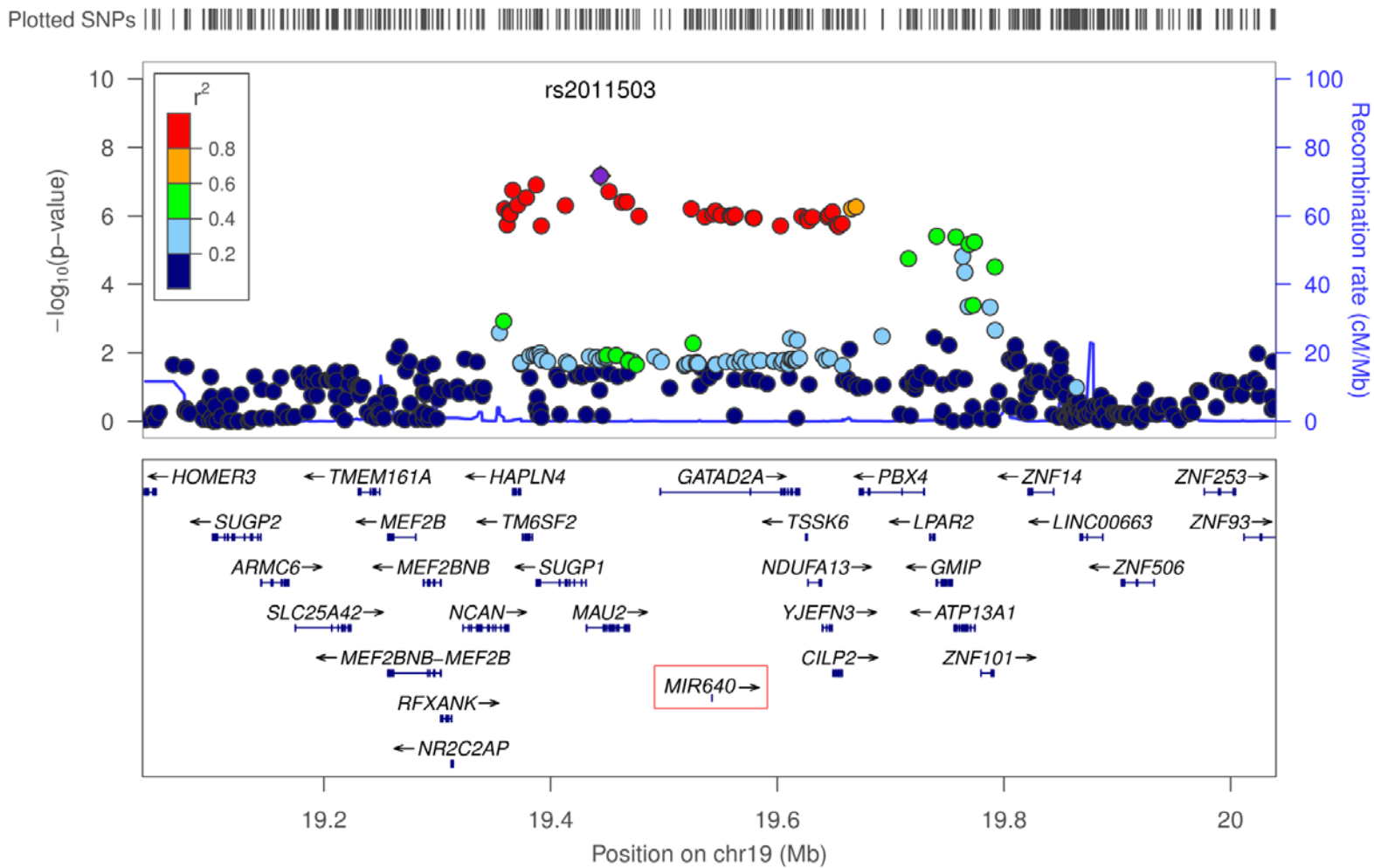Supplementary information: Forstner, Hofmann *et al.*, Genome-wide analysis implicates microRNAs and their target genes in the development of bipolar disorder
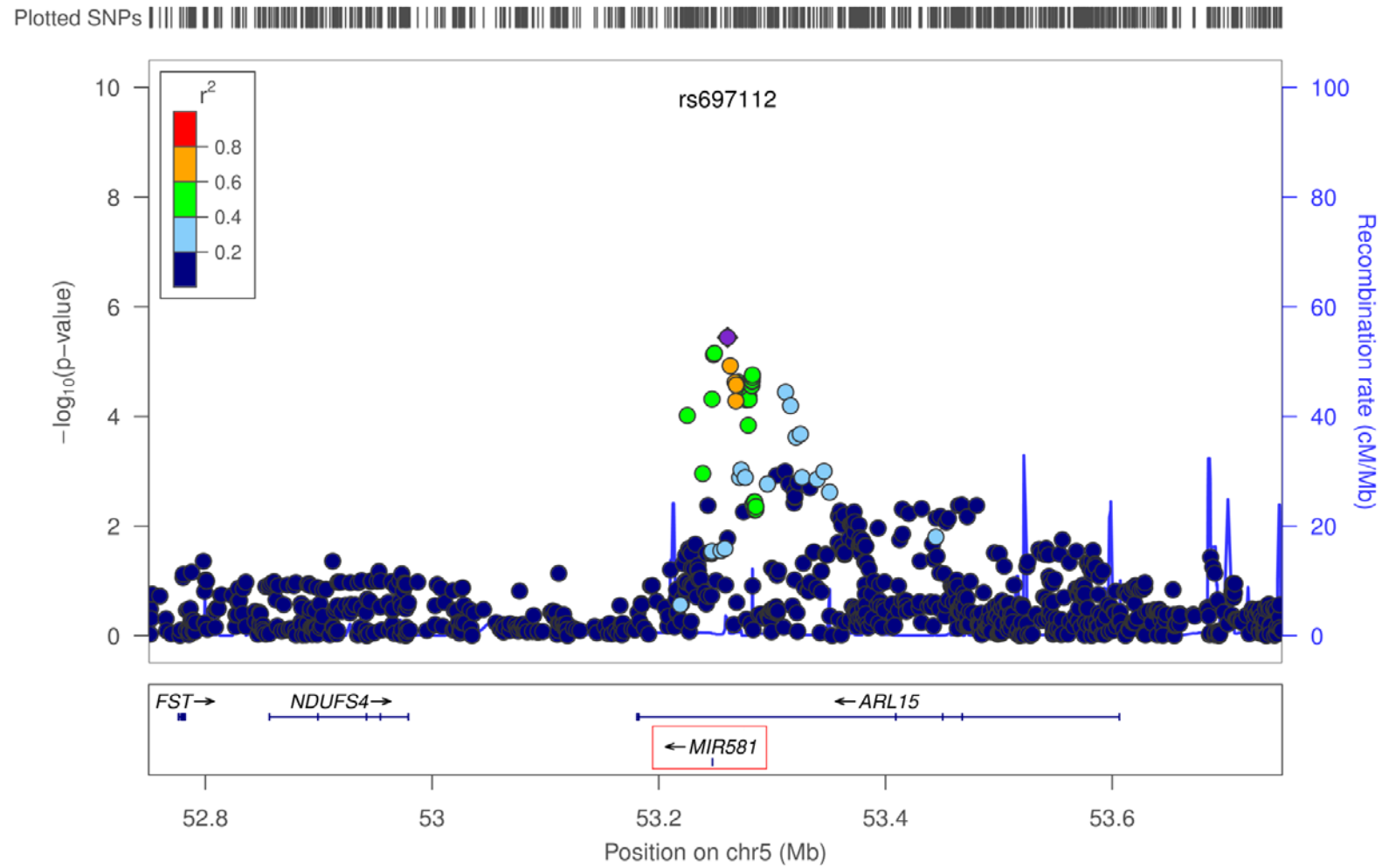
Table of contents

Supplementary Figure 1: Regional association plot of *miR-640*

Supplementary Figure 2: Regional association plot of *miR-581*

Supplementary Figure 3: Regional association plot of *miR-644* and *miR-499*
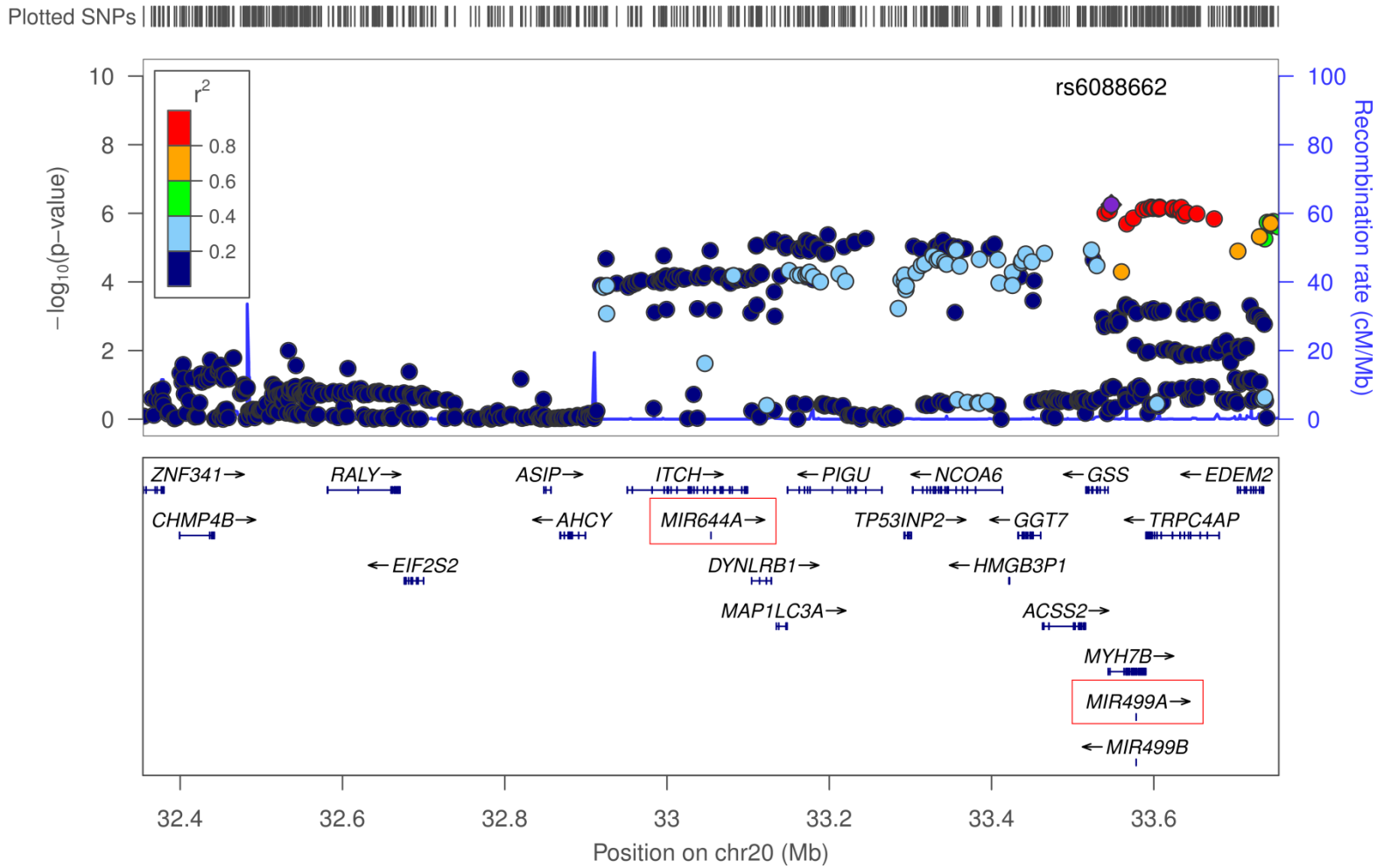
Supplementary Figure 4: Regional association plot of *let-7g* and *miR-135a-1*
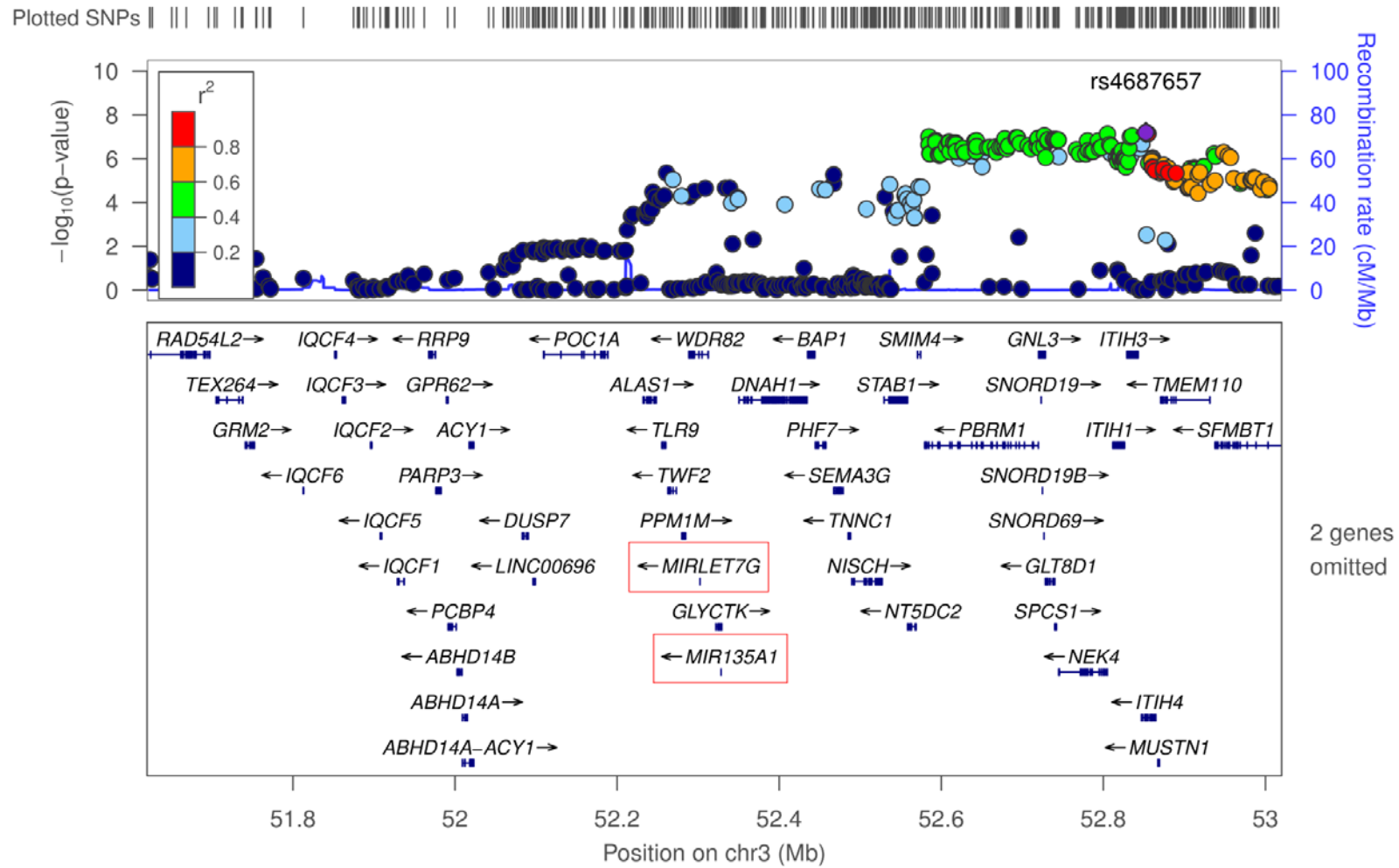
Legend Supplementary Figures 1-4: Regional association results for all associated miRNAs and their +/- 500 kb flanking regions were plotted using LocusZoom (Pruim et al., 2010). A signal was considered miRNA-associated if the Top SNP of the region was located at the miRNA locus or if it was in high or moderate LD (r^2>0.6) with the miRNA locus.

Supplementary Figure 5: Directed Acyclic Graphs (DAG) of the enriched GO categories

A
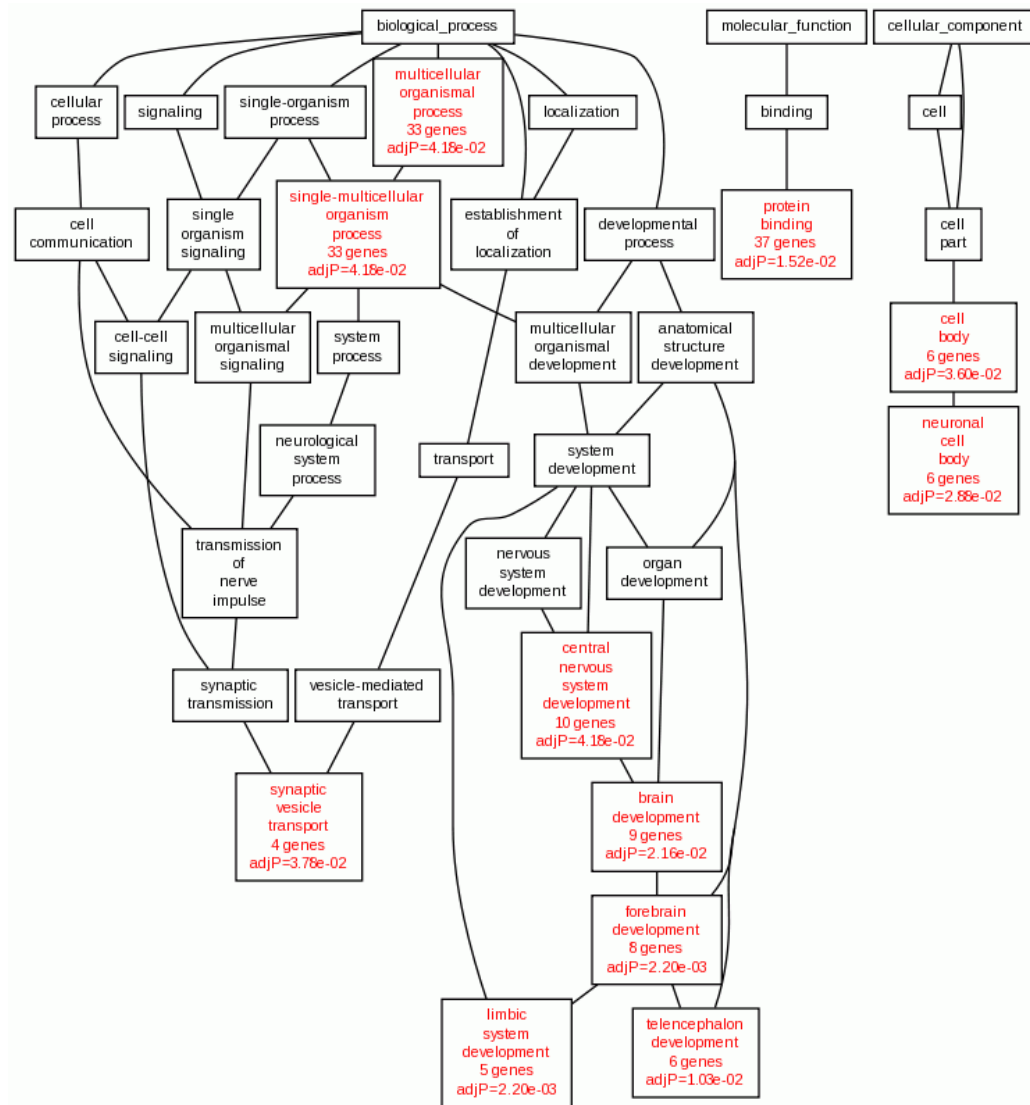
B
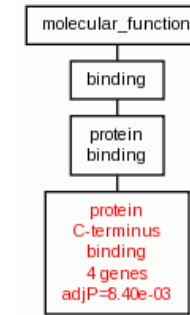
C
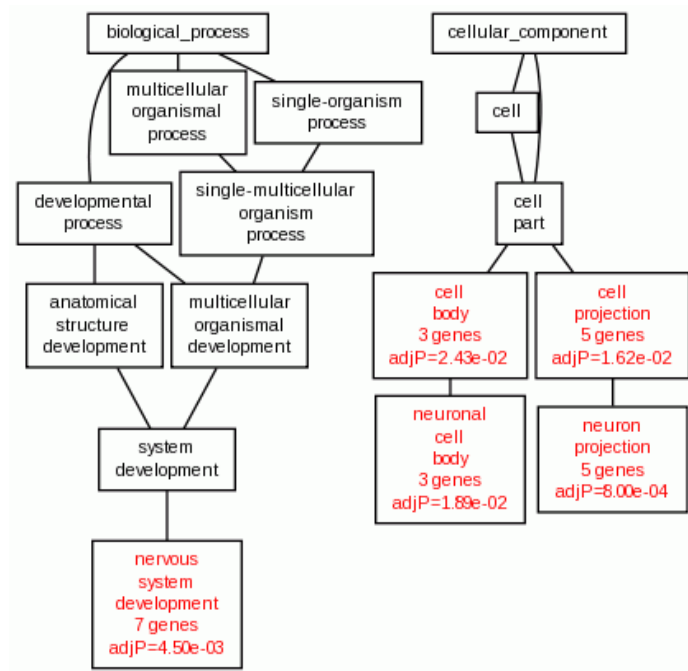
Legend Supplementary Figure 5: Target gene sets of the three microRNAs 499, 708 and 1908 were subjected to gene ontology (GO) analysis. For each microRNA the results of the GO analysis are presented as directed acyclic graphs. Significant results (p < 0.05, Bonferroni corrected) for pathways which contained at least three target genes are shown in red. **A** *miR-499* **B** *miR-708* and **C** *miR-1908*.

Supplementary Figure 6: Validation of *miR-499* and *miR-708* processing using a dual-Luciferase reporter assay in rat hippocampal neurons
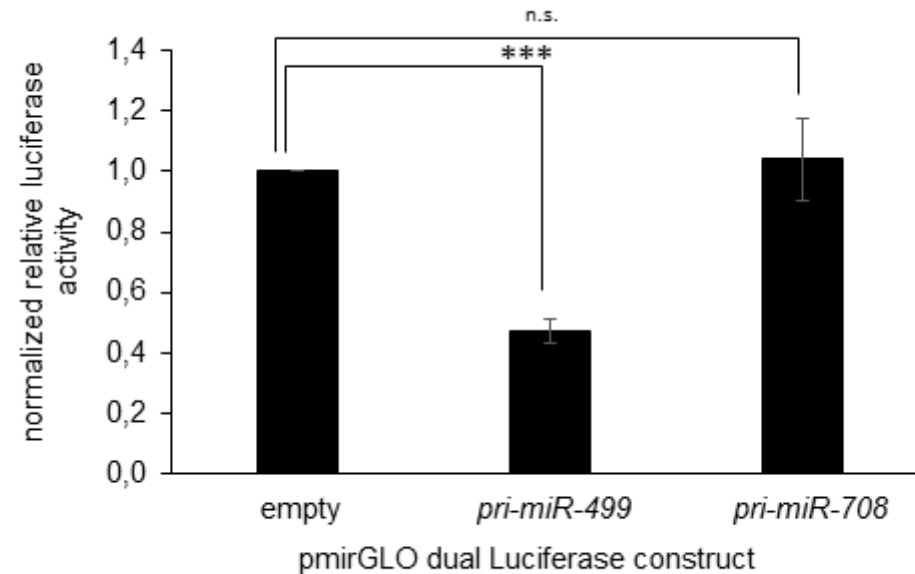
Legend Supplementary Figure 6:
Relative Luciferase activity (RLA) of DIV 10 primary rat hippocampal neurons transfected with the pmirGLO dual Luciferase reporter construct containing indicated primary-miRNA genes in the Firefly 3'UTR. Processing of the primary-miRNA genes leads to a loss of the protective poly-A tail, resulting in a fast degradation of the Firefly mRNA and therefore to reduced luciferase activity. Data are presented as the mean of three independent experiments normalized to the empty reporter construct ± standard deviation; n.s. = non significant $*p > 0.05$ $**p > 0.01$ $***p < 0.005$ (t-test).

Supplementary Table 1: Subcategory enrichment for different p value thresholds

p value threshold	SNPs in miRNA +/- 20 kb	SNPs in genes	Intergenic SNPs
$<1 \times 10^{-6}$	0.11	4.00×10^{-4}	> 0.99
$\geq 1 \times 10^{-6}$ & $<1 \times 10^{-4}$	0.037	2.39×10^{-7}	> 0.99
$\geq 1 \times 10^{-4}$ & <0.05	0.041	1.22×10^{-15}	> 0.99
≥ 0.05	0.98	> 0.99	5.53×10^{-18}

Legend Supplementary Table 1: Subcategory testing for different BD-association p value thresholds for the categories SNPs in miRNA loci (+/- 20 kb), SNPs in genes, intergenic SNPs. Enrichment was calculated using a one-sided fisher's exact test.

Supplementary Table 2: Comparison of the results of the gene-based tests for the nine microRNAs that withstood Bonferroni correction based on the LD structure derived from HapMap phase 2 or the 1,000 Genomes Project

MicroRNA	Chr	Top SNP	p Top SNP	nSNPs HM2	p Gene HM2	nSNPs 1000G	p Gene 1000G
miR-499	20	rs3818253	6.58×10^{-7}	27	2.00×10^{-6}	27	1.00×10^{-6}
miR-640	19	rs2965184	7.23×10^{-7}	21	2.00×10^{-6}	21	1.00×10^{-6}
miR-708	11	rs7108878	3.45×10^{-7}	72	2.00×10^{-6}	72	3.00×10^{-6}
miR-581	5	rs697112	3.61×10^{-6}	36	1.20×10^{-5}	34	1.40×10^{-5}
miR-644	20	rs7269526	1.22×10^{-5}	12	1.70×10^{-5}	12	8.00×10^{-6}
miR-135a-1	3	rs9311474	2.16×10^{-5}	20	2.00×10^{-5}	20	2.00×10^{-5}
let-7g	3	rs6445358	2.23×10^{-5}	9	5.00×10^{-5}	10	2.50×10^{-5}
miR-1908	11	rs174575	2.85×10^{-5}	16	5.80×10^{-5}	16	6.10×10^{-5}
miR-611	11	rs174535	5.03×10^{-5}	23	7.50×10^{-5}	23	7.20×10^{-5}

Legend Supplementary Table 2: Abbreviations: Chr = Chromosome; p Top SNP = p value of the Top SNP within gene; nSNPs = number of investigated SNPs; HM2 = based on HapMap phase 2 data, 1000G = based on the 1,000 Genomes Project data, p Gene = nominal gene-based p value.

Supplementary Table 3: Significant biological pathways in microRNA target gene data sets

microRNA	Type	Pathway	no. genes in subcategory	no. target genes	p corr.	gene symbol
miR-499	KEGG	Regulation of actin cytoskeleton	213	3	0.0032	*ENAH, VAV3, PFN2*
	GO	forebrain development	281	8	0.0022	*LMX1A, BCL11B, EPHA5, CNTNAP2, SLC8A3, ETS1, HOOK3, ZEB2*
	GO	limbic system development	73	5	0.0022	*LMX1A, EPHA5, CNTNAP2, ETS1, ZEB2*
	GO	telencephalon development	168	6	0.0103	*LMX1A, BCL11B, EPHA5, CNTNAP2, SLC8A3, ZEB2*
	GO	protein binding	7337	37	0.0152	*AAK1, H2AFZ, ENAH, CNTNAP2, VPS13A, PFN2, ETS1, AP3S1, EEA1, RIMS1, CACNB2, QKI, PIM1, MARCKS, TBC1D15, CPSF6, TOP1, VAV3, CHD9, SLC8A3, DYNLT1, HOOK3, HNRNPC, ILF3, PTCH1, EFHC1, WDR82, UHRF1BP1, GPC6, KCNN3, PURB, FKBP5, SOX5, PTPN14, SPAST, PRKAR1A, ZEB2*
	GO	brain development	502	9	0.0216	*BCL11B, SLC8A3, HOOK3, PTCH1, LMX1A, EPHA5, CNTNAP2, ETS1, ZEB2*
	GO	neuronal cell body	291	6	0.0288	*EPHA5, EFHC1, CNTNAP2, TANC1, SLC8A3, TOP1*
	GO	cell body	312	6	0.0360	
	GO	synaptic vesicle transport	68	4	0.0378	*RIMS1, AP3S1, PFN2, EEA1*
	GO	central nervous system development	688	10	0.0418	*BCL11B, SLC8A3, HOOK3, PTCH1, LMX1A, EPHA5, CNTNAP2, ETS1, SOX5, ZEB2*
	GO	multicellular organismal process	5644	33	0.0418	*BCL11B, ENAH, CNTNAP2, VPS13A, PFN2, ETS1, KCNQ5, AP3S1, EEA1, RIMS1, CACNB2, QKI, PIM1, TOP1, VAV3, SLC8A3, TMEM2, DYNLT1, JPH1, HOOK3, PTCH1, EPHA5, TANC1, KCNN3, LMX1A, PURB, PTPN14, SPAST, SOX5, ZEB2, PRKAR1A, RNF114, ROD1/PTBP3*
	GO	single-multicellular organism process	5612	33	0.0418	
miR-708	GO	protein C-terminus binding	158	4	0.0084	*ATXN1, SHANK3, PFKM, FOXN3*
miR-1908	GO	neuron projection	651	5	0.0008	*MINK1, SLC12A5, SLC17A7, NCDN, KLC2*
	GO	nervous system development	1724	7	0.0045	*MINK1, PCDHA1, MDGA1, OTX1, GDF11, NRGN, NCDN*
	GO	cell projection	1230	5	0.0162	*MINK1, SLC12A5, SLC17A7, NCDN, KLC2*
	GO	neuronal cell body	291	3	0.0189	*SLC12A5, NCDN, EEF1A2*
	GO	cell body	312	3	0.0243	

Legend Supplementary Table 3: Significant pathways with a minimum of three genes included per set are depicted in Supplementary Table 3. Abbreviations: KEGG = Kyoto Encyclopedia of Genes and Genomes; GO = Gene Ontology; no. genes in subcategory = number of reference genes in KEGG/GO subcategory; no. target genes = number of microRNA target genes contained in subcategory; p corr = Bonferroni corrected p value.

Supplementary Box 1: List of the 107 brain-expressed microRNA target genes associated with bipolar disorder at gene-based p < 0.05