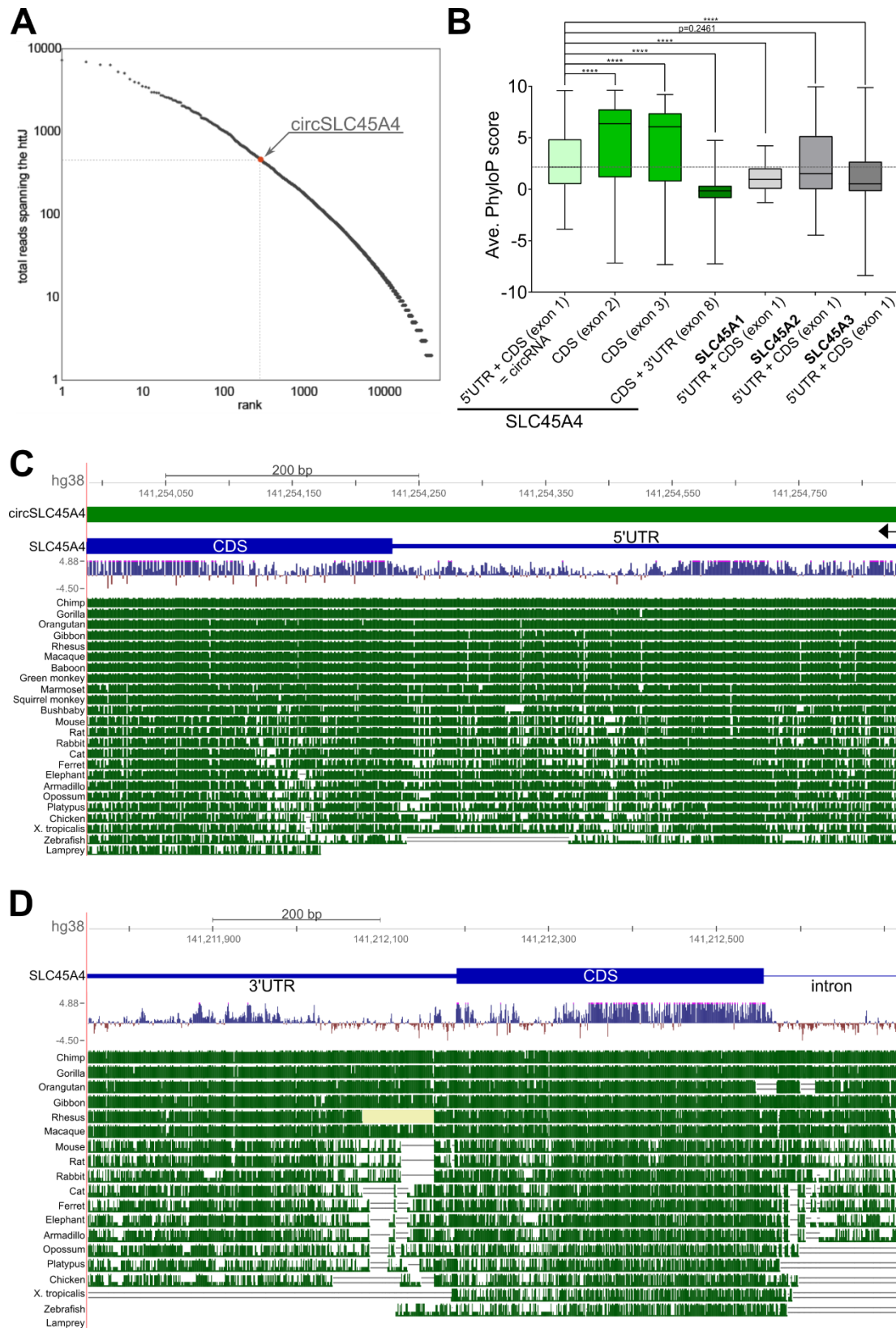


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**Supplemental Information**

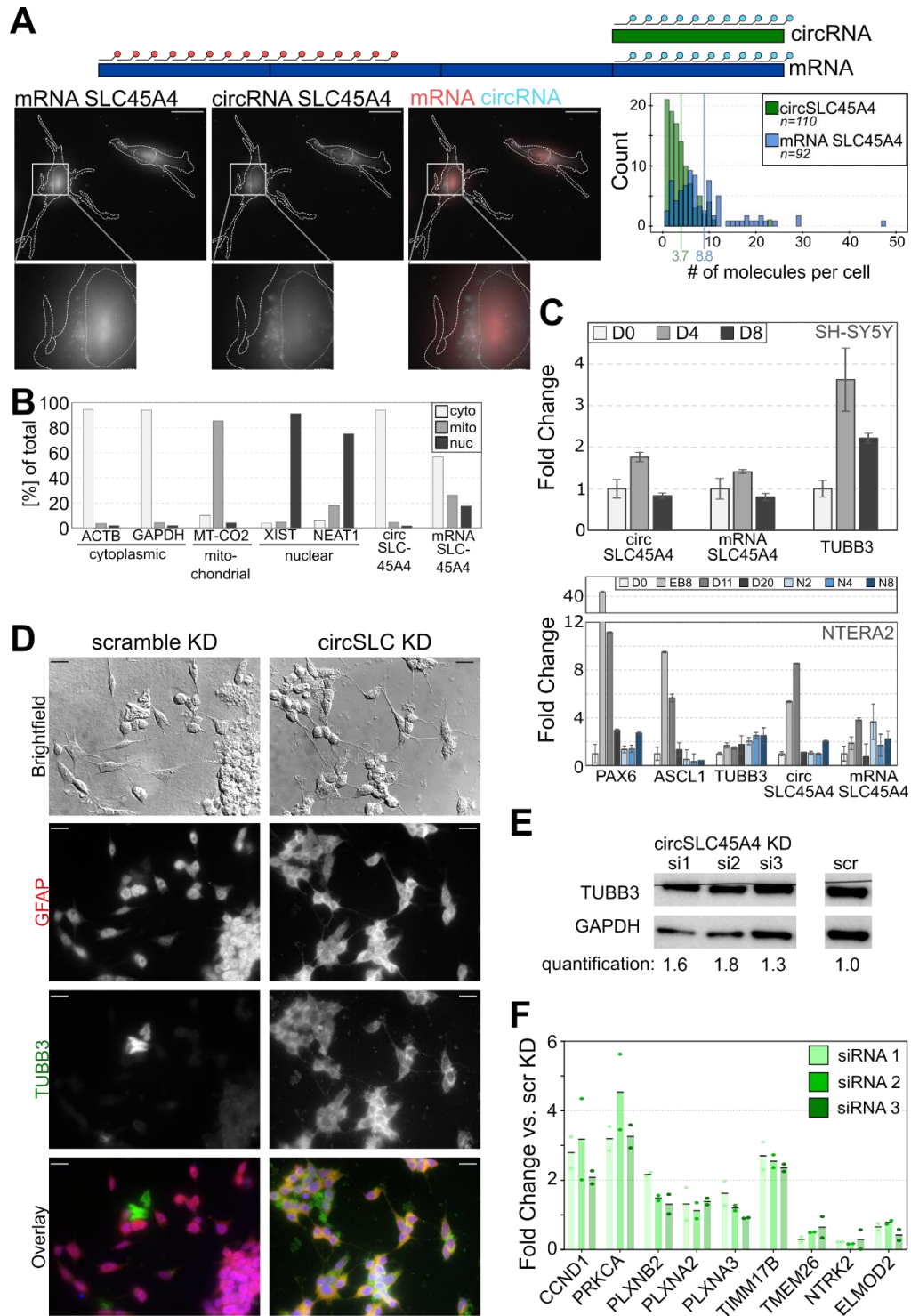
**A Highly Conserved Circular RNA Is Required  
to Keep Neural Cells in a Progenitor State  
in the Mammalian Brain**

**Christin Suenkel, Daniel Cavalli, Simone Massalini, Federico Calegari, and Nikolaus Rajewsky**



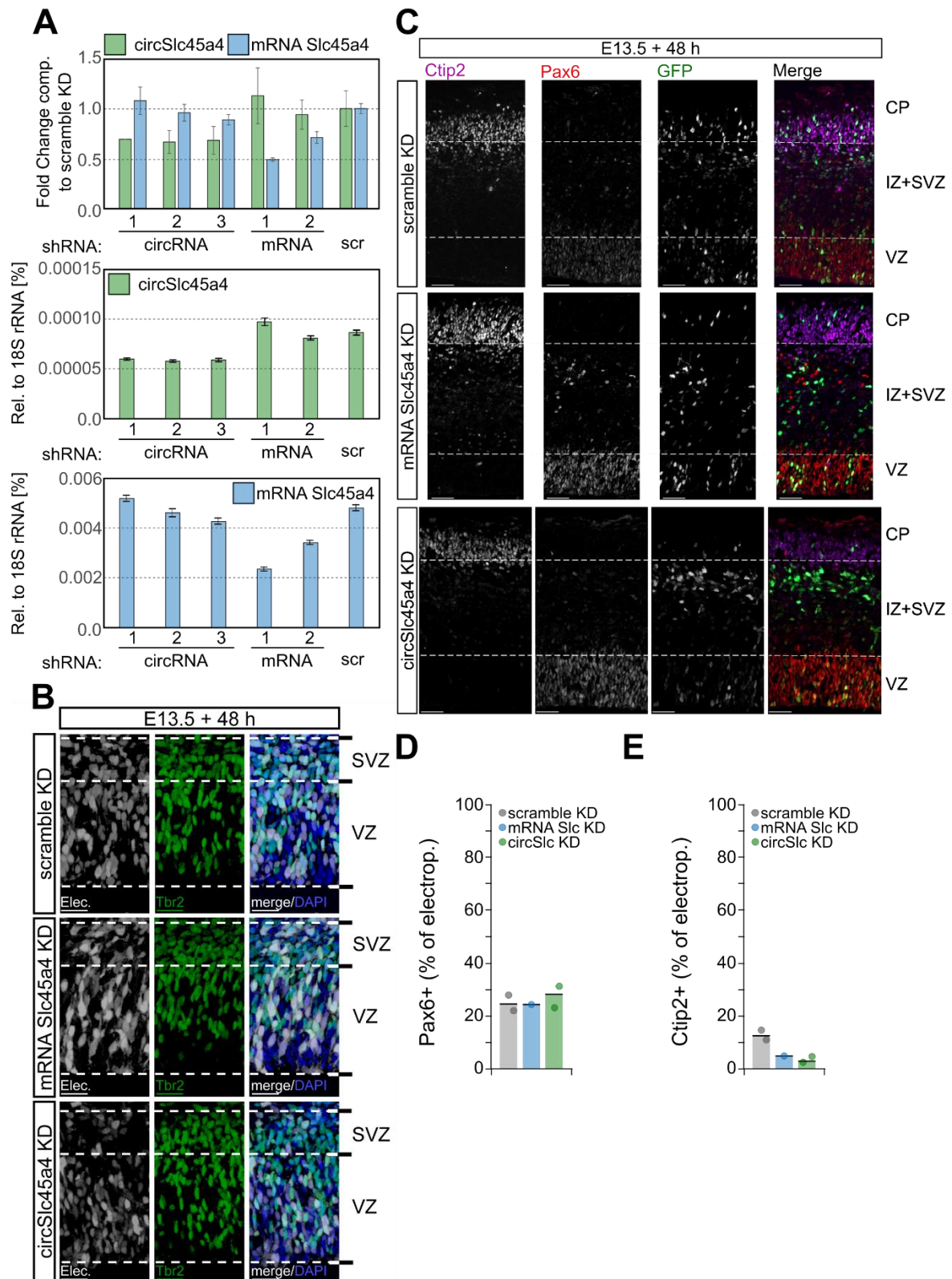
**Figure S1: related to Figure 1.**

(A) – Expression rank of circSLC45A4 in fetal human cortex, ranked by total reads spanning the head-to-tail splice junction (295<sup>th</sup>, top 1 %). (B) – Average PhyloP scores of multiple alignments of 100 vertebra, SLC45A4 exon1 (641 nt, mean = 2.86), 2 (190 nt, mean = 5.04), 3 (183 nt, mean = 4.52), and 8 (931 nt, mean = -0.25), SLC45A1 exon 1 (103 nt, mean = 1.13), SLC45A2 exon 1 (534 nt, mean = 2.64), SLC45A3 exon 1 (403 nt; mean = 1.73). T-test, p-value < 0.0001 is \*\*\*\*. (C) – Genome Browser conservation tracks visualizing the exceptional conservation of circSLC45A4 compared to the 3'UTR and CDS of the same gene, SLC45A4 (D).



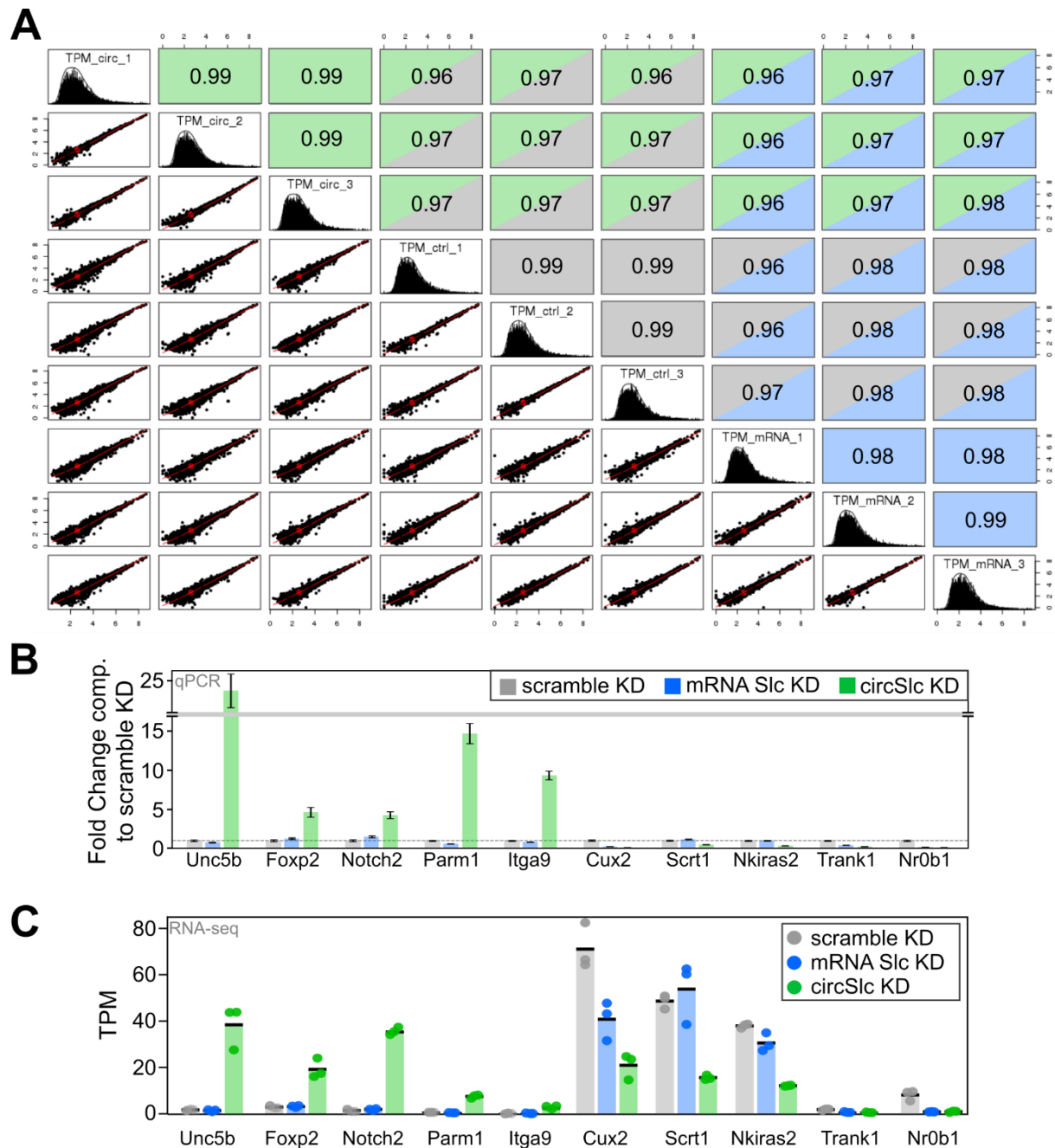
**Figure S2: related to Figure 2.**

(A) – Single-molecule FISH of circSLC45A4 and mRNA SLC45A4, probe design, representative pictures and quantification in SH-SY5Y. Scale bar corresponds to 10  $\mu$ m. Cell outlines are marked with dashes, nuclei outlines are marked with dots. Histogram based on n=110 cells for circSLC45A4 and n=92 for mRNA SLC45A4. (B) – Fractionation of SH-SY5Y cells shows that circSLC45A4 is largely localized to the cytoplasm, while mRNA SLC45A4 is found mostly in the cytoplasm, but also in mitochondria and in the nucleus. (C) – circSLC45A4 expression during SH-SY5Y and NTERA2 differentiation. Expression of important marker genes that highlight the progression of differentiation are shown. Error bars are standard deviation from three technical replicates. (D) – Immunofluorescence of SH-SY5Y cells 96 h after knockdown of circSLC45A4, staining for GFAP and TUBB3. Scale bar – 10  $\mu$ m. (E) – Western Blot quantification of TUBB3 protein levels after knockdown of circSLC45A4 with 1 of each of the 3 siRNAs and scramble KD. Fiji was used for quantification. All samples were run on the same membrane, an irrelevant lane was digitally deleted. (F) – qPCR validation of selected gene expression changes. Each dot represents one biological replicate.



**Figure S3: related to Figure 3.**

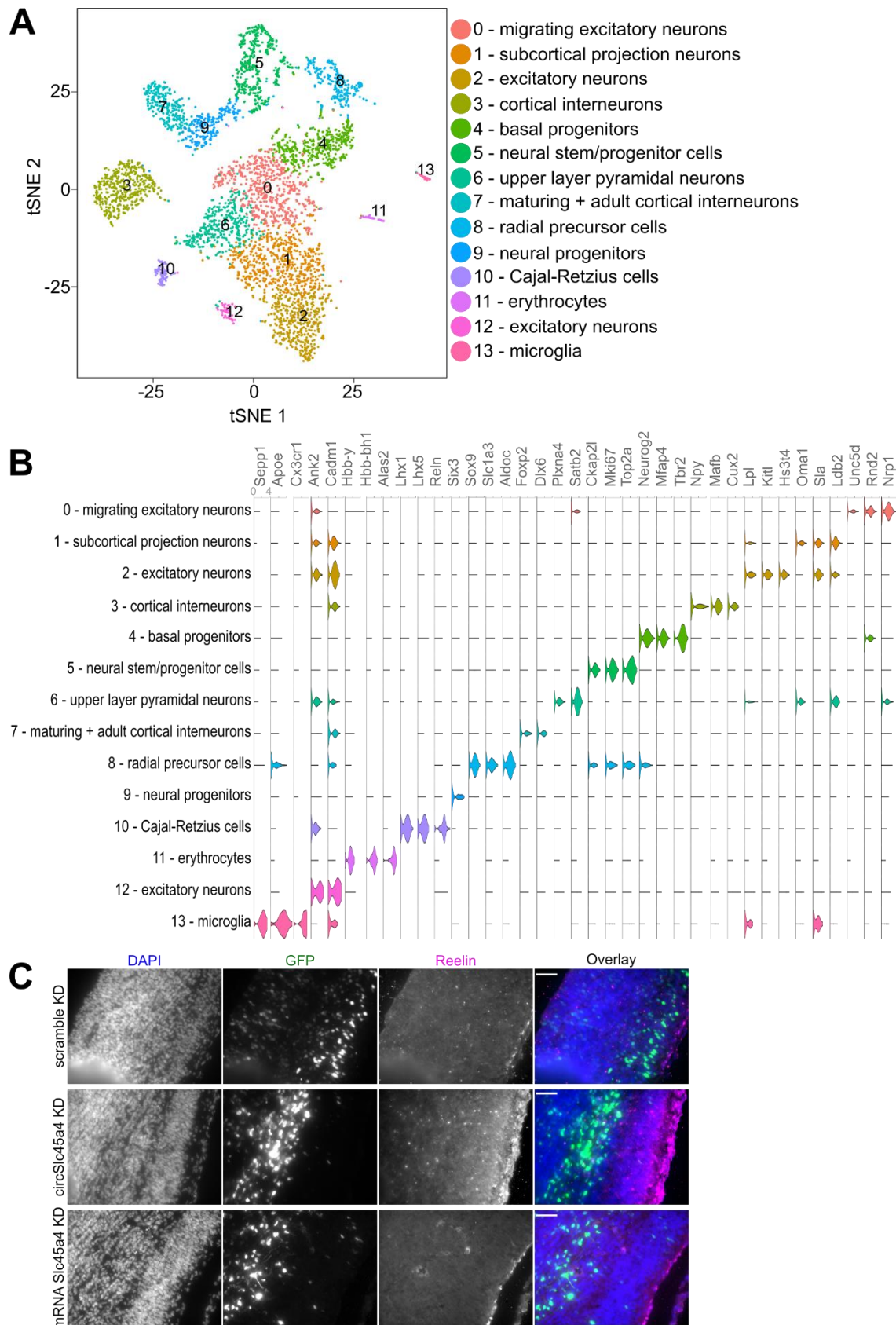
(A) – KD efficiency of the respective shRNAs against circSlc45a4 and mRNA Slc45a4 in mouse P19 cells. Error bars are standard deviation from three technical replicates. (B) – Manipulated cells (white – GFP+) in developing cortex at E15.5 for scrambled control KD, mRNA Slc45a4 and circSlc45a4 knockdown. Nuclei are DAPI stained (blue), co-staining with Tbr2. Scale bar is 50  $\mu$ m. (C) – Manipulated cells (GFP+), neurons (Ctip2+) and apical progenitors (Pax6+) in developing cortex at E15.5 for scrambled control KD, mRNA Slc45a4 and circSlc45a4 KD. Scale bar is 50  $\mu$ m. (D) – Quantification of electroporated cells that are Pax6+. (E) – Quantification of electroporated cells that are Ctip2+.



**Figure S4: related to Figure 4.**

(A) – Overall correlation between all biological replicates collected after knockdown of circSlc45a4, mRNA Slc45a4 and scramble KD. (B) – qPCR validation of induced expression changes after knockdown of circSlc45a4, mRNA Slc45a4 and scramble KD in embryonic mouse cortex. Error bars are standard error of the mean, 3 biological replicates. (C) – TPM (transcripts per million) values for various genes, illustrating that most changes are specific to circSlc45a4 knockdown.





**Figure S5: related to Figure 5.**

(A) – tSNE plot of embryonic mouse cortex at E15.5, data from (Yuzwa et al., 2017), reanalyzed. (B) – Subset of identified marker genes that were used for cell population analysis. All y-scales are set from 0 to 4. (C) – Representative images of Reelin staining after KD of circSlc45a4, mRNA Slc45a4 and scramble KD. Scale bar is 50  $\mu$ m.

**Table S1, related to Figure 2:** Results of GO term analysis of significantly deregulated genes after circSLC45A4 KD in SH-SY5Y

Upregulated genes					
	Term	Enrichment <sup>1</sup>	p-value <sup>2</sup>	p-adj <sup>3</sup>	Example genes
GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	14/65	6.22E-10	1.24E-05	DCHS1, CELSR3, CELSR2, TRO, CADM4
GO:0098742	cell-cell adhesion via plasma-membrane adhesion molecules	15/98	2.17E-08	2.05E-04	DCHS1, CELSR3, CELSR2, TRO, CADM4
GO:0007399	nervous system development	72/1573	2.76E-07	1.26E-03	SEMA6C, GATA2, PRKCA
GO:0045652	regulation of megakaryocyte differentiation	9/40	5.14E-07	1.35E-03	SETD1A, KMT2A/B/C, TNRC6B
GO:0050808	synapse organization	18/185	1.03E-06	1.35E-03	SHANK1, L1CAM, NLGN2, BSN
GO:0034968	histone lysine methylation	12/88	2.03E-06	2.35E-03	DOT1L, KMT2A/B/C/D
GO:0016571	histone methylation	13/107	2.88E-06	2.35E-03	DOT1L, KMT2A/B/C/D
GO:0030219	megakaryocyte differentiation	9/51	4.48E-06	2.92E-03	SETD1A, KMT2A/B/C, TNRC6B
GO:0048667	cell morphogenesis involved in neuron differentiation	27/412	5.89E-06	2.98E-03	DAG1, ULK1, SEMA6C, SZT2
GO:0018022	peptidyl-lysine methylation	12/98	6.39E-06	2.98E-03	SETD1A, KMT2A/B/C, TNRC6B
GO:0048812	neuron projection morphogenesis	28/453	1.18E-05	3.01E-03	DAG1, ULK1, SEMA6C, SZT2
GO:0031175	neuron projection development	36/675	1.78E-05	3.25E-03	DAG1, SEMA6C, NCAM1, LRP1
GO:0120039	plasma membrane bounded cell projection morphogenesis	28/465	1.91E-05	3.93E-03	DAG1, SEMA6C, NCAM1, LRP1
GO:0048858	cell projection morphogenesis	28/468	2.14E-05	3.94E-03	DAG1, SEMA6C, NCAM1, NEO1
GO:0048666	neuron development	38/744	2.69E-05	4.28E-03	DAG1, SEMA6C, NCAM1, NEO1
GO:0097485	neuron projection guidance	15/171	2.86E-05	4.54E-03	DAG1, SEMA6C, NCAM1, PLXNA2
GO:0007409	axonogenesis	22/328	3.09E-05	4.54E-03	DAG1, SEMA6C, NCAM1
GO:0045814	negative regulation of gene expression, epigenetic	10/80	3.16E-05	5.28E-03	PHF1, DOT1L, HIST2H2AA3/4
GO:0061564	axon development	23/353	3.22E-05	5.43E-03	DAG1, SEMA6C, NCAM1, PLXNA2
Downregulated genes					
GO:0060087	relaxation of vascular smooth muscle	4/6	5.97E-06	0.078	GUCY1A3, PRKG1, ADORA1, RGS2
GO:0045932	negative regulation of muscle contraction	5/14	1.73E-05	0.109	GUCY1A3, PRKG1, ADORA1, PDE5A
GO:0044557	relaxation of smooth muscle	4/8	2.68E-05	0.109	GUCY1A3, PRKG1, ADORA1, RGS2
GO:0045986	negative regulation of smooth muscle contraction	4/9	4.72E-05	0.161	GUCY1A3, PRKG1, ADORA1, RGS2
GO:0006041	glucosamine metabolic process	3/4	6.44E-05	0.188	GPNDA2, GPNPAT1, PGM3

<sup>1</sup> number of identified genes in the entire GO term; <sup>2</sup> Fisher's exact test; <sup>3</sup> adjusted with Benjamini-Hochberg method

**Table S2, related to Figure 4:** Results of GO term analysis of significantly deregulated genes after circSlc45a4 KD in mouse cortex.

<b>Upregulated genes</b>					
	Term	Enrichment <sup>1</sup>	p-value <sup>2</sup>	p-adj <sup>3</sup>	Example genes
GO:0009653	anatomical structure morphogenesis	305/1578	5.00E-19	8.61E-14	Unc5b, Notch2, Pag1, Gnaq, Rbpj
GO:0023052	signaling	513/3060	5.11E-19	8.61E-14	Unc5b, Notch2, Pag1, Gnaq, Rbpj
GO:0007165	signal transduction	466/2761	1.90E-17	3.91E-13	Unc5b, Notch2, Gas7, Foxp2, Fat3
GO:0007154	cell communication	511/3097	2.10E-17	7.12E-13	Unc5b, Notch2, Ldha, Pag1, Plcb1
GO:0023051	regulation of signaling	335/1880	2.41E-15	6.67E-12	Unc5b, Notch2, Plcb1, Gnaq, Igfbp5
GO:0010646	regulation of cell communication	331/1862	5.43E-15	1.38E-11	Unc5b, Notch2, Plcb1, Nell2, Timp2
GO:0048856	anatomical structure development	517/3236	1.01E-14	1.12E-10	Unc5b, Notch2, Gas7, Foxp2, Fat3
GO:0009966	regulation of signal transduction	299/1652	1.68E-14	1.12E-10	Unc5b, Notch2, Plcb1, Sfrp2, Rbpj
GO:0007155	cell adhesion	143/635	3.53E-14	1.99E-10	Fat3, Pag1, Plcb1, Ccdc80, Cyfip2, Vcam1
GO:0035295	tube development	140/617	3.59E-14	1.99E-10	Unc5b, Notch2, Foxp2, Sfrp2, Ptges3, Gna13
GO:0022610	biological adhesion	143/637	4.61E-14	1.12E-09	Fat3, Pag1, Plcb1, Ccdc80, Cyfip2, Vcam1
GO:0032502	developmental process	546/3486	5.57E-14	1.50E-09	Unc5b, Notch2, Gas7, Foxp2, Fat3, Plcb1
GO:0051240	positive regulation of multicellular organismal process	190/937	8.67E-14	1.75E-09	Notch2, Foxp2, Plcb1, Sfrp2, Timp2, Rbpj
GO:0051094	positive regulation of developmental process	174/838	1.27E-13	8.04E-09	Notch2, Foxp2, Plcb1, Sfrp2, Timp2, Rbpj
GO:0035239	tube morphogenesis	117/491	1.60E-13	9.53E-09	Unc5b, Notch2, Sfrp2, Hs2st1, Gna13
GO:0048731	system development	433/2659	3.06E-13	9.53E-09	Unc5b, Notch2, Gas7, Foxp2, Fat3, Plcb1
GO:0032879	regulation of localization	293/1654	5.56E-13	1.07E-08	Plcb1, Gnaq, Igfbp5, Sfrp2, Nell2, Gna13
GO:0051239	regulation of multicellular organismal process	289/1626	5.58E-13	1.67E-08	Notch2, Foxp2, Fat3, Plcb1, Igfbp5, Sfrp2
GO:0007166	cell surface receptor signaling pathway	246/1332	7.36E-13	1.89E-08	Unc5b, Notch2, Usp46, Pag1, Plcb1, Gnaq
<b>Downregulated genes</b>					
GO:0008088	axo-dendritic transport	16/55	9.06E-06	0.094	Tmem108, Nef1, Spg7, Snapin, Ap3m2, Trak1/2
GO:0006221	pyrimidine nucleotide biosynthetic process	9/20	1.69E-05	0.094	Dut, Umps, Cad, Tyms, Dctd, Nme6, Tbp1l
GO:1901607	alpha-amino acid biosynthetic process	14/46	1.85E-05	0.094	Park7, Shmt2, Asns, Atp2b4, Cad, Adi1, Asl
GO:0006220	pyrimidine nucleotide metabolic process	10/25	2.02E-05	0.094	Dut, Umps, Cad, Tyms, Nt5c, Dctd, Nme6, Tbp1l
GO:0008652	cellular amino acid biosynthetic process	14/47	2.43E-05	0.094	Park7, Shmt2, Asns, Atp2b4, Cad, Adi1, Asl
GO:0044281	small molecule metabolic process	131/1105	5.19E-05	0.157	Eno1b, Pgm211, Pfkfb, Abcg2, Wdct1, Atp5o
GO:0072527	pyrimidine-containing compound metabolic process	12/39	6.49E-05	0.157	Thtpa, Dut, Umps, Cad, Tyms, Nt5c, Dctd, Nme6
GO:0019752	carboxylic acid metabolic process	72/534	6.53E-05	0.157	Dut, Tyms, Dctd, Dnph1, Shmt1



GO:0072528	pyrimidine-containing compound biosynthetic process	9/24	9.62E-05	0.201	Dut, Umps, Tyms, Dctd, Shmt1
GO:0006082	organic acid metabolic process	74/561	1.06E-04	0.201	Eno1b, Pfkfb, Abcg2, Wdtd1, Cbfa2t3
GO:0006520	cellular amino acid metabolic process	32/187	1.15E-04	0.201	Park7, Arg2, Shmt2, Sars2, Asns, Gclc, Azin1
GO:0043436	oxoacid metabolic process	73/557	1.44E-04	0.201	Eno1b, Pfkfb, Abcg2, Wdtd1, Cbfa2t3, Park7
GO:0009162	deoxyribonucleoside monophosphate metabolic process	5/8	2.06E-04	0.231	Dut, Tyms, Dctd, Dnph1, Shmt1
GO:0009130	pyrimidine nucleoside monophosphate biosynthetic process	5/8	2.06E-04	0.253	Dut, Umps, Tyms, Dctd, Shmt1
GO:0022616	DNA strand elongation	8/21	2.09E-04	0.253	Parp1/2, Dna2, Rad50, Rnaseh2a, Nbn, PcnA
GO:0009219	pyrimidine deoxyribonucleotide metabolic process	6/12	2.29E-04	0.253	Dut, Tyms, Nt5c, Dctd, Tbp11, Shmt1
GO:0009157	deoxyribonucleoside monophosphate biosynthetic process	4/5	2.50E-04	0.253	Dut, Tyms, Dctd, Shmt1
GO:0009177	pyrimidine deoxyribonucleoside monophosphate biosynthetic process	4/5	2.50E-04	0.253	Dut, Tyms, Dctd, Shmt1
GO:0036265	RNA (guanine-N7)-methylation	4/5	2.50E-04	0.253	Trmt112, Mett11, Bud23, Wdr4

<sup>1</sup> number of identified genes in the entire GO term; <sup>2</sup> Fisher's exact test, <sup>3</sup> adjusted with Benjamini-Hochberg method