

microRNA profiling of mouse cortical progenitors and neurons reveals miR-486-5p as a novel regulator of neurogenesis

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1 **ABSTRACT**

2 MicroRNAs (miRNAs) are short (~22 nt) single-stranded non-coding RNAs that
3 regulate gene expression at the post-transcriptional level. Over the past years, many
4 studies have extensively characterized the involvement of miRNA-mediated
5 regulation in neurogenesis and brain development. However, a comprehensive
6 catalog of cortical miRNAs cell-specifically expressed in progenitor types of the
7 developing mammalian cortex is still missing. Overcoming this limitation, here we
8 exploited a double reporter mouse line previously validated by our group to allow the
9 identification of the transcriptional signature to neurogenic commitment and provide
10 the field with the complete atlas of miRNAs expression in proliferating neural stem
11 cells, neurogenic progenitors and newborn neurons during corticogenesis. By
12 extending the currently known list of miRNAs expressed in the mouse brain by over
13 two fold, our study highlights the power of cell type-specific analyses for the detection
14 of transcripts that would otherwise be diluted out when studying bulk tissues. We
15 further exploited our data by predicting putative novel miRNAs and validated the
16 power of our approach by providing novel evidence for the involvement of miR-486
17 as a novel player in brain development.

18 19 20 **INTRODUCTION**

21 MicroRNAs (miRNAs) are short (~22 nt) single-stranded non-coding RNAs that
22 regulate gene expression at the post-transcriptional level (1,2). Canonical miRNAs
23 derive from longer primary transcripts harboring a stem-loop that is processed by two
24 RNase III enzymes: Drosha in the nucleus and Dicer in the cytoplasm (3,4).
25 Eventually, mature miRNAs are loaded into the RNA-induced silencing complex

26 (RISC) (5,6) to destabilize or cleave complementary target messenger RNAs
27 (mRNAs) thereby inhibiting their translation (7).

28 miRNA-mediated regulation of translation is far more than an adjustment of
29 cellular protein levels, but rather an essential developmental mechanism. In fact, a
30 number of mouse lines mutant for miRNA-processing enzymes or individual miRNAs
31 showed dramatic phenotypes, ranging from impaired organogenesis to pre- and
32 perinatal lethality (8-11). The effects of interfering with miRNA function were found to
33 be particularly severe during brain development and leading to a decreased survival
34 of neural progenitors and newborn neurons and ultimately causing cortical
35 malformations (12-15). In addition, well-established regulatory loops are mediated by
36 miRNAs such as in the synergistic effect of miR-9 and *let-7b* inducing neural
37 progenitors differentiation by targeting the Tlx receptor (Nr2e1) (16,17) as well as
38 down-regulating Hes1 and CyclinD1 as critical gene hubs controlling cell-cycle exit
39 and enhancing differentiation (18,19). Moreover, it is well characterized the
40 interaction of miR-9 with miR-124 to target the RE-1 Silencing Transcript factor
41 (REST), a strong inhibitor of pro-neural genes (20-22). Many more examples are
42 known of miRNAs controlling neurogenesis and brain development (23,24)
43 highlighting the importance of studying their physiological expression patterns in
44 different cell types of the developing cortex as a crucial step to gain insights into the
45 pathways underlying their timely regulation and function. Remarkably, however, a
46 comprehensive catalog of cortical miRNAs cell-specifically expressed in progenitor
47 types and neurons is still missing.

48 The lack of a comprehensive catalog of miRNAs expression in specific populations
49 of neural progenitor cells is due to many factors including technical limitation in the
50 coverage of single-cell small RNA sequencing (25) and that essentially all previous
51 high-throughput miRNA studies on neurogenesis used either microarrays or total

52 brain lysates (26-30). As a consequence, the resolution of previous studies was
53 limited by the variety of probes printed on the microarrays or, alternatively, by the
54 coexistence in time and space of different cell types of the developing brain. To
55 overcome these limitations, we here exploited a previously described dual-reporter
56 mouse line, which allows the isolation of different neural progenitor types and
57 newborn neurons (31).

58 More specifically, with the progression of neurogenesis two distinct, lineage-
59 related populations of neural progenitors coexist in the developing cortex: radial glia,
60 proliferative progenitors (PP) that expand the stem cell pool by symmetric divisions
61 and neurogenic, differentiative progenitors (DP) that divide to generate neurons (N)
62 (32,33). In studying the fate and nature of each population, several studies have
63 identified the expression of defined molecular markers in each cell type. In particular,
64 and by taking advantage of *Btg2* and *Tubb3* expression, our group has generated a
65 combinatorial, double-reporter mouse line in which RFP and or GFP expression
66 allowed the isolation specifically of PP, DP and N based on their endogenous
67 fluorescence (RFP⁻, RFP⁺ and GFP⁺, respectively) (31).

68 Validation and use of this mouse line revealed to be very powerful in the
69 identification of several new genes and biological processes regulating cortical
70 development (31). This included the thorough characterization of the elusive class of
71 long non-coding (34) and circular (35) RNAs, novel transcription factors involved in
72 corticogenesis (36) and a comprehensive description of DNA methylation and
73 hydroxymethylation as epigenetic marks tuning brain development (37).

74 Given the previously validated power of our approach, we here exploited this
75 *Btg2::RFP/Tubb3::GFP* line to provide the field with a complete atlas of miRNAs
76 expression in cortical progenitors and neurons of the mouse brain at embryonic day
77 (E) 14.5 as a mid-stage of corticogenesis. Furthermore, and validating our approach,

78 we provide evidence for the involvement of miR-486 as a novel regulator of
79 corticogenesis.

80

81 **MATERIALS AND METHODS**

82 **Animals and embryos dissection**

83 Mice were housed into the Biomedical Services Facility (BMS) of the MPI-CBG under
84 standard conditions (12-hour light-dark cycle, $22 \pm 2^\circ\text{C}$ temperature, $55 \pm 10\%$
85 humidity, food and water supplied *ab libitum*). All experimental procedures were
86 performed according to local regulations and all animal experiments were approved
87 by local authorities (Landesdirektion Sachsen; 24D-9168.11-1/41, 2008-16, 2011-11,
88 TVV 39/2015, 13/2016 TVV and 16-2018). $\text{Btg2}^{\text{RFP}}/\text{Tubb3}^{\text{GFP}}$ males were time-mated
89 with C57BL/6J females, which were marked as E0.5 the morning that a spermatic
90 plug was observed. Pregnant females were anesthetized using Isoflurane (Baxter)
91 and sacrificed by cervical dislocation at E14.5. Brains of RFP/GFP double-positive
92 embryos were collected and lateral cortices isolated after removal of meninges and
93 ganglionic eminences. Plugged C57BL/6J females for *in utero* electroporation or
94 RNA extraction for Northern blot were purchased from Janvier Labs. Mice were
95 sacrificed at E14.5 or E15.5 and embryo brains and cortices were dissected as
96 above.

97

98 **Cell dissociation and FAC-sorting**

99 Lateral cortices of RFP/GFP double-positive embryos were dissociated using Papain-
100 based Neural Tissue Dissociation Kit (Miltenyi Biotech) according to the
101 manufacturer's protocol. Cells were resuspended in 1 ml of ice-cold PBS and 10 μl of
102 7-AAD (BD Pharmingen) were added for dead cells discrimination. Sorting was
103 performed by BD FACSAriaTM III (BD Biosciences) with previously described gating

104 (31,35). A minimum of 1×10^6 cells per sample was collected in PBS and centrifuged
105 (300 g, 10 min at 4°C) before RNA extraction.

106

107 **RNA extraction**

108 For miRNA deep-sequencing, total RNA was isolated using Quick RNA Mini Prep kit
109 (Zymo Research) from cells sorted as described above. RNA quality and integrity
110 were assessed by Bioanalyzer (Agilent Genomics). RNA integrity values (RIN) were
111 above 9.0. For Northern blots, total RNA was isolated by TRI Reagent (Sigma-
112 Aldrich). Briefly, lateral cortices of all E14.5 embryos of one litter were pooled and
113 lysed in 1 ml of TRI Reagent. Samples were added 200 µl of chloroform, mixed and
114 left at RT for 15 min before centrifugation at 12,000 g for 30 min at 4°C. Aqueous
115 phases were transferred to new tubes and RNAs were precipitated by adding 500 µl
116 of 2-propanol. RNA pellets were washed with 1 ml of 75 % ethanol and eventually
117 resuspended in 50 µl of nuclease-free water.

118

119 **Library preparation and small RNA deep sequencing**

120 Library preparation was performed on 1 µg of total RNA with NEB Next Small RNA
121 Library Prep Kit. All cDNA libraries were prepared according to the manufacturer's
122 specifications, including adapter ligation, first-strand cDNA synthesis, PCR
123 enrichment and size selection. cDNA purity and concentration after gel extraction
124 were measured by qPCR. Samples were sequenced on Illumina HiSeq 2500 and
125 single-end 75-bp reads were obtained.

126

127 **Bioinformatics and statistical analyses**

128 Sequencing data were obtained for PP, DP and N in 3 biological replicates. After
129 adapter removal, reads shorter than 30 bp were aligned to miRBase v.20 (38) using

130 gsnap (39). Alignment was performed in 3 consecutive steps: a) on mature miRNAs
131 sequences, b) unmapped reads were extracted and c) aligned on precursor-miRNA.
132 During all steps, no mismatches were allowed and multi-mapped reads discarded.
133 Eventually, a table of read counts per mature miRNA (read count ≥ 1) was
134 assembled. For novel miRNA prediction, all unmapped reads were extracted and
135 aligned using miRDeep2 (40) on mouse genome (mm10). The R package DESeq2
136 (41) was used for normalization of the read count table and further testing of
137 differential expression. Mean counts from replicates were used for fold change (FC)
138 calculations: \log_2FC values ≥ 0.58 or ≤ -0.58 were considered up- or down-
139 regulation, respectively. Benjamini–Hochberg procedure was applied for multiple t-
140 test adjustment and FDR values lower than 0.05 were considered significant. A
141 minimum of 3 biological replicates was used for any other assessment presented in
142 the manuscript. Statistical differences of mean values were calculated by two-tailed
143 student t-test, assuming $p < 0.05$ as significant.

144

145 **In utero electroporation**

146 LNA oligonucleotides (miRCURY LNA miRNA Inhibitors) were purchased from
147 Exiqon and co-electroporated with pDSV-mRFPnls reporter plasmid (Lange et al.,
148 2009). LNA sequences are reported in **Supplementary Table 1**. *In utero*
149 electroporation was performed as previously described (42,43): C57BL/6J pregnant
150 mice were anesthetized with isoflurane at E 13.5 and 1 μ l of DNA solution (10 μ M
151 LNA, 0.8 μ M RFP plasmid) was injected into the embryo left ventricle, followed by the
152 application of 6 electric pulses (30V and 50 ms each at 1 s intervals) through
153 platinum electrodes using a BTX-830 electroporator (Genetronics).

154

155

156 **Immunohistochemistry**

157 After dissection, brains were fixed in 4 % paraformaldehyde in 0.1M phosphate buffer
158 (PFA, pH=7.4) overnight at 4°C, cryoprotected in 30 % sucrose and cryosectioned
159 (10 µm thick slices). Immunohistochemistry was performed as previously described
160 (42) (**Supplementary Materials and Methods** and **Supplementary Table 2** for a list
161 of used antibodies). Nuclei were counterstained with DAPI. Sections were imaged
162 using an automated microscope (ApoTome; Carl Zeiss), pictures digitally assembled
163 using Axiovision software (Carl Zeiss) and composites analyzed using Photoshop
164 CS6 (Adobe).

165

166 **RESULTS**

167 **The comprehensive miRNome of neurogenic commitment** Aiming to profile
168 global miRNA expression during cortical development, we isolated PP, DP and N
169 (each in three biological replicates) from the lateral cortices of
170 Btg2::RFP/Tubb3::GFP mouse embryos at E14.5, as previously described (31,35)
171 (**Figure 1a**). Total RNA was used for cDNA library preparation and small RNAs were
172 isolated by size selection, followed by 75-bp high-throughput sequencing. To
173 assemble the catalog of cortical miRNAs, we aligned reads with gsnap (39) and used
174 miRBase (v.20) as the most complete reference available to date (38) yielding an
175 average of 1.5 million unique-mapped reads (51% of total). Within the mapped reads,
176 we detected (defined to as reads ≥ 1) 1,058 mature miRNAs derived from 703
177 precursor transcripts (pre-miRNA) corresponding to 55% and 59% of the 1,908
178 mature and 1,186 pre-miRNAs reported in the reference, respectively. More
179 specifically, 640 mature miRNAs were in common to all 3 cell-types while 49 (4.6%),
180 58 (5.5%) and 129 (12.2%) were specific to PP, DP and N, respectively (**Figure 1a**).
181 Notably, when compared to a previous study which reported 294 pre-miRNAs (read

182 count ≥ 1) expressed in the whole E15.5 mouse brain (30), our dataset included
183 essentially all (96%) of these previously known cortical miRNAs and further doubled
184 this list by including additional 421 pre-miRNAs. In turn, this highlights the power of
185 cell type-specific analyses for the detection of transcripts that would otherwise be
186 diluted out when studying bulk tissues.

187 Furthermore, given that 42% of our reads did not align to any known miRNA and
188 recent studies reported the detection of novel miRNAs in both mice and humans
189 (44,45), we hypothesized that some of our reads might derive from novel miRNAs not
190 annotated in any database and used miRDeep2 (40) to investigate this possibility.
191 The prediction performed by this tool is based on the putative miRNA primary
192 structure and how reads are aligning to the precursor based on their biogenesis. With
193 this assumption, reads coming from a putative novel miRNA will fall into three main
194 categories: the mature sequence, the hairpin loop and the star sequence (22nt
195 sequence resulting from the removal of the loop that is not loaded into Ago and
196 degraded). If the combination of a possible hairpin precursor and mapping of the
197 sequencing reads is not following this expected pattern, those reads are discarded.
198 This resulted in the prediction of 163 putative novel miRNAs sequences (read count
199 ≥ 1) that for convenience were labeled as miR-n- followed by a progressive number
200 as identifier (**Figure 1a** and **Supplementary File S1**).

201 Next, we sought to select and validate some of these predicted miRNAs. To this
202 end, we first chose those showing a higher consistency in detection among biological
203 replicates (i.e. at least 2 out of 3 samples from the same cell type) reducing our initial
204 list of 163 to 22 candidates. Next, we rank-ordered this refined cohort of putative
205 novel miRNAs based on their average expression across cell populations selecting
206 the top 8 for validation by Northern blot with radioactive probes (**Supplementary**
207 **Table 1**). Among these, we confirmed the expression of 5 showing a size in the

208 range of 90-150 nt (**Supplementary Figure S1a**; note that in some lanes two miR-n
209 are probed with identical sequence but derived from different loci) which is
210 inconsistent with the known size of either mature or pre-miRNA (20-25 nt and ~60 nt,
211 respectively) and more in line with that of other small RNAs including t-, sn- or sno-
212 RNAs. Although not excluding the possibility that other novel miRNAs might be
213 present in our list, this exclusion of 8 out of 8, top-ranking putative novel miRNAs made
214 us conclude that our catalog of mouse cortical miRNAs is virtually complete.

215 As a next step, we validated the robustness of our datasets following a two-step
216 approach. First, we normalized read numbers using DESeq2 (median-ratio
217 normalization) (41) to account for differences in sequencing depth. Upon
218 normalization, principal component analysis (PCA) showed a clear separation of the
219 three cell types, which distributed according to lineage differentiation (PP→DP→N)
220 for the component displaying the highest variance (PC1) (**Figure 1b**). Second, we
221 selected 6 miRNAs known to play key roles in neurogenesis and compared their
222 normalized expression measured by deep sequencing with their tissue distribution
223 assessed by *in situ* hybridization (ISH) data from Eurexpress (46). We observed a
224 nearly perfect overlap between our sequencing and ISH data in all cases, regardless
225 of whether the miRNA was uniformly expressed throughout the cortex (miR-9-5p and
226 miR-17-5p), enriched in either progenitors (miR-92b-3p, miR-92a-3p and let-7b-5p)
227 or neurons (miR-124-3p) (**Figure 1c** and **Supplementary Figure S1b**).

228 Taken together, our results provide evidence for an overall complete catalog of
229 miRNA expression in cortical cell types during mouse development, more than
230 doubling the previously known list of 292 cortical miRNA precursors (30) by detecting
231 421 additional ones and for a total of 703 transcripts.

232

233

234 **Differentially expressed miRNAs**

235 The fine resolution of our system gave us the opportunity to assess differential
236 miRNA expression at single population level during lineage commitment
237 (**Supplementary File S2**). Therefore, by comparing the PP-DP and DP-N transitions,
238 we identified miRNAs that were up- or down-regulated by >1.5-fold (i.e. log₂ fold
239 change ≥ 0.58 or ≤ -0.58 , respectively, FDR <5%) in one cell type compared to its
240 parental population. As observed previously for linear and circular transcripts (31,35),
241 only a small fraction of miRNAs showed a significant change between PP-DP (7%)
242 and DP-N (17%) while the majority of those up- or down-regulated between PP-DP
243 continued to follow the same trend of up- or down-regulation, respectively, between
244 DP-N (**Figure 2**).

245 Furthermore, by analyzing coding and long non-coding transcripts, our group
246 previously concluded that a transient up- or down-regulation specifically in DP
247 compared to both their PP progenitors and N progeny (on- and off-switch transcripts,
248 respectively) represented a hallmark of functional commitment to the neurogenic
249 lineage (31,36). Intriguingly, the subset of miRNAs displaying this on-/off-switch
250 pattern of expression was strongly underrepresented, accounting for only 0.5% of the
251 total and suggestive of a highly specific expression pattern. In fact, we only found 2
252 on-switch (let-7b-5p and miR-135a-2-3p) and 2 off-switch (miR-486a-5p and miR-
253 486b-5p) miRNAs (**Figure 2** and **Figure 3**). Supporting our conclusion that on-/off-
254 switch transcripts are functionally involved in neurogenic commitment, both let-7b
255 and miR-135a-2 are well known to be key regulators of neurogenesis (17,47). In
256 contrast, while it has been shown that miR-486a and miR-486b promote myoblast
257 differentiation (48) and are involved in regulatory pathways of ectodermal-derived
258 tissues (49,50), no neurogenesis-related function has ever been reported for these
259 two miRNAs to date.

260 With regard to their genomic location, we observed that all 4 switch miRNAs were
261 intragenic. In particular, let-7b-5p and miR-135a-2-3p were processed, respectively,
262 from lncRNAs AC162302.2 and Rmst (which mediates Sox2-dependent progenitors
263 proliferation) (51). Similarly, miR-486a-5p and miR-486b-5p were processed from
264 Ankirin1 (Ank1) and the predicted gene Gm15816, respectively. Interestingly, miR-
265 486a-5p and miR-486b-5p shared the same mature sequence, despite originating
266 from different pre-miRNAs transcribed from opposite strands of the same genomic
267 locus. Unsurprisingly, when analyzing the expression pattern of the host genes of
268 switch miRNAs (data retrieved from (31)), we found a high degree of overlap in their
269 differential expression within different cell populations (**Figure 3**). This was consistent
270 with previous observations of our group on long non-coding and circular RNAs (cite-
271 cite) in which intragenic, switch genes were found to be regulated together in a
272 similar fashion at the level of a common “switch locus”.

273 274 **The off-switch miR-486a/b-5p is a novel regulator of neurogenesis**

275 The absence of any known function for miR-486a/b-5p in neural stem cells or brain
276 development, together with their intriguing switch expression pattern, drove us to
277 investigate their potential functional role during corticogenesis and, by this, also
278 attempting to validate the power of our miRnome atlas of cortical cell types. After
279 validating the expression of miR486a/b-5p by qRT-PCR on FAC-sorted PP, DP and
280 N of the E14.5 cortex (**Supplementary Figure 2a**), we used locked nucleic acids
281 (LNA) to inhibit their activity and, hence, address their functional role. First, we
282 confirmed the silencing efficacy of LNA-486 by luciferase assay on two validated
283 targets of miR-486a/b-5p (Foxo1 and Pten) (52,53) (see Materials and Methods and
284 **Supplementary Figure S2b and c**). Then, to investigate the effect of miR-486a/b-5p
285 inhibition on cortical progenitors, we *in utero* electroporated E13.5 mouse embryos

286 with LNA-486 or LNA-control together with an RFP-reporter plasmid. Brains were
287 collected 48h later and distribution of electroporated cells (identified as RFP+) was
288 assessed as a readout of neurogenesis and neuronal migration.

289 LNA-486 significantly altered cell distribution across all cortical layers. Particularly
290 affected were the subventricular zone and the cortical plate that showed a 1.6 fold
291 increase (from 12 ± 1 to $20\pm 2\%$; $p<0.01$), and a comparable decrease (from 20 ± 3 to
292 $12\pm 2\%$; $p<0.01$), in RFP+ cells after delivery of LNA-486 relative to brains
293 electroporated with control LNAs, respectively (**Figure 4a**). By using Tbr2 as a
294 marker to identify basal from apically progenitors, we observed a significant increase
295 in both cell types at the expense of neurons. In particular, apical progenitors
296 increased by 1.2 fold (from 14 ± 0.2 to $18\pm 2\%$; $p<0.05$), whereas basal increased by
297 1.3 fold (from 21 ± 2 to $28\pm 4\%$; $p<0.05$). This was paralleled by a comparable, 1.2 fold
298 decrease in neurons found in the neuronal layers (from 66 ± 2 to $54\pm 2\%$; $p<0.001$)
299 (**Figure 4b**). Notably, no major effect was found neither at the level of cell survival
300 nor migration of newborn neurons as assessed by activated-caspase
301 immunoreactivity or upon 24h birthdating with BrdU (data not shown) and hinting at a
302 cell fate-specific effect upon inhibition of miR-486a/b-5p activity.

303 Taken together, characterization of the miRNome of progenitor cell types and
304 neurons revealed a powerful tool to identify new miRNAs involved in cortical
305 development allowing us to describe for the first time the functional effects of
306 interfering with the activity of miR-486a/b.

307

308 **DISCUSSION**

309 Here we provided a complete catalog of miRNAs expression in neural progenitors
310 and newborn neurons during cortical development and validated our resource by

311 identifying a new player in neural stem cell fate specification: the switch miR-486a/b-
312 5p.

313 Since the discovery of miRNAs as critical regulators of translation (7), many
314 groups attempted to obtain atlases of their expression during brain development. The
315 use of microarrays offered a first approach toward this goal (26-29) but was limited
316 by a previous knowledge about the sequence of such miRNAs. Next-generation
317 sequencing overcame this limitation and significantly increased the number of known
318 miRNAs (30). However, previous studies remained limited either to the use of cell
319 cultures or to analyses of whole brain lysates due to a lack of systems to discriminate
320 between different cellular subtypes coexisting in time and space during
321 corticogenesis. Even with the advent of single-cell sequencing, the study of small
322 RNAs remains hindered by two major technical limitations that a) drop-seq is
323 currently applicable only to poly(A)-RNAs and b) library preps with <1,000 cells
324 display extremely poor coverage (25).

325 Here we exploited the Btg2::RFP/Tubb3::GFP mouse line as a well-established
326 tool used by our group in previous studies to characterize the molecular signature of
327 neurogenic commitment (34-37). By doing so, our group identified switch transcripts
328 belonging to several classes of RNAs and including coding, long non-coding and
329 circular RNAs and in most cases showing their functional roles in brain development
330 (31,35,36). Continuing this line of research, here not only we provided the field with a
331 validated and overall complete catalogue of cortical miRNAs at single-population
332 level but also identified in miR-486a/b-5p a novel regulator of neurogenesis. We hope
333 that future studies will be able to dissect the molecular mechanisms underlying this
334 novel cortical, switch miRNA and that the field in general will profit from this novel
335 resource.

336

337 **ACCESSION NUMBERS**

338 Sequencing data generated during the current study are available at GEO repository
339 (GSE142253)

340

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344

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349

350 **AUTHORS CONTRIBUTION**

351 FC, MD and DC conceived the project; MD carried out the bioinformatic analyses
352 (supported by ML and AD) and DC performed experiments with the help of and SM,
353 LHAA and BCT. SK and GS performed the Northern Blot. FC, MD and DC wrote the
354 manuscript. All authors approved the manuscript.

355

356 **CONFLICTS OF INTEREST**

357 Authors declare no conflicts of interests.

358

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539 **FIGURE LEGENDS**

540 **Figure 1.** Assembly and validation of cortical miRNome. **a)** Outline of the steps taken
541 to generate cortical miRNome: sorting of E14.5 PP, DP and N, followed by small
542 RNA sequencing. Mature miRNAs were identified through alignment on miRBase
543 and novel miRNAs were predicted by miRDeep2. **b)** Principal component analysis of
544 DESeq2-normalized 100 most diverse miRNAs between biological replicates (1-3)
545 and cell populations (proliferative progenitors, grey; differentiative progenitors, red;
546 neurons, green). **c)** Sagittal sections of E14.5 cortices downloaded from Eurexpress.
547 Magnifications of the lateral cortex are shown (bottom-right) to appreciate the extent
548 of the overlap with miRNA expression data measured by deep sequencing
549 (histograms). Error bars = s.d. N = 3.

550 **Figure 2.** Differential expression analysis. Representation of differentially expressed
551 miRNAs in the three cell types (PP: grey; DP: red; N: green). Numbers indicate the
552 number of miRNA in each group and percentages are calculated over the parental
553 population. miRNAs not detected in PP or never detected in any cell type are also
554 reported (top left and bottom left, respectively). Oblique lines represent a > 50%
555 change (\log_2 fold change ≥ 0.58 or ≤ -0.58) and FDR <5%, whereas horizontal lines a
556 < 50% change or an FDR >5%. Bold lines are depicting on- and off- switch patterns.

557 **Figure 3.** Genes hosting switch miRNAs are depicted (black): blue arrows represent
558 the direction of transcription, whereas black boxes and lines constitute exons and
559 introns, respectively. Position and mature sequence of switch miRNAs are indicated
560 in red. Expression patterns of miRNAs and host genes are reported (graphs) on the
561 left.

562 **Figure 4.** Manipulation of miR-486a/b-5p. **(a,b)** Coronal sections of electroporated
563 lateral cortices stained for RFP (electroporated cells, white), DAPI (all nuclei, blue)

564 (a) and Tbr2 (BP, green) (b). Histograms represent quantifications of cells distribution
565 48 hours after electroporation of LNA-control (white) or LNA-486 (black)
566 (histograms).

567 VZ: ventricular zone; SVZ: sub-ventricular zone; IZ: intermediate zone; CP: cortical
568 plate. AP: apical progenitor (Tbr2⁻ cells in VZ); BP: basal progenitor (Tbr2⁺ cells in
569 VZ and SVZ). Error bars = s.d. N ≥ 3. Individual dots represent biological replicates.

570 *p<0.05 ; **p<0.01; ***p<0.001. Scale bar = 25 μm.

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Figure 1 Dori M. & Cavalli D. *et al.*,

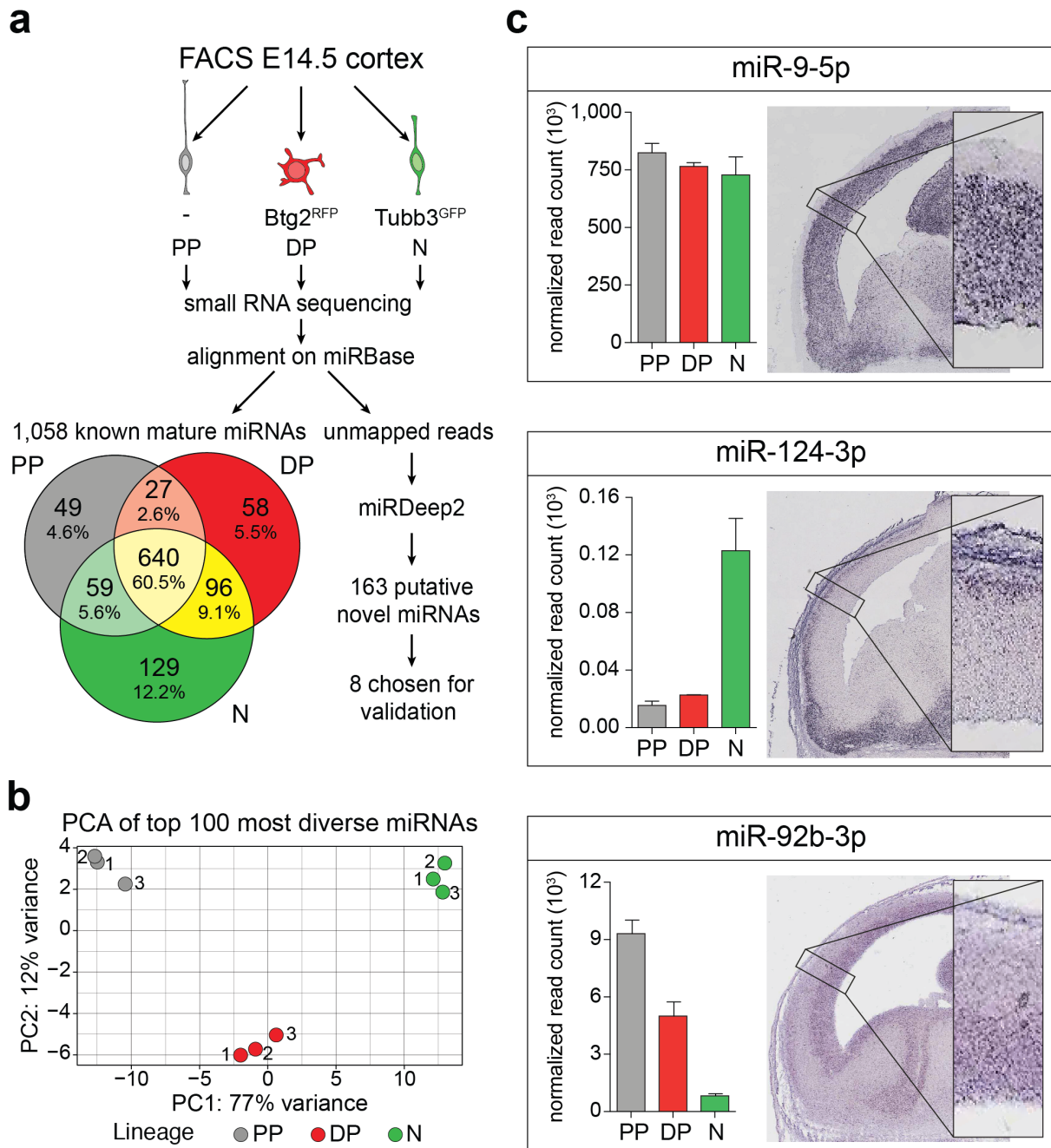
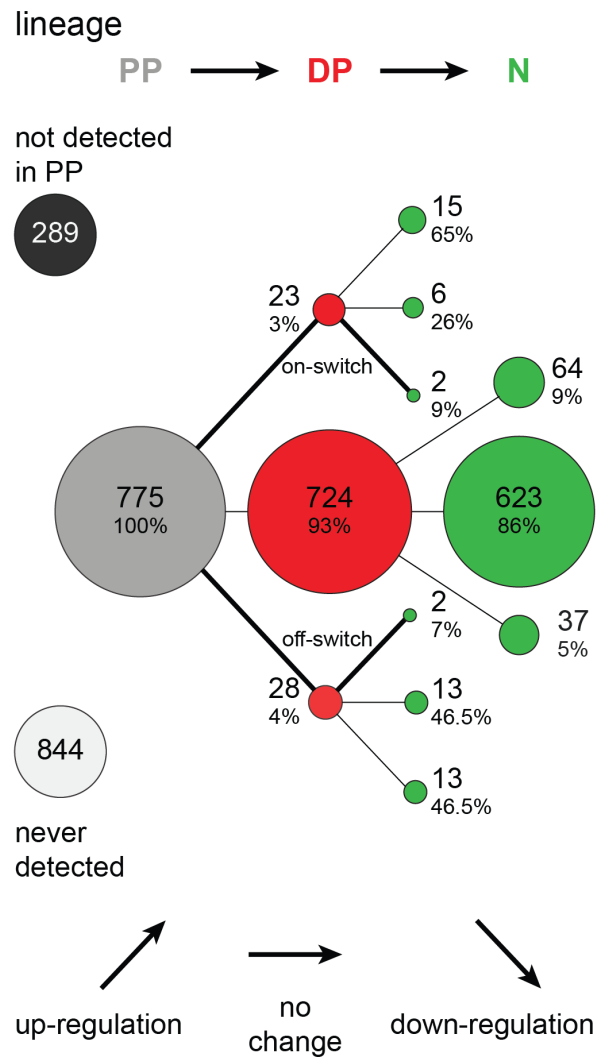


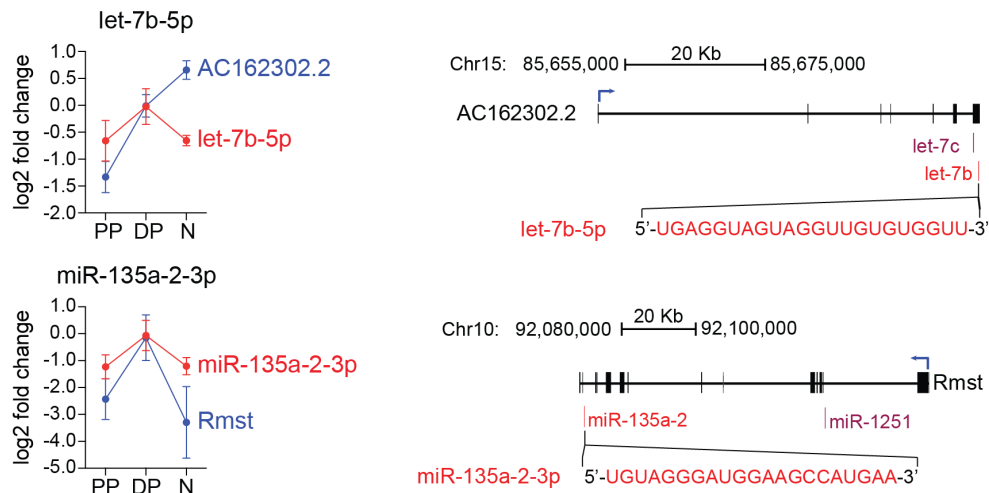
Figure 2 Dori M. & Cavalli D. *et al.*,



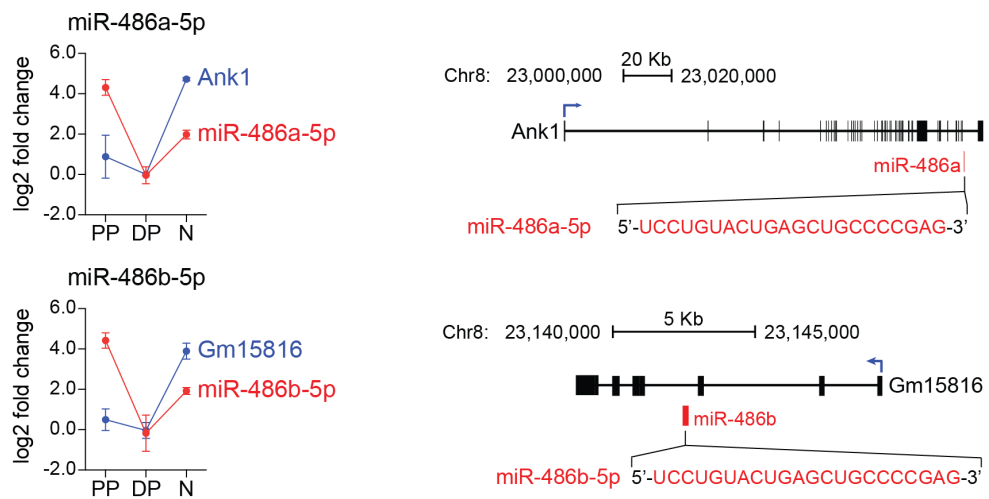
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Figure 3 Dori M. & Cavalli D. *et al.*,

on-switch miRNAs

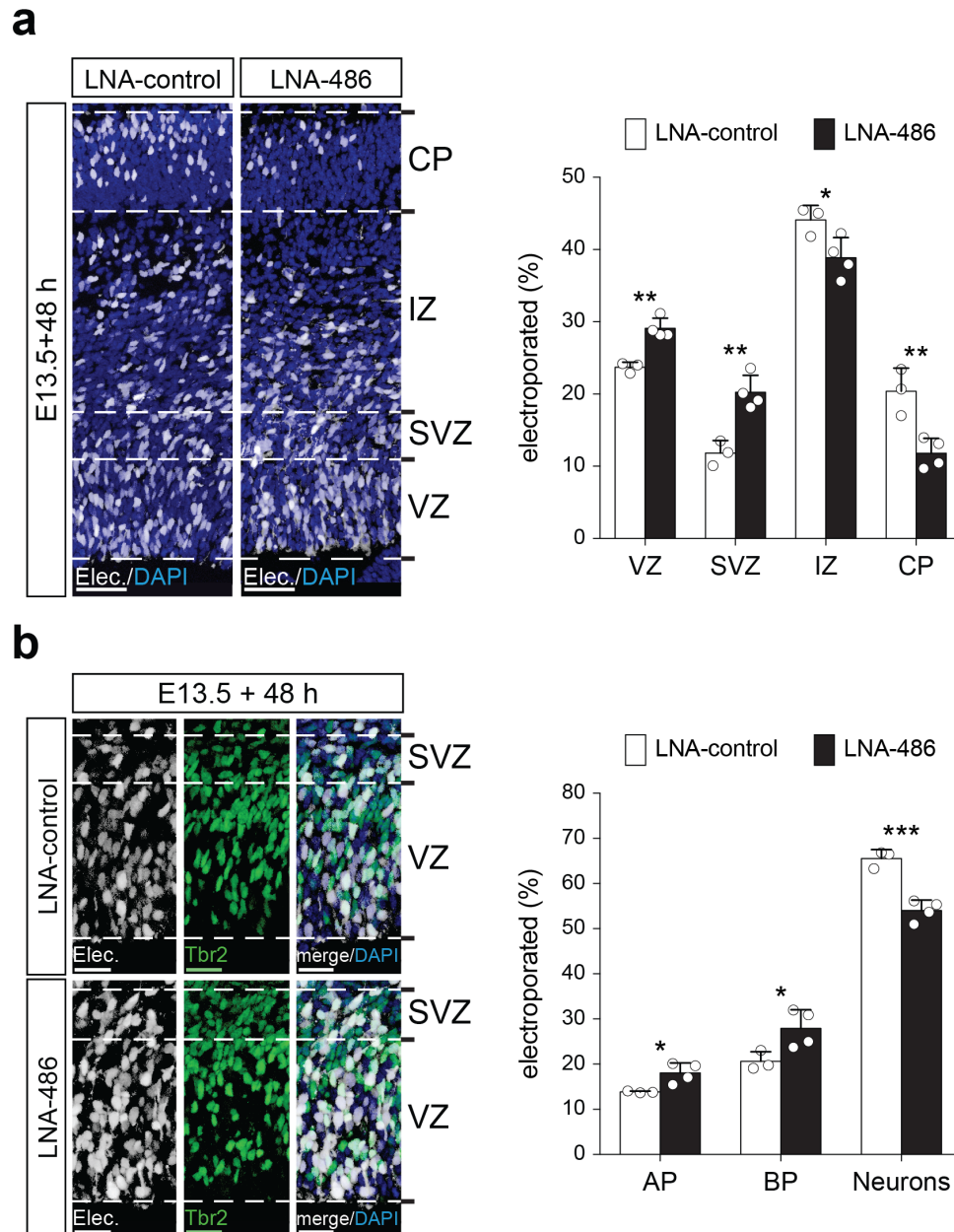


off-switch miRNAs



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Figure 4 Dori M. & Cavalli D. *et al.*,



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