Genetic studies of accelerometer-based sleep measures yield new insights into human sleep behaviour

Jones, van Hees, Mazzotti et al

Supplementary Methods

Sensitivity analysis details

<u>Lifestyle factors for sensitivity analysis (6)</u>
In the sensitivity analysis that included "lifestyle" factors, we adjusted models for multiple variables measured at baseline that may have had an impact on sleep timing, quality and/or quantity. These were:
- Townsend Deprivation Index (TDI; field 189)
- Alcohol intake frequency (field 1558; categories treated as ordinal scale – "Never"=0 to "Daily or almost daily"=5)
- Smoking status (field 20116; categories treated on a nominal scale)
- Snoring (field 1210; nominal scale)
- Nap during day (field 1190; nominal scale)
- Had menopause (field 2724; nominal scale; included both "Not sure" answers as separate categories; males were recoded as an additional category to avoid exclusion)
- Caffeine intake (continuous scale; calculated from fields 1498, "Coffee intake", and 1488, "Tea intake" using the formula caffeine_intake=137*coffee_intake + 47*tea_intake; individuals were assumed to have zero caffeine contribution from coffee if they answered "Decaffeinated coffee (any type)", "Do not know" or "Prefer not to answer" in field 1508, "Coffee type")
- Sleep apnoea (matching code 1123 in "Non-cancer illness code, self-reported", field 20002; treated as binary variable)

Individuals responding "Do not know" or "Prefer not to answer" for "Alcohol intake frequency", "Smoking status", "Snoring", "Nap during day" and "Had menopause" were excluded from this sensitivity analysis. Caffeine intake was representative of habitual intake and did not consider dietary recall questions, which were available in less than half of the cohort at the time of analysis.

<u>Exclusion criteria for sensitivity analysis (7)</u>
Shift or night shift work
Two baseline questionnaire variables were used to assess whether individuals were performing either shift work or night shift work at the time of assessment. Individuals answering anything other than "Never/rarely" for either field 826 ("Job involves shift work") or field 3426 ("Job involves night shift work") were considered to be shift or night shift workers.

Self-report mental health and sleep disorders
We excluded participants if they had answered yes in field 2090 ("Seen doctor (GP) for nerves, anxiety, tension or depression") or had reported "sleep apnoea" (coded as 1123), "insomnia" (1616), "depression" (1286), "schizophrenia" (1289) or "mania/bipolar disorder/manic depression" (1291) at the baseline verbal interview. These data are stored in field 20002 ("Non-cancer illness code, self-reported").

Hospital episode statistics (HES) mental health and sleep disorder diagnoses
Individuals were excluded if they had one or more of the following diagnosis codes (or their respective sub-classifications) in either field 41202 ("Diagnoses – main ICD10") or 41204 ("Diagnoses – secondary ICD10"):
- Major depressive disorder (MDD) – ICD10 codes F32 and F33

- Schizophrenia – F20 to F29
- Bipolar disorder – F30 and F31
- Anxiety disorders – F40 to F43
- Mood disorders – F30 to F39
- Sleep disorders – F51 (nonorganic) and G47

Relevant self-report medications
We excluded any individuals reporting one or more of the following medications (field 20003) at baseline. These include drug ingredients and brand names:
Sleep medications: oxazepam, meprobamate, medazepam, bromazepam, lorazepam, clobazam, chlormezanone, temazepam, nitrazepam, lormetazepam, diazepam, zopiclone, triclofos, methyprylone, prazepam, triazolam, ketazolam, dichloralphenazone, clomethiazole, zaleplon, butobarbital, diphenhydramine product, nytol, sonata
Antidepressants: amitriptyline, citalopram, fluoxetine, sertraline, venlafaxine, dosulepin, paroxetine, mirtazapine, escitalopram, trazodone, prozac, seroxat, cipralex, duloxetine, lofepramine, clomipramine, nortriptyline, imipramine, dothiepin, cipramil, amitriptyline, prothiaden, trimipramine, lustral, reboxetine, zispin, cymbalta, anafranil, doxepin, moclobemide, phenelzine, fluvoxamine, yentreve, triptafen, surmontil, tranylcypromine, allegron, edronax, molipaxin, mianserin, nardil, faverin, nefazodone, amitriptyline+chlordiazepoxide, isocarboxazid, manerix, maoi, sinequan, tranylcypromine+trifluoperazine, ludiomil, norval, tryptizol, fluphenazine hydrochloride+nortriptyline.
Antipsychotics: prochlorperazine, olanzapine, quetiapine, risperidone, chlorpromazine, trifluoperazine, amisulpride, sulpiride, seroquel, haloperidol, aripiprazole, stelazine, depixol, flupentixol, clozapine, promazine, risperdal, modecate, fluanxol, flupenthixol, zyprexa, zuclopenthixol, clopixol, largactil, abilify, fluphenazine, haldol, serenace, clozaril, cpz, perphenazine, levomepromazine, pericyazine, dolmatil, fentazin, fluphenazine, benperidol, pimozide, zaponex, denzapine, neulactil, thioridazine, dozic, fluspirilene, panadeine, sertindole.
Anxiolytics: zopiclone, diazepam, temazepam, zolpidem, nitrazepam, lorazepam, hydroxyzine, zimovane, phenergan, promethazine, buspirone, atarax, oxazepam, loprazolam, chlordiazepoxide, lormetazepam, ucerax, stilnoct, diazepam, buspar, alprazolam, librium, xanax, meprate, dalmane, clomethiazole, meprobamate, welldorm, amitriptyline+chlordiazepoxide, flurazepam, heminevrin, medazepam, neulactil, sinequan, almazine, atensine, carisoma, chloractil, chloral, dichloralphenazone, dormonoct, methyprylone, mogadon, rohypnol, tryptizol.

Supplementary Tables

Supplementary Table 1. Spearman's rank correlation statistics for activity monitor derived sleep traits and self-report hours slept and self-report chronotype (coded for increased morningness).

Trait	M10 timing	L5 timing	Sleep midpoint	Sleep duration	Sleep duration (SD)	Sleep efficiency	Diurnal inactivity	Number of sleep episodes	Self-report hours slept
L5 timing	0.460								
Sleep midpoint	0.383	0.591							
Sleep duration	0.012	-0.023	-0.039						
Sleep duration (SD)	0.059	-0.007	-0.102	-0.303					
Sleep efficiency	0.009	0.035	0.119	0.566	-0.165				
Diurnal inactivity	0.043	0.083	-0.105	0.269	0.098	-0.200			
Number of sleep episodes	0.036	-0.023	-0.054	0.142	-0.182	-0.250	0.202		
Self-report hours slept	0.025	0.010	0.008	0.189	-0.043	0.133	0.040	0.082	
Self-report chronotype	-0.277	-0.293	-0.237	0.027	-0.042	0.018	-0.044	-0.006	-0.005

Supplementary Table 2. Averages of the mean number of sleep episodes detected within individuals in the UK Biobank split by APOE Alzheimer's disease risk haplotypes and lower/upper age groups.

Haplotype	All Subjects		Lower age group (<63.5 years)		Upper age group (=>63.5 years)	
	N	Average Mean Sleep Episodes (SD)	N	Average Mean Sleep Episodes (SD)	N	Average Mean Sleep Episodes (SD)
APO-e2/e2	479	17.40 (3.70)	236	18.07 (3.59)	243	16.75 (3.69)
APO-e2/e3	8,922	17.32 (3.62)	4,386	17.64 (3.66)	4,536	17.00 (3.55)
APO-e2/e4	1,795	17.17 (3.59)	913	17.74 (3.67)	882	16.58 (3.40)
APO-e3/e3	42,679	17.26 (3.60)	21,210	17.63 (3.63)	21,469	16.90 (3.52)
APO-e3/e4	17,049	17.14 (3.60)	8,686	17.52 (3.65)	8,363	16.74 (3.49)
APO-e4/e4	1,660	17.02 (3.56)	862	17.49 (3.71)	798	16.52 (3.31)

Supplementary Table 3. MAGMA Gene-Set Analysis for SNPs associated with disturbed sleep based on number of sleep episodes reaching Bonferroni significance.

Gene Set	N genes in gene set	Gene set beta*	Gene set beta STD**	SE***	P-value	P_Bonferroni
GO_bp:go_serotonin_metabolic_process	10	1.43	0.0335	0.264	3.10E-08	3.4E-04
GO_bp:go_primary_amino_compound_metabolic_process	13	1.06	0.0283	0.232	2.34E-06	0.026

* Regression coefficient of the gene set
** Gene set beta STD: semi-standardized regression coefficient, corresponding to the predicted change in Z-value given a change of one standard deviation in the predictor gene set
*** Standard error of regression coefficient

Supplementary Figures

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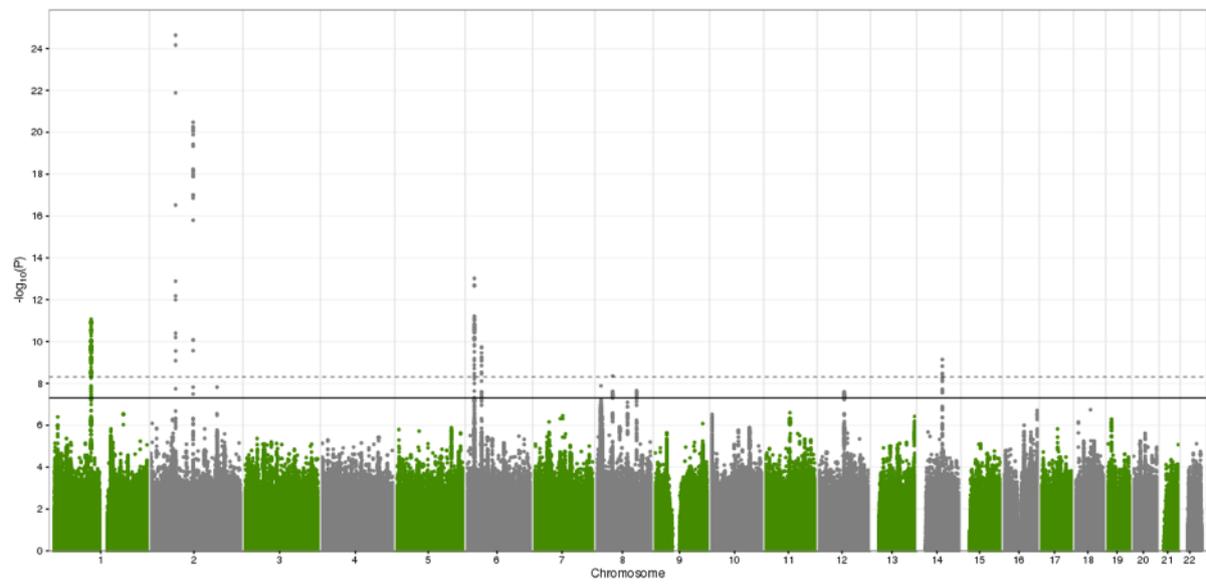
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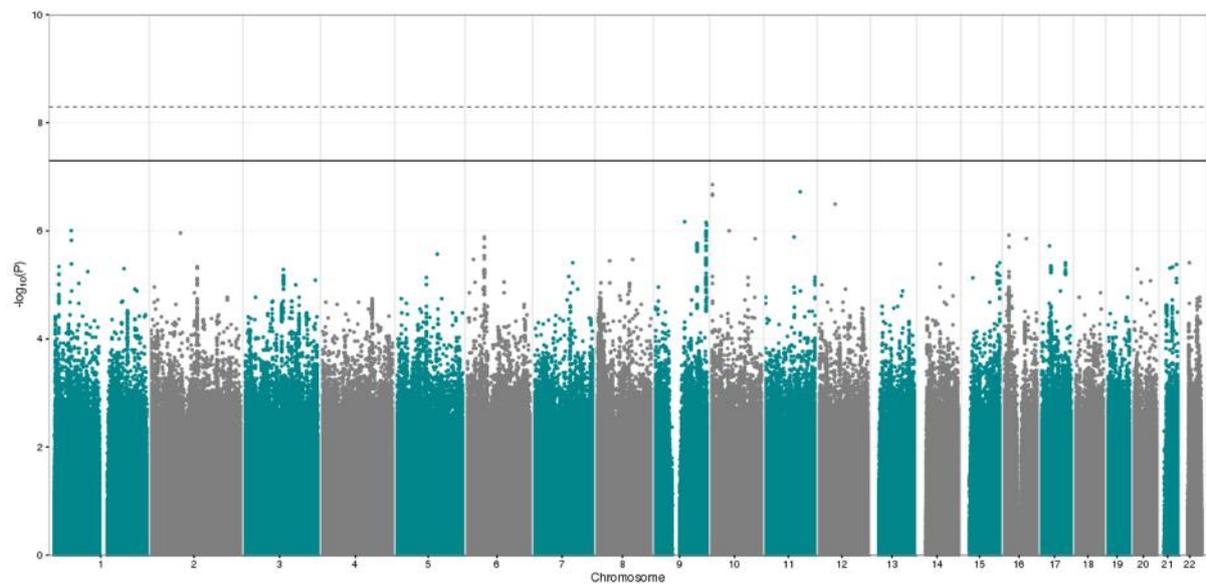
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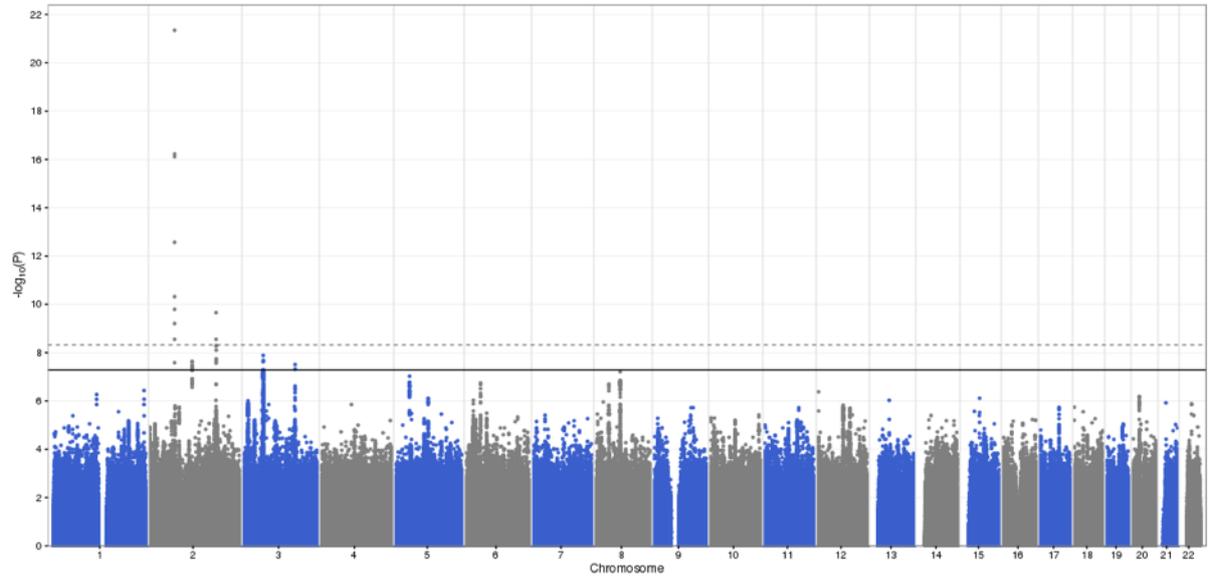
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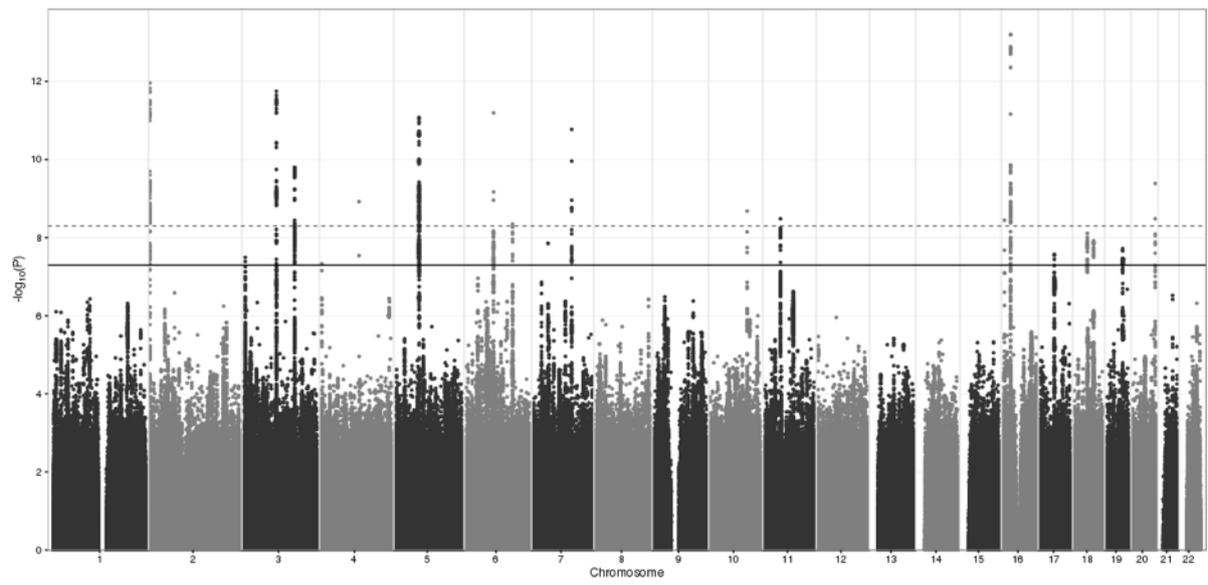
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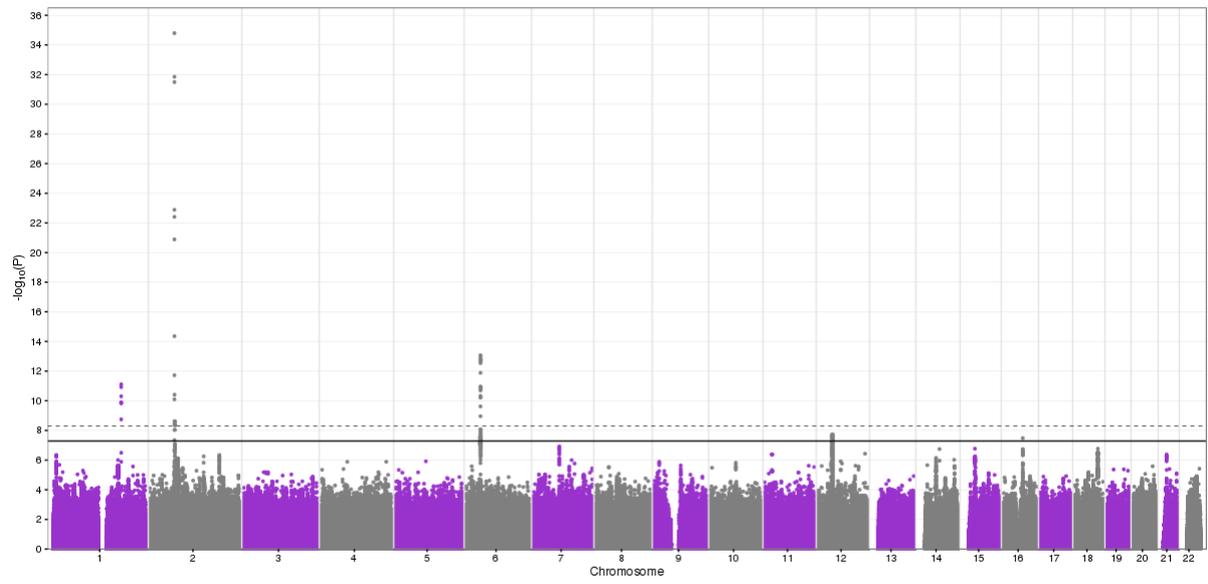
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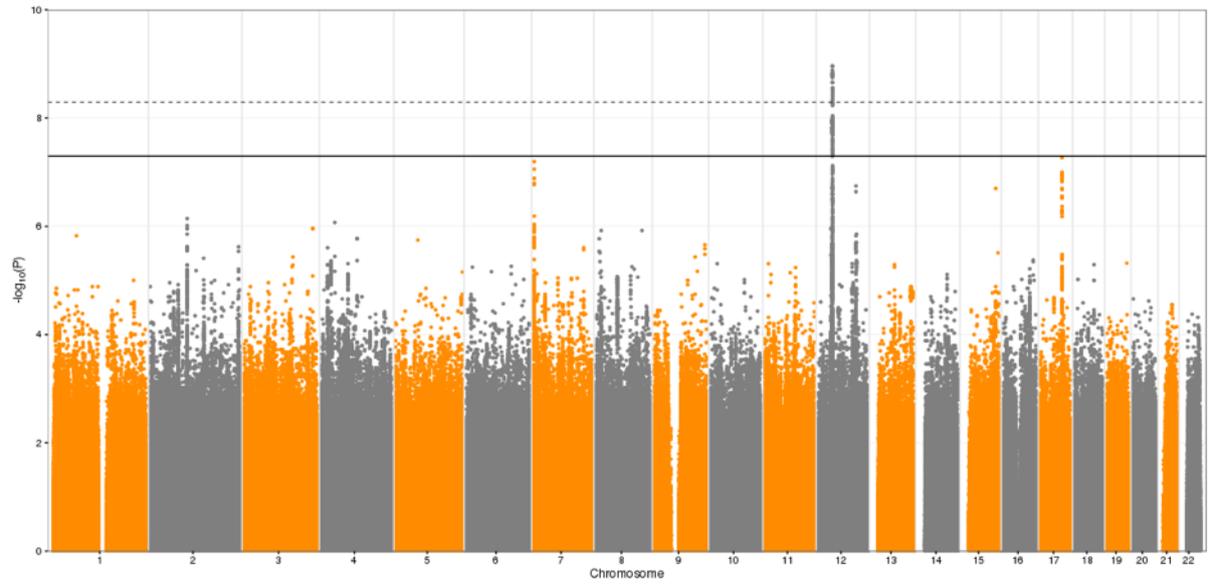
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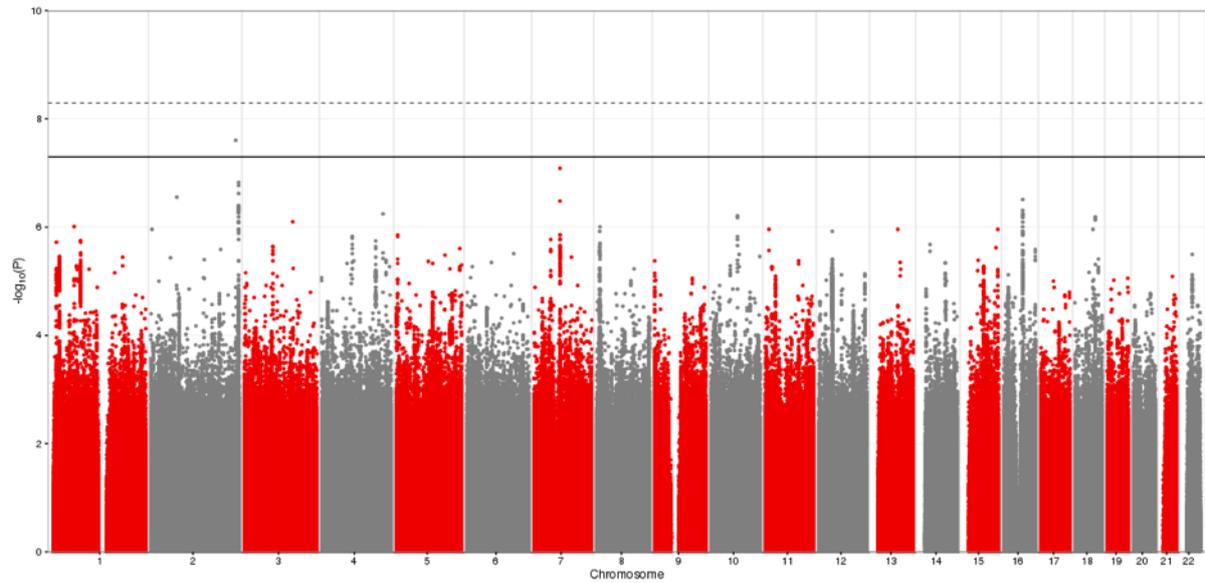
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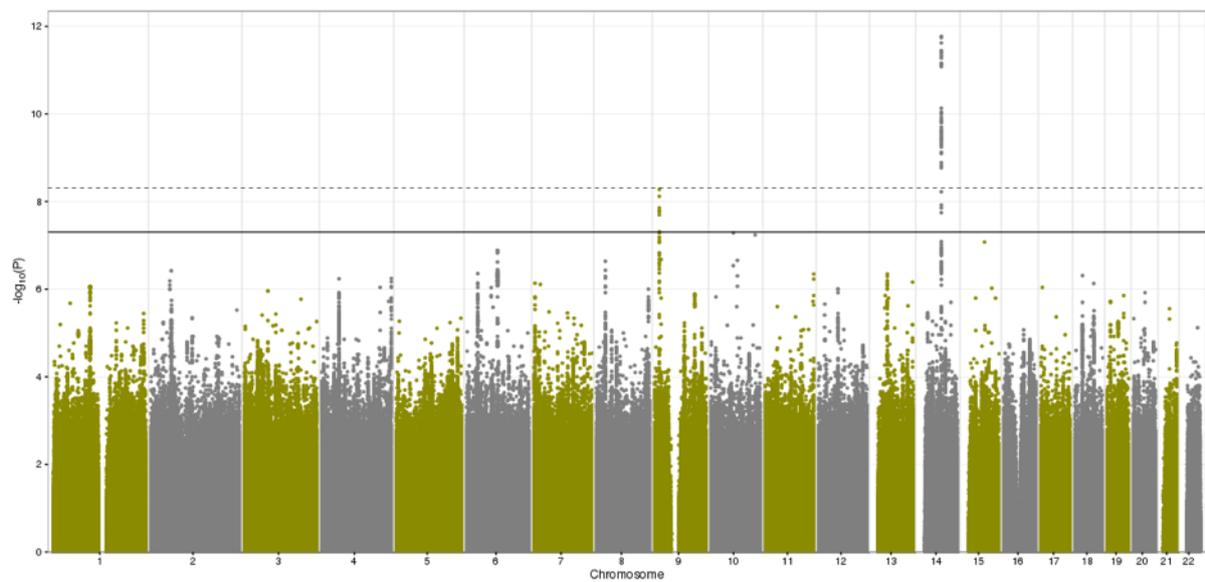
Supplementary Figure 1. Manhattan plots for the eight accelerometer-derived phenotypes. Plots show GWAS results for a) sleep duration, b) sleep duration variability, c) sleep efficiency, d) number of sleep episodes, e) L5 timing, f) M10 timing, g) sleep midpoint and h) diurnal inactivity.

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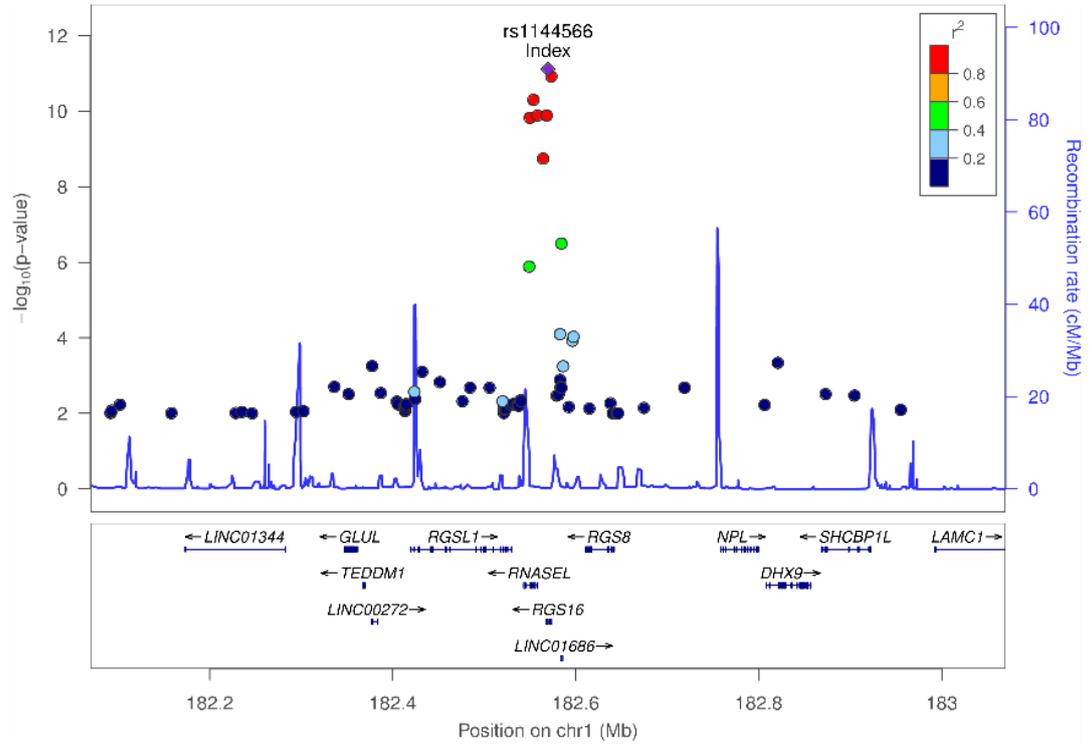
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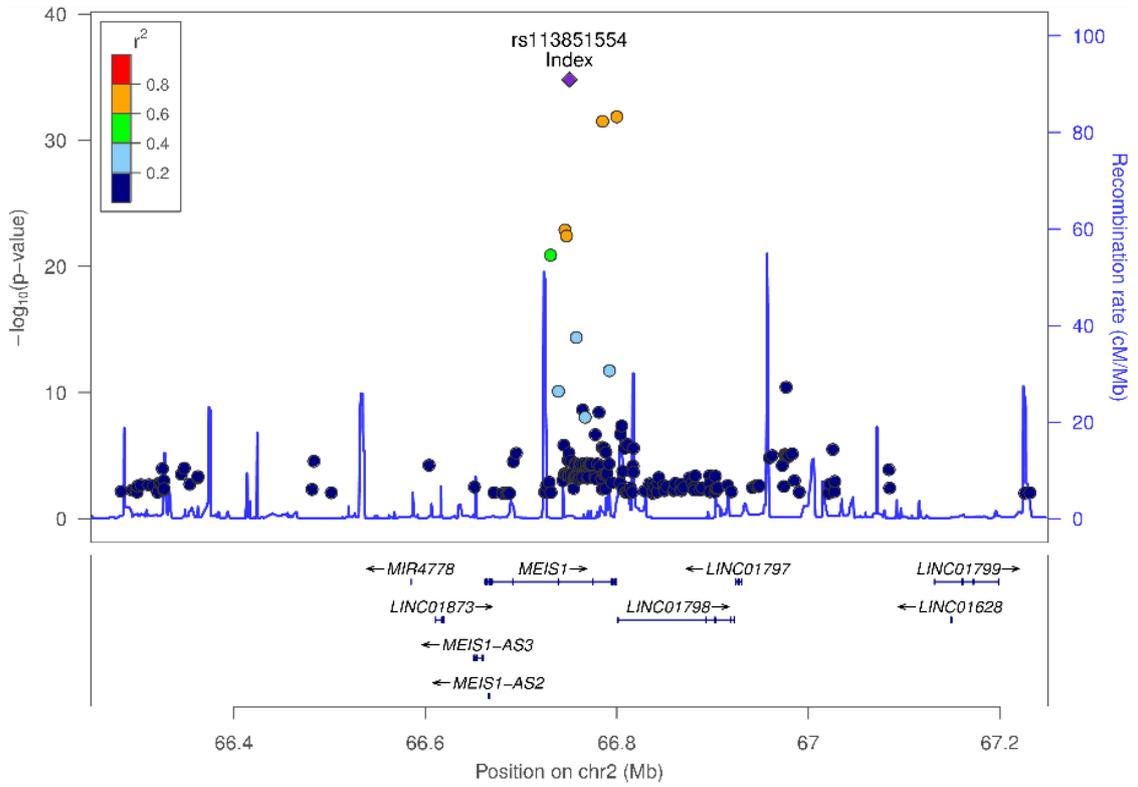
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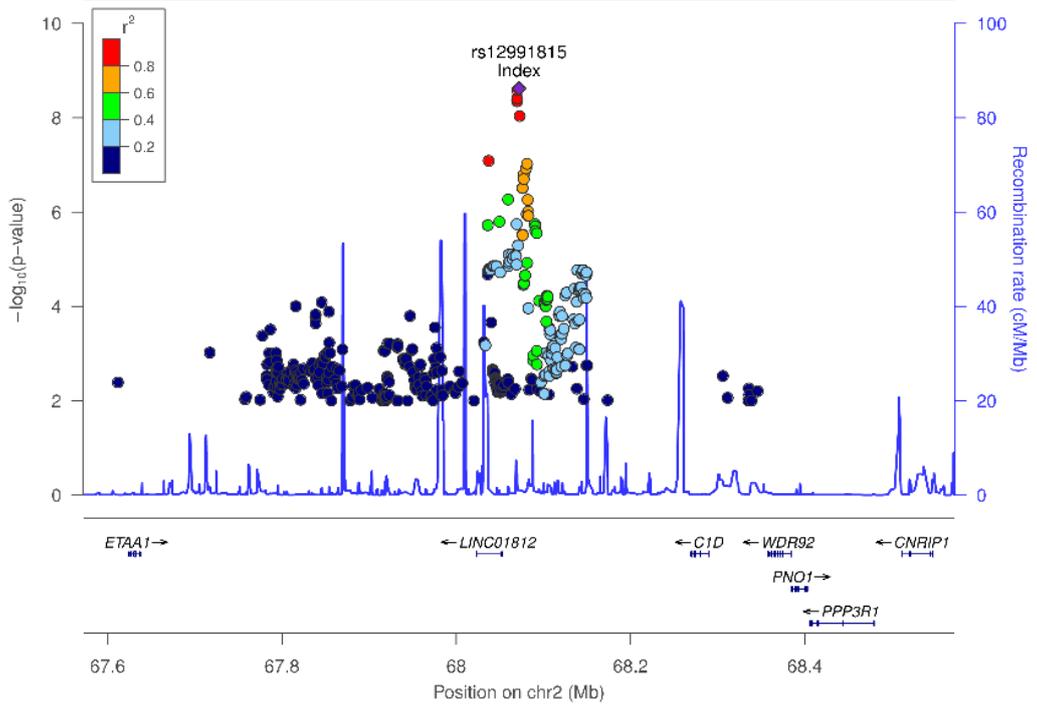
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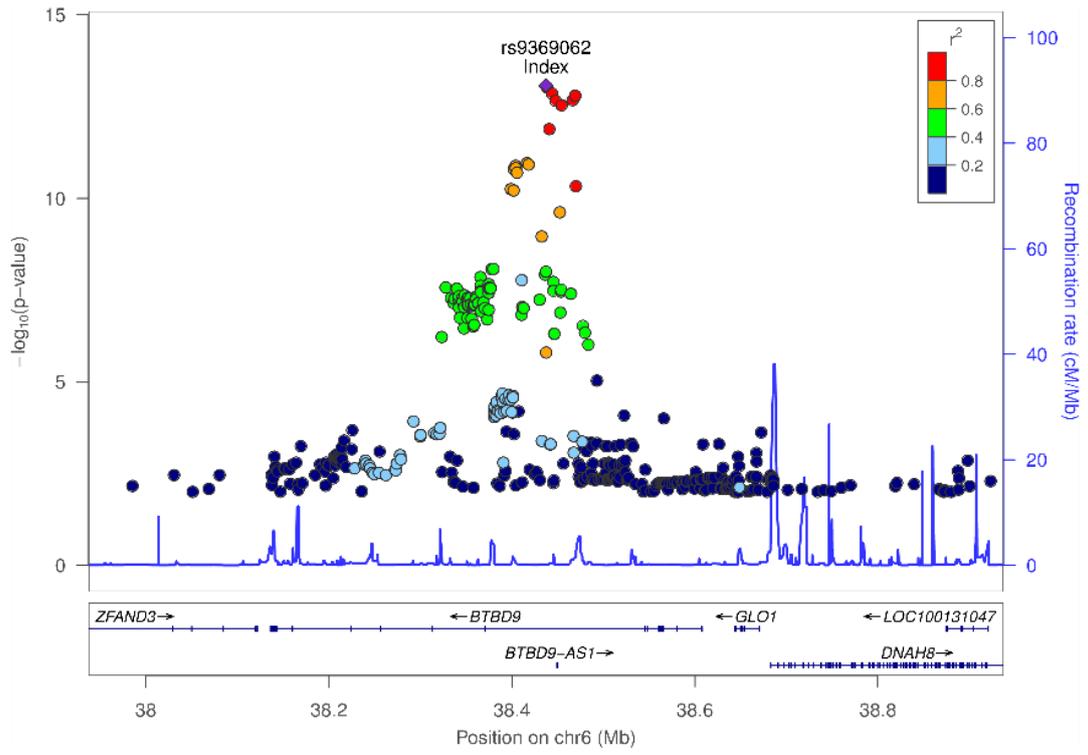
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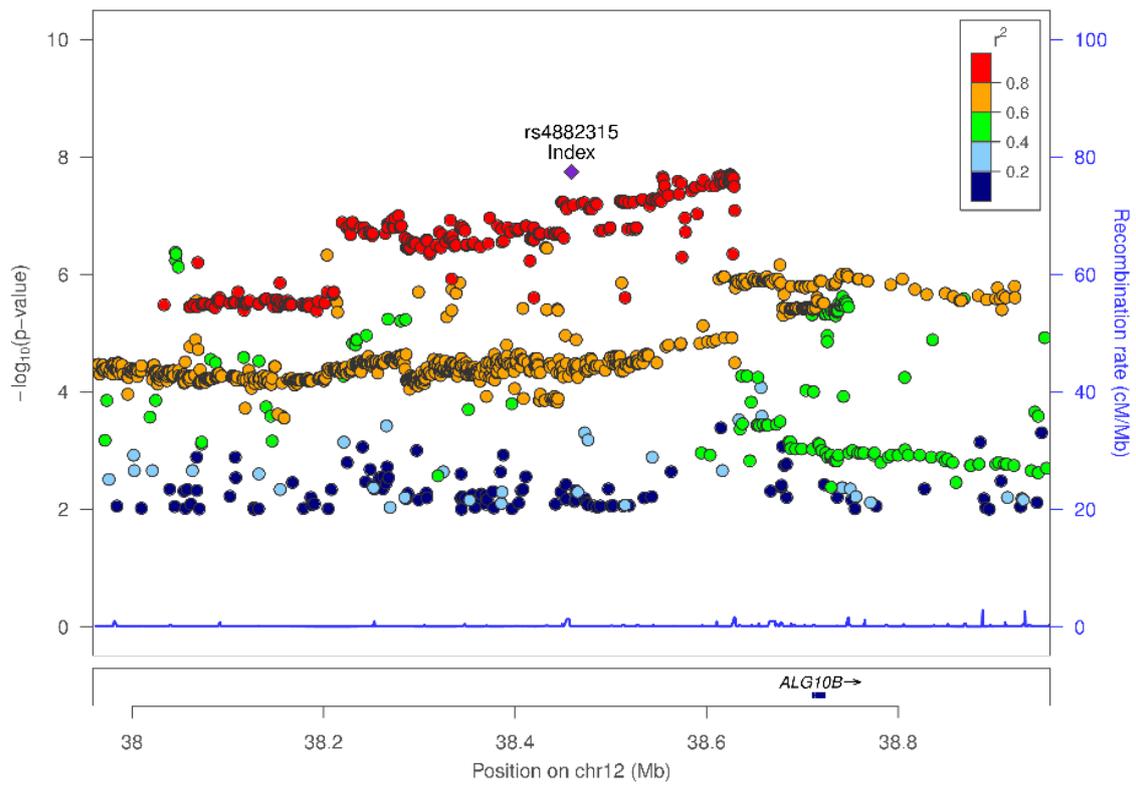
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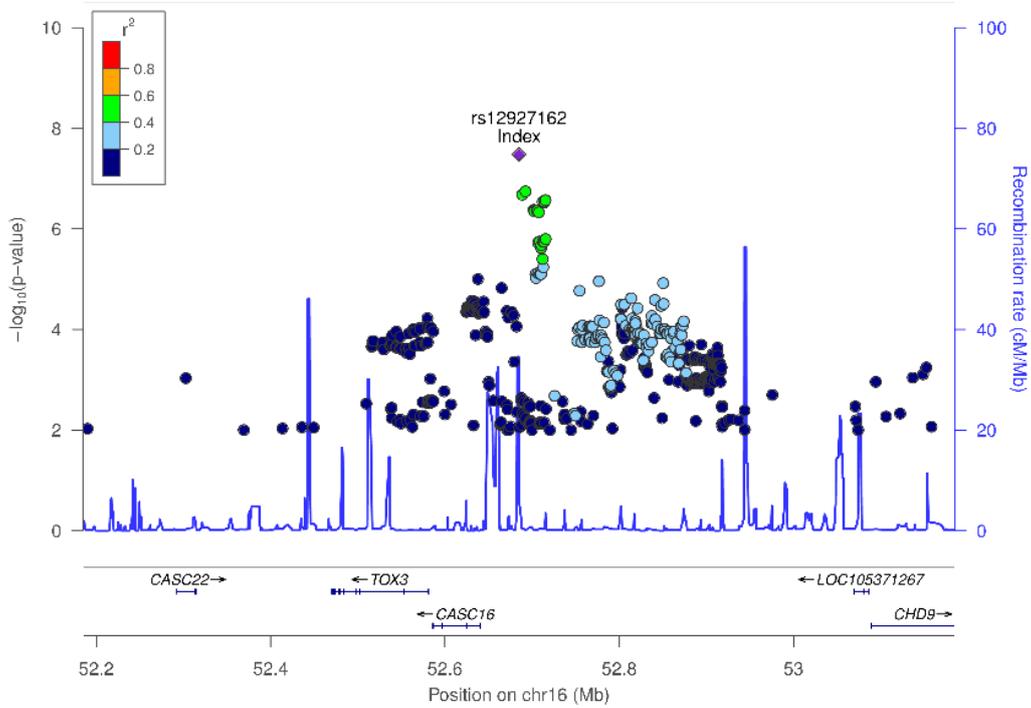
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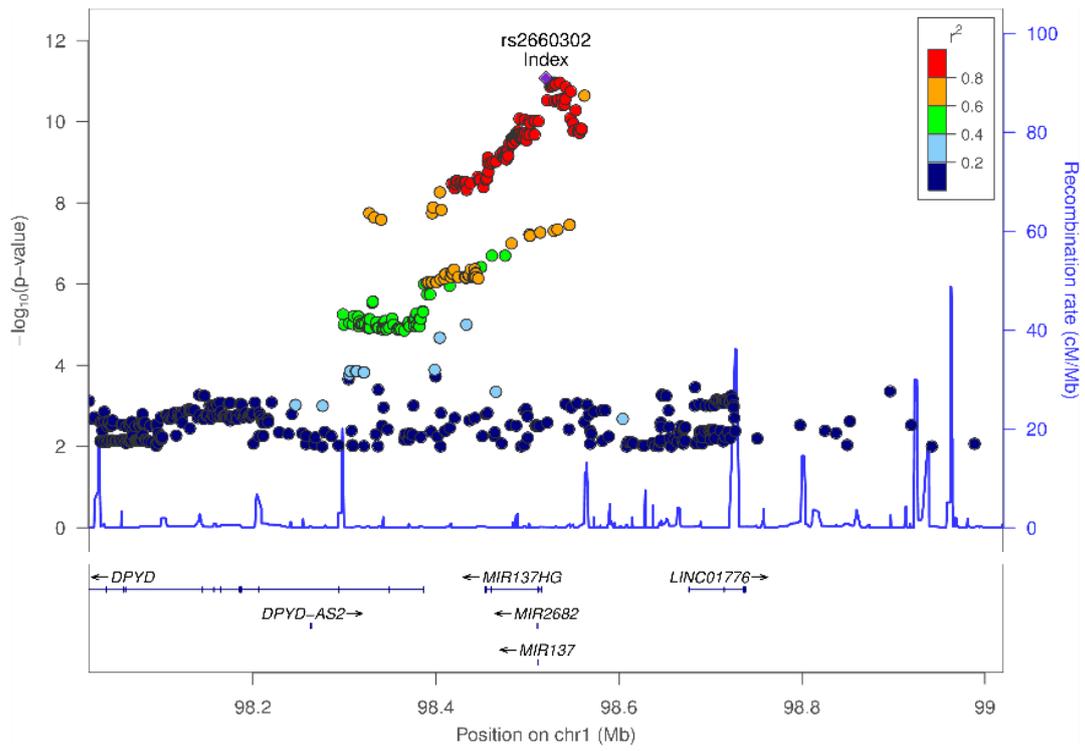
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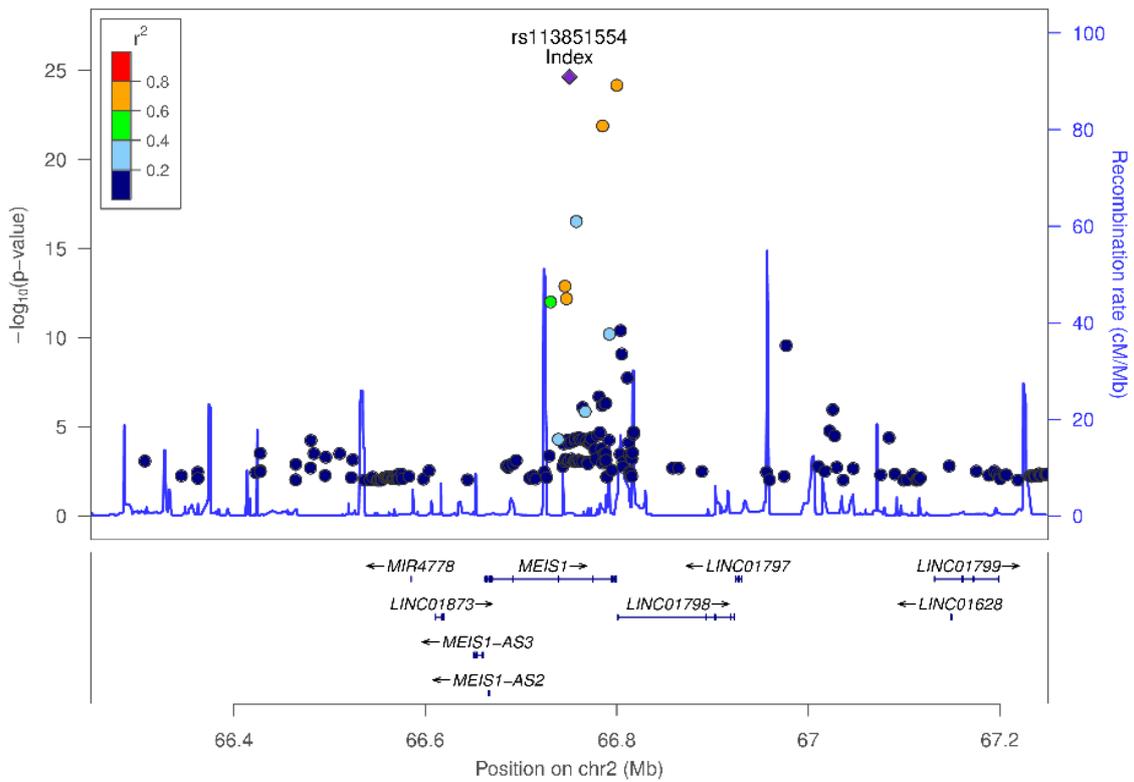
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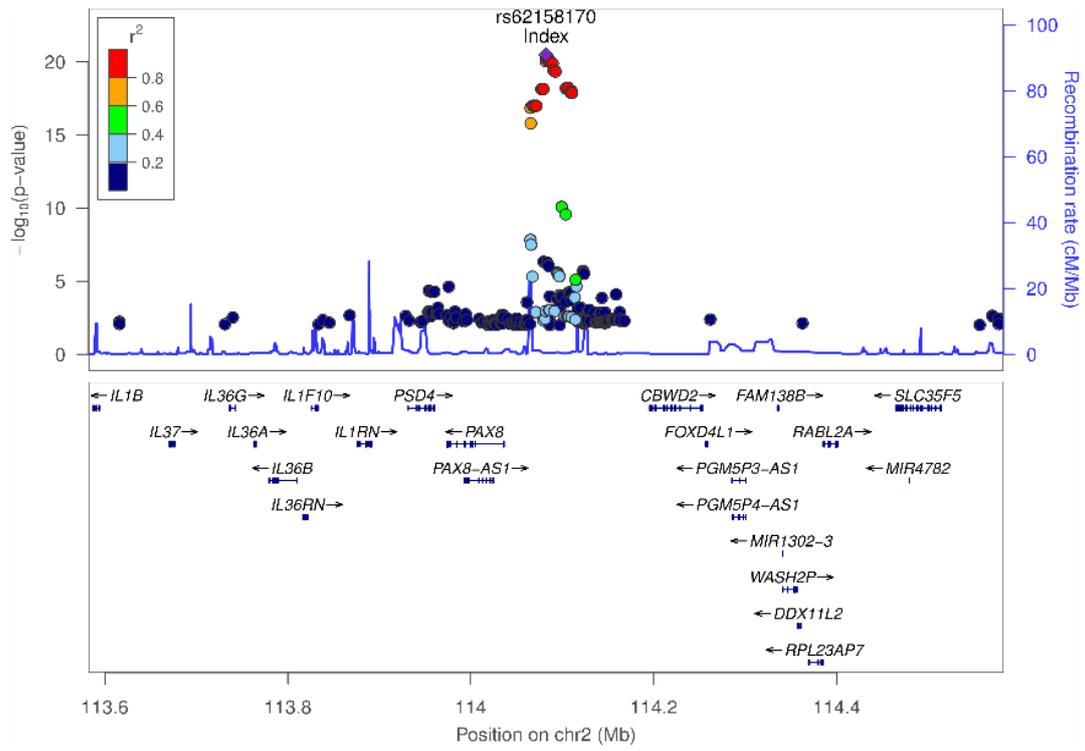
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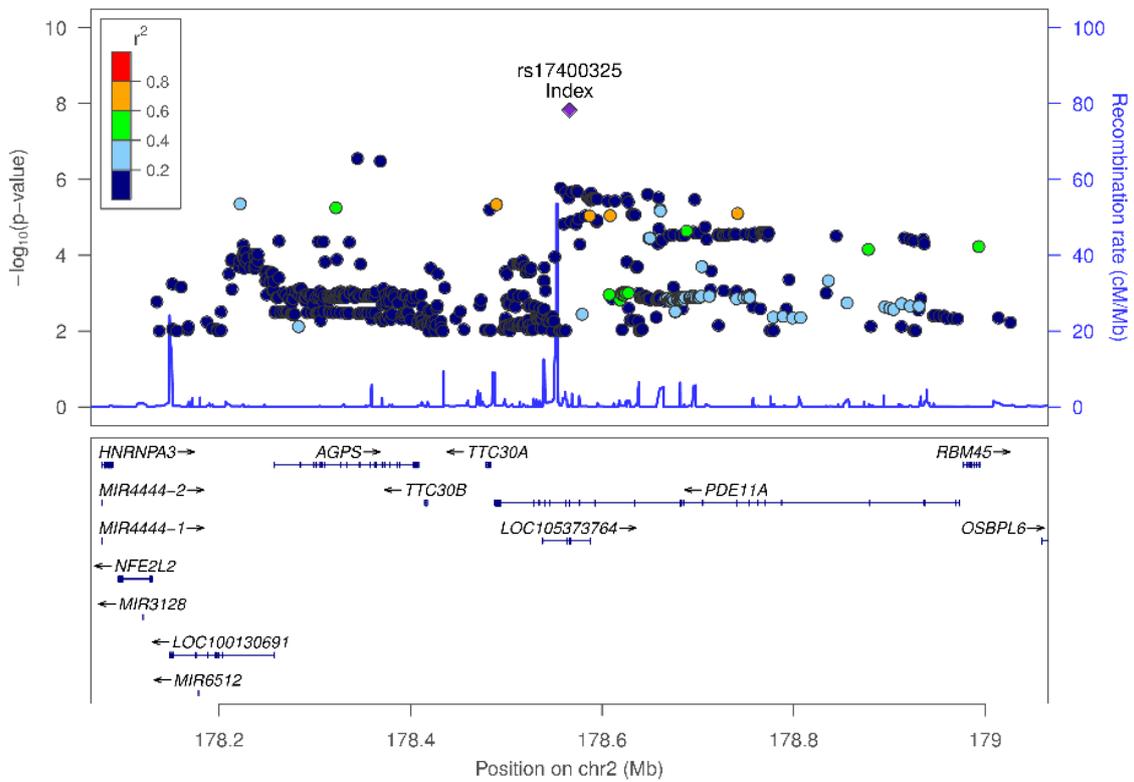
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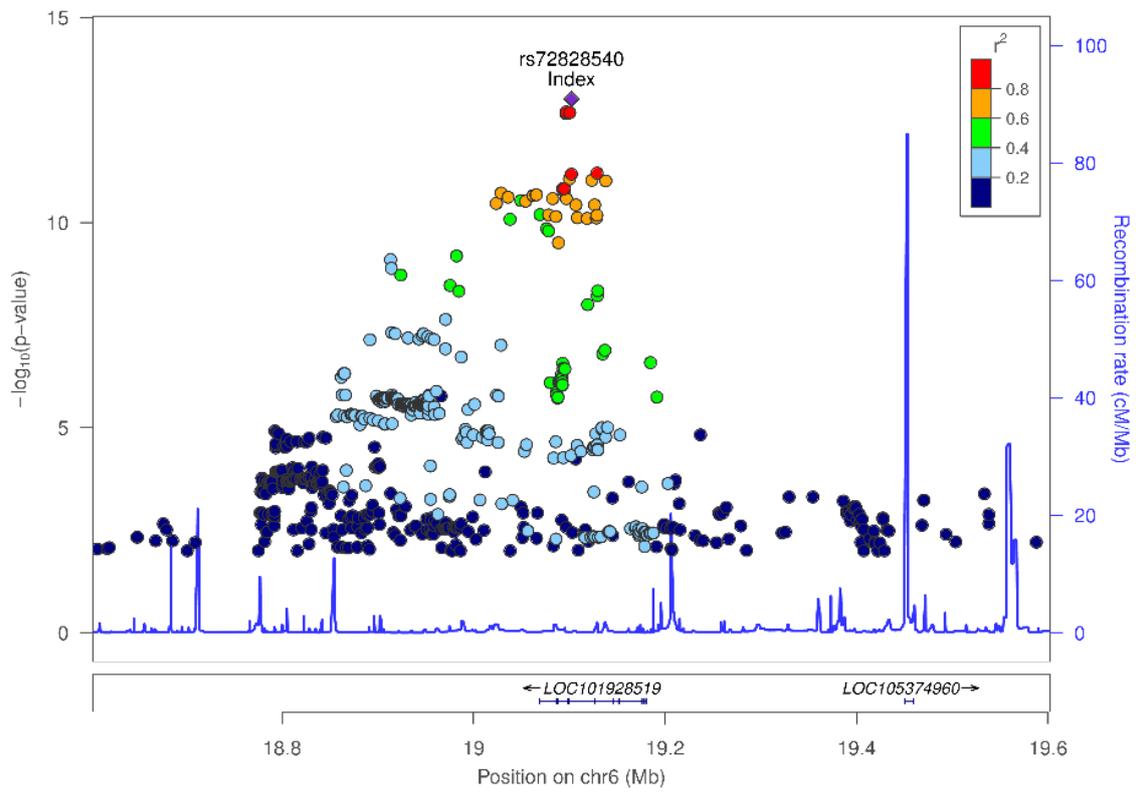
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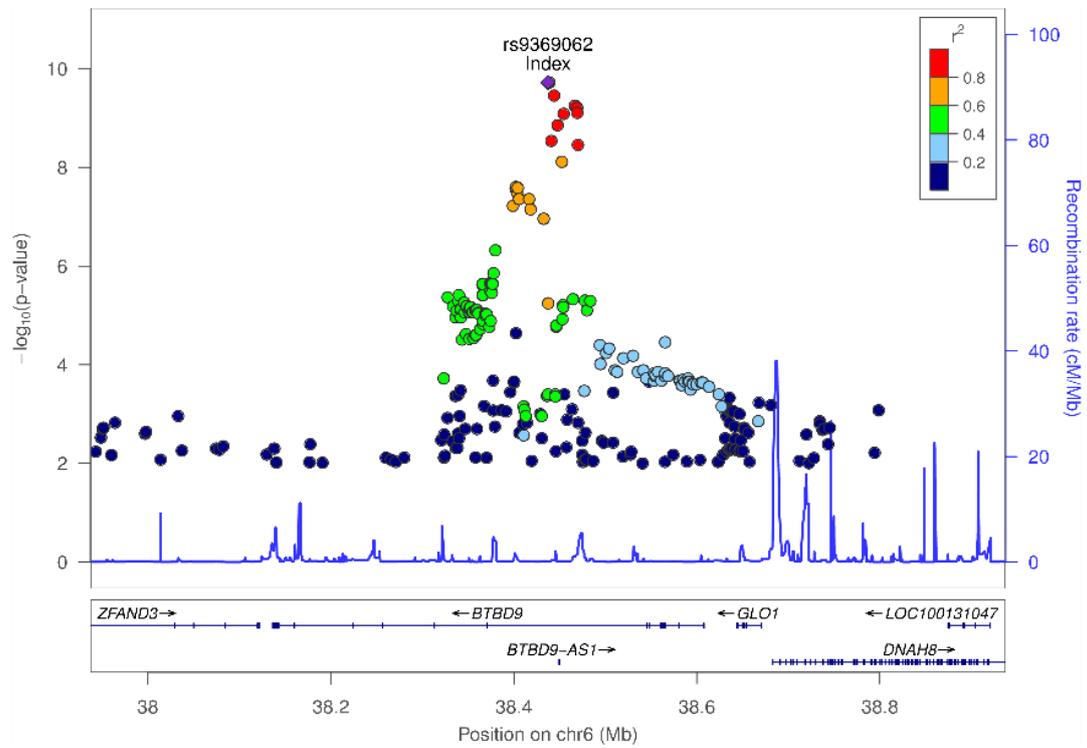
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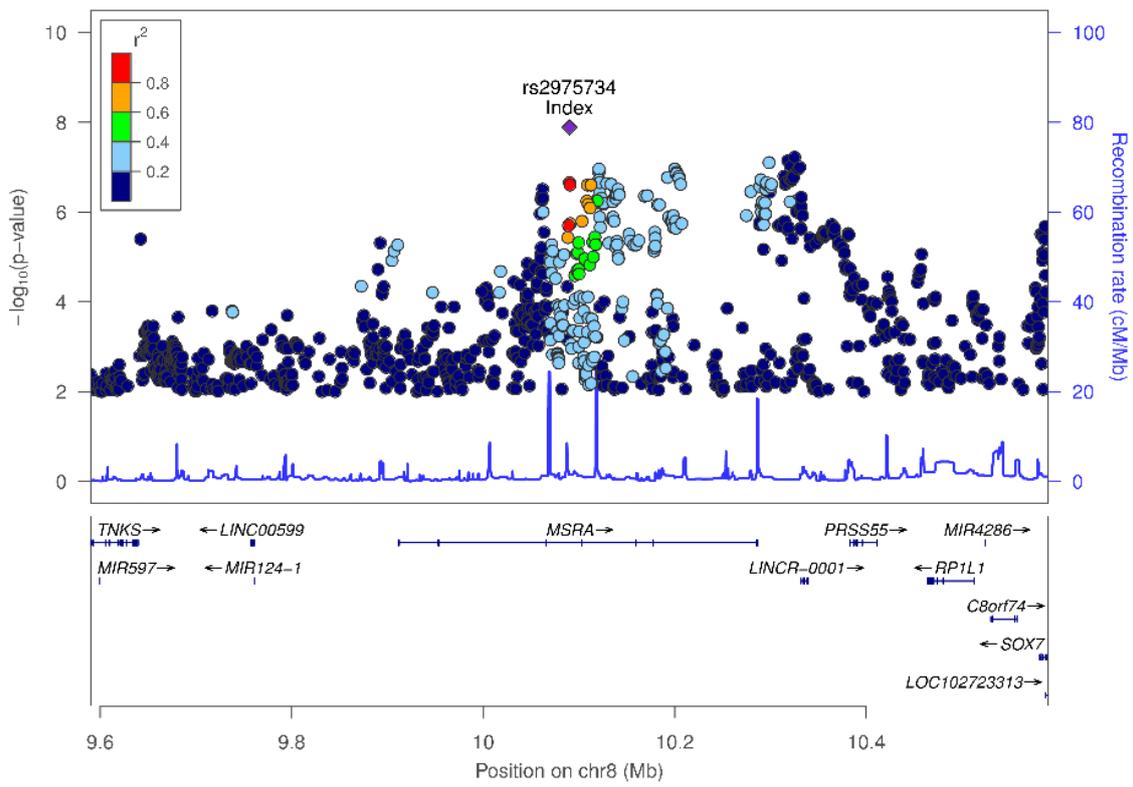
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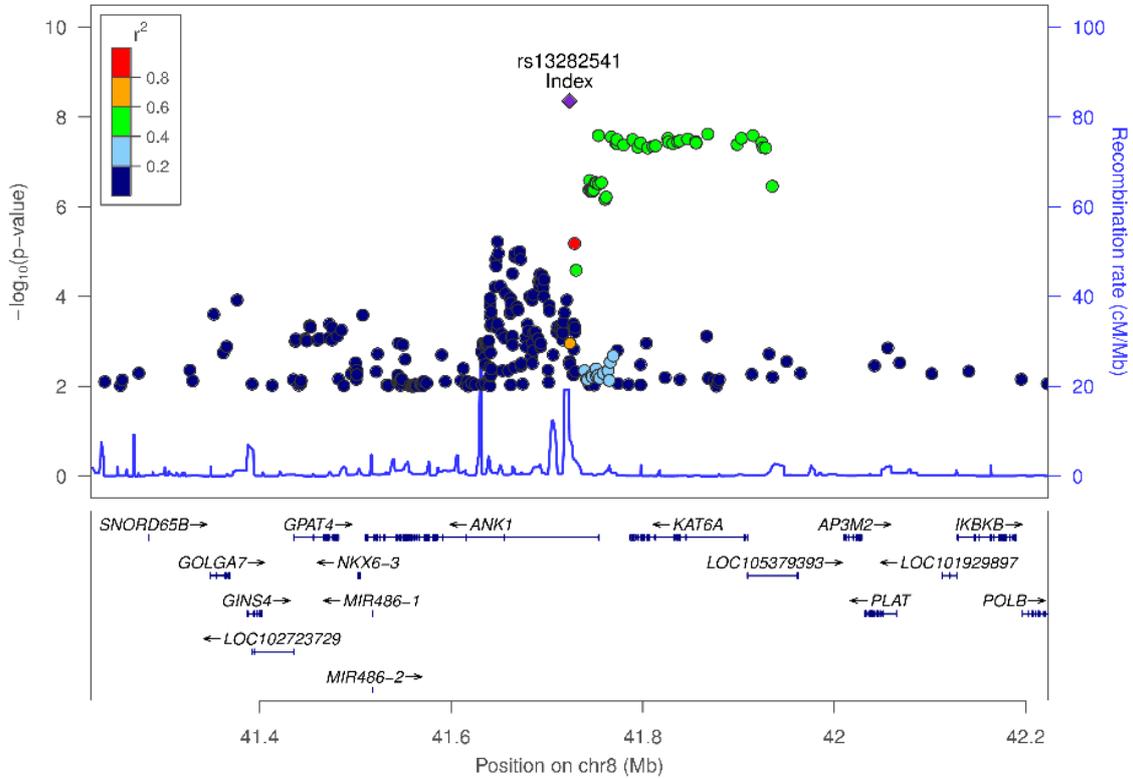
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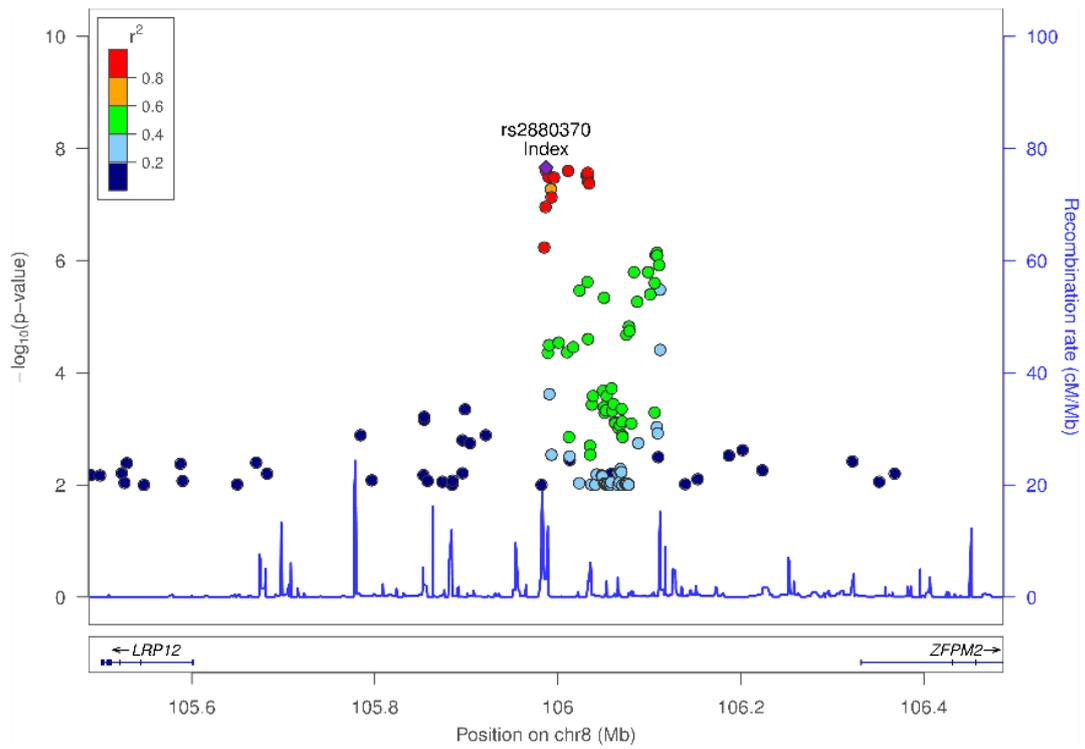
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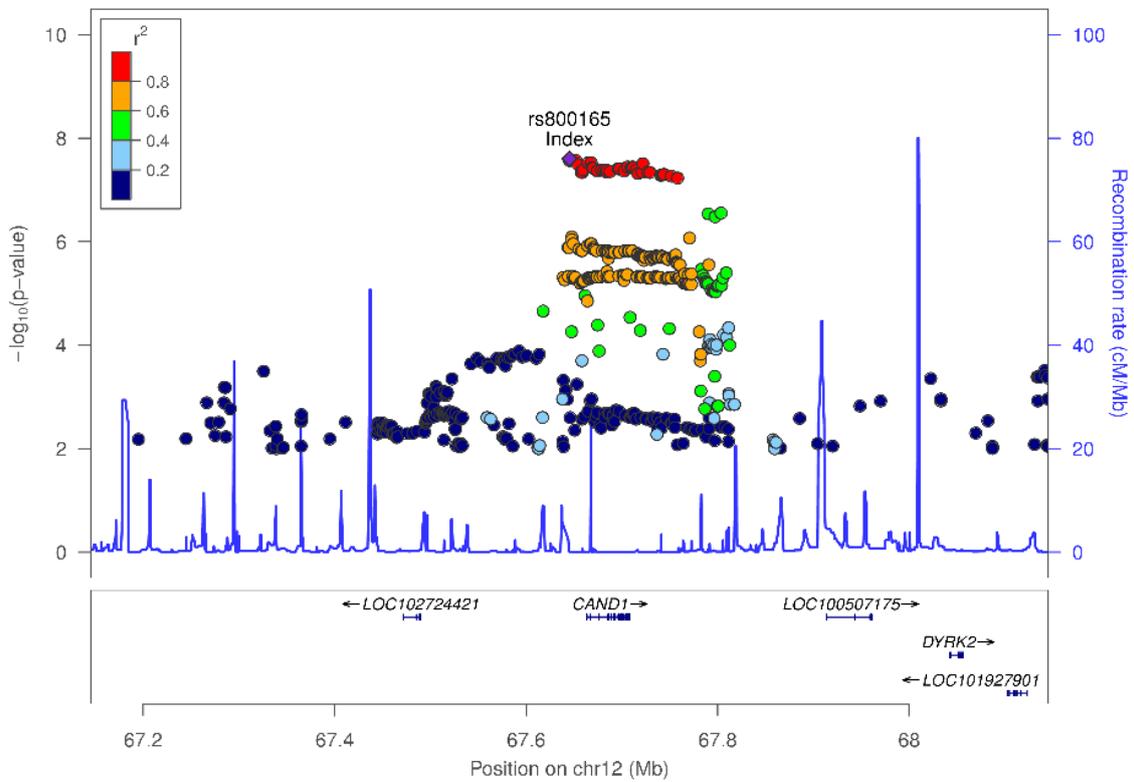
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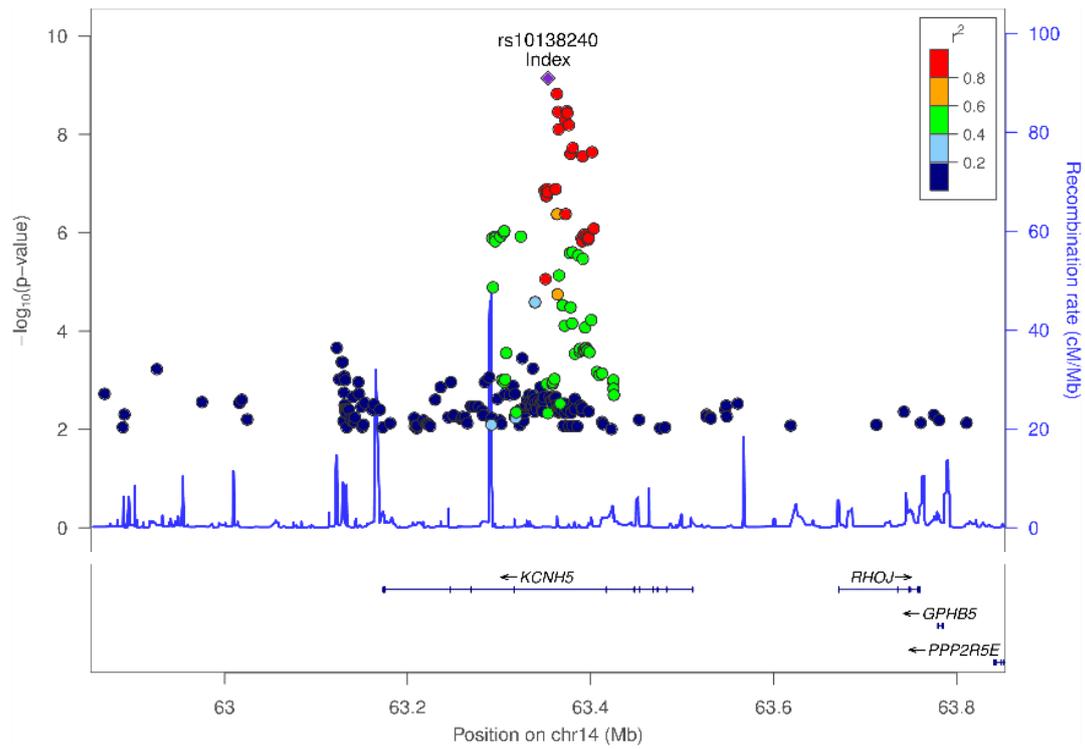
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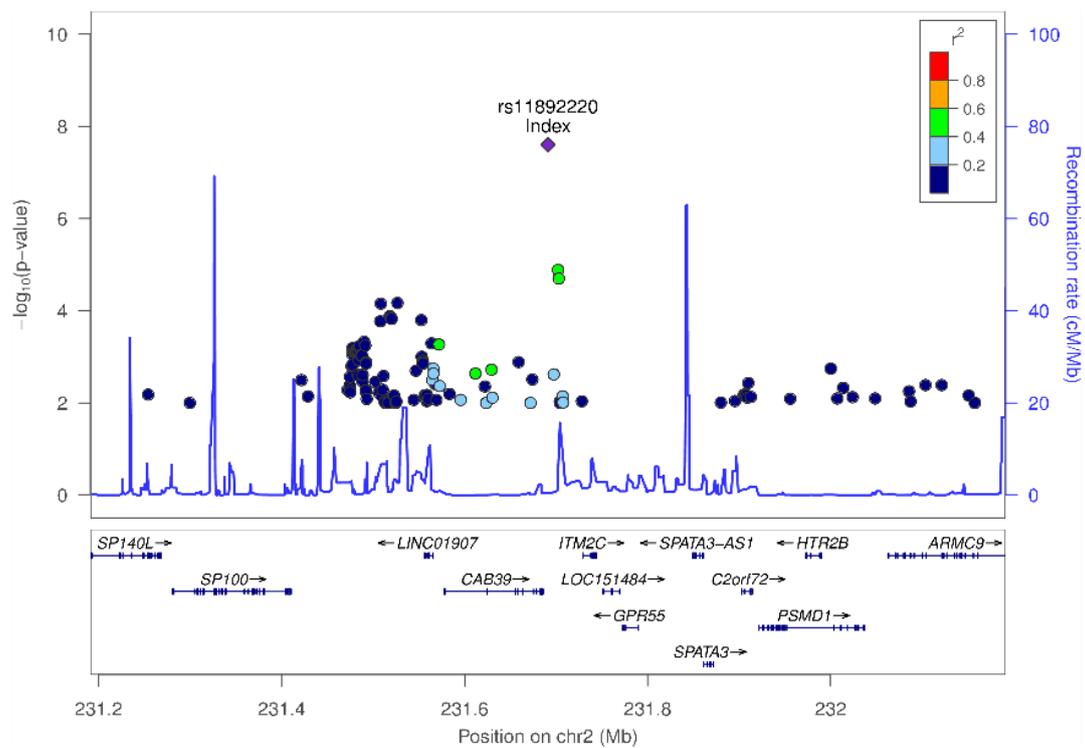
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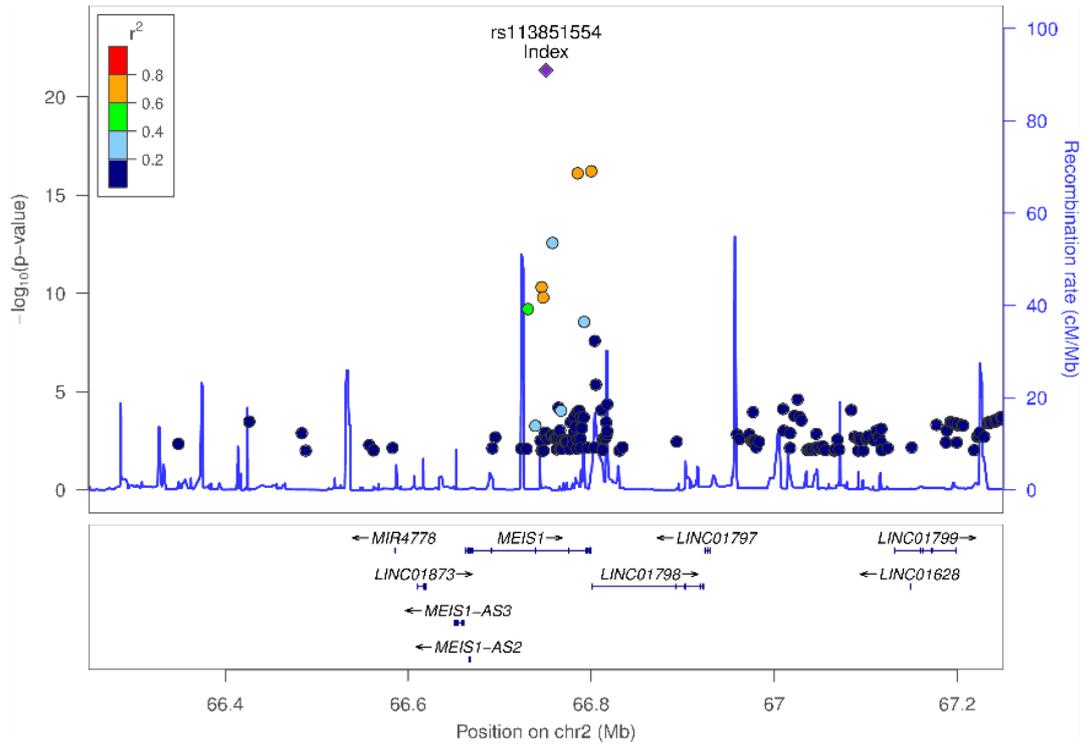
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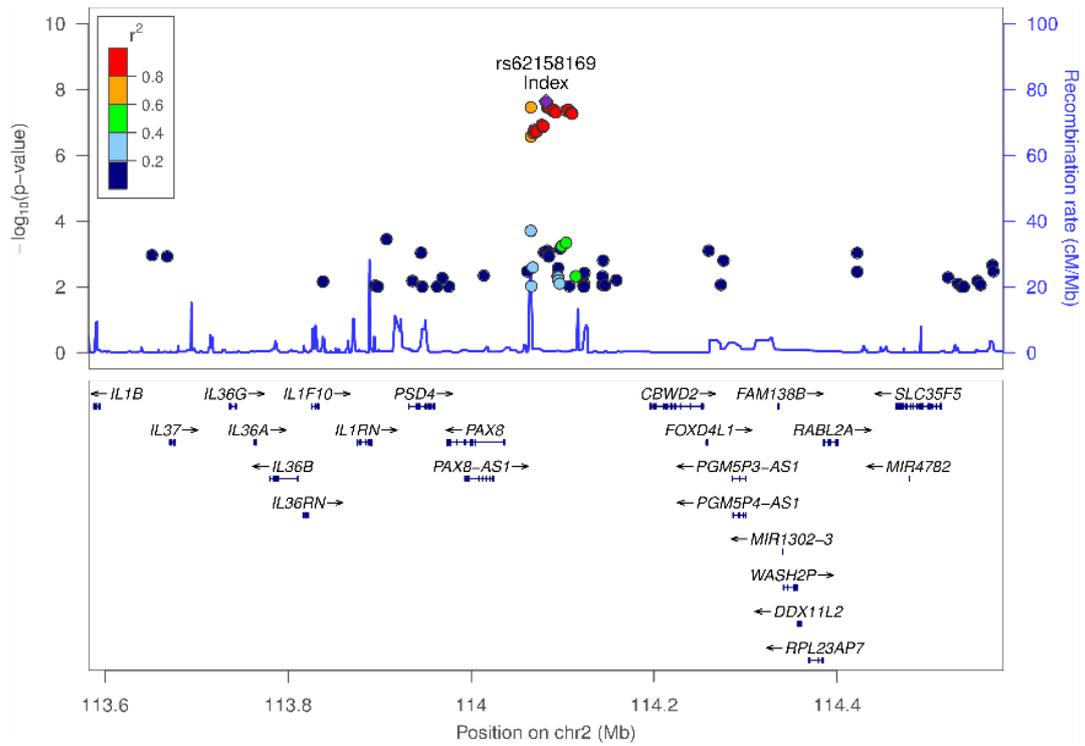
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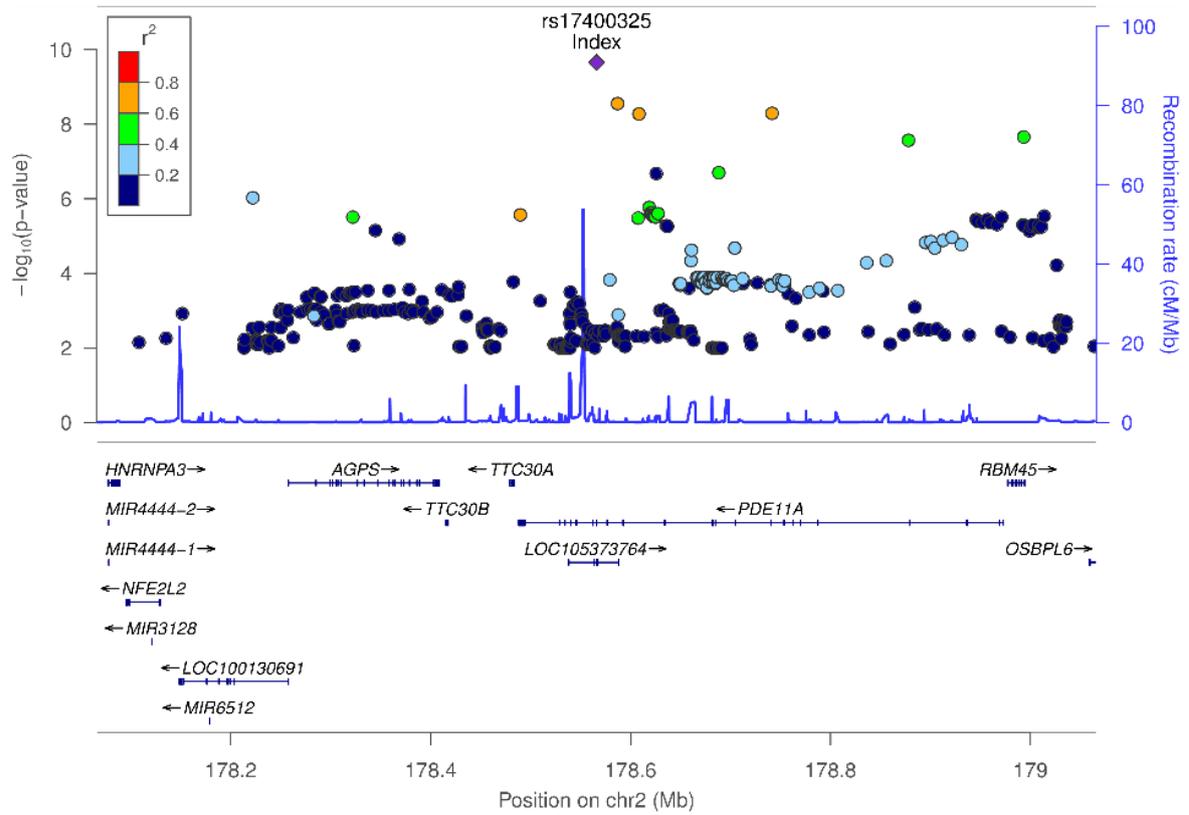
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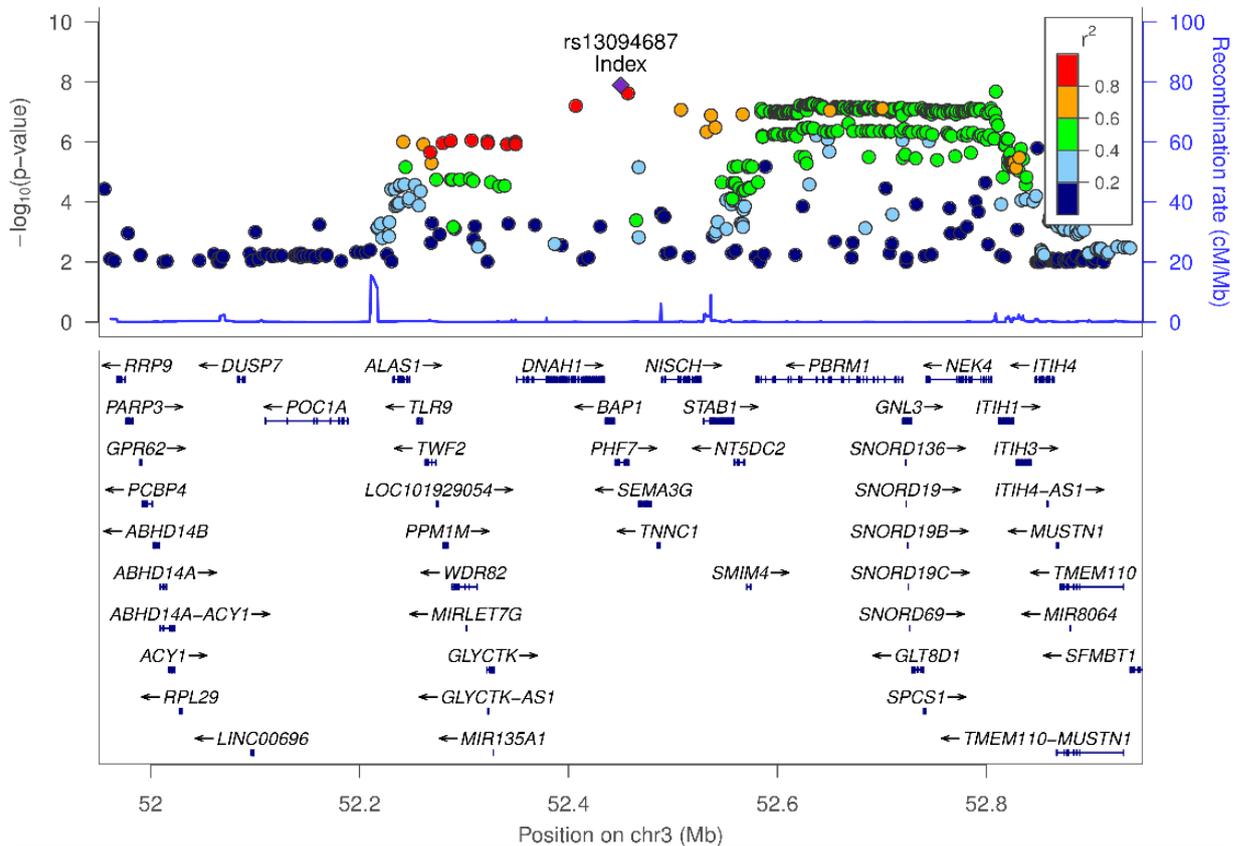
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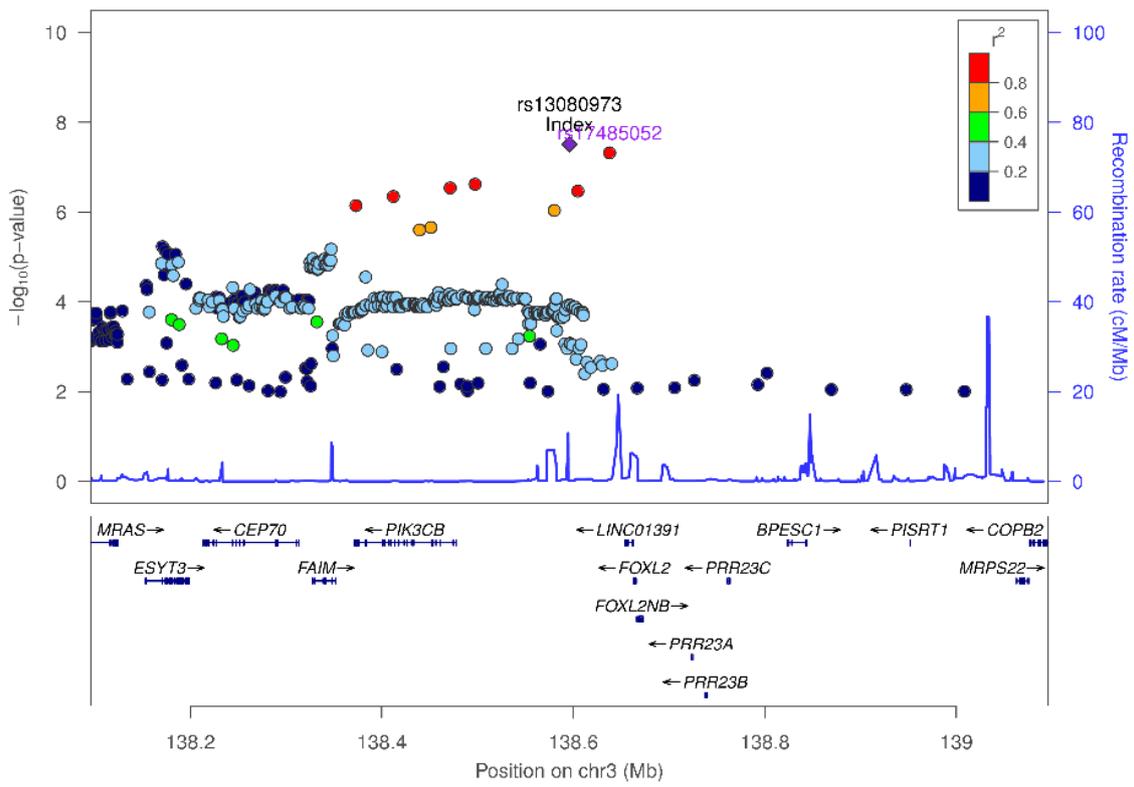
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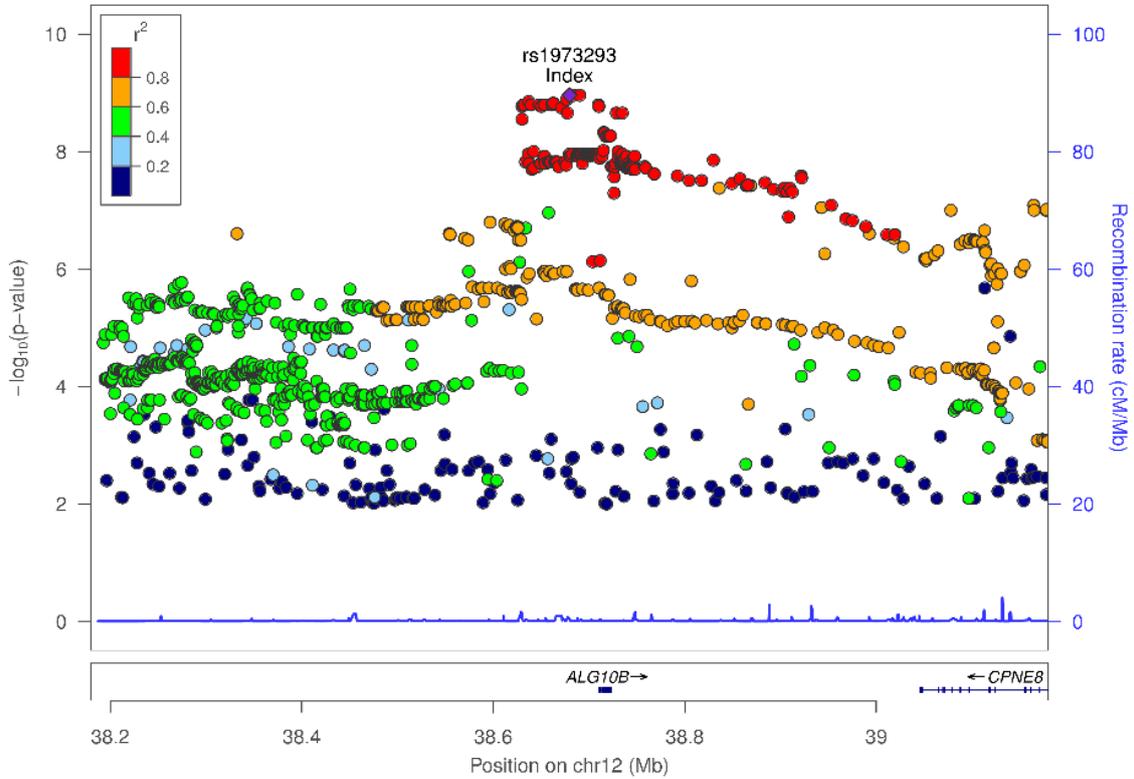
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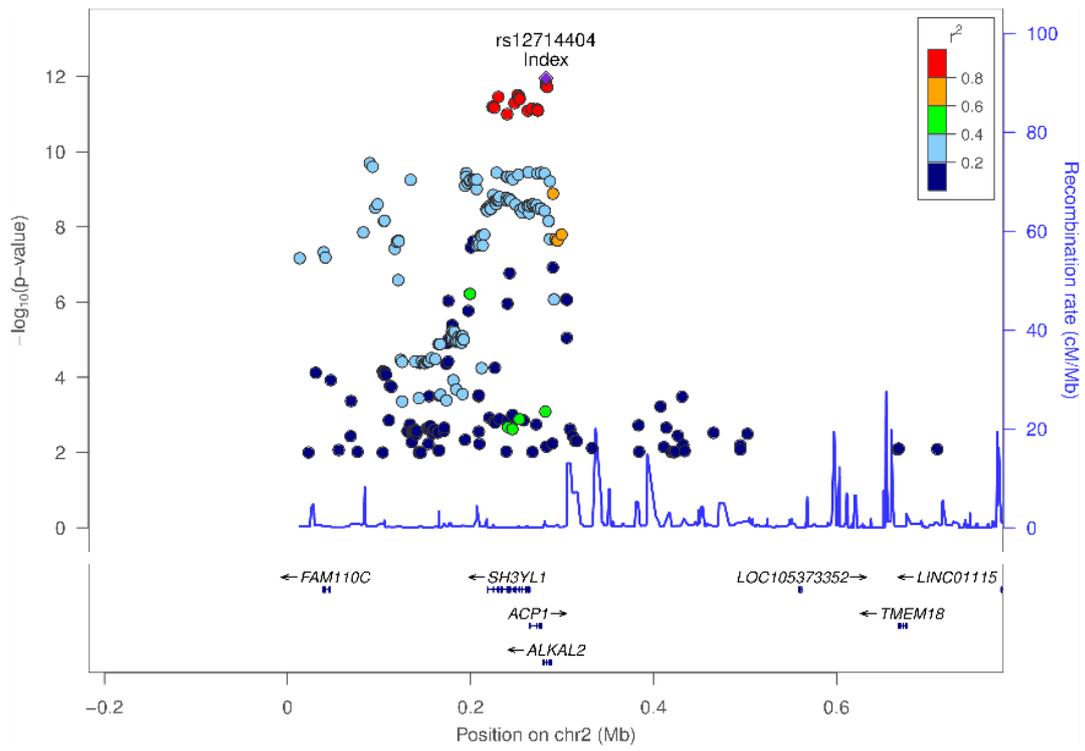
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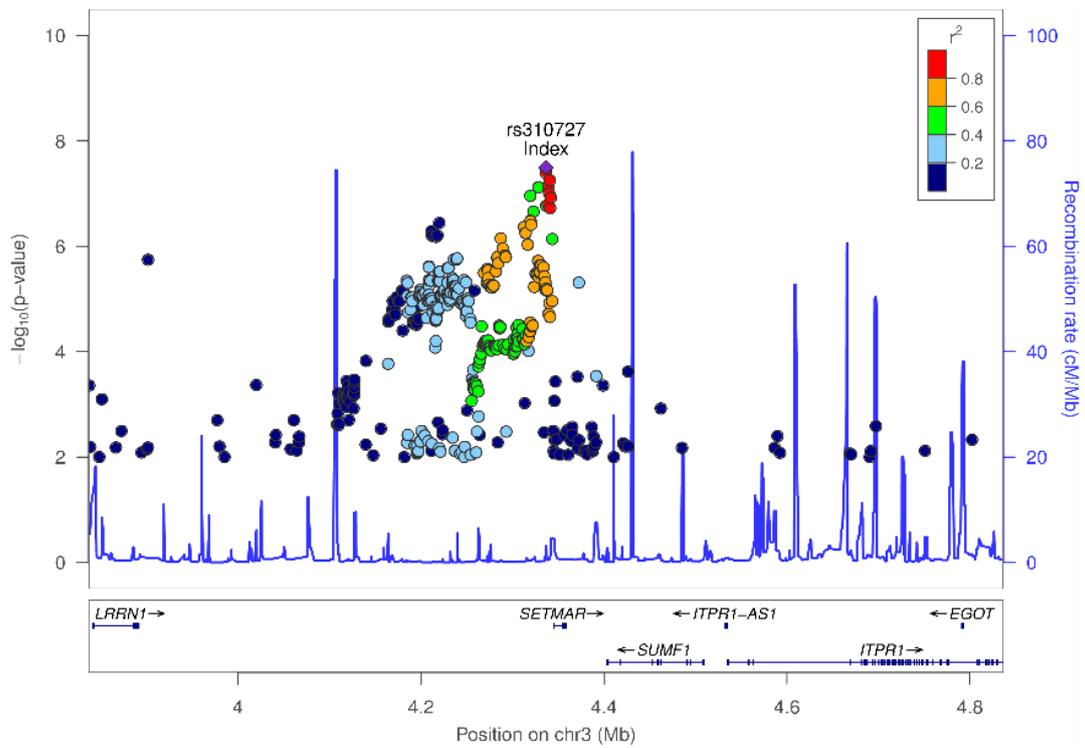
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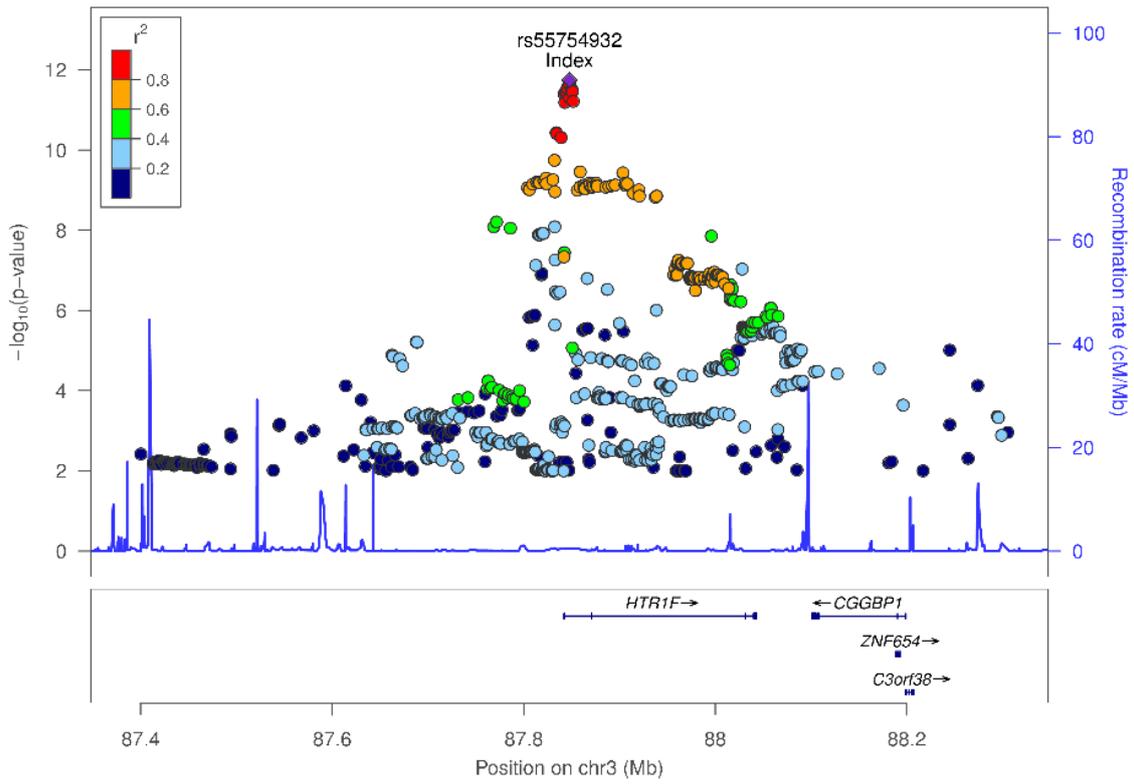
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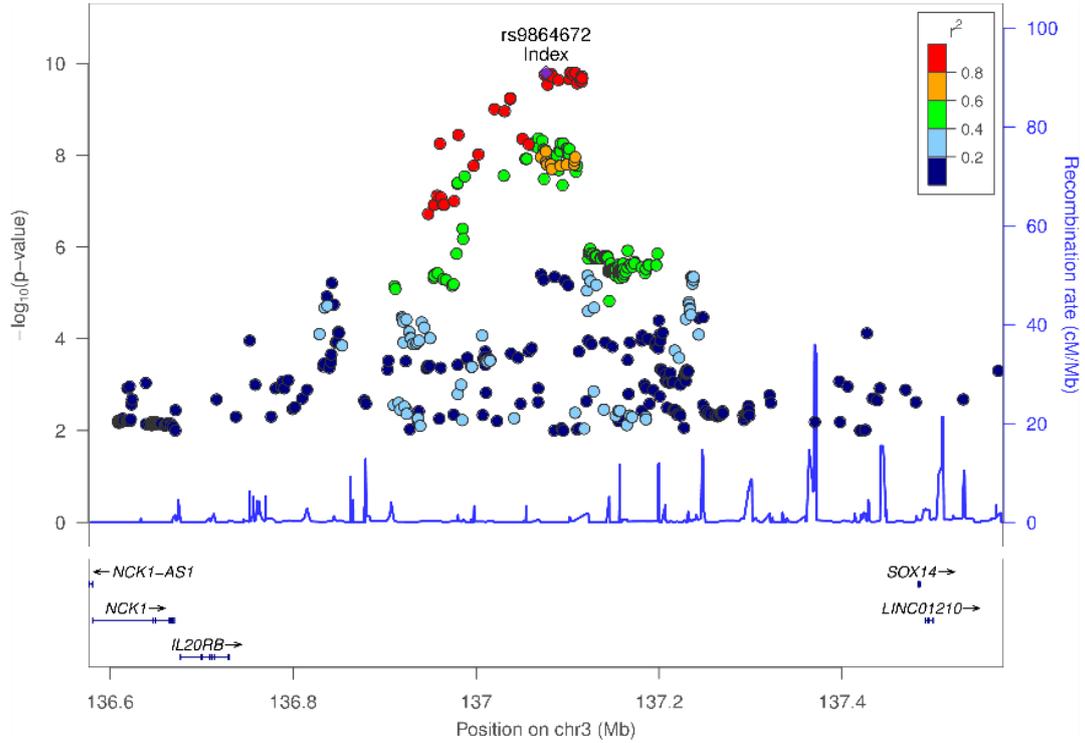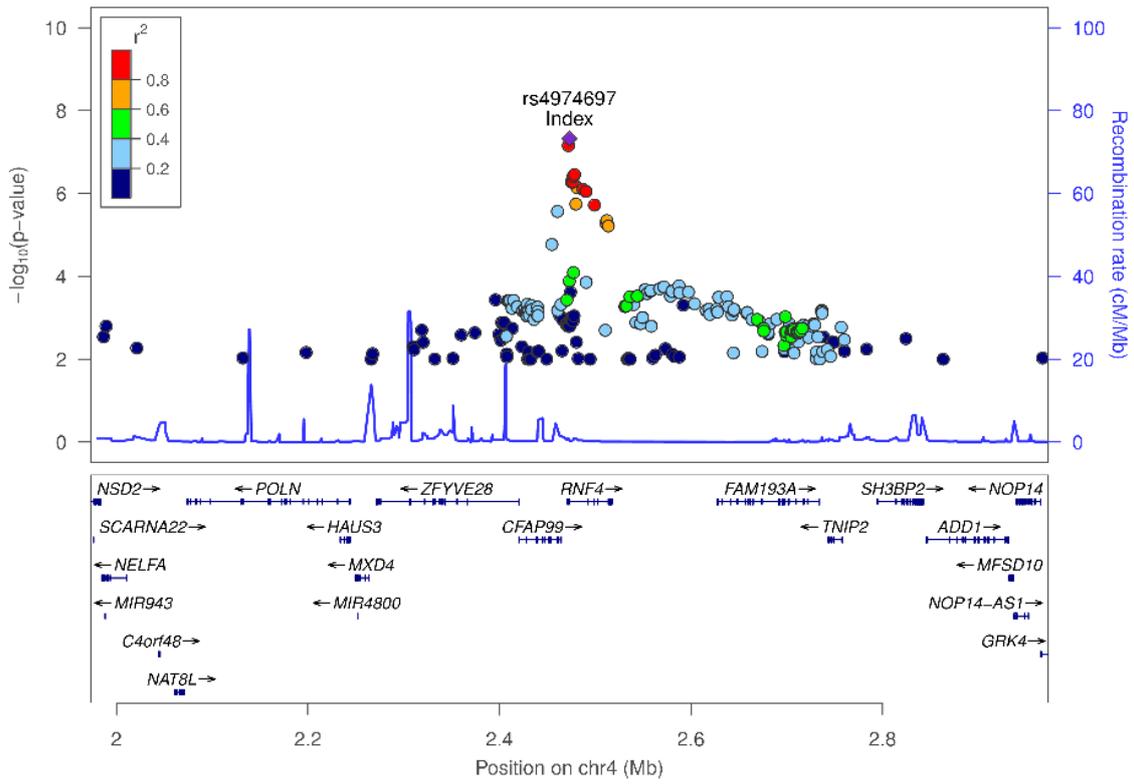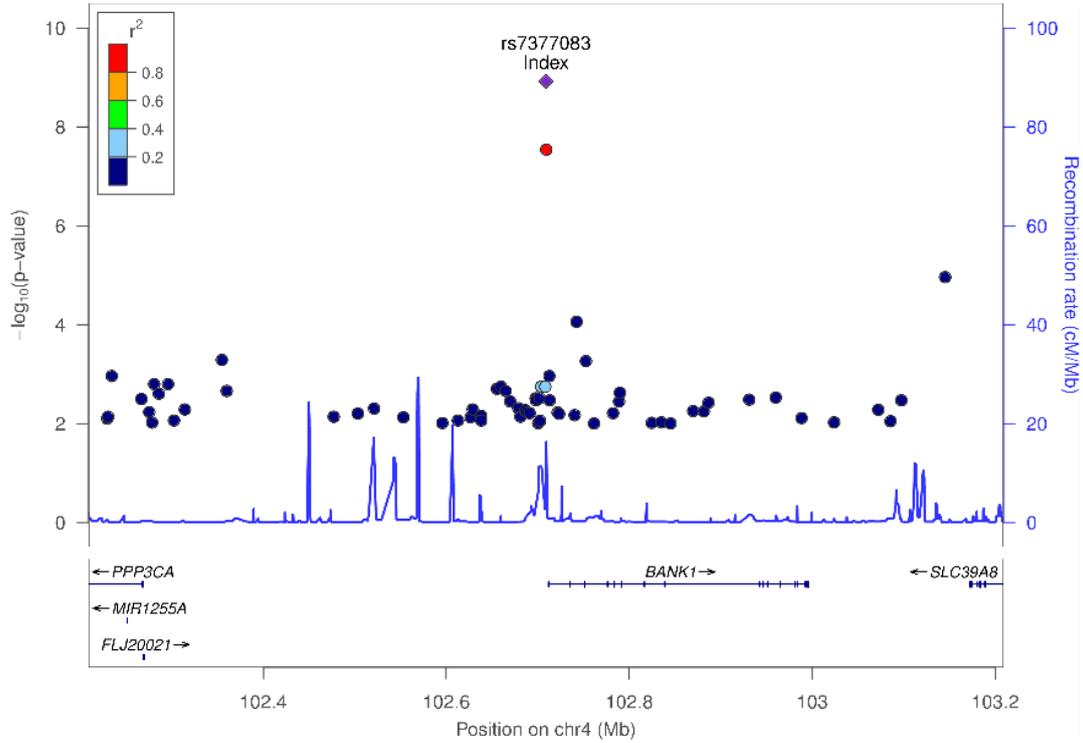
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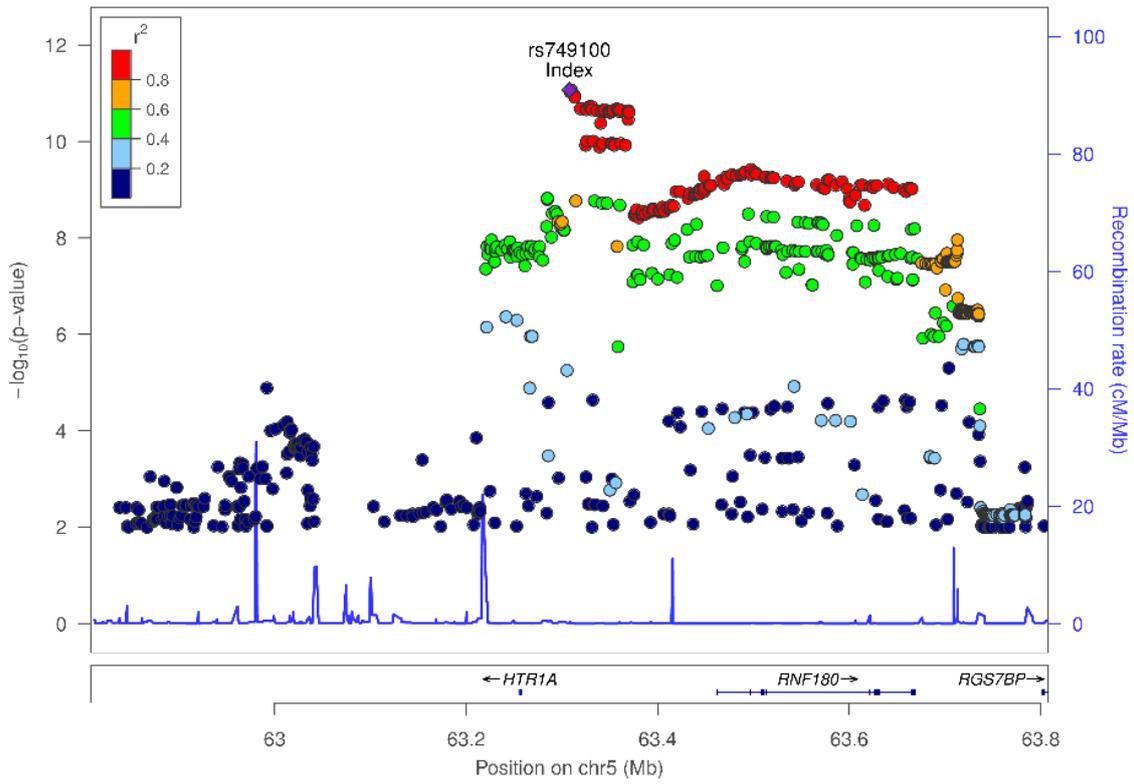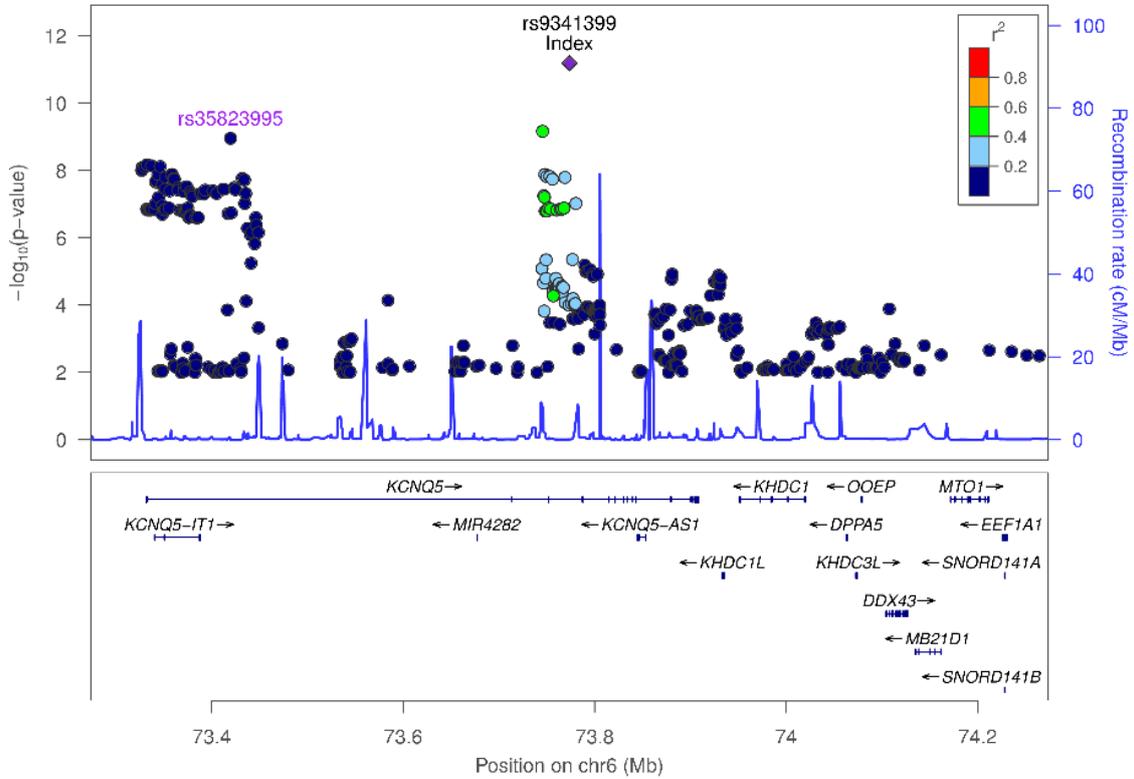
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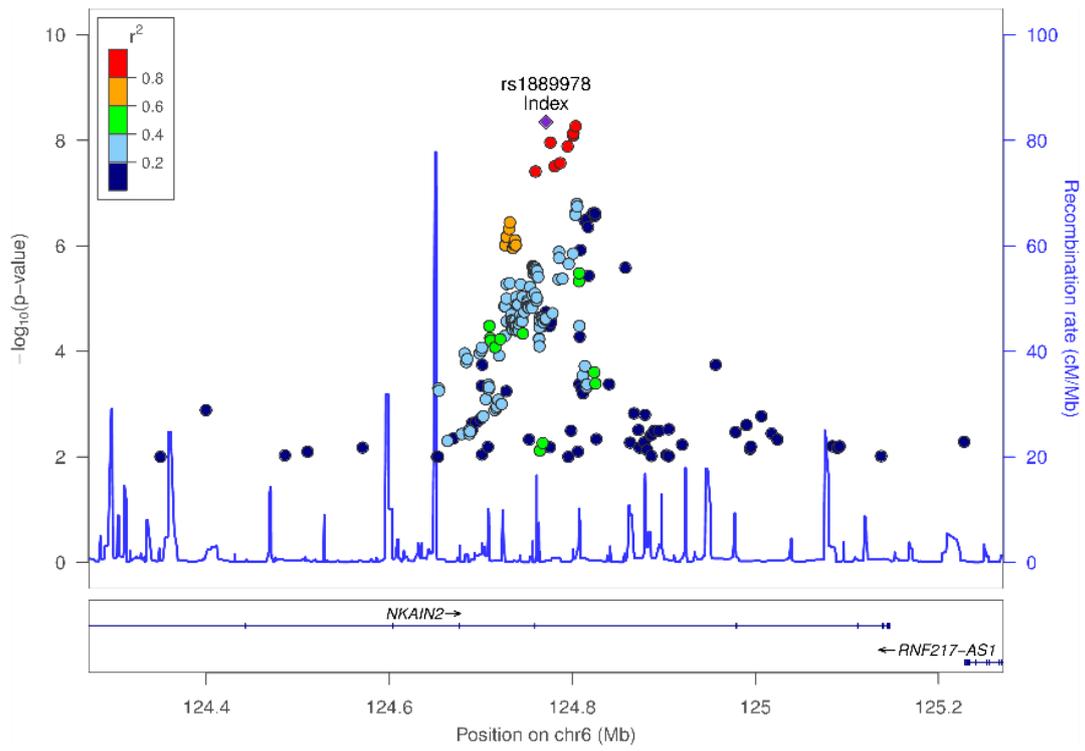
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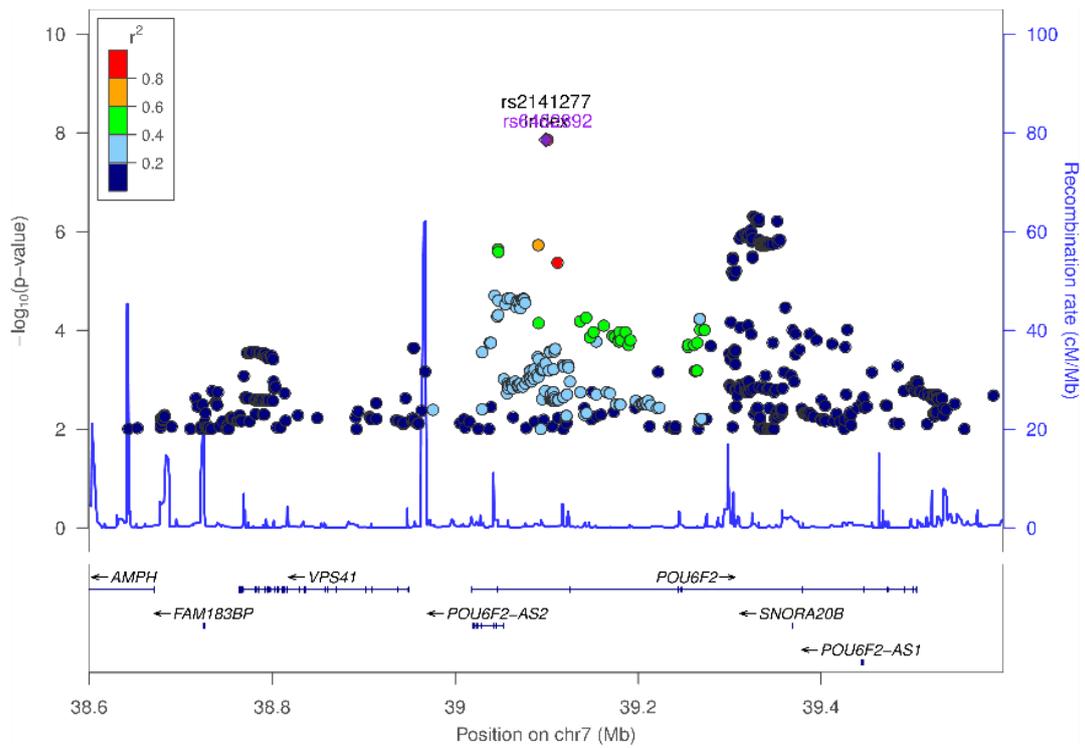
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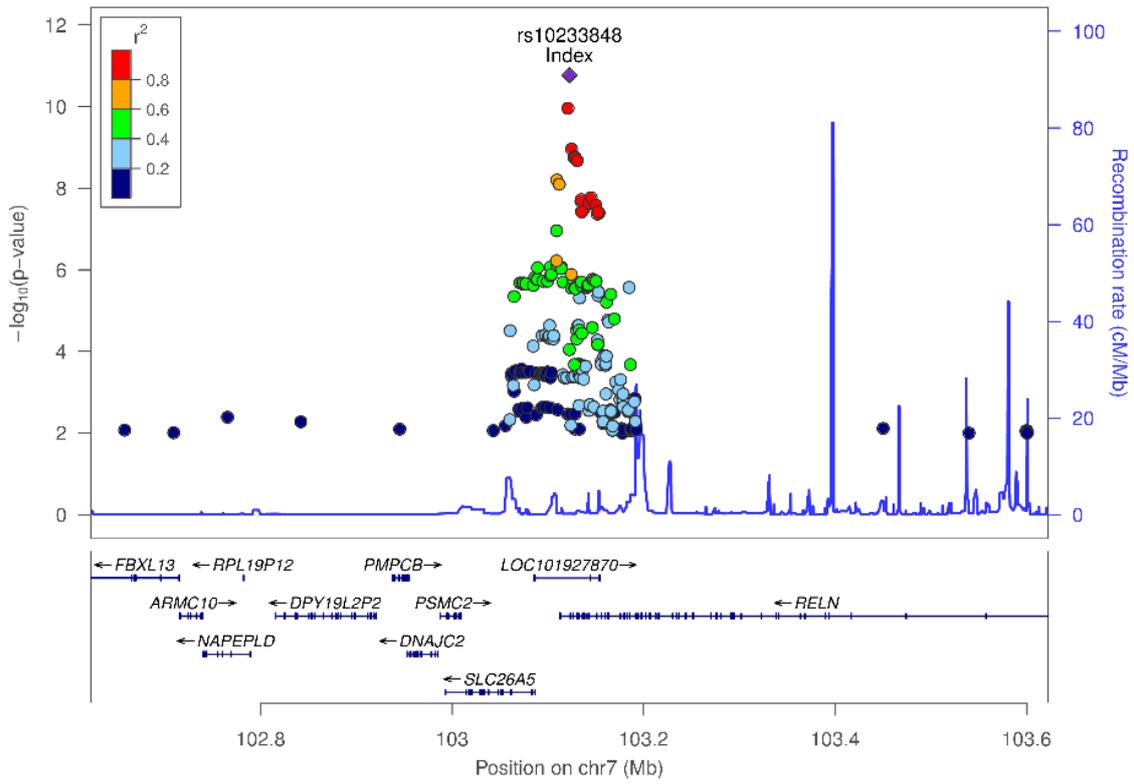
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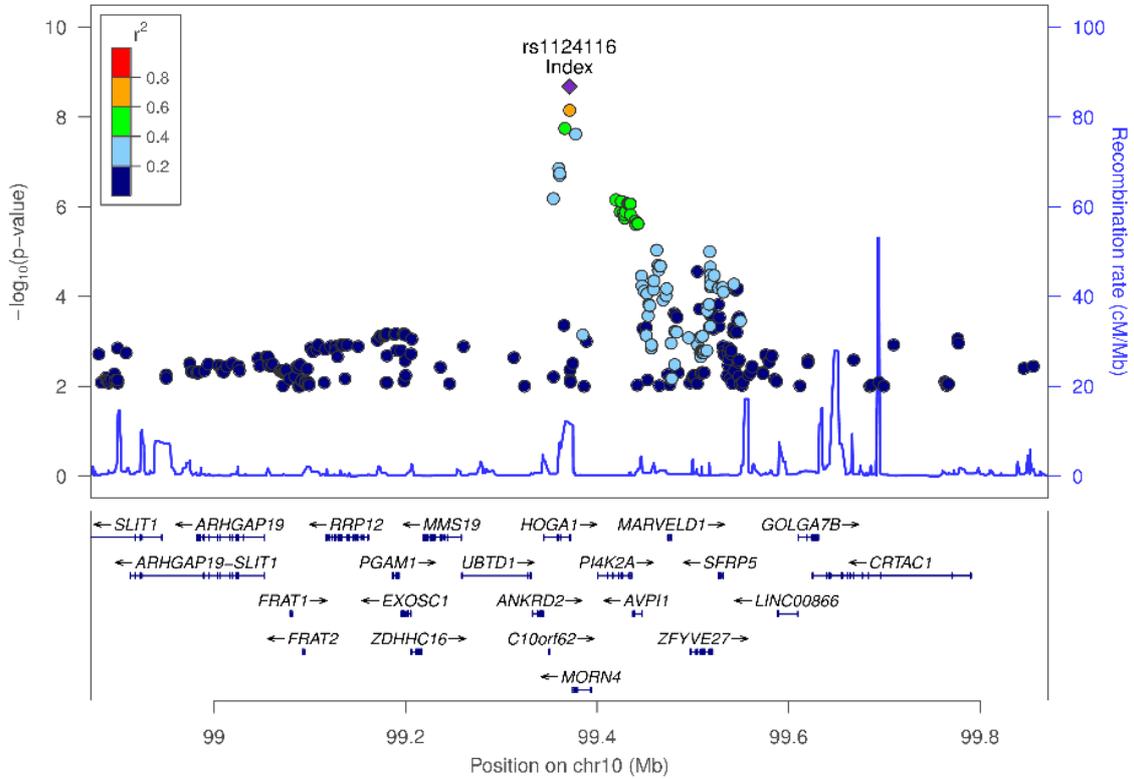
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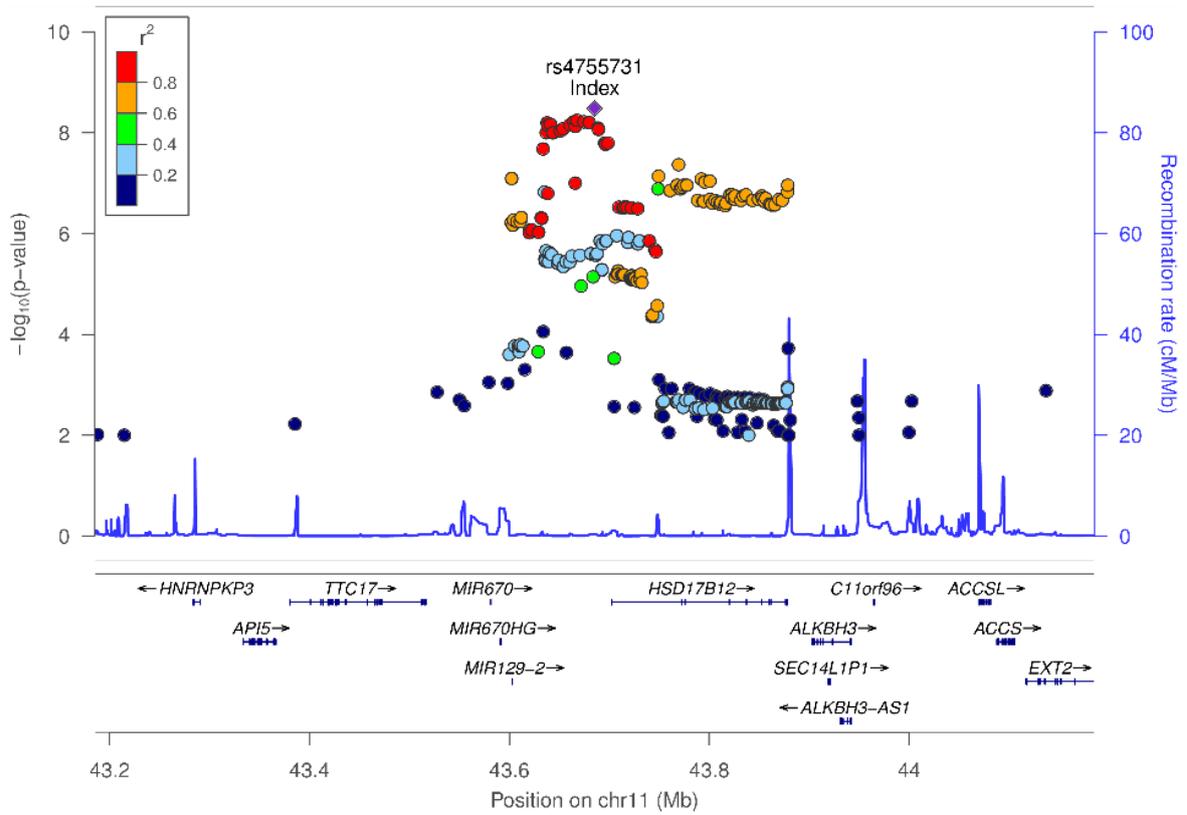
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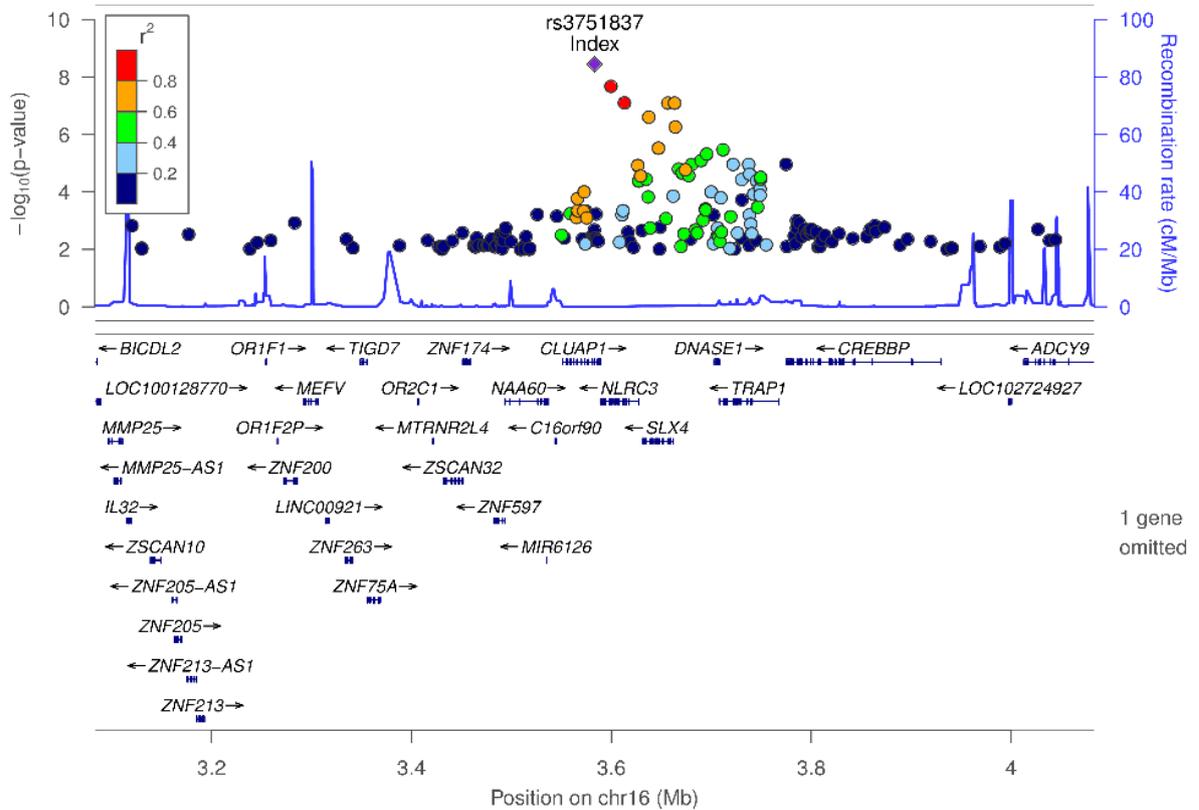
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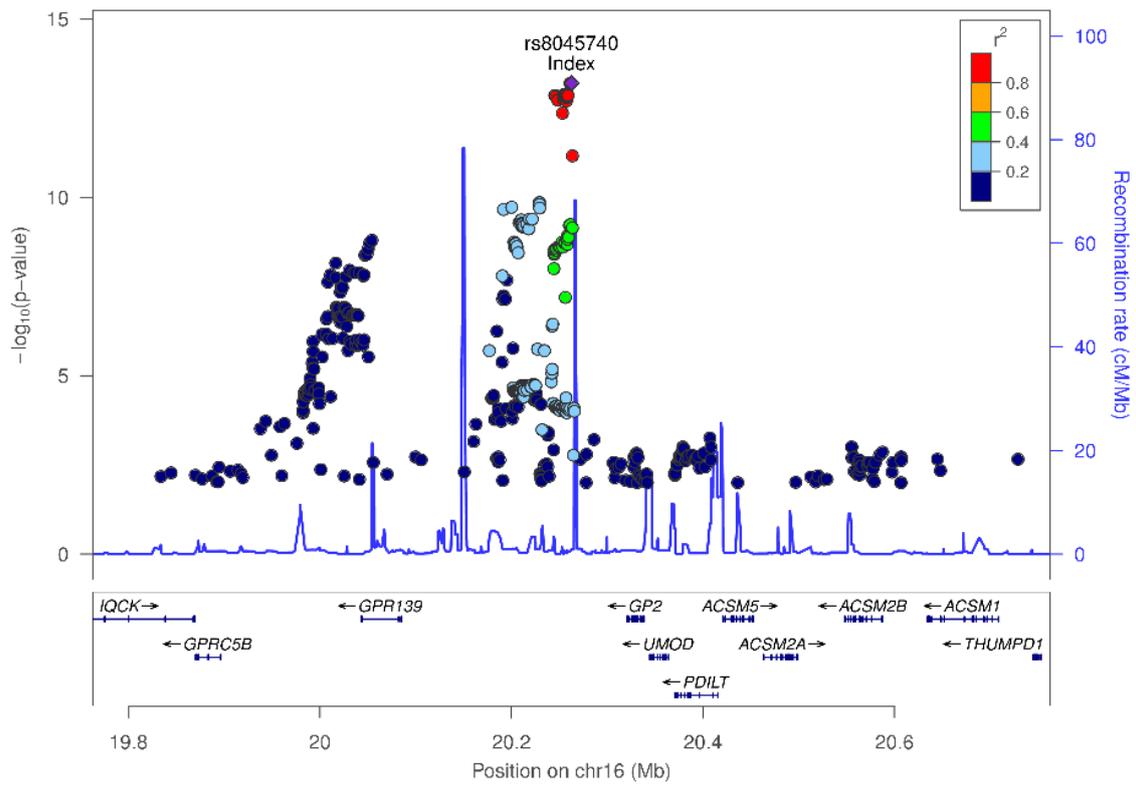
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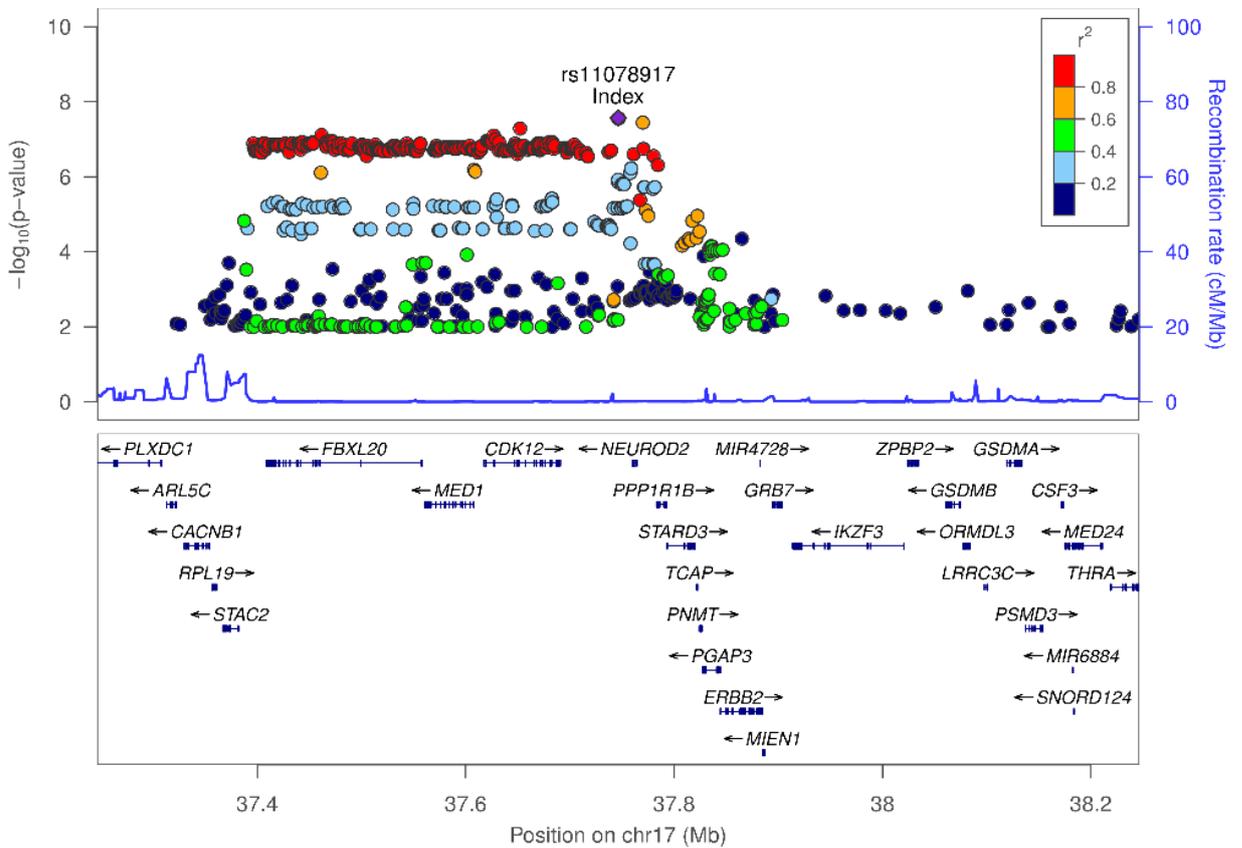
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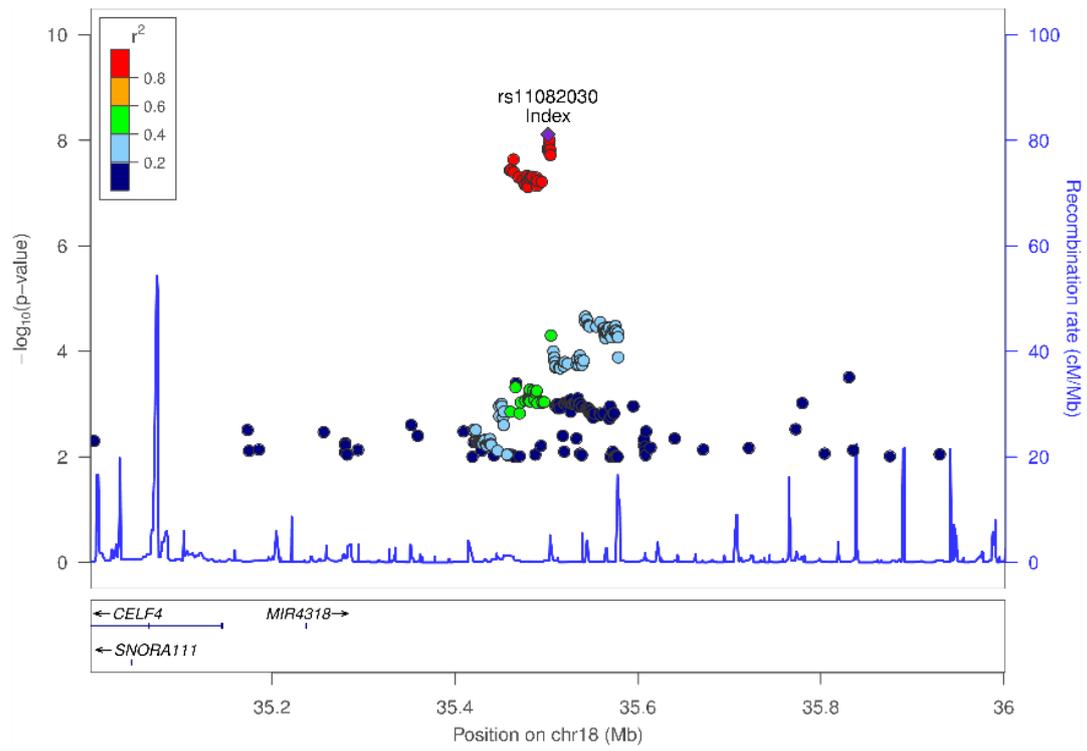
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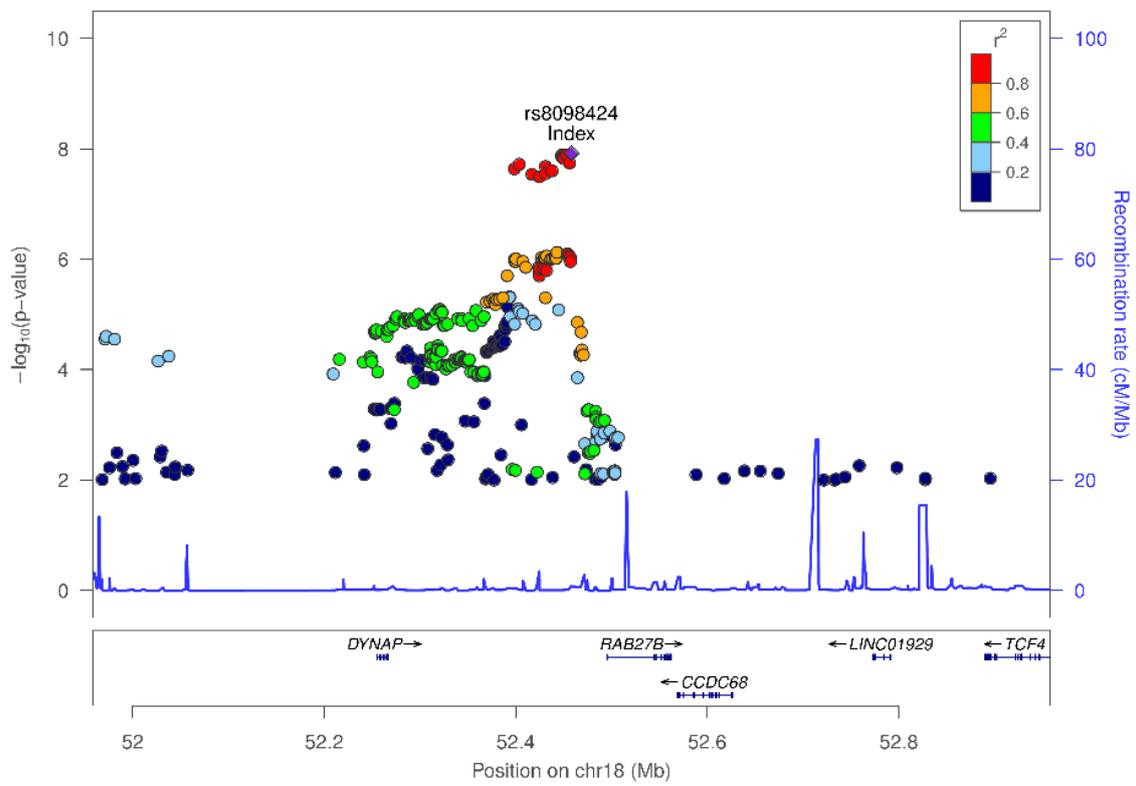
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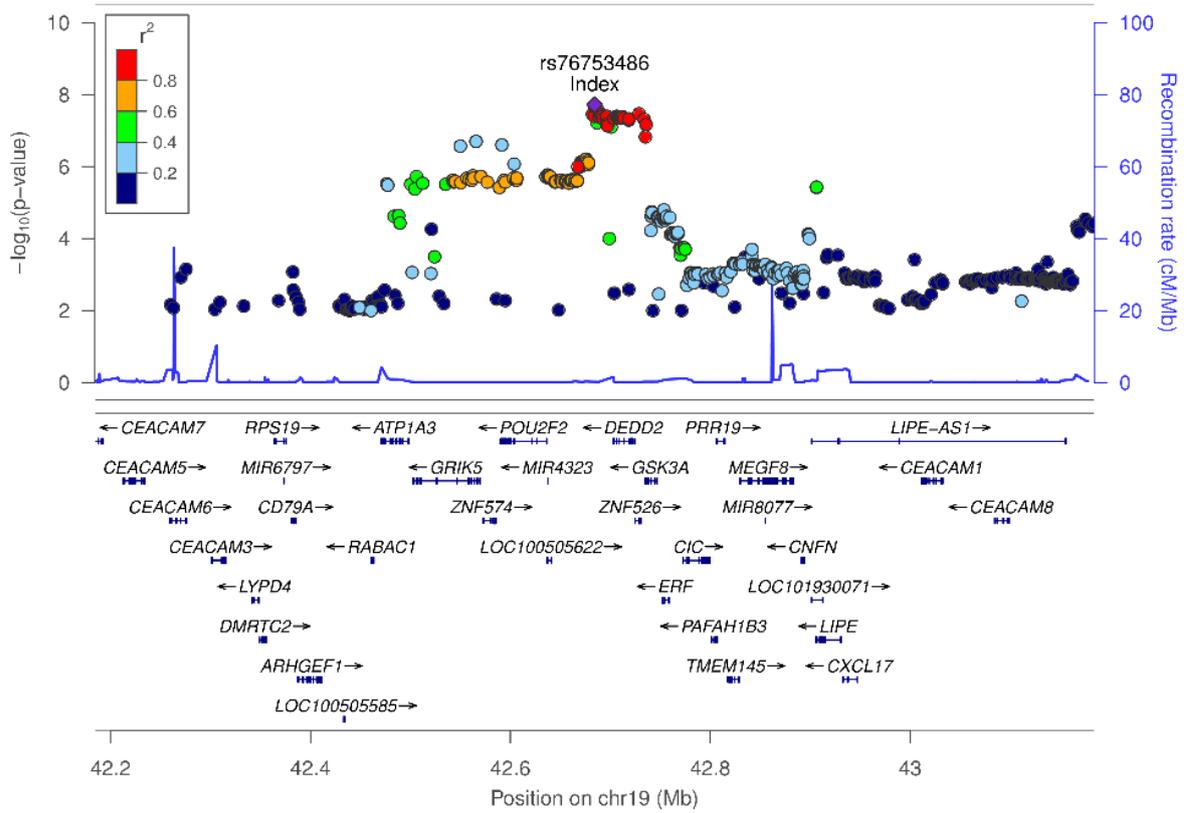
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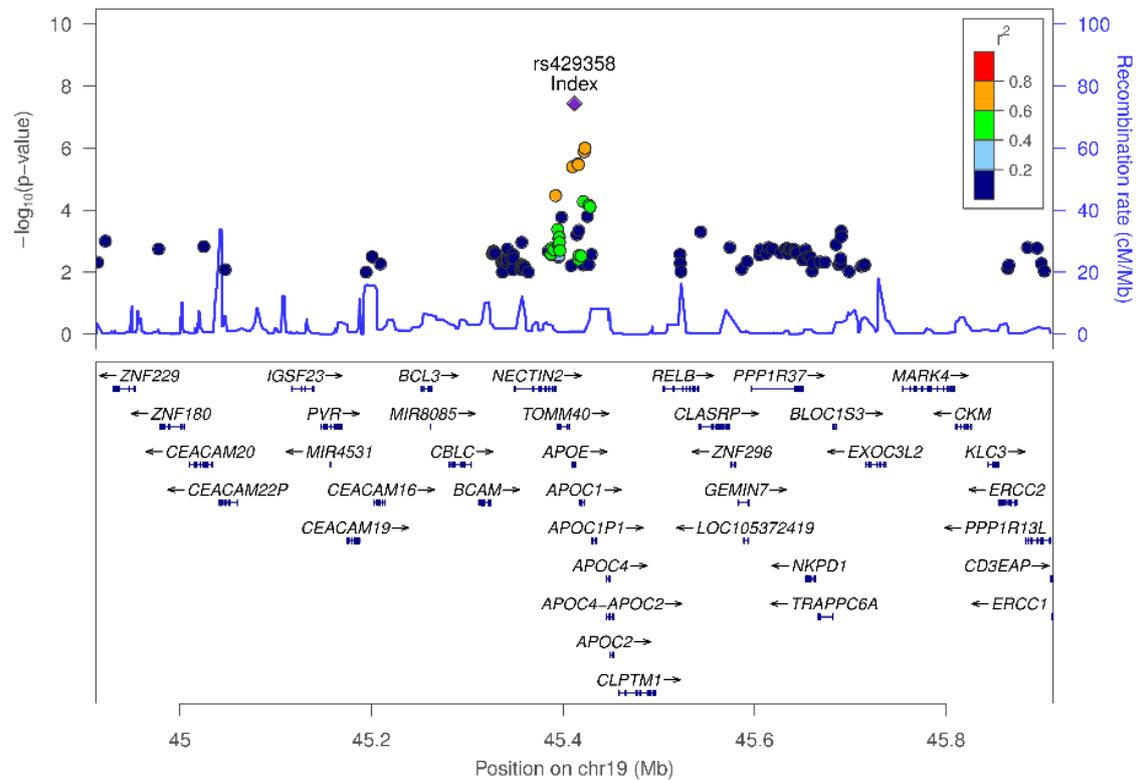
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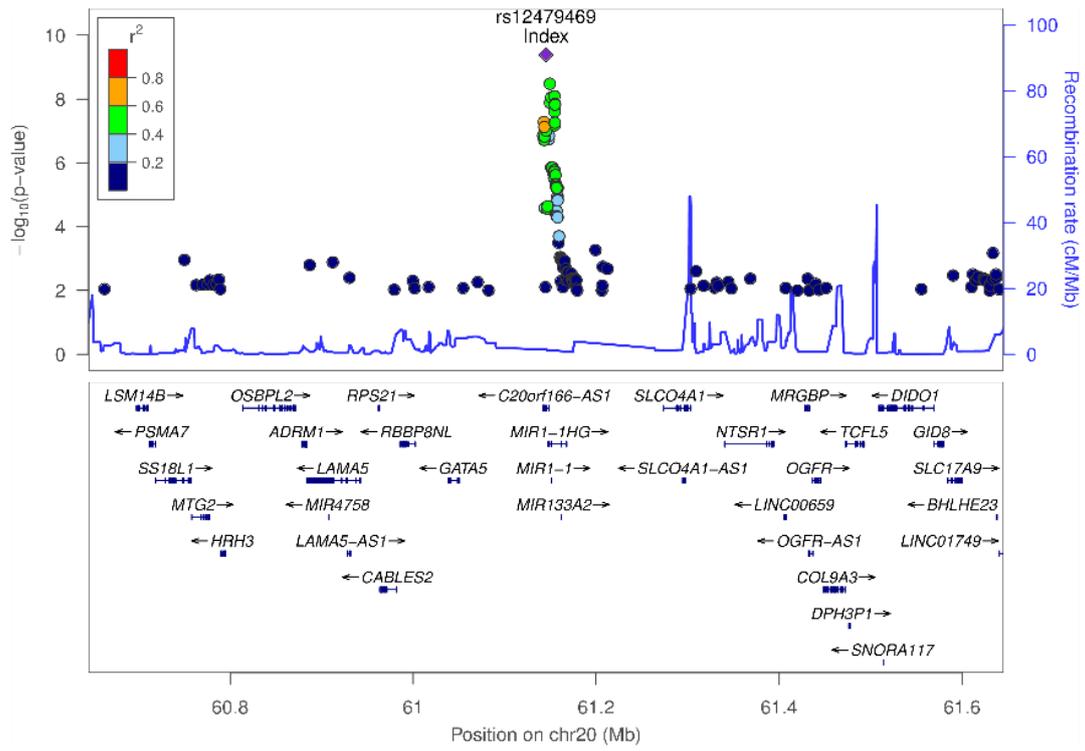
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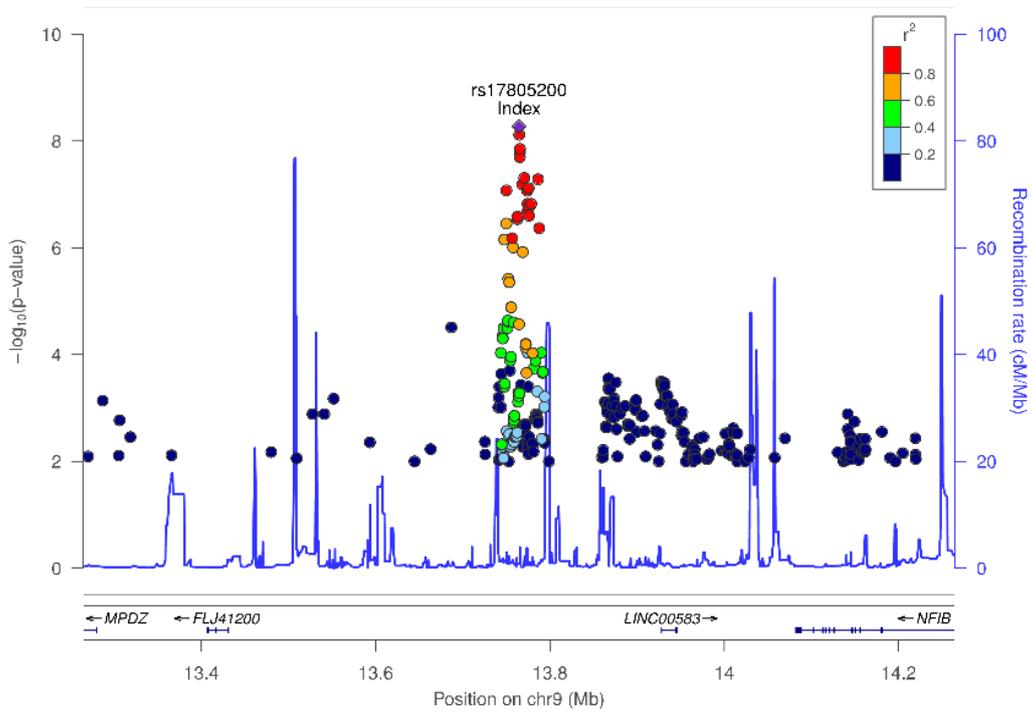
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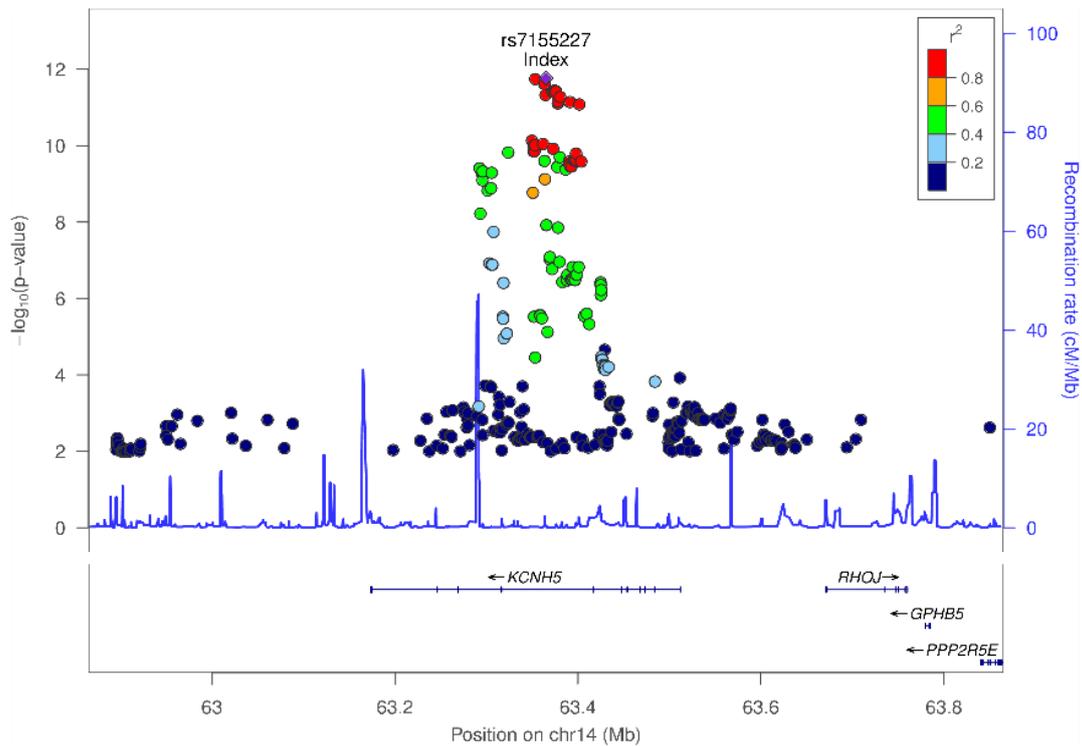
Supplementary Figure 2. Locus zoom plots for associated loci across all traits. Plots show the i) *RGS16*/*RNASEL*, ii) *MEIS1*, iii) *C1D*, iv) *BTBD9*, v) *CPNE8*/*ALG10B* and vi) *TOX3* loci for L5 timing, vii) *DPYD*, viii) *MEIS1*, ix) *PAX8*, x) *PDE11A*, xi) *LOC101928519*, xii) *BTBD9*, xiii) *MSRA*, xiv) *ANK1*, xv) *LRP12*/*ZFPM2*, xvi) *CAND1* and xvii) *KCNH5* loci for sleep duration, xviii) *CAB39* locus for sleep midpoint, xix) *MEIS1*, xx) *PAX8*, xxi) *PDE11A*, xxii) *PHF7* and xxiii) *FOXL2* loci for sleep efficiency, xxiv) *CPNE8*/*ALG10B* locus for M10 timing, xxv) *ACP1*/*SH3YL1*, xxvi) *SUMF1*/*SETMAR*, xxvii) *HTR1F*, xxviii) *IL20RB*/*SOX14*, xxix) *RNF4*, xxx) *BANK1*, xxxi) *HTR1A*/*RNF180*, xxxii) *KCNQ5*, xxxiii) *NKAIN2*, xxxiv) *POU6F2*, xxxv) *RELN*, xxxvi) *HOGA1*/*MORN4*, xxxvii) *HSD17B12*, xxxviii) *CLUAP1*, xxxix) *GPR139*, xl) *NEUROD2*, xli) *CELF4*, xlii) *RAB27B*, xliii) *DEDD2*/*ZNF526*, xliv) *APOE* and xlv) *MIR133A2* loci for number of sleep episodes, and the xlvi) *MPDZ*/*NFIB* and xlvii) *KCNH5* loci for diurnal inactivity. Index (most significant) variants are labelled in black text and credible causal variants identified by FINEMAP (if any) are labelled in purple text. Variants with *P*>0.01 were excluded from the plots for clarity.
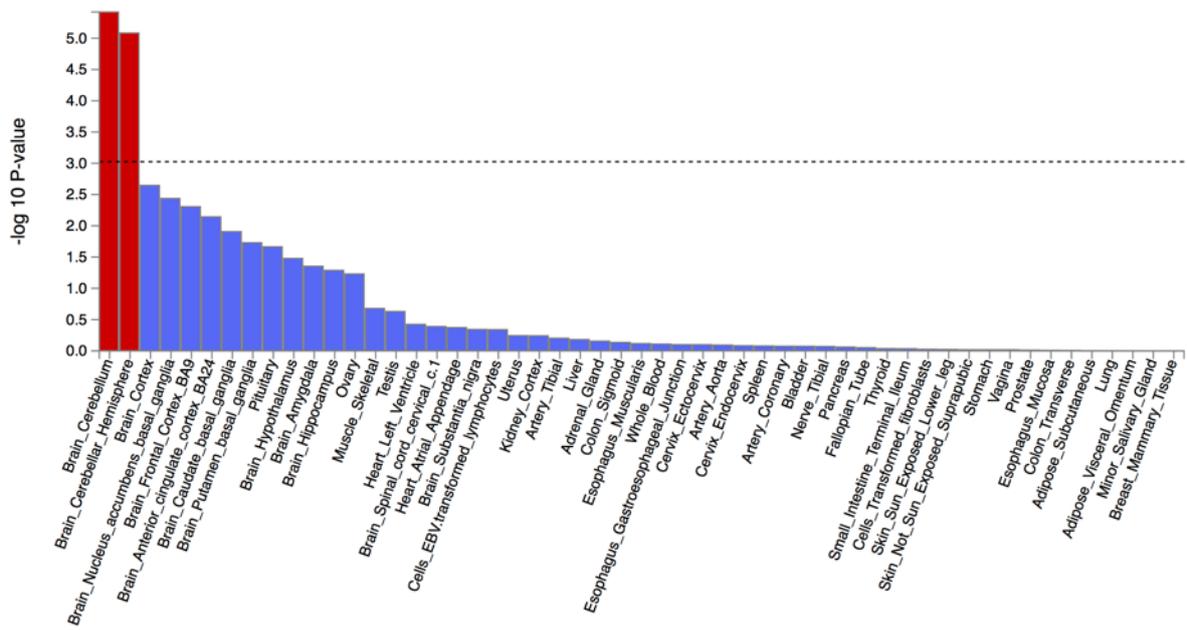
Supplementary Figure 3. Tissue enrichment of genes at associated loci for accelerometer-based sleep duration. Genes were identified using MAGMA and then analysed for enrichment using the GTEx RNA-seq database (v6) to investigate expression across 53 tissue types.
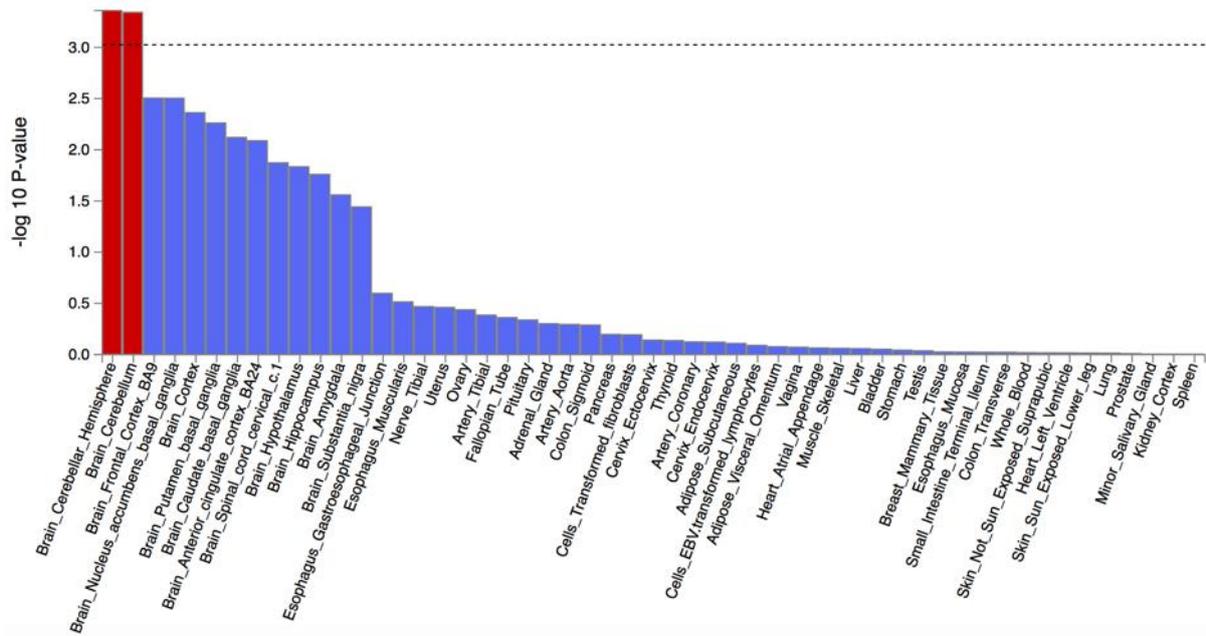
Supplementary Figure 4. Tissue enrichment of genes at associated loci for the number of sleep episodes. Genes were identified using MAGMA and then analysed for enrichment using the GTEx RNA-seq database (v6) to investigate expression across 53 tissue types.