

**C Downregulated genes**

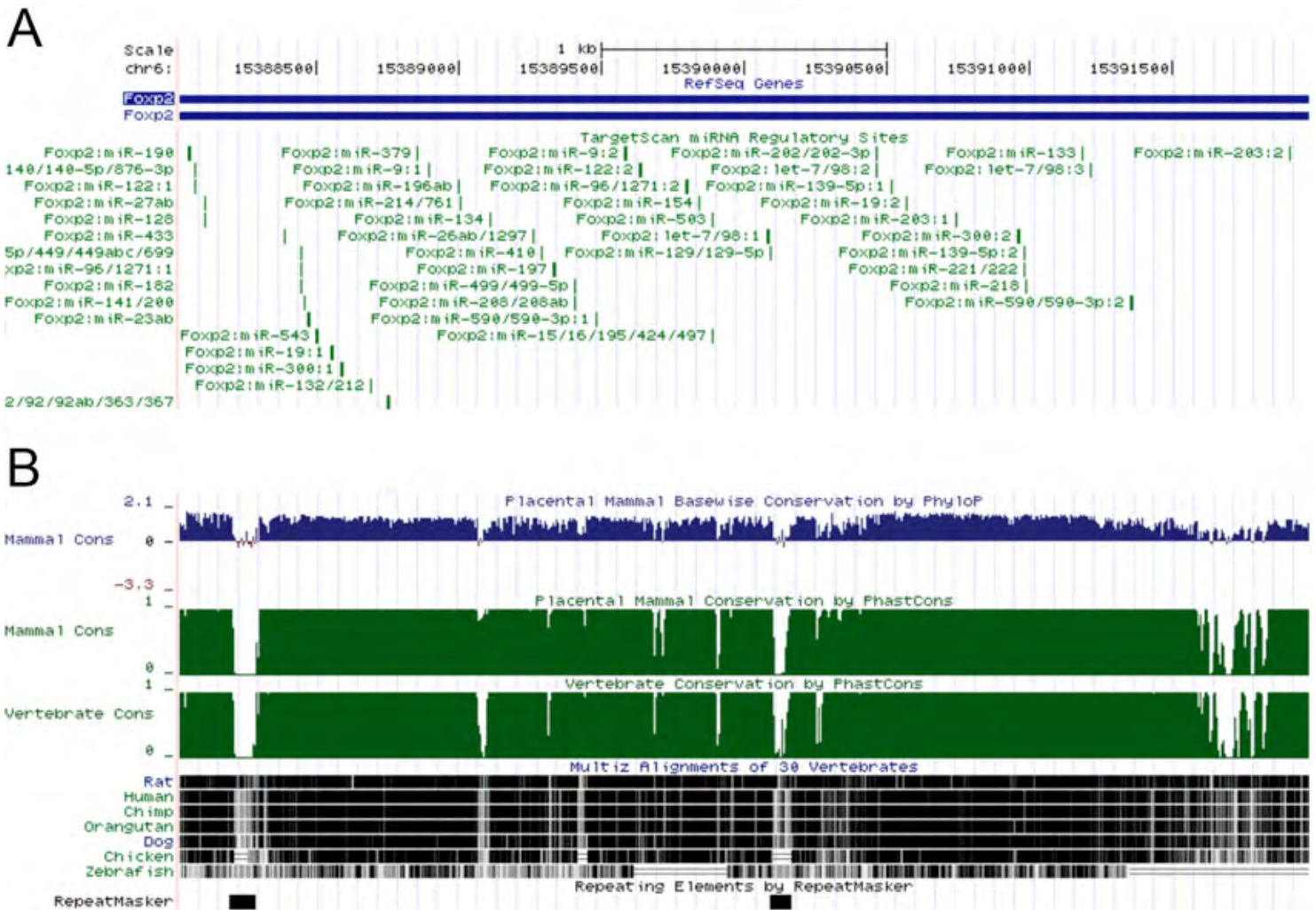
Ensembl Gene ID	Description	Associated Gene Name	log2	FDR
ENSMUSG00000026227	RIKEN cDNA 2810459M1.1 gene Gene [Source:MGI Symbol;Acc:MGI:1920042]	2810459M1.1Rik	-1.4857009	0.0332855
ENSMUSG00000059325	HOP homeobox Gene [Source:MGI (curated);Acc:MGI:1916782]	Hopx	-1.2917070	0.00560871
ENSMUSG00000004892	brevican Gene [Source:MGI Symbol;Acc:MGI:1096385]	Bcan	-1.2824554	0.0486312
ENSMUSG000000062345	serine (or cysteine) peptidase inhibitor, clade B, member 2 Gene [Source:MGI (curated);Acc:MGI:97609]	Serpinh2	-1.150748	0.00243955
ENSMUSG000000045257	MORN repeat containing 2 Gene [Source:MGI Symbol;Acc:MGI:2674071]	Morn2	-1.1392899	0.00644425
ENSMUSG000000035642	RIKEN cDNA 1810020D17.7 gene Gene [Source:MGI Symbol;Acc:MGI:1913523]	1810020D17Rik	-1.0959095	0.00340925
ENSMUSG000000068205	MACRO domain containing 2 Gene [Source:MGI (curated);Acc:MGI:1920149]	Macro2d	-1.0710821	0.00237502
ENSMUSG000000070394	RIKEN cDNA 1810027O10.0 gene Gene [Source:MGI Symbol;Acc:MGI:1916436]	1810027O10Rik	-1.0336299	0.00645658
ENSMUSG000000042198	coiled-coil-helix-coiled-coil-helix domain containing 7 Gene [Source:MGI (curated);Acc:MGI:1913683]	Chchd7	-1.0225972	0.00513
ENSMUSG000000041718	asparagine-linked glycosylation 13 homolog (S. cerevisiae) Gene [Source:MGI (curated);Acc:MGI:1914824]	Alg13	-1.0084264	0.00434832
ENSMUSG000000019761	keratin 10 Gene [Source:MGI (curated);Acc:MGI:96685]	Krt10	-1.0062412	0.00252591

**Upregulated genes**

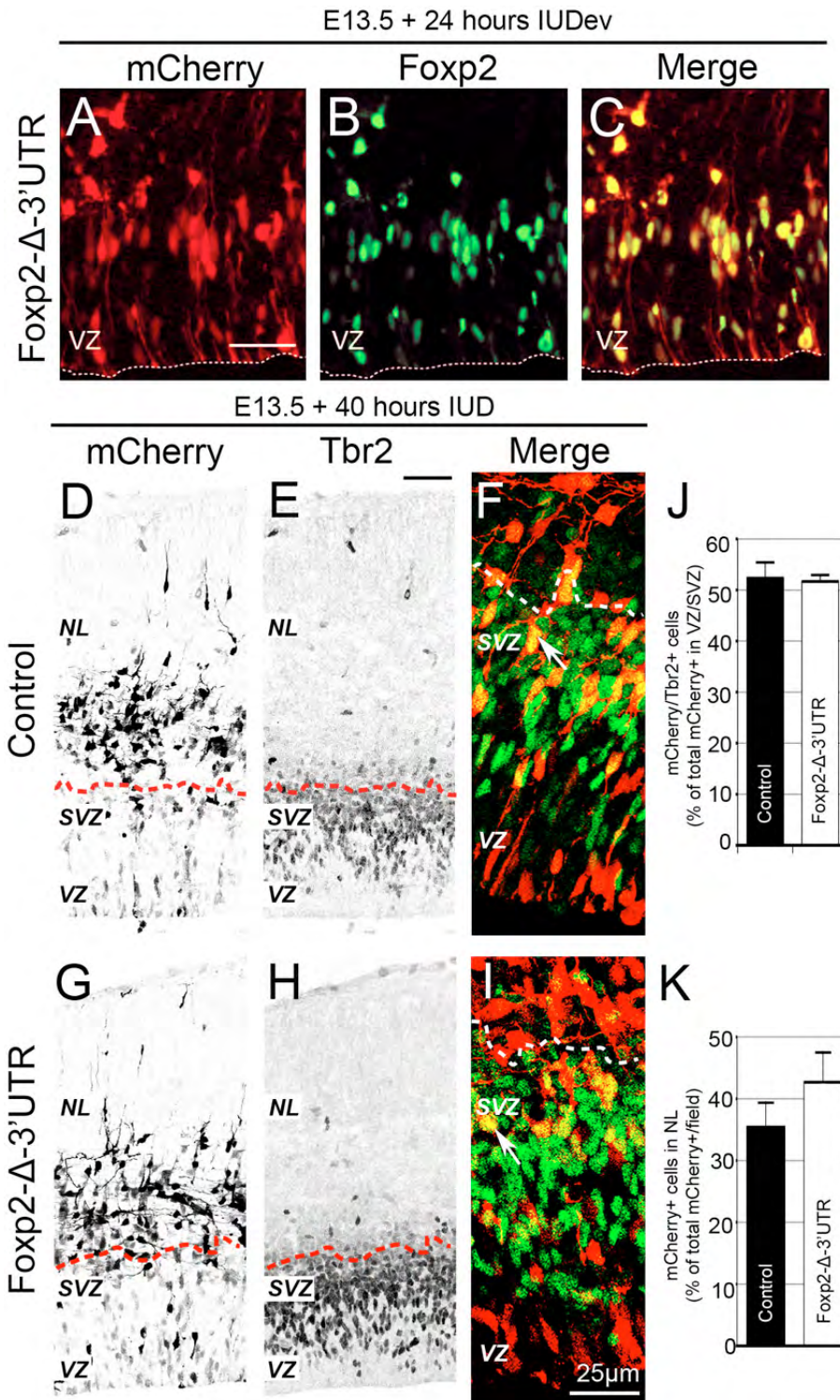
Ensembl Gene ID	Description	Associated Gene Name	log2	FDR
ENSMUSG000000050240	hypermethylated in cancer 2 Gene [Source:MGI Symbol;Acc:MGI:1929869]	Hic2	1.01787592	0.0048853
ENSMUSG000000024642	transducin-like enhancer of split 4, homolog of Drosophila E(spl) Gene [Source:MGI Symbol;Acc:MGI:104633]	Tie4	1.04046809	0.0015253
ENSMUSG000000036279	POU domain, class 2, transcription factor 1 Gene [Source:MGI (curated);Acc:MGI:101898]	AC120871.2	1.04080651	0.0071940
ENSMUSG000000057098	early B-cell factor 1 Gene [Source:MGI (curated);Acc:MGI:95275]	Ebf1	1.04404118	0.0019040
ENSMUSG000000028364	tenascin C Gene [Source:MGI (curated);Acc:MGI:101922]	Tnc	1.0449761	0.014687
ENSMUSG000000022483	collagen, type II, alpha 1 Gene [Source:MGI (curated);Acc:MGI:88452]	Col2a1	1.05067295	0.013134
ENSMUSG000000014592	calmodulin binding transcription activator 1 Gene [Source:MGI (curated);Acc:MGI:2140230]	Camta1	1.0510997	0.0063325
ENSMUSG000000043753	doublesex and mab-3 related transcription factor like family A1 Gene [Source:MGI (curated);Acc:MGI:2653627]	Dmrt1a	1.05503611	0.0018790
ENSMUSG000000052105	RIKEN cDNA 1110012J17.7 gene Gene [Source:MGI Symbol;Acc:MGI:1915867]	1110012J17Rik	1.06303596	0.0028119
ENSMUSG000000019230	LM homeobox protein 9 Gene [Source:MGI (curated);Acc:MGI:1316721]	Lhx9	1.07297162	0.0034404
ENSMUSG000000026365	POU domain, class 2, transcription factor 1 Gene [Source:MGI (curated);Acc:MGI:101898]	Pou2f1	1.0911457	0.0024488
ENSMUSG000000068740	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila) Gene [Source:MGI (curated);Acc:MGI:1858235]	Celsr2	1.09662841	0.0041055
ENSMUSG000000030494	rhophilin, Rho GTPase binding protein 2 Gene [Source:MGI (curated);Acc:MGI:1289234]	Rhpn2	1.10353949	0.0011148
ENSMUSG000000061393	activin receptor IIB Gene [Source:MGI Symbol;Acc:MGI:87912]	Acrv2b	1.1067165	0.0023734
ENSMUSG000000038718	pre B-cell leukemia transcription factor 3 Gene [Source:MGI (curated);Acc:MGI:97496]	Pbx3	1.10773954	0.023161
ENSMUSG000000033581	insulin-like growth factor 2 mRNA binding protein 2 Gene [Source:MGI (curated);Acc:MGI:1890358]	Igf2bp2	1.12862099	0.0073122
ENSMUSG000000073019	Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:Q3UHE8]	AL669964.2	1.13430238	0.0014193
ENSMUSG000000009418	neuron navigator 1 Gene [Source:MGI Symbol;Acc:MGI:2183683]	Nav1	1.13450356	0.0081533
ENSMUSG000000029238	circadian locomotor output cycles kaput Gene [Source:MGI Symbol;Acc:MGI:99698]	Clock	1.13611199	0.0026813
ENSMUSG000000029193	cholecystokinin A receptor Gene [Source:MGI Symbol;Acc:MGI:99478]	Cckar	1.13784167	0.0081569
ENSMUSG000000066440	zinc finger, FYVE domain containing 26 Gene [Source:MGI Symbol;Acc:MGI:1924767]	Zfyve26	1.15225607	0.0044533
ENSMUSG000000028527	adenylate kinase 4 Gene [Source:MGI (curated);Acc:MGI:87979]	Ak3l1	1.15663948	0.0023365
ENSMUSG000000048562	trans-acting transcription factor 8 Gene [Source:MGI Symbol;Acc:MGI:2443471]	Sp8	1.16568607	0.0022085
ENSMUSG000000039830	oligodendrocyte transcription factor 2 Gene [Source:MGI (curated);Acc:MGI:1355331]	Olig2	1.16628652	0.038565
ENSMUSG000000038872	zinc finger homeobox 3 Gene [Source:MGI Symbol;Acc:MGI:99948]	Zfhx3	1.17879595	0.011001
ENSMUSG000000073202	Putative uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:Q3TDD0]	AC158586.1	1.1822875	0.0084865
ENSMUSG000000017453	pipecolic acid oxidase Gene [Source:MGI (curated);Acc:MGI:1197006]	Pipox	1.23898109	0.0016235
ENSMUSG000000050558	prokinectin receptor 2 Gene [Source:MGI (curated);Acc:MGI:2181363]	Prokr2	1.25049806	0.040107
ENSMUSG000000035834	polymerase (RNA) III (DNA directed) polypeptide G Gene [Source:MGI Symbol;Acc:MGI:1914736]	Polr3g	1.25318266	0.0033569
ENSMUSG000000029714	GRB10 interacting GYF protein 1 Gene [Source:MGI Symbol;Acc:MGI:1888677]	Gygf1	1.25498525	0.0029667
ENSMUSG000000025571	trinucleotide repeat containing 6C Gene [Source:MGI (curated);Acc:MGI:2443265]	Trnrc6c	1.25694333	0.0025115
ENSMUSG000000000214	tyrosine hydroxylase Gene [Source:MGI (curated);Acc:MGI:98735]	Th	1.25905654	0.0075223
ENSMUSG000000006205	Htra serine peptidase 1 Gene [Source:MGI (curated);Acc:MGI:1929076]	Htra1	1.2922388	0.022002
ENSMUSG000000074415	RIKEN cDNA 2610203C20.0 gene Gene [Source:MGI Symbol;Acc:MGI:1917705]	2610203C20Rik	1.29480563	0.0024315
ENSMUSG0000000031762	metallothionein 2 Gene [Source:MGI Symbol;Acc:MGI:97172]	Mt2	1.30157757	0.0062098
ENSMUSG000000022708	zinc finger and BTB domain containing 20 Gene [Source:MGI (curated);Acc:MGI:1929213]	Zbtb20	1.30669793	0.0060576
ENSMUSG000000013415	insulin-like growth factor 2 mRNA binding protein 1 Gene [Source:MGI (curated);Acc:MGI:1890357]	Igf2bp1	1.30704626	0.0024119
ENSMUSG0000000061762	tachykinin 1 Gene [Source:MGI Symbol;Acc:MGI:98474]	Tac1	1.3214311	0.016411
ENSMUSG000000049313	sortilin-related receptor, LDLR class A repeats-containing Gene [Source:MGI (curated);Acc:MGI:1202296]	Sorl1	1.3322444	0.0072966
ENSMUSG000000022054	neurofilament, medium polypeptide Gene [Source:MGI (curated);Acc:MGI:97314]	Nefm	1.34915044	0.0086085
ENSMUSG000000025241	FYVE and coiled-coil domain containing 1 Gene [Source:MGI Symbol;Acc:MGI:107277]	Fyco1	1.36460089	0.021586
ENSMUSG000000027347	RAS guanyl releasing protein 1 Gene [Source:MGI (curated);Acc:MGI:1314635]	Rasgrp1	1.38439901	0.011016
ENSMUSG000000025019	ligand dependent nuclear receptor corepressor Gene [Source:MGI Symbol;Acc:MGI:2443930]	Lncor	1.42571491	0.002951
ENSMUSG000000029563	forkhead box P2 Gene [Source:MGI (curated);Acc:MGI:2148705]	Foxp2	1.45595582	0.0090679
ENSMUSG000000047888	trinucleotide repeat containing 6b Gene [Source:MGI Symbol;Acc:MGI:2443730]	Trnrc6b	1.52056734	0.0024994
ENSMUSG000000022055	neurofilament, light polypeptide Gene [Source:MGI (curated);Acc:MGI:97313]	Nefl	1.58024378	0.0084437
ENSMUSG000000032394	immunoglobulin superfamily, DCC subclass, member 3 Gene [Source:MGI Symbol;Acc:MGI:1202390]	Igdc3	1.582201	0.0030652
ENSMUSG000000052707	trinucleotide repeat containing 6a Gene [Source:MGI Symbol;Acc:MGI:2385292]	Trnrc6a	1.65042927	0.002168
ENSMUSG000000030729	phosphoglucomutase 2-like 1 Gene [Source:MGI (curated);Acc:MGI:1918224]	Pgm2l1	1.91167672	0.0023632
ENSMUSG000000075324	fdgufin Gene [Source:MGI (curated);Acc:MGI:1890647]	Fign	1.91470256	0.0041970
ENSMUSG000000029546	UNC homeobox Gene [Source:MGI Symbol;Acc:MGI:108013]	Uncx	2.07100304	0.0013406

Targeting miRNA	Cell type, Tissue	Reference
*1 miR-335	human breast cancer metastasis	Tavazoie SF, Nature, 2008
*2 miR-129	human hematopoietic stem cells	Liao R, Cell Biochem, 2008
*3 miR-17-3p	mouse embryonal spinal chord	Chen JA, Neuron, 2011
*4 miR-130	human mesenchymal stem-cell-derived neuronal cells	Greco SJ, Proc Natl Acad Sci USA, 2007
*5 miR-21	mouse splenic CD4+T cells	Murthy RG, Brain Behav Immun, 2008 Pan W, J Immunol, 2010

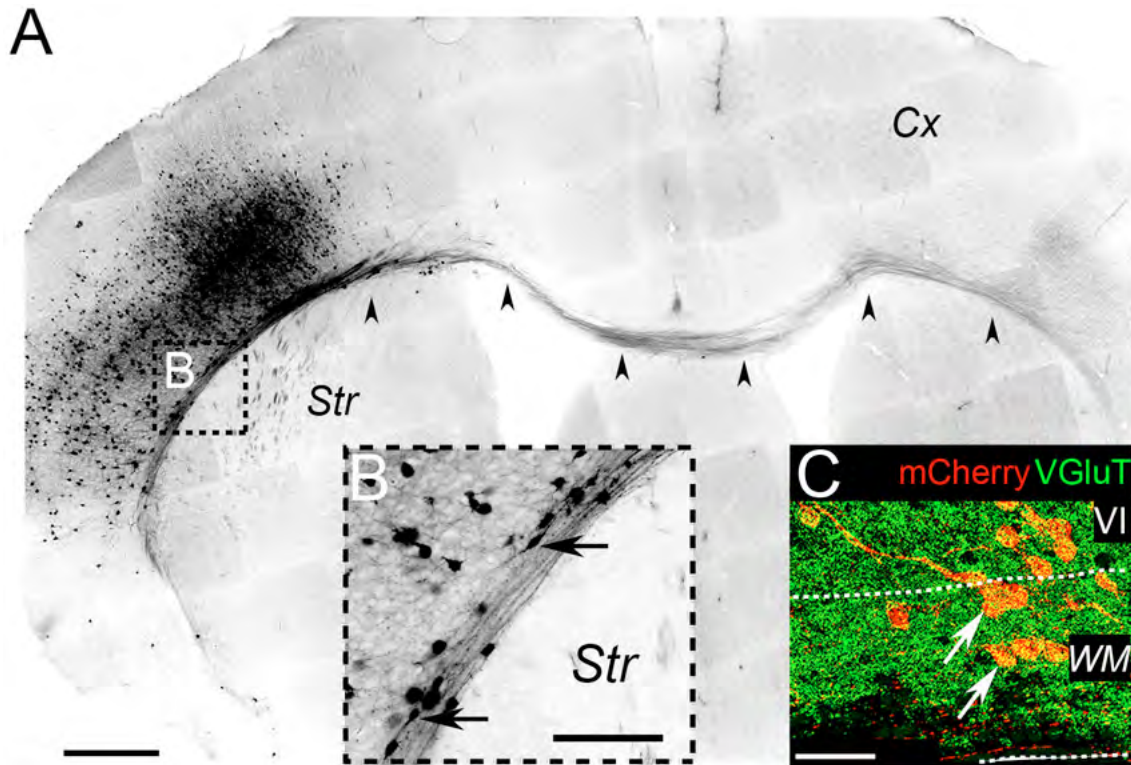
**Fig. S1. Gene Ontology (GO) categories and differentially expressed genes in embryonic neocortex of conditional *Dicer* knockout (*Dicer* KO) mice.** (A,B) Enrichment of high-ranking ( $P < 0.001$ ) and low-ranking ( $P = 0.001$ ) genes in GO categories (Prüfer et al., 2007) in the differentially expressed genes (upregulated, A; downregulated, B) in embryonic neocortex of conditional *Dicer* knockout (*Dicer* KO), using 1000 gene permutations and a cutoff of at least 20 annotated genes per GO category. (C) List of the 62 detected and annotated genes analyzed in *Dicer* KO samples, with a difference of more than twofold ( $\log_2$  scale) over control samples and false discovery rate (FDR)  $< 5\%$  [corresponding to  $P < 0.02198$  (Student's *t*-test)], normalized to mean 0 and standard deviation of 1. Asterisks and numbers indicate genes previously validated as miRNA targets (references listed in the bottom panel).



**Fig. S2. *Foxp2* 3' UTR is highly conserved in vertebrates and contains several predicted miRNA-binding sites. (A)** Higher magnification view of the 3' end of the mouse *Foxp2* gene showing the 3'UTR region (blue lines), miRNAs and their respective predicted binding sites in mouse *Foxp2* 3'UTR (green). **(B)** Conservation of *Foxp2* 3'UTR in placental mammals by PhyloP and Phastcons (blue and green, respectively), and in vertebrates by Phastcons (green); multiple alignment (black) of *Foxp2* 3'UTR region from various vertebrates (indicated in blue or green).



**Fig. S3. Ectopic expression of Foxp2 in neural progenitors does not impair their subtype specification and differentiation in embryonic neocortex.** (A-C) Fluorescence images from section through the dTel of E14.5 wild type (WT) mouse embryo co-electroporated *in utero* at E13.5 with pCAGGS-mCherry along with pCAGGS-Foxp2- $\Delta$ -3'UTR, illustrating intrinsic fluorescence of mCherry (A,C, red) and Foxp2 immunostaining (B,C, green). VZ, ventricular zone; dotted lines, edge of the lateral ventricle. Scale bar: 25  $\mu$ m. (D-I) Confocal images from sections through the telencephalon of E15 wild-type mouse embryo co-electroporated *in utero* at E13.5 with pCAGGS-mCherry and either pCAGGS-empty (control, D-F), or pCAGGS-Foxp2- $\Delta$ -3'UTR (Foxp2- $\Delta$ -3'UTR; G-I), showing intrinsic mCherry fluorescence (D,F,G,I) and Tbr2 immunostaining (E,F,H,I). Arrows indicate mCherry and Tbr2 double-positive cells. SVZ, sub-ventricular zone; NL, neuronal layers; dashed lines, top boundary of the SVZ. Scale bars: 100  $\mu$ m in D,E,G,H; 25  $\mu$ m in F,I). (J) Quantification of Tbr2 and mCherry double-positive progenitors (F,I, arrows) in the VZ and SVZ of cortices electroporated with control (black bar) or pCAGGS-Foxp2- $\Delta$ -3'UTR (white bar) plasmids, expressed as percentage of total mCherry-positive cells in VZ and SVZ per field (as shown in F-I). (K) Quantification of mCherry-positive neurons in NL of cortices electroporated with control (black bar) or pCAGGS-Foxp2- $\Delta$ -3'UTR (white bar) plasmids, and expressed as percentage of total mCherry-positive cells per field (as shown in D,G). Data are mean $\pm$ s.e.m. of at least three brains counted per condition (two or three sections along the rostrocaudal axis were counted per brain).



**Fig. S4. Ectopic expression of *Foxp2* does not impair cortical projection neuron differentiation.** (A-C) Fluorescence microscopy images of sections through the cerebral cortex of a wild-type (WT) P15 mouse, co-electroporated in utero at E13.5 with pCAGGS-mCherry and pCAGGS-*Foxp2*- $\Delta$ -3'UTR, showing intrinsic mCherry fluorescence (A,B, black; C, red) and vGluT immunostaining (C, green). Str, striatum; Cx, cortex; Dashed box in A indicates the region shown at higher magnification in B and a similar region shown in C. Arrowheads indicate fibers of electroporated cells innervating the contralateral cortex; black arrows indicate ectopic cells with tangential morphology extending neural processes; white arrows indicate mCherry and vGluT double-positive neurons. Scale bar: 500  $\mu$ m in A; 100  $\mu$ m in B; 50  $\mu$ m in C.

<b>LNA-Probe Name</b>	<b>Probe Sequence (5'&gt;3')</b>
Mmu-miR-132	CGACCATGGCTGTAGACTGTTA
Mmu-miR-9	TCATACAGCTAGATAACCAAAGA
<b>PCR primer Name</b>	<b>Primer sequence (5'&gt;3', Cloning sites are shown in <b>bold</b>)</b>
Foxp2-3'UTR (first 2Kb) forward	CATCG <b>GAATTC</b> TTAGAACAATTACGGCAGA
Foxp2-3'UTR (first 2Kb) reverse	GCAT <b>GAATTC</b> TTATTTGTA CTT CAGAAATGTAA
Foxp2-3'UTR (last 2Kb) forward	ATGC <b>GAATTC</b> GAACGAACTTGTGACACCT
Foxp2-3'UTR (last 2Kb) reverse	GCAT <b>GAATTC</b> CAGAGGTAGAAGCTTTCAGAAAG
Foxp2 ORF forward	ATGCG <b>CTAGCT</b> TATTAAGTCATGATGCAGGAATC
Foxp2 ORF reverse	GCAT <b>GAATTC</b> GTTCTCATTCCAGGTCCTCA
Foxp4 ORF forward	ATG <b>GCTAGC</b> GGCAATGATGGTGGAG
Foxp4 ORF forward	CGTAG <b>GAATTC</b> TGCCCTTAGGACATGTCCTC

CLOVIS *et al.*, Table S1