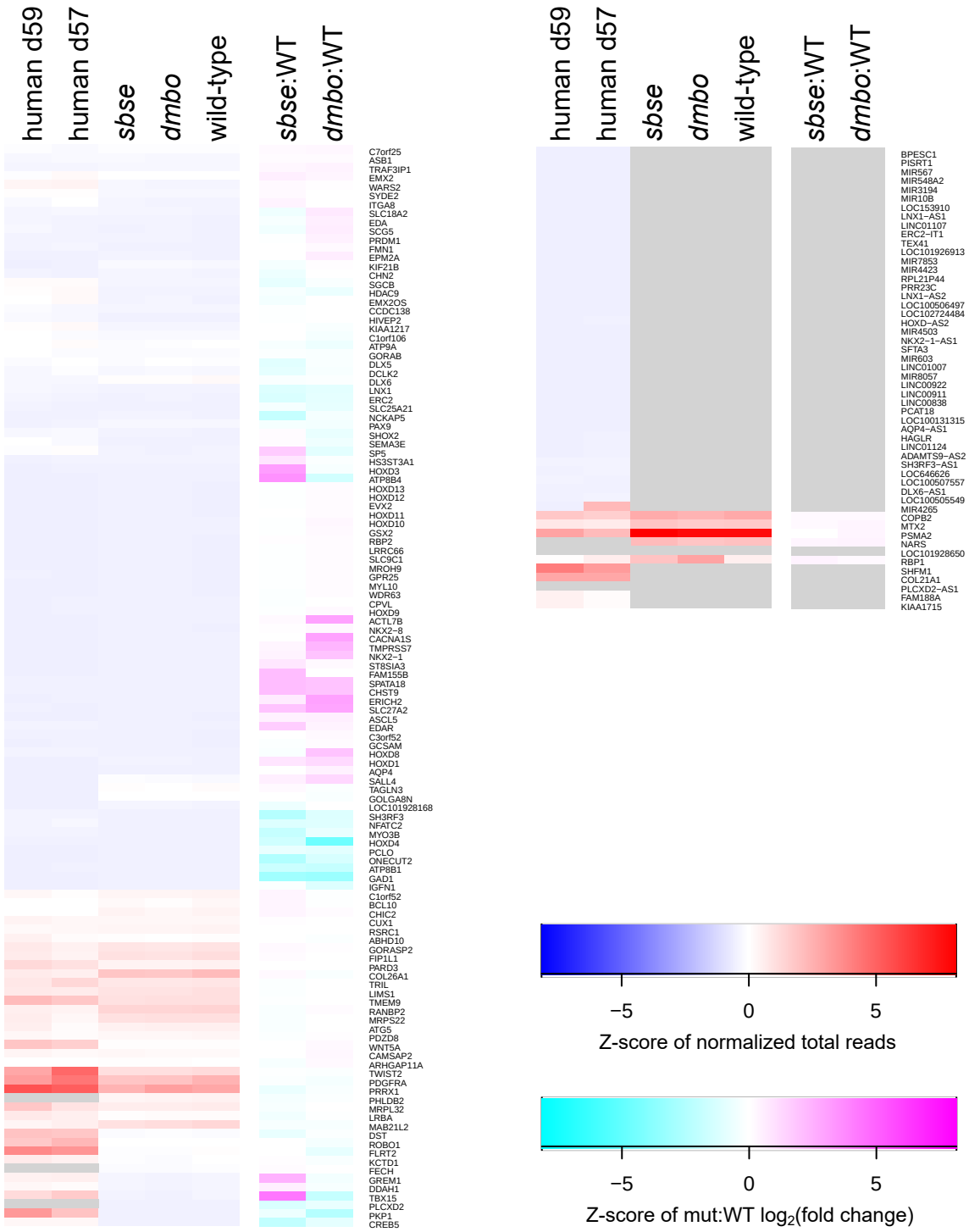


Figure S4: Heatmaps of gene expression in fetal human pinna (at days 59 and 57) and embryonic mouse second branchial arch tissue (in *sbse* mutants, *dmbo* mutants, and wild-type), and fold change of mutant mouse compared to wild-type mouse for 174 genes within 250 kb of the 49 lead SNPs observed in the genetic association meta-analysis across all cohorts. Genes are clustered by expression patterns with the bottom of the heatmap wrapping around to a second column. The shading scale is shown for Z-scores of expression and fold change. Genes for which expression data were not measured are shown in gray.

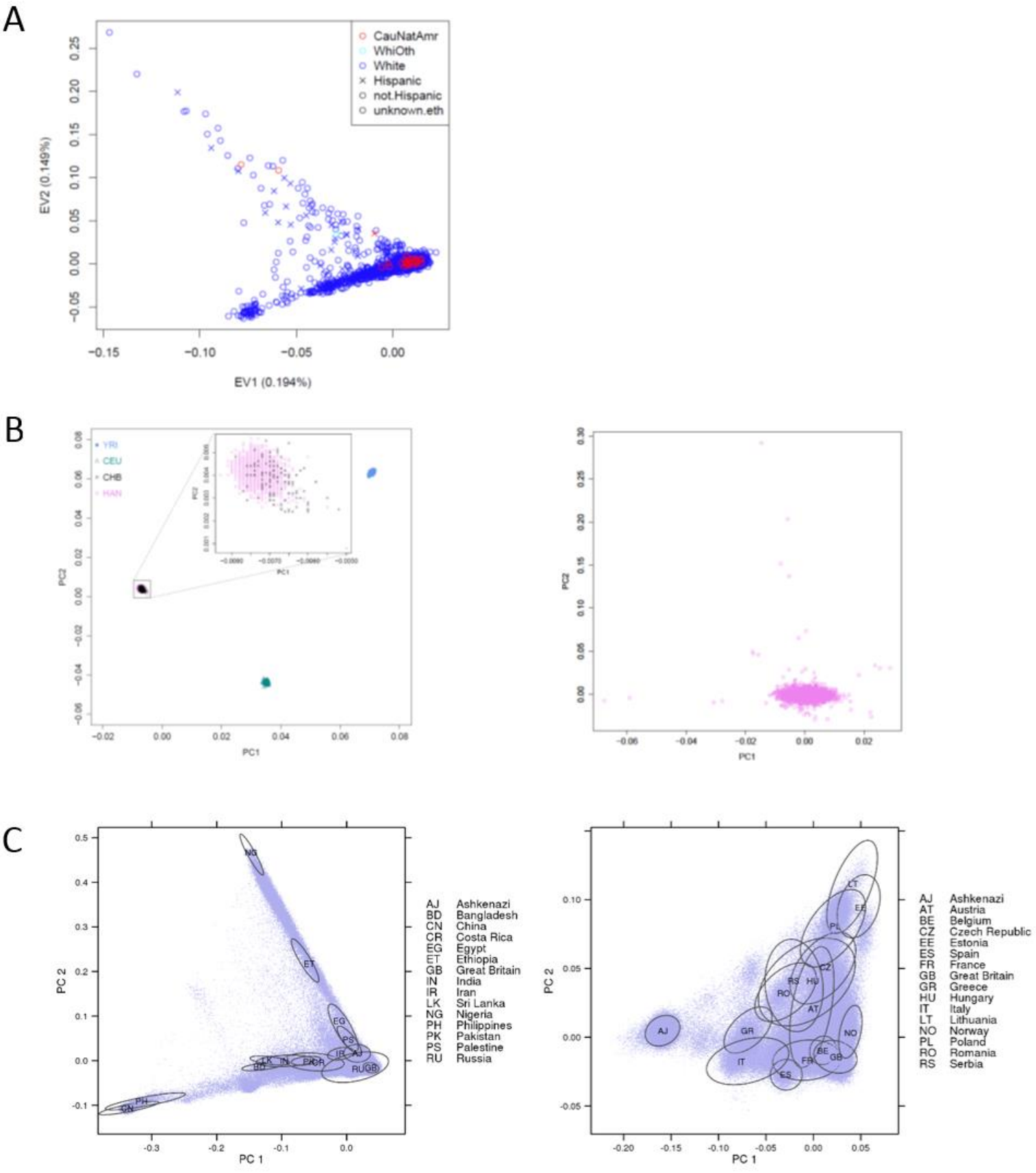


Figure S5: Population structure of cohorts is indicated by principal components of ancestry: (A) European American, (B) Chinese (left, with HapMap controls, right, analysis sample), and (C) 23andMe (left, entire sample, right, European American sample used in the analysis)

Table S1: Characteristics of the cohorts

	European American	Latin American	Chinese	23andMe European ancestry
N	1,791	5062	2,857	64,950
sex (% female)	59.46	52.7	63.67	51.01
age (mean [range])	22.35 [3-40]	24.4 [18 – 43]	56.14 [31-87]	
earlobe attachment (%)				
free	82.52	11.7	3.12	73.75
partially attached	9.94	75.6	31.92	
attached	7.54	12.7	64.96	26.25
ancestry	European	Admixed	East Asian	European
genotyping platform	Illumina HumanOmni Express+Exome	Illumina HumanOmni Express	Illumina HumanOmni Zhonghua-8	Illumina HumanHap550, HumanOmni Express, or custom array
# genotyped SNPs passing QC	653,629	671,772	795,597	1,044,759
# imputed SNPs	10,156,807	9,143,600	7,383,741	15,573,758
regression analysis	linear	linear	linear	logistic
covariates	Age, sex	age, sex, height, BMI	age, sex, BMI	age, sex, genotyping platform
principal components of ancestry included	3	5	0 [a]	5
genomic inflation factor	0.994	1.02	1.037	1.254 [b]

[a] Principal components analysis indicated negligible population structure, therefore no adjustments were made for principal components of ancestry in the Chinese sample.

[b] Genomic inflation for a subset of 1000 individuals with attached vs. 1000 with unattached earlobes was 1.002.

Table S3: Genetic data quality control filtering criteria

Filter	SNPs omitted	SNPs cumulatively retained
European American		
None (all SNPs)		968,515
Technical filters	8,470	960,045
Missing call rate ≥ 2%	9,675	950,370
>1 discordant calls in 69 duplicates [a]	26	950,344
>1 Mendelian error across 8 HapMap trios	122	950,222
HWE p-value < 10^{-4}	2,038	948,184
Allele frequency difference ≥ 0.2 between sexes [b]	274	947,910
Heterozygosity difference ≥ 0.3 between sexes [b]	41	947,869
Positional duplicates	19,597	928,272
Monomorphic (MAF = 0)	108,485	819,787
MAF < 0.01	164,959	654,828
non-autosomal or X	1,199	653,629
Latin American		
None (all SNPs)		730,525
Chromosome 0 (SNPs without assigned location)	1230	729,295
Missing call rate ≥ 5%	3550	725,745
MAF < 0.01 (includes monomorphic)	53973	671,772
Chinese cohort		
None (all SNPs)		887,270
Technical filters	278	886,992
Missing call rate ≥ 2%	18,628	868,364
Minor Allele Frequency < 0.01	67,873	800,491
HWE p-value < 10-3	2,588	797,903
Positional duplicates	2,306	795,597

[a] one duplicate was removed from QC filters due to a chromosomal anomaly

[b] filter applied to SNPs on autosomes and XY pseudo-autosomal region

Table S4: Summary of imputation methods and quality control filters

	European American	Latin American	Chinese	23andMe
Reference data source	1000 Genomes Project Phase 3	1000 Genomes Project Phase 1	1000 Genomes Project Phase 3	1000 Genomes Project Phase 1
Pre-phasing	SHAPEIT2	SHAPEIT2	SHAPEIT2	custom tool based on BEAGLE
Imputation	IMPUTE2	IMPUTE2	IMPUTE2	Minimac2
Other quality control procedures	masked variant analysis	masked variant analysis0	-	-
Imputation filters				
genotype probability (per SNP per person)	> 0.9	> 0.8	> 0.9	-
INFO score (per SNP)	> 0.5	> 0.4	> 0.6	-
MAF	> 0.01	> 0.01	> 0.02	-
Missing rate	-	-	< 0.05	-
HWE	-	-	≥ 10e-6	-
concordance (concord_type0) for masked variant analysis	-	> 0.7	-	-
info_type0-concord_type0 (chip genotype quality measure)	-	> 0.1	-	-