

Fig. S1. Expression of neuronal and hippocampal identity markers in hiPSC-derived neural cultures.

(A) WiBiTi and CHIR cells were immunostained at DIV60 for neurocytoskeletal and pallial-identity markers.

(B) Quantification of immunofluorescence for neuronal markers (β III-TUBULIN, N-Acetylated TUBULIN, MAP2, DCX, NEUN) and pallial markers (PAX6, FOXG1) as in (A). (C-E). CHIR cells at DIV28 were seeded on a carpet of mESC-derived hippocampal cells (Terrigno et al., 2018), as such (C) or after transduction with membrane-GFP (m-GFP) lentiviral vector and treatment with DAPT 24 hours prior to reseeding (D). After 40 days in culture, cells were fixed and immunostained for the markers indicated in labels. (E) Quantification of immunofluorescence for the markers shown in (C,D). Statistical analysis in (B) was performed by One-Way ANOVA followed by post-hoc Kruskal-Wallis test whereas statistical analysis in (E) was performed using an unpaired t-test assuming unequal variances; asterisks above plotted values indicate comparison against control whereas above solid black bars indicate between groups. In the box and whisker plots, the horizontal line indicates the median, the upper and lower edges of the boxes represent the upper and the lower quartile and the whiskers represent the 99% confidence interval. *-p-val<0.05, **-p-val<0.01.

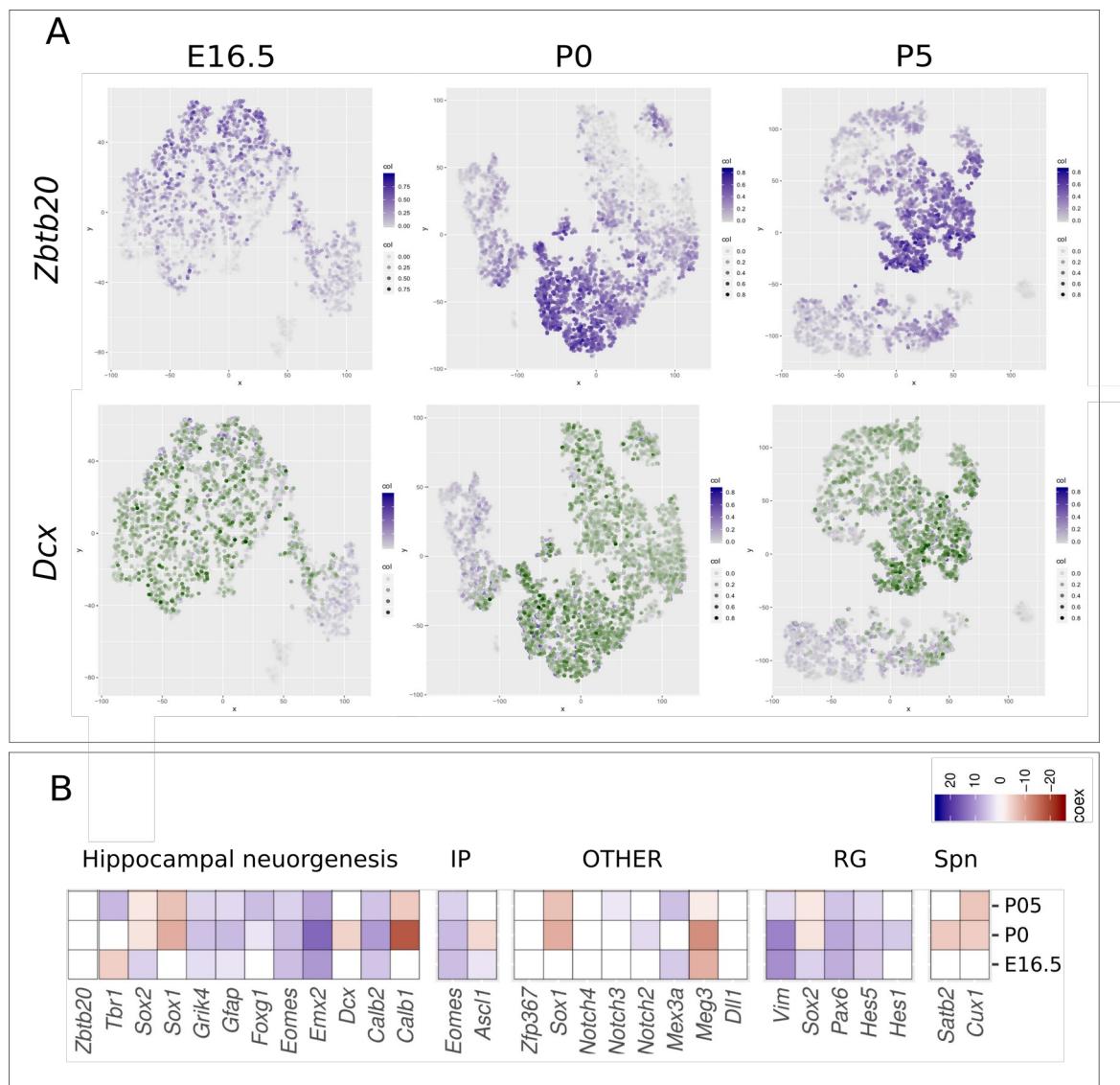
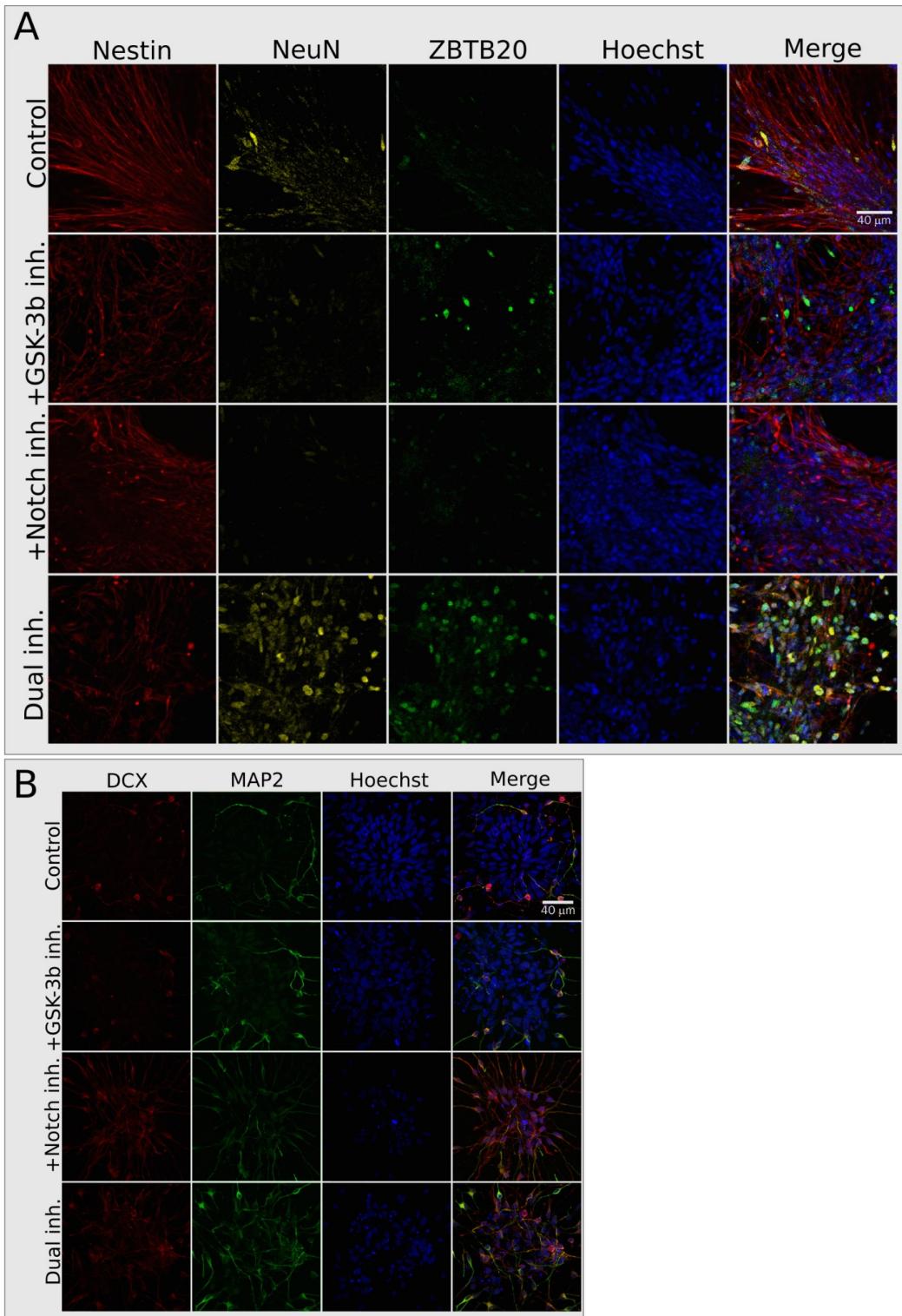


Fig. S2. Analysis of co-expression of ZBTB20 and selected markers of hippocampal and cortical neurogenesis.

(A,B) scRNA-seq analysis of *in vivo* mouse hippocampal cells over distinct developmental time points, mined from (Hochgerner et al., 2018). (A) single cell transcripts of *Zbtb20* and *Dcx* from mouse hippocampus at embryonic day 16.5 (E16.5), post-natal day 0 (P0) and 5 (P5) were analyzed. Cell-type clustering of *Zbtb20*⁺ and *Dcx*⁺ populations overlap and in a gradient along the differentiation axis from neural stem cell population to neuronal populations, though not all *Dcx*⁺ cells co-express *Zbtb20* at early stages. Scale bars indicate relative normalized expression. (B) Values of gene co-expression (coex) with *Zbtb20* as calculated by COTAN (Galfr et al., 2021). Positive and negative coex values indicate the probability of gene co-expression and anti-co-expression, respectively. The analysis revealed *Zbtb20* co-expression or anti-coexpression with established markers of hippocampal neurogenesis, intermediate progenitors (IP) radial glia cells (RG), cortical superficial projecting neurons (Spn) or other markers of neural progenitors (Other). Meg3, maternally expressed gene 3 (McLaughlin et al., 2006).

**Fig. S3. Differentiation of hippocampal neural progenitors by NOTCH inhibition.**

(A) Confocal images and staining of experiments quantified in Fig. 2D. Nestin staining showed no quantitative differences between conditions. (B) Confocal images of experiments as quantified in Fig. 2D-E, depicting DCX-positive and MAP2-positive positive cells.

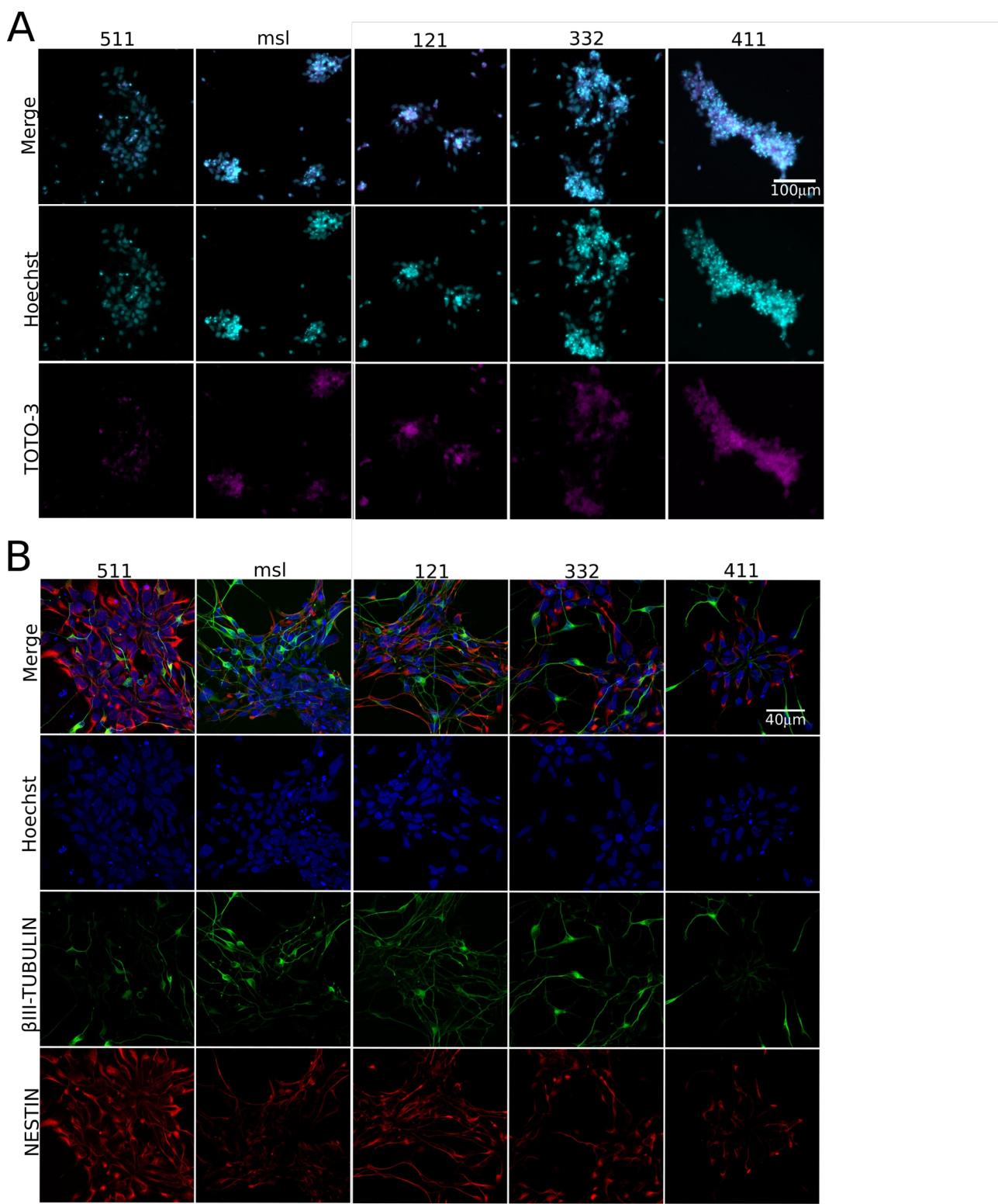


Fig. S4. Laminin isoform 511 promotes membrane health and prevents NSC differentiation *in vitro*.(A) Imaging of cells of the experiments quantified in Fig. 3C, depicting membrane blebbing by TOTO-3 imaging and Hoechst⁺ nuclear staining. (B) Confocal images of experiments quantified in Fig. 3D,E depicting NESTIN and βIII-TUBULIN immunodetection.

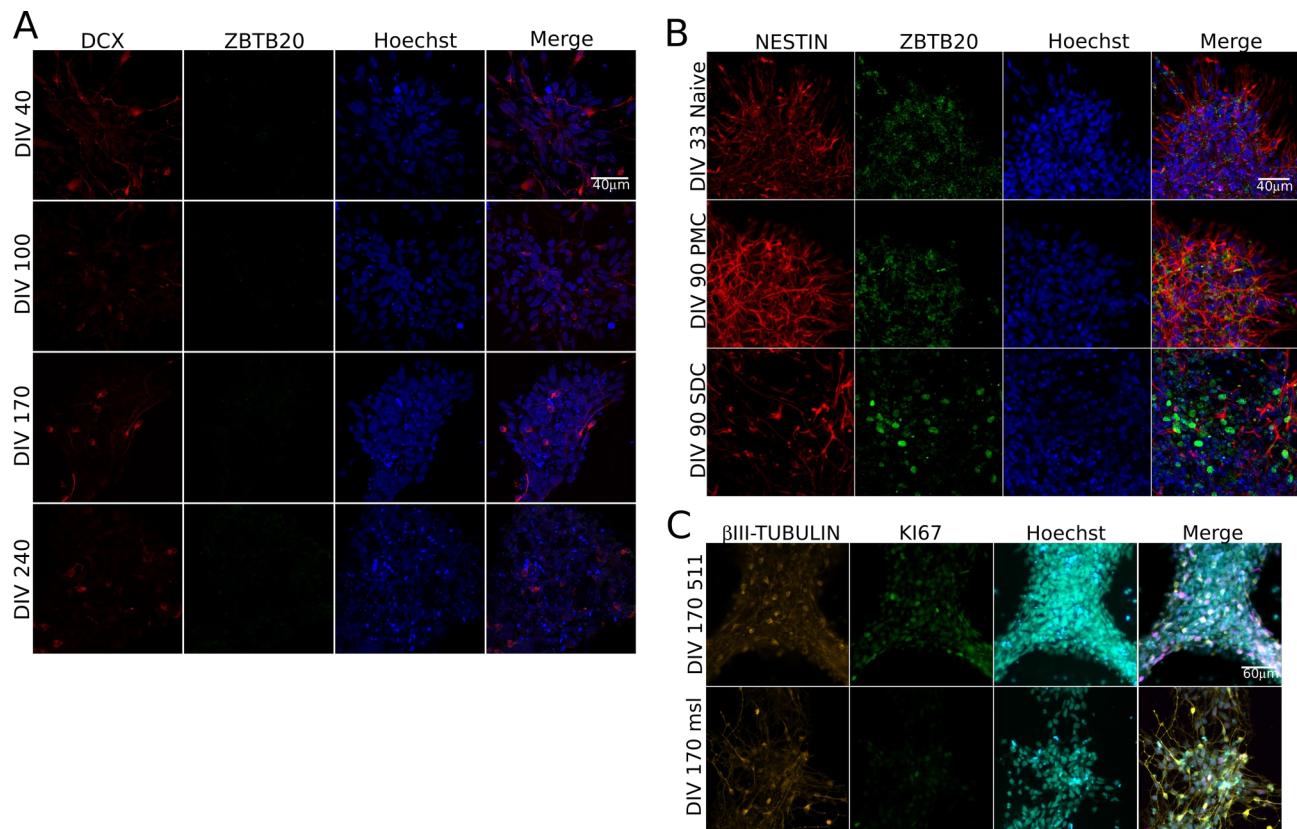


Fig. S5. Hippocampal NSC proliferation and differentiation are affected by laminin isoform.

(A) Imaging of the longitudinal experiments quantified in Fig. 3I-J, depicting DCX-positive and ZBTB20-positive cells. (B,C) Imaging of the experiments quantified in Fig. 4B,C showing NESTIN-positive and ZBTB20-positive cells (B), Ki67-positive and βIII-TUBULIN-positive cells (C).

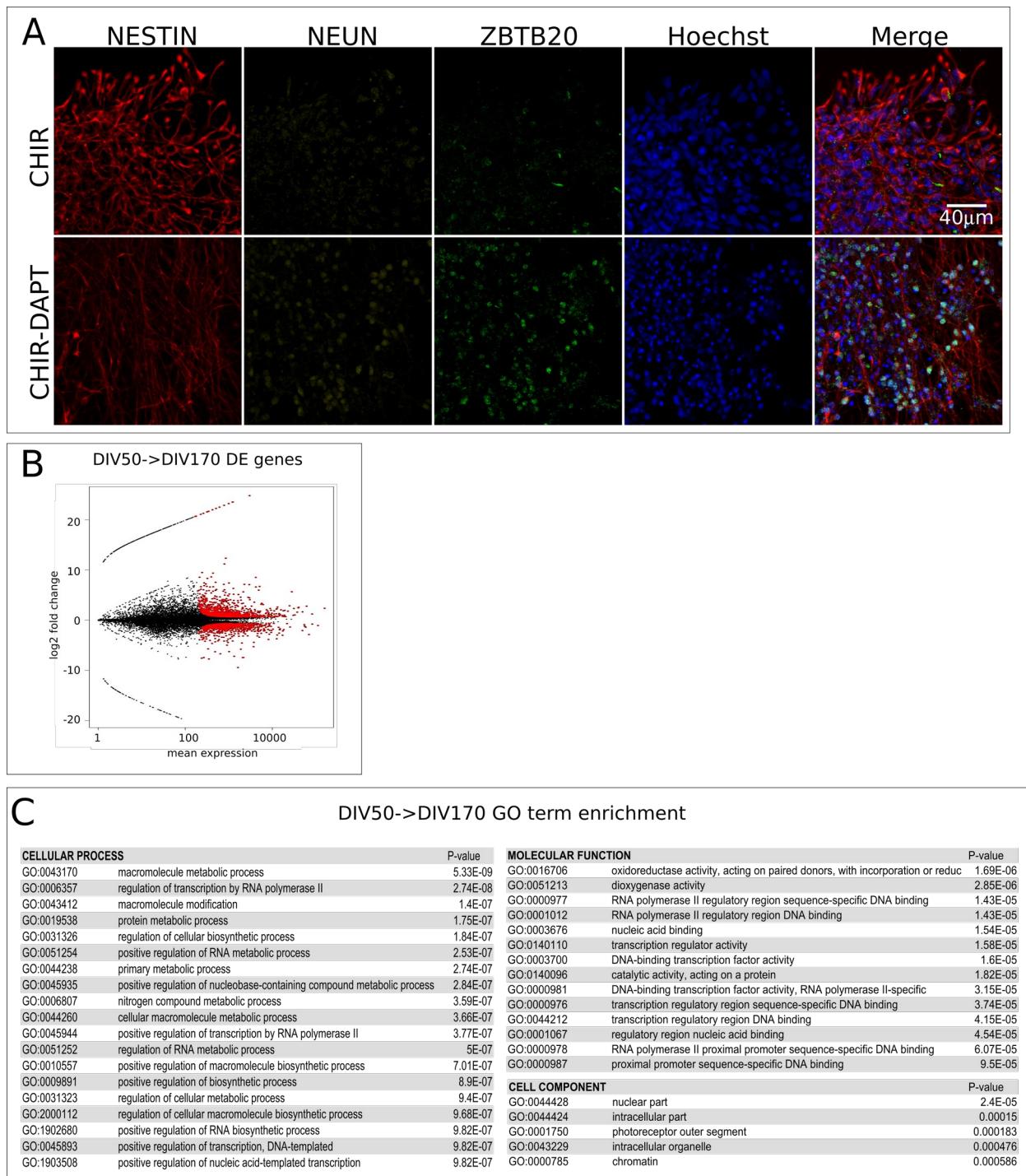


Fig. S6. Gene expression comparison between early and late CHIR cells. (A) Imaging of NESTIN, DCX and ZBTB20 in DIV170 cells. (B) Plot of RNA-seq analysis of DIV50 versus DIV170 cells ($n=3$ biological replicates) upon continuous CHIR treatment; red dots show DE genes as evaluated by NOIseq analysis ($q>0.8$). (C) GO terms most significantly enriched in the DE genes shown in (B).

Table S1. GO analysis. The table shows the most significantly enriched PROCESS GO terms in the genes differentially expressed between hiPSCs and DIV28 cells (reported in Fig. 1 C).

GO Term	Description	FDR q-value
GO:0048856	anatomical structure development	4.82E-20
GO:0032502	developmental process	2.15E-17
GO:0048731	system development	1.08E-13
GO:0045165	cell fate commitment	5.82E-12
GO:0048869	cellular developmental process	2.52E-11
GO:0007389	pattern specification process	9.24E-11
GO:0006357	regulation of transcription by RNA polymerase II	3.87E-10
GO:0003002	regionalization	0.00000000477
GO:0051960	regulation of nervous system development	0.00000000813
GO:0007399	nervous system development	0.00000000835
GO:0030154	cell differentiation	0.0000000112
GO:0030182	neuron differentiation	0.0000000139
GO:0050767	regulation of neurogenesis	0.0000000442
GO:0045664	regulation of neuron differentiation	0.0000000883
GO:0060284	regulation of cell development	0.0000000984
GO:0045944	positive regulation of transcription by RNA polymerase II	0.000000256
GO:0051252	regulation of RNA metabolic process	0.000000282
GO:0010975	regulation of neuron projection development	0.000000301
GO:0006355	regulation of transcription, DNA-templated	0.000000339
GO:1903506	regulation of nucleic acid-templated transcription	0.000000339
GO:0048665	neuron fate specification	0.000000348
GO:2001141	regulation of RNA biosynthetic process	0.000000343
GO:0009653	anatomical structure morphogenesis	0.000000365
GO:0097485	neuron projection guidance	0.000000733
GO:0001708	cell fate specification	0.000000867
GO:0007411	axon guidance	0.00000141
GO:0032501	multicellular organismal process	0.00000163
GO:0048468	cell development	0.00000188

Table S2. Hippocampal identity markers. The table shows the gene names of hippocampal identity markers selected from literature (Abellán et al., 2014; Cembrowski et al., 2016; Grove and Tole, 1999; Grove et al., 1998; Lee et al., 2000; Lein et al., 2004; Yu et al., 2014).

Gene id	Gene name	Description
ENSG00000138795	LEF1	lymphoid enhancer binding factor 1
ENSG00000106689	LHX2	LIM homeobox 2
ENSG00000089116	LHX5	LIM homeobox 5
ENSG00000143355	LHX9	LIM homeobox 9
ENSG00000048540	LMO3	LIM domain only 3
ENSG00000143013	LMO4	LIM domain only 4
ENSG00000117707	PROX1	prospero homeobox 1
ENSG00000075290	WNT8B	Wnt family member 8B
ENSG00000176165	FOGX1	forkhead box G1
ENSG00000170370	EMX2	empty spiracles homeobox 2
ENSG0000007372	PAX6	paired box 6
ENSG00000163064	EN1	engrailed homeobox 1
ENSG00000122877	EGR2	early growth response 2
ENSG00000165588	OTX2	orthodenticle homeobox 2
ENSG00000127152	BCL11B	BAF chromatin remodeling complex subunit BCL11B
ENSG00000119042	SATB2	SATB homeobox 2
ENSG00000257923	CUX1	cut like homeobox 1
ENSG00000153266	FEZF2	FEZ family zinc finger 2
ENSG00000068078	FGFR3	fibroblast growth factor receptor 3
ENSG00000117318	ID3	"inhibitor of DNA binding 3 HLH protein"
ENSG00000181722	ZBTB20	zinc finger and BTB domain containing 20
ENSG00000077279	DCX	doublecortin
ENSG00000136535	TBR1	"T-box brain 1"
ENSG00000163508	EOMES	eomesodermin
ENSG00000181449	SOX2	SRY-box 2
ENSG00000134595	SOX3	SRY-box 3
ENSG00000176887	SOX11	SRY-box 11
ENSG00000197921	HES5	hes family bHLH transcription factor 5
ENSG00000178403	NEUROG2	neurogenin 2
ENSG00000181965	NEUROG1	neurogenin 1
ENSG00000162992	NEUROD1	neuronal differentiation 1
ENSG00000151322	NPAS3	neuronal PAS domain protein 3
ENSG00000118260	CREB1	cAMP responsive element binding protein 1
ENSG00000185920	PTCH1	patched 1
ENSG00000118689	FOXO3	forkhead box O3
ENSG00000114861	FOXP1	forkhead box P1
ENSG00000128573	FOXP2	forkhead box P2
ENSG00000180613	GSX2	GS homeobox 2
ENSG00000115844	DLX2	distal-less homeobox 2
ENSG00000136352	NKX2-1	NK2 homeobox 1
ENSG00000016082	ISL1	ISL LIM homeobox 1
ENSG00000164330	EBF1	EBF transcription factor 1
ENSG00000148737	TCF7L2	transcription factor 7 like 2
ENSG00000175745	NR2F1	nuclear receptor subfamily 2 group F member 1
ENSG00000135638	EMX1	empty spiracles homeobox 1
ENSG00000138083	SIX3	SIX homeobox 3
ENSG00000107831	FGF8	fibroblast growth factor 8
ENSG00000134245	WNT2B	Wnt family member 2B
ENSG00000154342	WNT3A	Wnt family member 3A
ENSG00000154764	WNT7A	Wnt family member 7A
ENSG00000188064	WNT7B	Wnt family member 7B
ENSG00000157240	FZD1	frizzled class receptor 1
ENSG00000180340	FZD2	frizzled class receptor 2
ENSG00000155760	FZD7	frizzled class receptor 7
ENSG00000104290	FZD3	frizzled class receptor 3
ENSG00000163251	FZD5	frizzled class receptor 5
ENSG00000177283	FZD8	frizzled class receptor 8
ENSG00000188763	FZD9	frizzled class receptor 9
ENSG00000111432	FZD10	frizzled class receptor 10

Table S3. DE genes between laminin 511 and msl cultures evaluated by NOLseq analysis.

[Click here to download Table S3](#)

Table S4. Primary antibodies used for immunodetection.

Antibody	Host	Dilution		Company	Catalog No
		<i>culture</i>	<i>section</i>		
ZBTB20	Rat	1:200	-	BD Biosciences	565453
ZBTB20	Rabbit	1:500	-	Novus	NBP1-84146
MAP2	Chicken	1:6000	-	Novus	NB300-213
MAP2 (A-4)	Mouse	1:100	-	Santa Cruz	SC-74421
GFP	Chicken	1:1000		Aves	GFP-1020
GFP	Goat	-	1:800	Abcam	ab5450
VGluT1	Guinea Pig	-	1:500	Synaptic Systems	135304
Cherry	Rat	1:2000	-	Invitrogen	M11217
FOXG1	Rabbit	1:500	-	Abcam	ab18259
SATB2	Mouse	1:200	-	Abcam	ab51502
CTIP2	Rabbit	1:1000	-	Abcam	ab28448
Ki67	Chicken	1:1000		EnCor	CPCA-Ki-67
Nestin	Mouse	1:500	-	Santa Cruz	SC23927
DCX	Rabbit	1:6000		Abcam	ab18723
Human Nuclei	Mouse	1:500	1:500	Merck	MAB1281
Human NCAM	Mouse	1:500	1:800	Santa Cruz	SC-106
PSD-95	Rabbit	1:500	1:800	Abcam	ab18258
Synaptophysin	Guinea Pig	1:1000	-	Synaptic Systems	101-004
PAX6	Rabbit	1:1000	-	Covance	PRB278P
TUJIII	Rabbit	1:1000	-	Covance	MRB435P
NeuN	Guinea Pig	1:500	-	Millipore	ABN90

Supplementary references

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