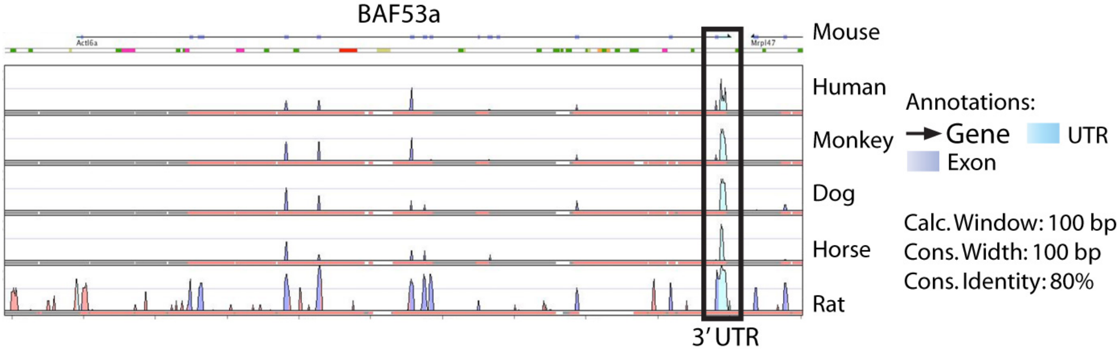
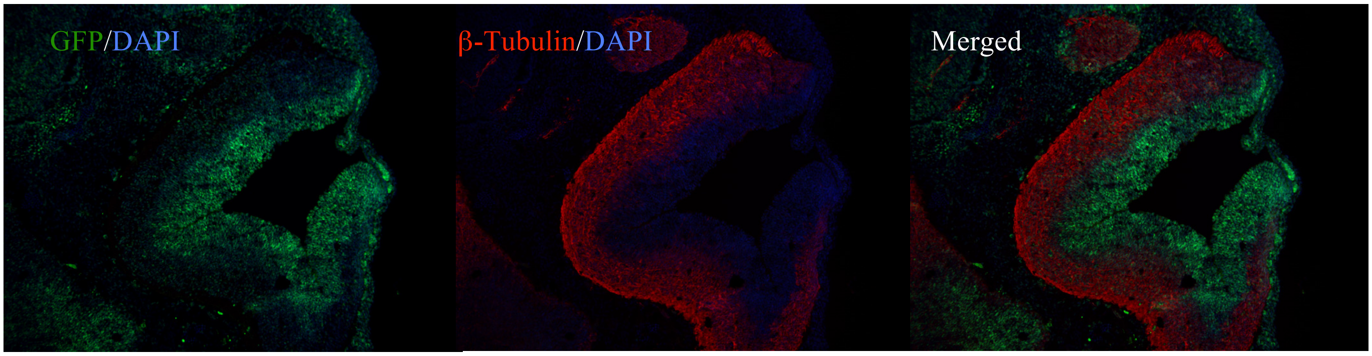
Supplementary Figure 1



Cross-species alignment of BAF53a genomic regions of mammals by VISTA[1]**.** The peaks represent conserved sequences in a minimal 80% identity in a scanning window of 100 base pairs. The peaks are present at the coding regions while the most conserved region resides in the 3' UTR (shown by the light blue peaks in the rectangular enclosure). The genomic region shown above is approximately 18 kb.



BAF53a BAC d2nucEGFP-
BAF53a **WT** UTR

Sagittal sections of the E11.5 brain of BAF53a BAC reporter near the 4th ventricle. Downregulation of the BAF53a reporter expression is consistent in β-Tubulin-positive neurons in the brain, demonstrating the pan-neuronal specificity of BAF53a repression.

Supplementary Figure 2

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   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
           10        20        30        40        50        60        70        80        90       100
   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
mm ------GGGCTCCA------CCCTGCCTGCC-C--GTCACCT-CAACGTCTGTAGCTTTAGTACACTCAGGAA-AAGA-TGACCATCTTTTGTAGAATGT     82
rn -----AGGGCTCCA------CCCTGCCTGCC-C--ATCACCT-CA-CGTCTGTAGCTTTAGGACACTCAGGAA-AAGA-TGACCATCTTTTGTAGAATGT     82
oc ---AAGAGT-CCCAAA---CCTTTACCTTCCGGT-GTCACCT-TACGTTTCATAGCTTTAGTCTACTCAGGAA-AAGAATGACCATCTTTTGTAGAATGT     90
hs -GAAAGAGTTCCCAAG---CTTCTACCTTCCTTTTGTCACCT-TACGTTTCATAGCTTTAGTATACTCAGGAA-AAGAATGACCATCTTTTGTAGAATGT     94
pt -GAAAGAGTTCCCAAG---CTTCTACCTTCCTTTTGTCACCT-TACGTTTCATAGCTTTAGTATACTCAGGAA-AAGAATGACCATCTTTTGTAGAATGT     94
cf --AGAGAGTTCCCAAA---GCTCTTCCTTCCTGT-GTCACCT-TAAGTTTCATAGCTTTAGTATACTCAGGAA-AAGAATGACCATCTTTTGTAGAATGT     92
bt ----TAAGTTCCCAAA---CCTCTACCTTCTAT--GTCACCT-TACGTTTCATAGCTTTAGTATACTCAGGAA-AAGAACCACCATCTTTTGTAGAATGT     89
la ----AGAGTTCCCAAA---CTTCTGCCTTCCCCGTGTCACCT-TATGTTTCATAGCTTTAGAATACTCAGGAA-AAGAATAACCATCTTTTGTAGAAAGT     91
et ----AGAGTTTCCAAG---CCTCCACCTTCTGT--GTCACCT-TCCGTTTCATAGCTTTAGTAAACTCAGGAA-AAGAATGACCATCTTTTGTAGAAAAT     89
md --AACCAATTCTGAAAAAACTGTGGCCTTCCCTATGATCCCT-TATGTTTCATAGCTTTAGTATACTCAGGAACAAAAATGAACGTCTTTTGTAGAATGT     97
gg AAATTTCTTACTGTGAGGACTGCTGCTTCCTCAGCGCTTCTTCTGACTCTCATAGCTTTAGCATACTCAGGAATGGGA-TGGAC-TTTTTGTAGAAAGT     98

   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
          110       120       130       140       150       160       170       180       190       200
   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
mm TTATACA----TGTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAGGCTTTAACTGGCTCTATAAATTAAA-TGAGTTTGTGCTT    164
rn TTATACA----TGTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAGGCTTTAACTGGCTCTATAAATTAAA-TGAGTTTGTGCTT    164
oc TTATACA----TTTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAGGCTTTAACTGGCTCTATAAATTAAA-TGAGTTTGTGCTT    172
hs TTATACA----TTTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAAGCTTTAACTGGCTCTATAAATTAA-----GTTTGTGCTT    172
pt TTATACA----TTTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAAGCTTTAACTGGCTCTATAAATTAA-----GTTTGTGCTT    172
cf TTATACA----TTTTTGCATATTTCAATTTCCACTT--AA----------AATTTTTAAGGCTTTAACTGGCTCTATAAATTAAGATAAGTTTGTGCTT    175
bt TTATACA----TTTTTGCATATTTCAATT-CCACTT--AA----------ATTTTTTAAGGCTTTAACTGGTTCTATAAATTAAAATAAGTTTGTGCTT    171
la TTATACA----TTTTTGCATATTTCAATTTCCACTT--AA----------ATTTTTTAAGGCTTTAACTTGTTCTATAAATTGA-----GTTTGTGCAT    169
et TTATACA----TTTTTGCATATTTCAATTTCCACTG--CA----------ATTTTTTAAGGCTTTAACTTGCTCTATAAATTAAAATGAGTTTGTGCTT    172
md TTATACA----TTTTTACATATTTCAATTTCCAGTG--AACA---------AATTTTAAAGCCTTCAACTGGCCATATAACTTAAAATGAGTTTTTGCCT    182
gg TTATATTGGATTTTTTGCATAATTCAAGTTCCATTTTTAACAAACCTTAGAAATTTTGAGAGACCTAATTGGTACTATCATAGAAAAAGGATGCTTATGT    198

   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
          210       220       230       240       250       260       270       280       290       300
   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
mm TCCTTGAAATGCACTTATTCTTATTACAGGCATTTTATAATTTTGTATGAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAG-TACTCTCCAGCT-    262
rn TCCTTGAGATGCACTTATTCTTATTACAGGCATTTTATAATTTTGTATGAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAG-TACTTTCCAGCT-    262
oc TCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATGTCTATTTTCTCTAAATATTTTGCTTTTAGTAAAATGCTTTTCAAC--    270
hs TCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAAATGCTTTCCAACT-    271
pt TCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAAATGCTTTCCAACT-    271
cf TCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAAA-GCTTTCCAACT-    273
bt TCCTTGAAATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATGTCTATTTTCTCTAAATATTTTGCTTTCAGTAAAATGCTTTCCAACT-    270
la TCCTTGAGATGCACTTATTCTTATTATAAGCATTTTATAATTTTGTATAAATATCTATTTTCTCTAAATATTT                                242
et TCCTTGAGATGCACTTATTCTTATTACAAGCATTTTATAATTTTGTATAAATATCTATTTTCTCTAAATATTTTGCTTTCAGTAAAATGCTTTCCAACT-    271
md TCCTTGTAAAGCAATAACTCCTAT---AAGCATTTTATAATTTTGTATAAATATCTATTTTCTCTAAATATTTTCCTTTCAGTAAACTGCTTTCAACCCT    279
gg CCTTTGTAAAGCAATAATTCATATAACAAGTATTTTATAATTTTGTATAAATGTCTATTTTCTCTAG-TATCTTTTCCTGGGAACA--GCTTTACAGG--    293

   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
          310       320       330       340       350       360       370       380       390       400
   ---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+
mm CTCCT-------------GGGGGTTGGTTGGTGGAATTACTCTGTATTGACAAGTACAAGTTACTGCCTATGCTTTGTACCTTAGGCT-ACAAAACTAAA    348
rn CTCCT-------------GTGGGTT----GGTGGAATTACTCTTTATTGACTAGTAAAAGTTACTGCCTATGCTTTTTACCTTAGGCTTACAAAATTAAA    345
oc -TGTTTAGTATATTAATTACCAGTGGATTGGTAGAATTGCTTTTTATTGACTAGTAAAAGTTACTGCCTATGCTTTTTACCTTAGGCTTACAAAATTCAA    369
hs CTGTTTAGTGTATTAATTACCAGTGGATTGGTAGAACTGCTTTTTATTGACTAGTAAAAGTTACTGCCTATGCTTTTTACCTTAGGCTTACAGAATTAAA    371
pt CTGTTTAGTGTATTAATTACCAGTGGATTGGTAGAATTGCTTTTTATTGACTAGTAAAAGTTACTGCCTATGCTTTTTACCTTAGGCTTACAGAATTAAA    371
cf CTGTTTAGTATGTTAATTACCAGTC-ATTGGTAGAATTGGTTTTTATTGACTAGTAAAACTTACTGCCTATGCTTTTTATCTTAGGCTTGCAAAATTAAA    372
bt CTGTTTAGTATATT----ACCAGTGGATTGGTAGAATTGCTTTTTATTGACTAGTAAAACTTATTGCCTATACTTTTTACATTAGGCTTTTAAAATTAAA    366
la                                                                                                         242
et CTGTTTAGTATATTAATTATAAGTGGATTGGTAGAATTGCTTTTTATTGACTAGTAAAACTTACTGCCTATGCTTTTTATCTCAGGCTTGCAAAGTTAAA    371
md CTGATTAGTATACTGATTTTCAAATGATTGGTAGAATTGATTTTTACTGACTAGTAAAACTTACTGCCTATACCTT-TACCTCATACTTGCAAAACTAAA    378
gg ------------------TTAGCTAAT-AGAGGCATAACCTTGCAAATGCCTGCACAATTT                                          335

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mm TAAAAATC                                                                                                356
rn TAAAAATC                                                                                                353
oc TAAAAATT                                                                                                377
hs TAAAAATT                                                                                                379
pt TAAAAATT                                                                                                379
cf TAAAAATT                                                                                                380
bt TAAAAATT                                                                                                374
la                                                                                                         242
et TAAAAATG                                                                                                379
md TAATAAAG                                                                                                386
gg                                                                                                         335
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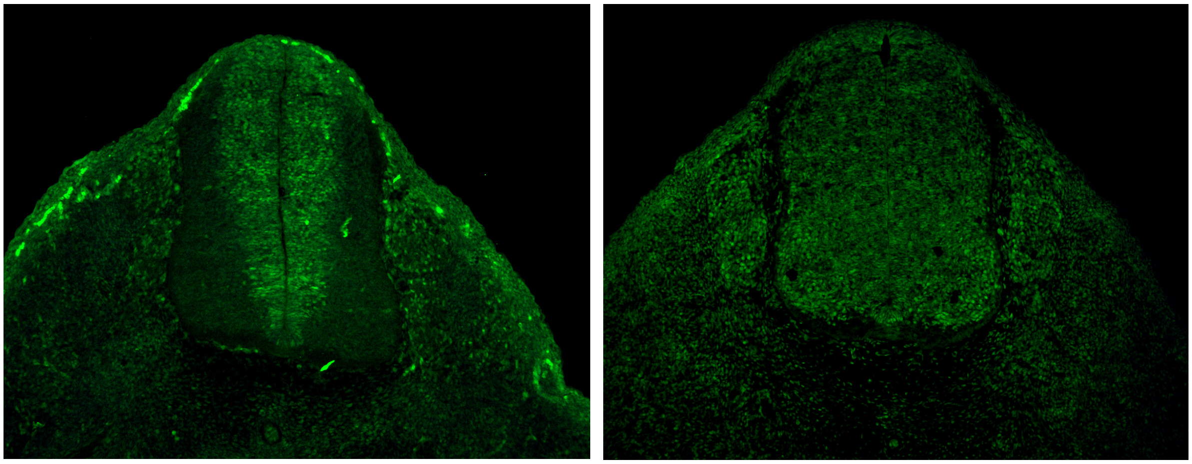
Cross-species alignment of 3' UTRs of BAF53a. The conserved regions corresponding to the predicted binding sites of miR-9, miR-9* and miR-124 are shown in highlights in yellow, green and blue, respectively. The predicted configurations of the microRNAs binding to target sites in the 3' UTR of BAF53a are shown in Fig. 2a.

Supplementary Figure 3

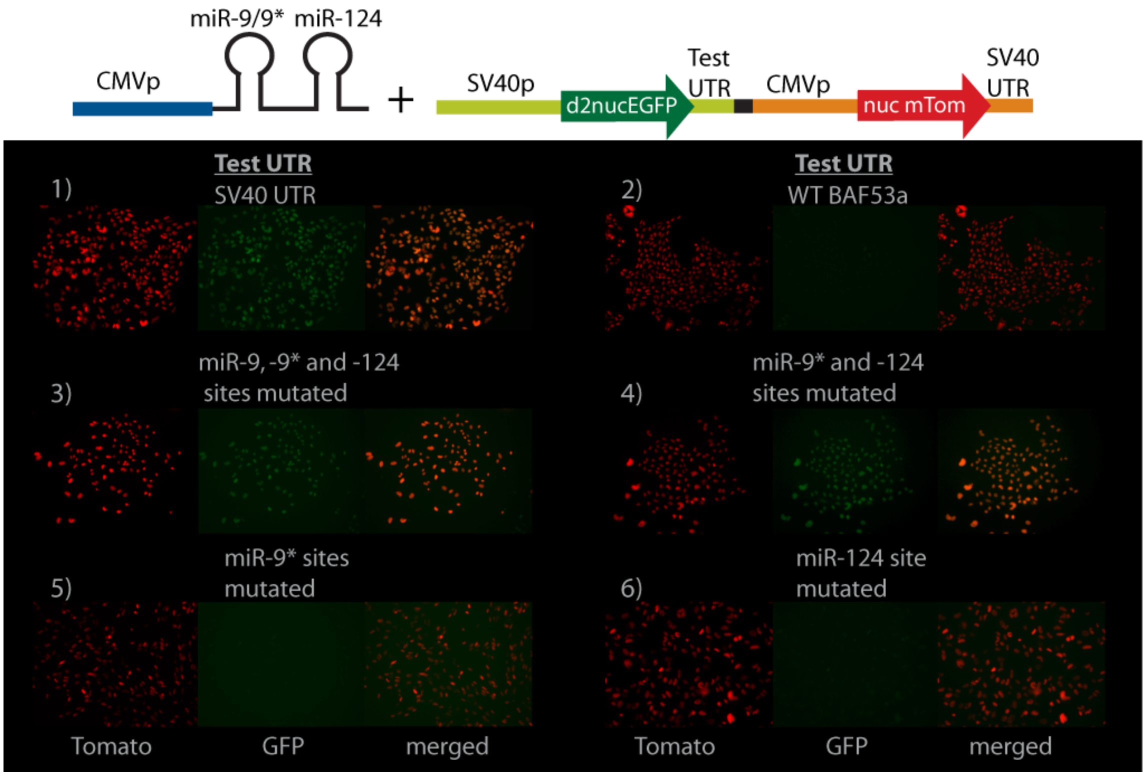


BAF53a BAC d2nucEGFP-
BAF53a **WT** UTR

BAF53a BAC d2nucEGFP-
BAF53a **MUT** UTR

Repression of BAF53a BAC reporter is specific for differentiated neurons. Top panels show wide views of GFP signals from the cross sections of transgenic embryos for the wild type BAF53a BAC reporter and the mutant BAF53a BAC reporter (containing mutations in BAF53a 3'UTR of d2nucEGFP). The de-repression of the reporter expression seen with the mutant reporter (right panel) appears to be specific for differentiated neurons in the neural tube and the neural crest-derived neurons.

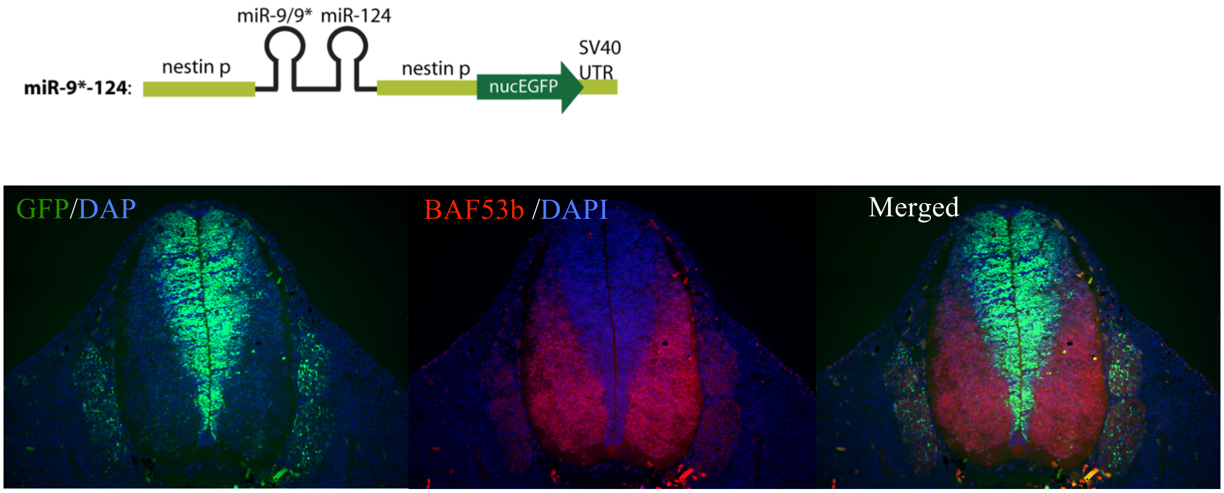
Supplementary Figure 4



Schematic diagram of microRNA expression and sensor constructs. We synthesized a cluster of miR-9* and miR-124 precursors driven by CMV promoter (top diagram). The production of mature miR-9* and miR-124 was confirmed by quantitative RT-PCR (data not shown). The sensor (d2nucEGFP) and marker (nuclear Tomato) reporters were driven by SV40 and CMV promoters, respectively. The photographs show representative fields of stable CHO lines that contain the microRNA overexpression and sensor constructs (quantified in Fig.2c). Note that stable CHO lines showed significantly reduced EGFP (sensor) expression with 3' UTR of BAF53a. This EGFP downregulation was abolished with mutations in miR-9* and miR-124 binding sites.

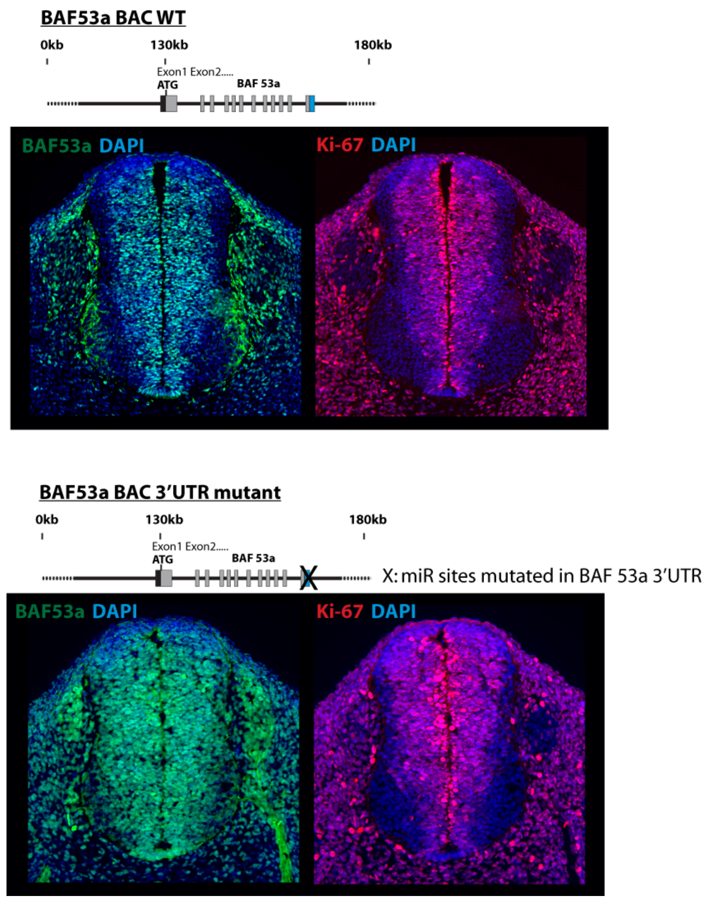
Test UTRs: 1) SV40 3' UTR, 2) wild type BAF53a UTR, 3) BAF53a 3' UTR with mutations in miR-9, -9* and -124 sites, 4) mutations in miR-9* and -124 sites, 5) mutation in miR-9* site only, and 6) mutaion in miR-124 site only.

Supplementary Figure 5



Overexpression of miR-9*/-124 in neural progenitors and reduction of BAF53a does not activate BAF53b expression in E11.5 transgenic embryos. The top drawing represents a schematic diagram of the miR-9*/-124 expression construct. GFP-positive progenitors show decreased population of BAF53a and Ki-67-positive cells, indicative of proliferative defects (Fig. 4b). Although the progenitors exit from cell cycle, there appears to be no overlapping expression between GFP and BAF53b as shown in the photos.

Supplementary Figure 6



Ki-67 expression in wild type and mutant BAF53a BAC-transgenic embryos.
The top panel shows E11.5 embryos transgenic with wild-type BAF53a BAC which showed normal downregulation of BAF53a and a lack of Ki-67 expression (a marker for proliferation) in the neuronal zone. Mutating the 3' UTR of BAF53a lead to extended expression of BAF53a in E11.5 transgenic embryos. Extended expression of BAF53a was associated with disorganized mitosis and extended Ki-67 expression. This phenotype may be related to the possible expression of miR-9* (Fig. 2d) and miR-124[2] along the ventricular surface of the neural tube, which has been proposed to a site of neurogenic asymmetric division of progenitors to produce neurons [3-5].

References

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