Stage-specific expression patterns and co-targeting relationships among miRNAs in the developing mouse cerebral cortex

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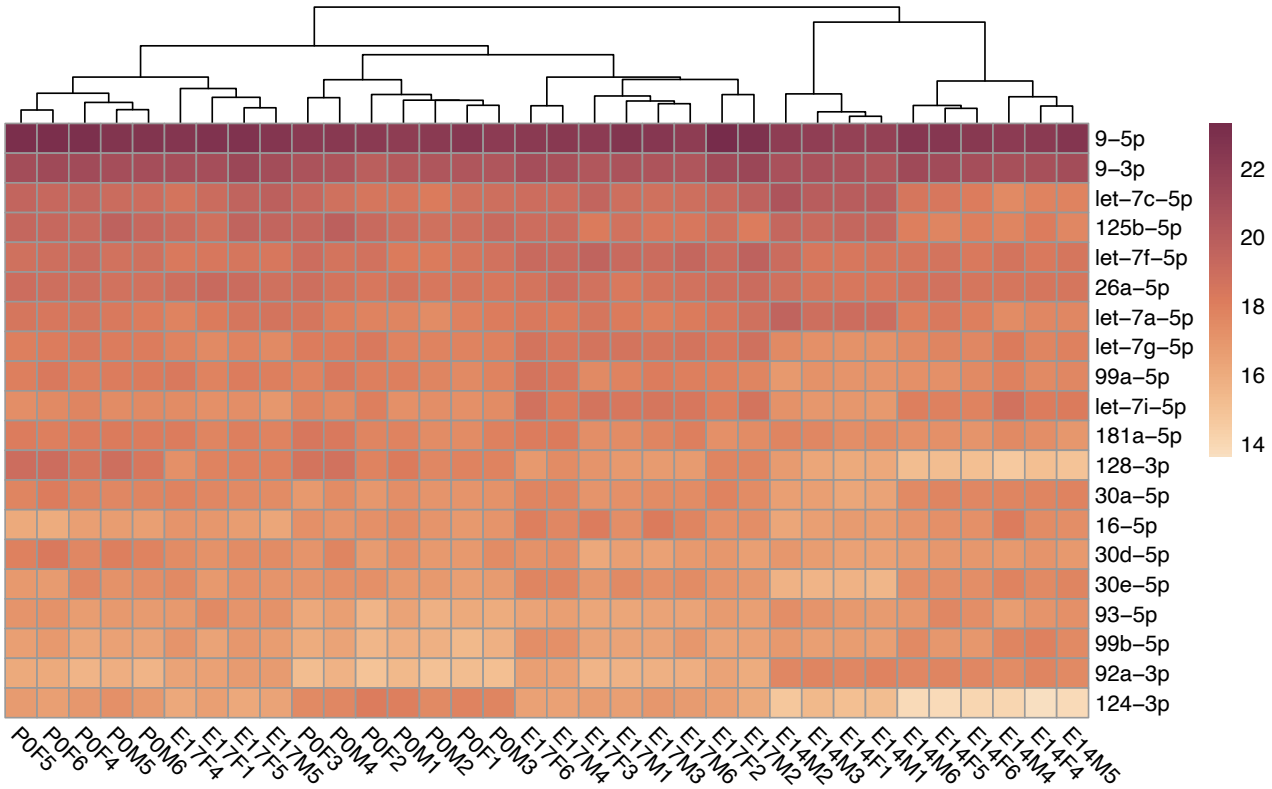
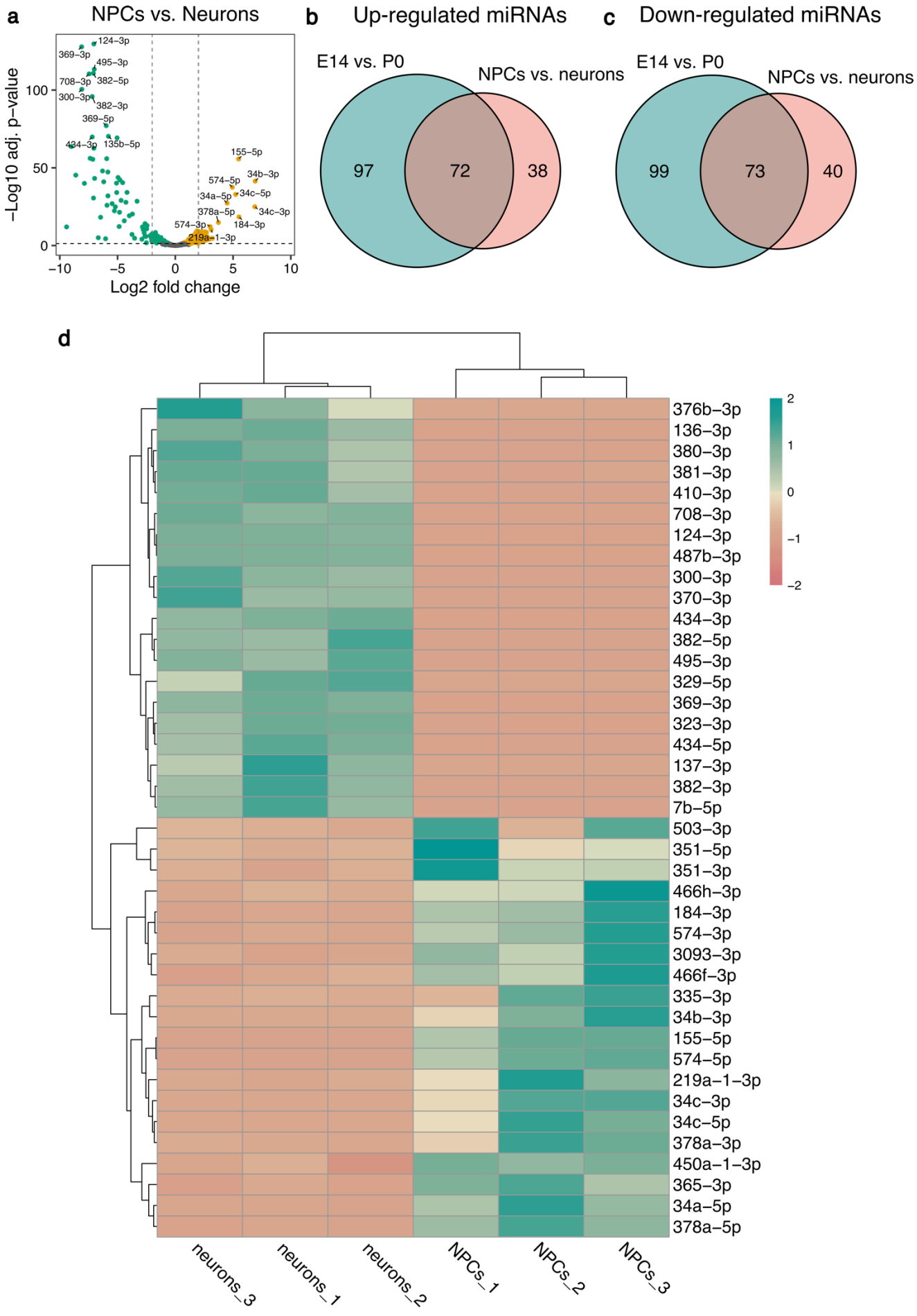
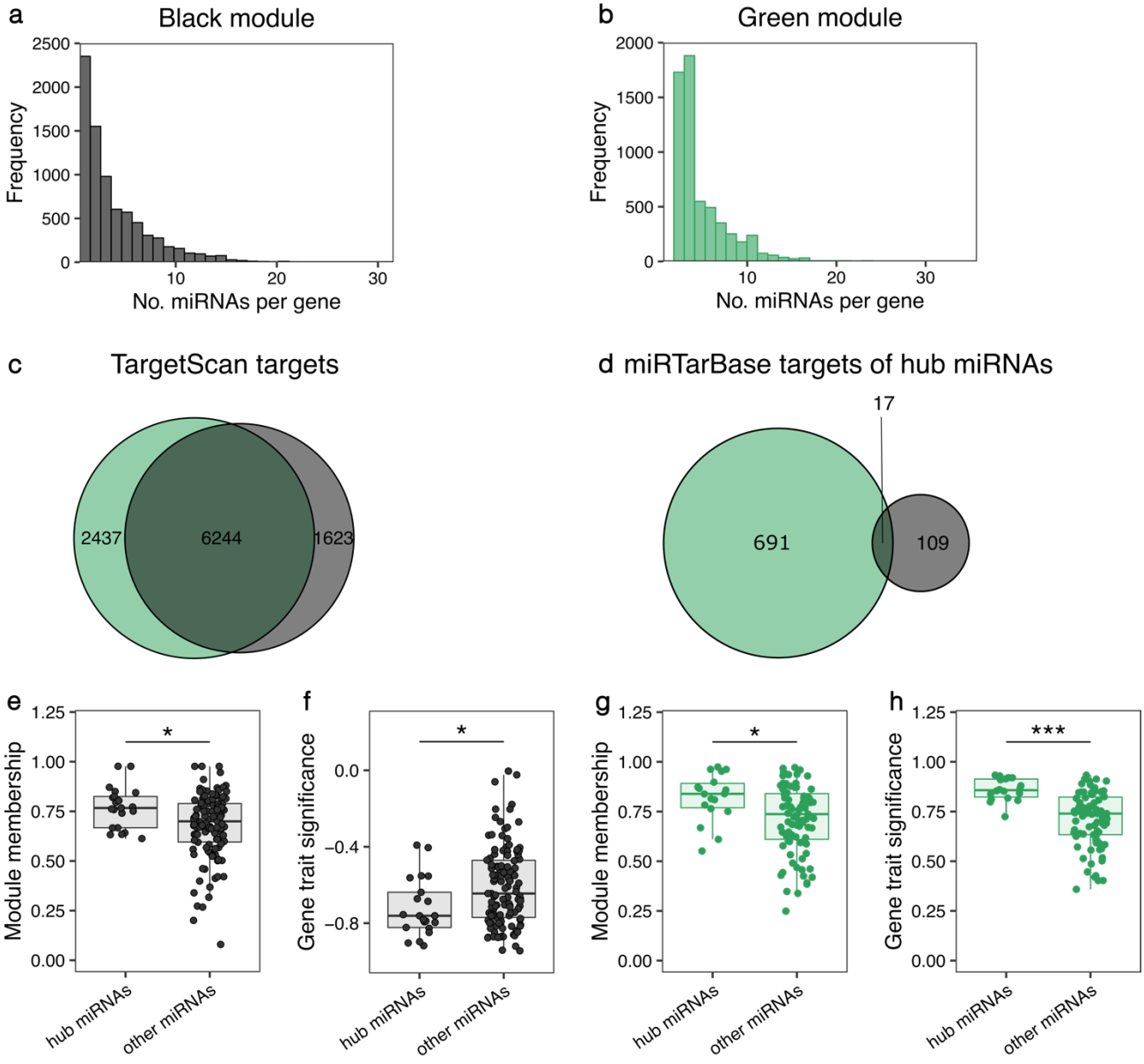


Supplementary Figure 1. Top 20 most highly expressed miRNAs in E14, E17 and P0 mouse cortical samples. Values in the heatmap correspond to log2-transformed normalized counts. M – males, F – females.

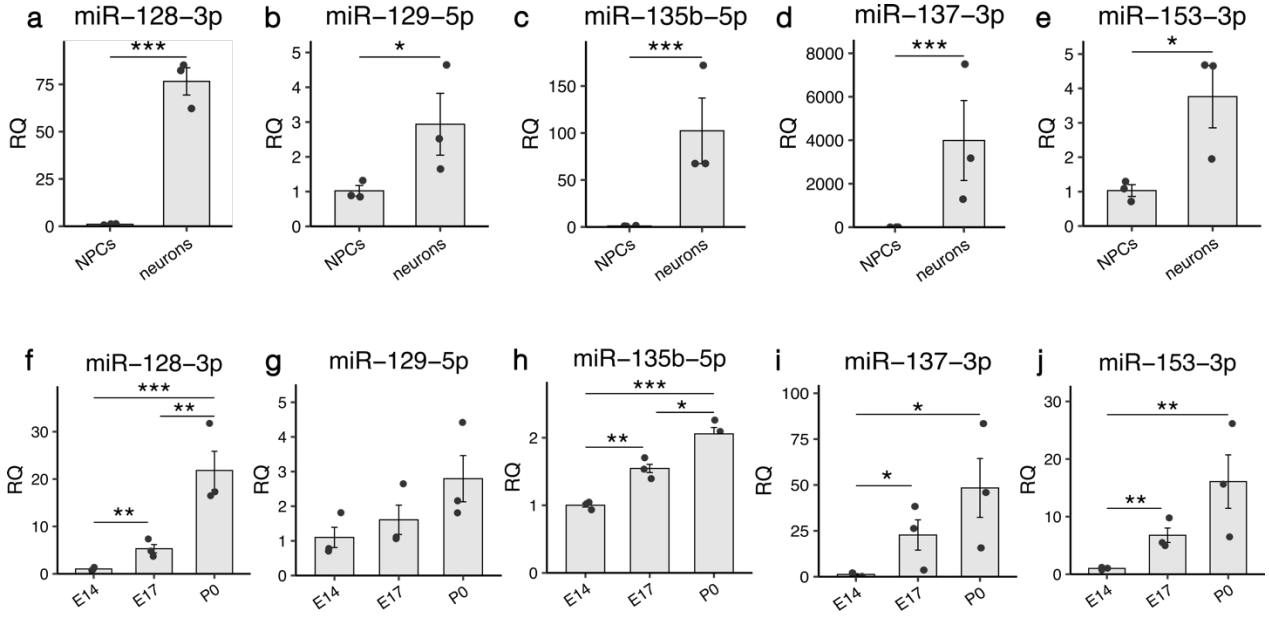

a
NPCs vs. Neurons
−Log10 adj. p−value
Log2 fold change
124−3p
369−3p
495−3p
708−3p
382−5p
300−3p
382−3p
369−5p
434−3p
135b−5p
155−5p
34b−3p
574−5p
34a−5p
34c−5p
378a−5p
34c−3p
574−3p
184−3p
219a−1−3p
b
Up-regulated miRNAs
E14 vs. P0
NPCs vs. neurons
97
72
38
c
Down-regulated miRNAs
E14 vs. P0
NPCs vs. neurons
99
73
40
d
376b−3p
136−3p
380−3p
381−3p
410−3p
708−3p
124−3p
487b−3p
300−3p
370−3p
434−3p
382−5p
495−3p
329−5p
369−3p
323−3p
434−5p
137−3p
382−3p
7b−5p
503−3p
351−5p
351−3p
466h−3p
184−3p
574−3p
3093−3p
466f−3p
335−3p
34b−3p
155−5p
574−5p
219a−1−3p
34c−3p
34c−5p
378a−3p
450a−1−3p
365−3p
34a−5p
378a−5p
neurons_3
neurons_1
neurons_2
NPCs_1
NPCs_2
NPCs_3

Supplementary Figure 2. Differential expression analysis of miRNAs in neuronal progenitor cells (NPCs) vs. neurons. a Volcano plot of differentially expressed miRNAs. Orange dots correspond to miRNAs up-regulated in NPCs, blue dots signify miRNAs that are up-regulated in neurons. Non-significant results are shown in gray, n = 3 biological replicates per group. **a-c** Venn diagrams showing the overlap of miRNAs that were up- or down-regulated in bulk cortical samples from E14 vs. P0 and NPCs vs. neurons. **d** Heatmap showing the expression levels of the top 20 down- and top 20 up-regulated miRNAs in NPCs vs. neurons. Values correspond to z-scores of normalized miRNA expression.



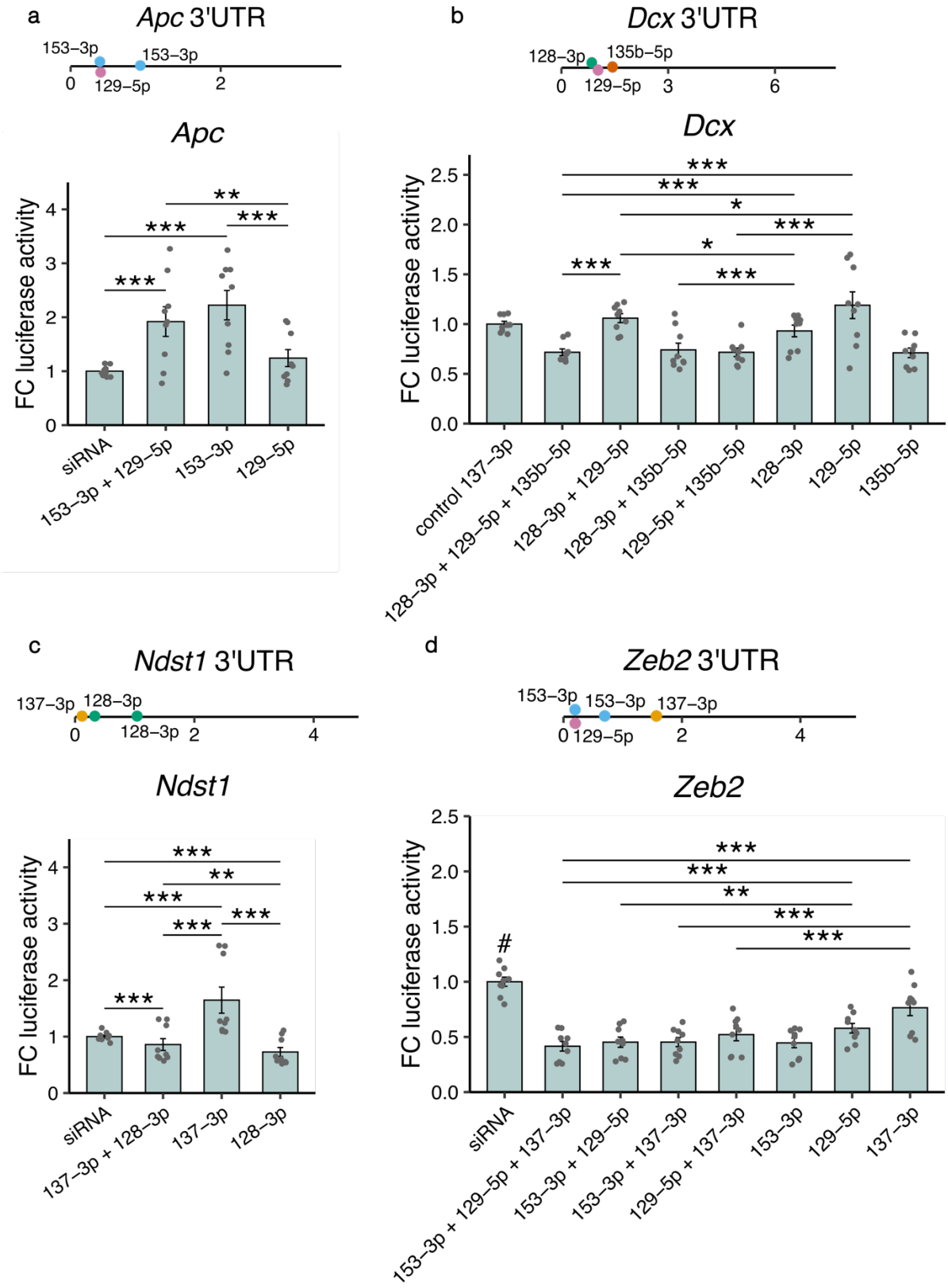
Supplementary Figure 3. Weighted gene co-expression network analysis. Histograms show the frequency distribution for the number of miRNAs targeting a gene for the black (**a)** and green (**b**) modules. **c** Venn diagram with the overlap of common genes targeted by miRNAs in the black and green modules. Target predictions were obtained from TargetScan using conserved miRNA families and binding sites. **d** Venn diagram with validated targets for conserved hub miRNAs from the black and green module obtained from miRTarBase. **e-h** Box

plots with the module membership and gene-trait significance values for the hub miRNAs and the remaining miRNAs in the black and green modules. ***p<0.001, * p<0.05, unpaired t-test.



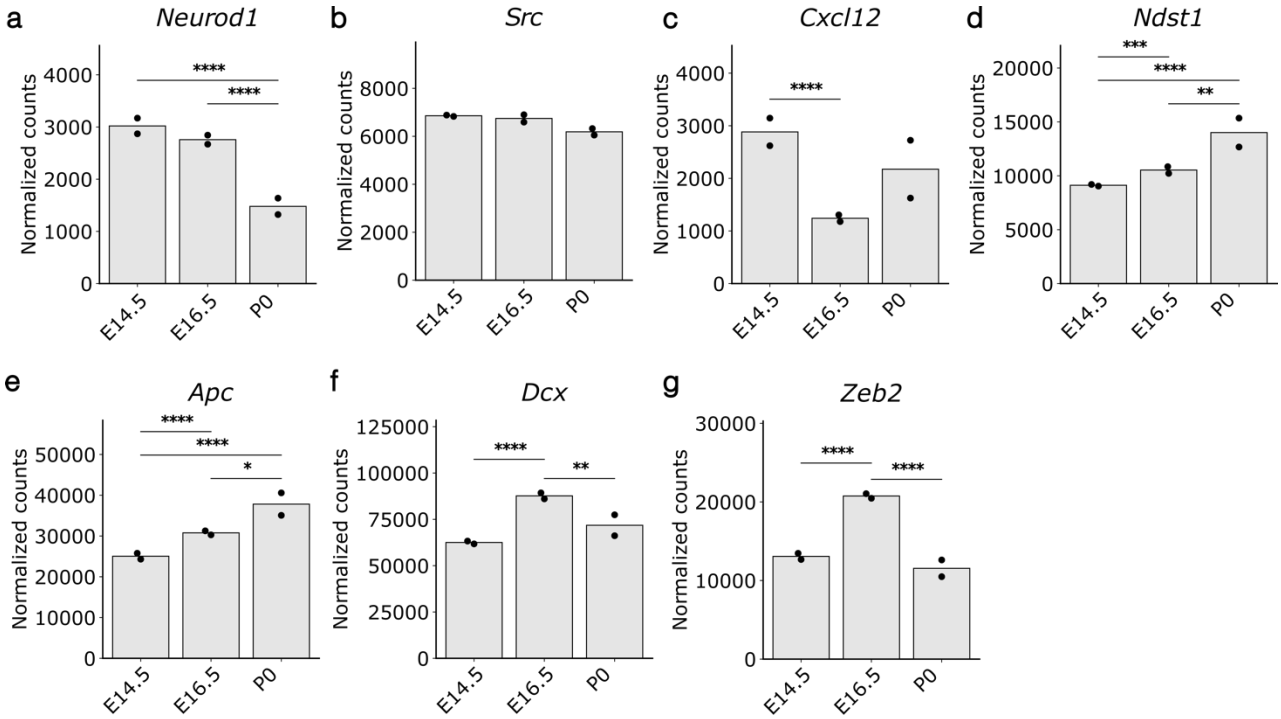
Supplementary Figure 4. Relative quantification of miRNA expression using RT-qPCR. **a-e** Expression levels of miRNAs selected for luciferase experiments in neuronal progenitor cells (NPCs) versus neurons. Relative quantification (RQ) values were normalized to the mean expression in NPCs.**f-j** Expression levels in cortical samples at E14, E17 and P0. RQ values were normalized to the mean expression in the E14 group.

Data are shown as mean ± standard error of the mean and individual values, n=3 biological replicates per group. *p<0.05, ** p<0.01, *** p<0.001, unpaired t-test of log-transformed RQ values.



Supplementary Figure 5. Luciferase activity in lysates of HEK293 cells transfected with plasmids containing 3' UTR fragments of target genes. Lysates were co-transfected with different combinations of miRNA mimics. Fold change (FC) of luciferase activity was obtained by calculating the ratio of *Renilla* luciferace and firefly luciferase activity and then normalizing to the mean of the control siRNA or miRNA group. Locations of the binding sites in the 3' UTR of the respective gene are represented by colored dots. The length of the 3' UTRs is indicated in kbp. Data are shown as mean ± standard error of the mean and individual values, n=9

replicates per group. *p<0.05, ** p<0.01, *** p<0.001, only statistical comparisons containing groups with at least two miRNA mimics are shown, #p<0.05 siRNA control vs. all other groups, two-way analysis of variance followed by Tukey's post-hoc test.



Supplementary Figure 6. Expression of target genes from the luciferase assay at different developmental time points. Bar plots show DESeq2-normalized counts for target genes that were used in the luciferase experiments at E14.5, E16.5 and P0 of cortical development. Data are depicted as mean and individual values. ** p<0.01, ***p<0.001, ***p<0.0001, Wald test from DESeq2, n=2 biological replicates per group. The expression values of genes were obtained by reprocessing data from the bulk RNA-seq study of the developing cerebral cortex by Weyn-Vanhentenryck et al. [1]

Supplementary Table 1. Primers used for PCR and nested PCR of target genes used in luciferase assays.

Primer name	Sequence 5' to 3'	PCR product size
Neurod1_XhoI_forw	ACGTCTCGAGGCCTTTGGAAGAAACAGGGG	274 bp
Neurod1_NotI_rev	ACGTGCGGCCGCGGTCACAGGTAGTAAAATGCTGG	
Neurod1_lo_XhoI_for	ACGTCTCGAGCGTCAGTTTCACTATTCCCGG	1188 bp
Neurod1_lo_NotI_rev	ACGTGCGGCCGCCAGCACTTATTCTGGACTGCA	
Zeb2_XhoI_forw	ACGTCTCGAGCCAGGAAGCTGTAGAGAGGG	1555 bp
Zeb2_NotI_rev	ACGTGCGGCCGCCAGGATCAGTTGAGAAAAGCTGT	
Dcx_XhoI_forw	ACGTCTCGAGGTTTGGGGTACATGATGTCACA	1022 bp
Dcx_NotI_rev	ACGTGCGGCCGCCATCAGCAATGCCACCAAGT	
Ndst1_XhoI_forw	ACGTCTCGAGCTTGTGTTCGCAGGGATGTC	1221 bp
Ndst1_NotI_rev	ACGTGCGGCCGCAGGACCCTTCAAGACTTCGC	
Src_XhoI_forw	ACGTCTCGAGCCCTGTGTGTGTGTGTTTGT	484 bp
Src_NotI_rev	ACGTGCGGCCGCTACAACAAGTCTGGGTCCCC	
Cxcl12_XhoI_forw	ACGTCTCGAGACAGTGGGGATTCTGGGTTC	407 bp
Cxcl12_NotI_rev	ACGTGCGGCCGCACGGTAGGAGGTTTACAGCA	
Nipbl_XhoI_forw	ACGTCTCGAGACATGCAGCCAAATTTACAGG	748 bp
Nipbl_NotI_rev	ACGTGCGGCCGCAAGTCAGCCTGTACAAACTGT	
Cited2_XhoI_forw	ACGTCTCGAGCACAAACTGCCATCTCGCTT	311 bp
Cited2_NotI_rev	ACGTGCGGCCGCCCTAAAAAGCTTTCAACACAGTAG	
Nfib_1_XhoI_forw	ACGTCTCGAGACATTACGTGCCTTGCCTTG	2529 bp
Nfib_1_NotI_rev	ACGTGCGGCCGCCTTCTCTCTCCTCGCAGCTT	
Nfib_2_XhoI_forw	ACGTCTCGAGATGCATTCTTCATCGAGGGC	957 bp
Nfib_2_NotI_rev	ACGTGCGGCCGCGTCTGTAGCATAGCTCATTT	
Apc_nested_forw	GAGCCCAAAGTCCTAAACGC	1304 bp

Apc_nested_rev	CGGAGAAGATGACGGGGTAA	
Apc_XhoI_forw	ACGTCTCGAGCTGGTCATTTTGGGAGGCAC	962 bp
Apc_XhoI_forw	ACGTGCGGCCGCGCCCTTATGTCCAGTGCCTA	

Supplementary Table 2. Primers used for targeted in vitro mutagenesis of miRNA binding sites.

Primer name	Sequence 5' to 3'
Neurod1_Mut137_forw	CTGATCGGGATAAAAAAAATCACAAACGATAATTAGGATC
Neurod1_Mut137_rev	GATCCTAATTATCGTTTGTGATTTTTTTTATCCCGATCAG
Neurod1_Mut153_forw	TAATTAGGATCTGTACAATTTTTAAACTAGTAATGGGCC
Neurod1_Mut153_rev	GGCCCATTACTAGTTTAAAAATTGTACAGATCCTAATTA
Cxcl12_Mut135b_forw	ATATATTTGAAGTGGAGCTACAGTAATGCCAGTAGAT
Cxcl12_Mut135b_rev	ATCTACTGGCATTACTGTAGCTCCACTTCAAATATAT
Cxcl12_Mut137_forw	CTGTGACATTATATGCACTAACGATAAAATGCTAATTGTTTC
Cxcl12_Mut137_rev	GAAACAATTAGCATTTTATCGTTAGTGCATATAATGTCACAG
Src_Mut137_forw	CCATTGCCCCATCACAACGATAATGTCCCCGCTACTGG
Src_Mut137_rev	CCAGTAGCGGGGACATTATCGTTGTGATGGGGCAATGG
Src_Mut153_forw	GTAGATTTCAGATGACTGATCAGAGGCCTTGGGGACC
Src_Mut153_rev	GGTCCCCAAGGCCTCTGATCAGTCATCTGAAATCTAC

References

1 Weyn-Vanhentenryck, S. M. *et al.* Precise temporal regulation of alternative splicing during neural development. *Nature Communications* **9**, 2189, doi:10.1038/s41467-018-04559-0 (2018).