

Supplemental information

Murine cerebral organoids develop network of functional neurons and hippocampal brain region identity

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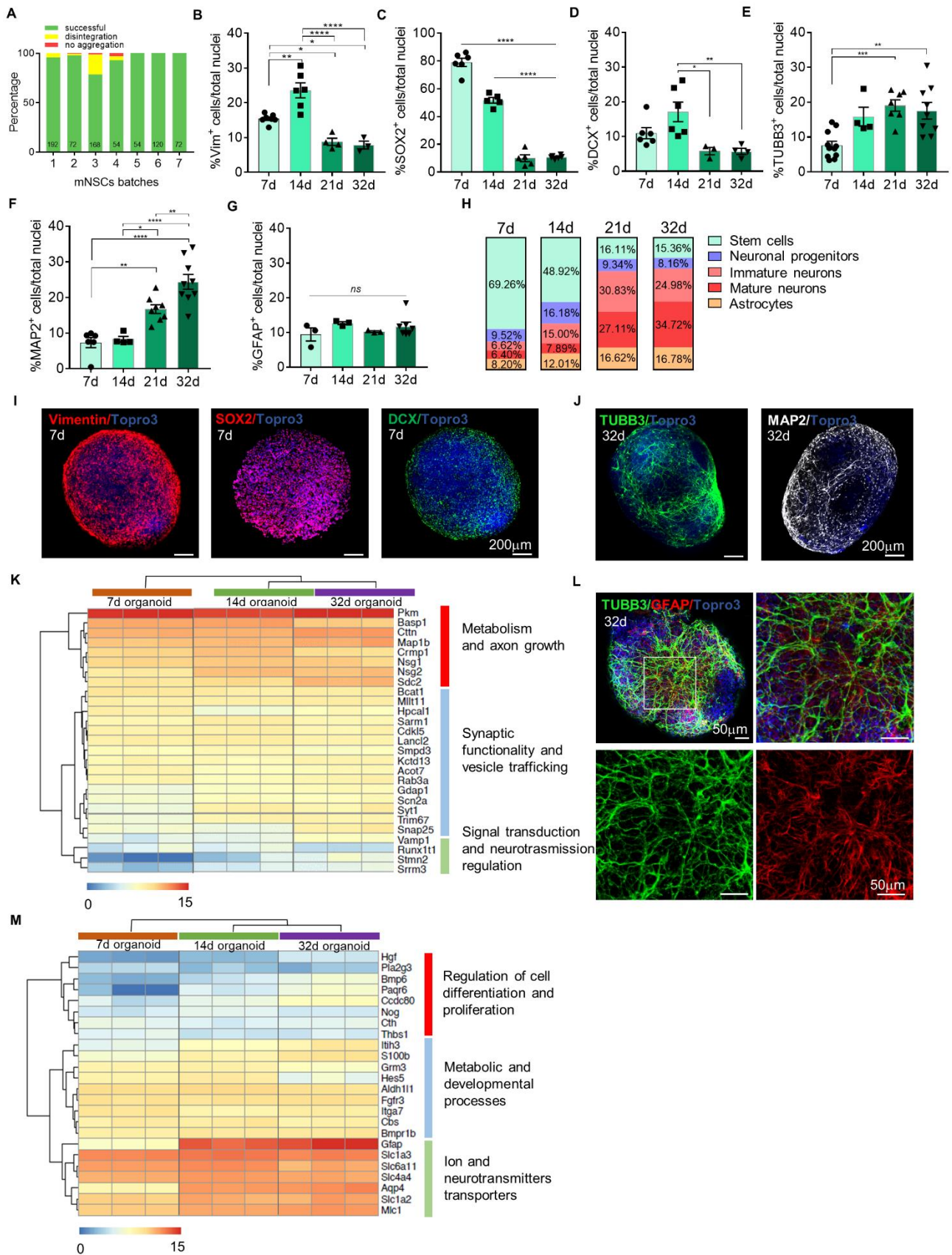


Figure S1 – PROGRESSIVE MATURATION OF MOUSE SGZ-NSCs-DERIVED ORGANOID. RELATED TO FIGURE 2. (A) Percentage of successful organoids generation, disintegration or no aggregation up to 32d *in vitro* for 7 different mNSCs batches (n=732, number inside each bar indicates the number of organoids evaluated for each mNSCs batch). **(B-G)** Graphs representing the percentage of

Vimentin (B), SOX2 (C), DCX (D), TUBB3 (E), MAP2 (F) and GFAP (G) positive cells over total cell nuclei at different time points. Data in all graphs are expressed as mean \pm SEM. Analysis was performed on $n \geq 3$ different organoids and at least on 3 entire sections for each organoid. $p < 0.05$ was considered statistically significant. Statistical differences between marker expression at different time point were calculated by ordinary one-way ANOVA followed by Tukey's multiple comparison test. **** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$; *ns* not statistically significant. **(H)** Part-to-whole graphs showing the percentage of cells belonging to different cell populations over the total cells populations' number. *Stem cells* refers to SOX2 positive cells, *Neuronal progenitors* refers to DCX positive cells, *Immature neurons* refers to TUBB3 positive cells, *Mature neurons* refers to MAP2 positive cells and *Astrocytes* identify GFAP positive cells. **(I)** Representative confocal immunofluorescence Z stack-projection image of whole mount early (7d) NSCs-derived organoid showing the presence and widespread distribution of Vimentin, SOX2, and DCX positive cells. Total nuclei are visible in blue (TOPRO3). **(J)** Representative confocal immunofluorescence Z stack-projection image of whole mount mature (32d) NSCs-derived organoid showing the distribution of TUBB3 and MAP2⁺ positive cells. **(K)** Heat map of RNAseq data from organoid samples at different stages of differentiation (7 days: n=3; 14 days: n=3; 32 days: n=3) generated based on mature neurons gene list dataset. **(L)** Representative confocal immunofluorescence Z stack-projection image of whole mount mature (32d) NSCs-derived organoid showing the presence of neuronal cells (TUBB3, in green) connected with astrocytes (GFAP, in red). Total nuclei are visible in blue (TOPRO3). The white box highlights a magnified zone reported also in the green and red split channels. **(M)** Heat map of RNAseq data from organoid samples at different stages of differentiation (7 days: n=3; 14 days: n=3; 32 days: n=3) generated based on astrocytes gene list dataset.

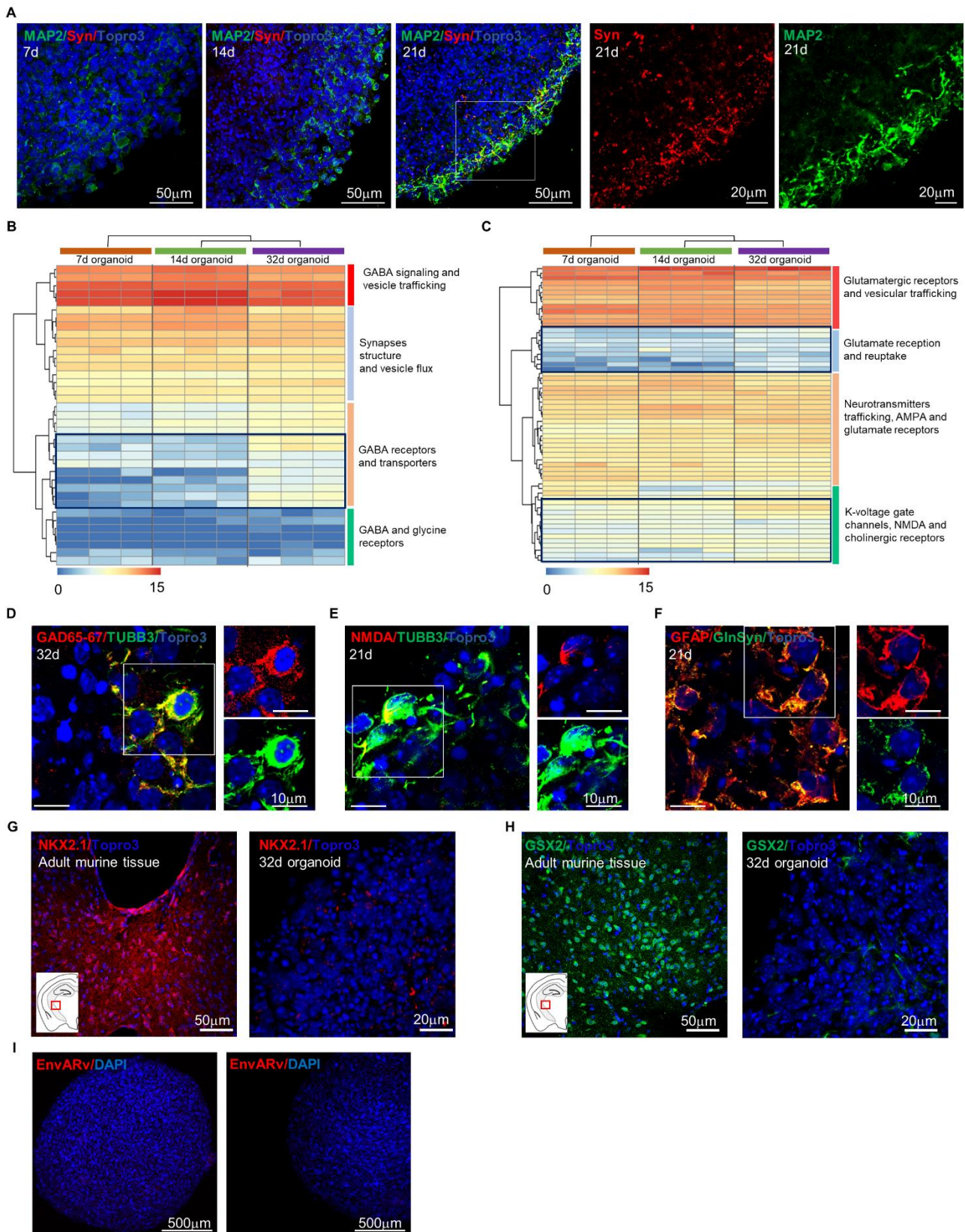


Figure S2 – SYNAPSE DEVELOPMENT WITHIN MATURE MURINE BRAIN ORGANIDS. RELATED TO FIGURES 4 AND 6. (A) Representative maximum Z-projection of confocal images of sliced organoids immunostaining performed at 7d, 14d and 21d for the Synaptophysin (red) and the MAP2 (green) markers, showing colocalization between neuronal cells and the synaptic marker starting from 21d. No evident expression of Syn was found at 7d and 14d. White box highlight the colocalization

between Syn and MAP2 at 21d, also reported in enlarged images. **(B-C)** Heat maps of RNAseq data from organoid samples at different stages of differentiation (7 days: n=3; 14 days: n=3; 32 days: n=3) generated based on an inhibitory (B) and excitatory (C) synapses gene lists. Blue boxes highlight the most upregulated genes in mature organoids (32d) respect to early organoid (7d and 14d). Specifically, the most up regulated inhibitory synapses genes in 32d organoids respect to 7d and 14d organoids are related to GABA receptors and transporters (i.e. *Gabra4*, *Gabrb1*, *Camk4*, *Gabrg3*, *Gabra1*, *Slc32a1*, *Gabra5*, *Gabrg2*, *Gad2*). The most up regulated excitatory synapses genes in 32d organoids respect to 7d and 14d organoids are related to glutamate reception and reuptake (i.e., *Grin2b*, *Grin1*, *Grm4*, *Grin2a*, *Grik1*) and K-voltage gate channels, NMDA and cholinergic receptors (i.e., *Kcnc4*, *Chrm3*, *Kcna3*, *Chrm1*, *Chrm4*). **(D)** Representative confocal images of sliced organoid immunostaining performed at 32 days for the GAD65-67 and TUBB3 markers. The white box highlights the double positive cells reported also in the red and green split channels. **(E)** Representative confocal images of sliced organoid immunostaining performed at 21 days for the NMDA and TUBB3 markers. The white box highlights the double positive cells reported also in the red and green split channels. **(F)** Representative confocal images of sliced organoid immunostaining performed at 21 days for the GFAP and Glutamine Synthetase (Gln Syn) markers. The white box highlights the double positive cells reported also in the red and green split channels. **(G-H)** Representative confocal immunofluorescence maximum intensity Z stack projections images of sliced adult (8-weeks) murine brain tissue and mature (32d) murine brain organoids showing the expression of NKX2.1 (G) and GSX2 (H) ganglionic eminences markers. White insets represent a sketch of coronal mouse brain sections while red boxes highlight the brain region of the murine tissue staining. **(I)** Representative maximum Z-projection of confocal images of organoids transfected only with the EnvARv (rabies-virus) showing no expression of red spots.

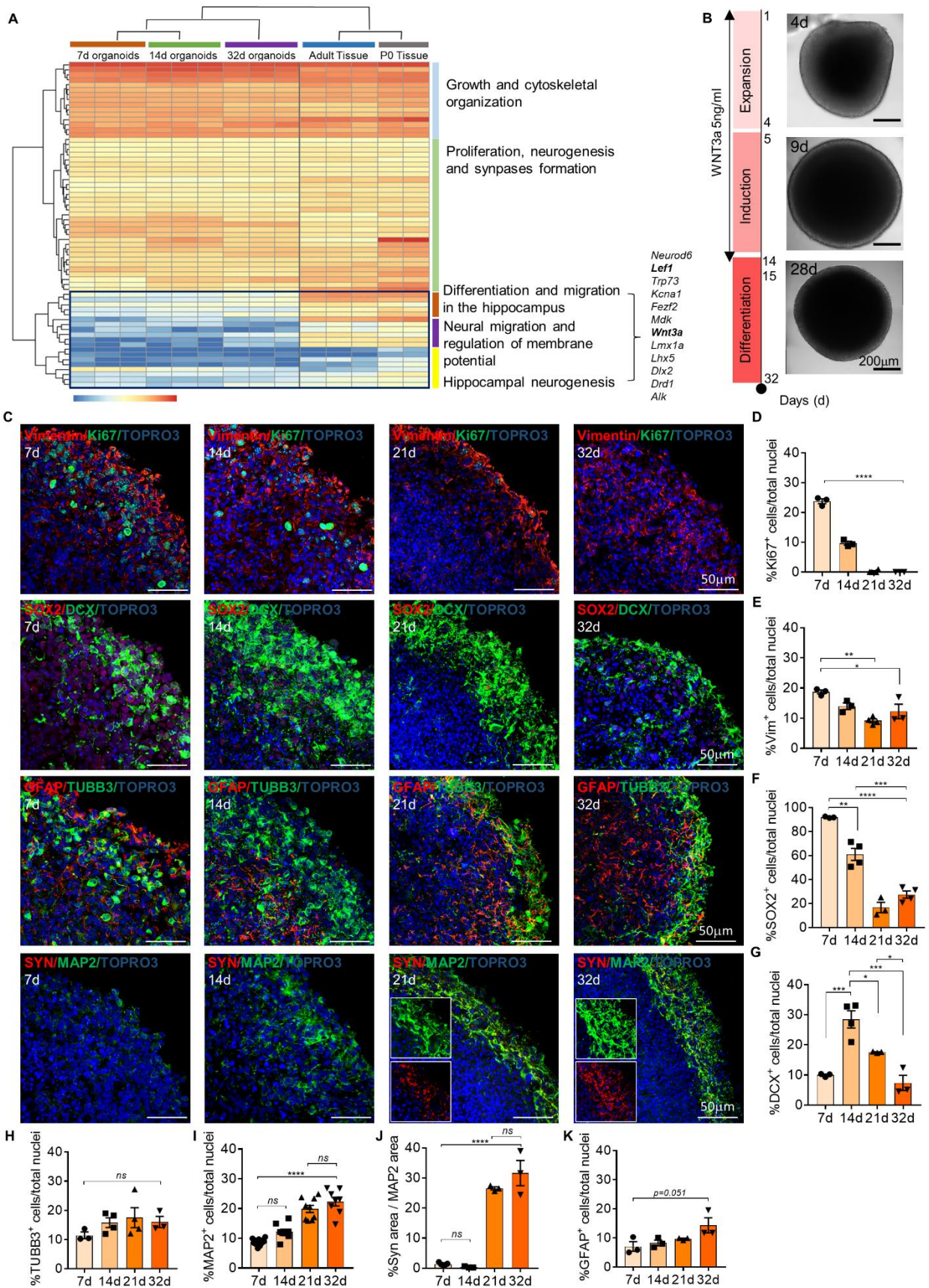


Figure S3 – ORGANIZATION AND SPATIAL DISTRIBUTION OF CELLS IN MURINE BRAIN ORGANOIDS AFTER WNT3A SUPPLEMENTATION. RELATED TO FIGURE 7. (A) Heat map of RNAseq data generated on selected hippocampal gene dataset. By evaluating the genes expression profile on murine P0 and adult cerebral tissues and on organoids at different developmental stages, the analysis

reveals no expression of some crucial genes required for the hippocampal genesis (*Lef1*, *Wnt3a*) into the 3D structures at all the evaluated time points. Blue box highlights the most down regulated hippocampal related genes (*Neurod6*, *Lef1*, *Trp73*, *Kcna1*, *Fezf2*, *Mdk*, *Wnt3a*, *Lmx1a*, *Lhx5*, *Dlx2*, *Drd1*, *Alk*) in organoids respect to the tissue. **(B)** Schematic rendition of the three-phases organoids generation protocol showing the WNT3a 5ng/ml administration period (expansion and induction phases). In all the three phases, organoids were maintained on an orbital shaker (dynamic culture). Pictures in **(B)** are representative brightfield images of WNT3a-treated organoids at different stage of the protocol. **(C)** Representative confocal immunofluorescence images of sliced NSCs-derived Wnt3a-treated organoids at 7d, 14d, 21d and 32d showing cells positive for Ki67, Vimentin, Sox2, DCX, TUBB3, GFAP, MAP2 and Synaptophysin. **(D-K)** Graphs showing the percentage of Ki67 (D), Vimentin (E), SOX2 (F), DCX (G), TUBB3 (H), MAP2 (I) positive cells over total cell nuclei, the synaptic density (% Syn⁺ area in MAP2⁺ cells area) (J) of Wnt3a-treated organoids and GFAP (K) positive cells at different time points. Analysis was performed on n≥3 different organoids and at least on 3 entire sections for each organoid. Data in all graphs are expressed as mean ± SEM. Statistical differences between marker expression at different time point were calculated by ordinary one-way ANOVA followed by Tukey's multiple comparison test. ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05; ns not statistically significant.

Table S1

Neural Stem Cells			Neuronal Progenitors	Mature Neurons
<i>Abcg2</i>	<i>Acot1</i>	<i>Slc4a1</i>	<i>Tbr2/Eomes</i>	<i>Neun</i>
<i>Bmi-1</i>	<i>Lxn</i>	<i>Gm12222</i>	<i>Mash1/Ascl1</i>	<i>Snap25</i>
<i>Chd2</i>	<i>Mut</i>	<i>Asrgl1</i>	<i>Neurog2</i>	<i>Syt1</i>
<i>Cxcr4</i>	<i>Tpbg</i>	<i>Etv4</i>	<i>Neurog1</i>	<i>Scn2a</i>
<i>Ctnnb1</i>	<i>Decr1</i>	<i>Abcd3</i>	<i>Tacc2</i>	<i>Vamp1</i>
<i>Fabp7</i>	<i>Cldn12</i>	<i>Slc15a2</i>	<i>Blbp</i>	<i>Cdkl5</i>
<i>Fzd-1</i>	<i>Slc9a3r1</i>	<i>Mfge8</i>	<i>Grm5</i>	<i>Rab3a</i>
<i>Fzd-2</i>	<i>Slitrk2</i>	<i>Acadl</i>	<i>Ly6h</i>	<i>Basp1</i>
<i>Hes5</i>	<i>Cpne2</i>	<i>Gas1</i>	<i>Lrp8</i>	<i>Runx1t1</i>
<i>Msi 1</i>	<i>Polr3h</i>	<i>Grm3</i>	<i>Prox1</i>	<i>Hpcal1</i>
<i>Msi 2</i>	<i>Luzp2</i>	<i>Lrrc16a</i>	<i>Nes</i>	<i>Pkm</i>
<i>Nes</i>	<i>Paqr7</i>	<i>Cnih2</i>	<i>Vim</i>	<i>Acot7</i>
<i>Notch1</i>	<i>Bcl2</i>	<i>Ctnnd2</i>	<i>Neurod1</i>	<i>Trim67</i>
<i>Nr2f1</i>	<i>Prom1</i>	<i>Trim9</i>	<i>Sox11</i>	<i>Bcat1</i>
<i>Neurod4</i>	<i>Ccdc80</i>	<i>Traf3</i>	<i>Foxg1</i>	<i>Sdc2</i>
<i>Pax6</i>	<i>Sardh</i>	<i>Adgrb1</i>	<i>Dcx</i>	<i>Sarm1</i>
<i>Pax3</i>	<i>Cdc42ep1</i>	<i>Sorbs1</i>	<i>Tbr1</i>	<i>Lancl2</i>
<i>Prom1</i>	<i>Tspan12</i>	<i>Ezr</i>	<i>Aplp1</i>	<i>Gdap1</i>
<i>Rest</i>	<i>Lfng</i>	<i>Fabp7</i>	<i>Stathmin 1</i>	<i>Smpd3</i>
<i>Slc1a3</i>	<i>Fam181b</i>	<i>Ptprz1</i>	<i>Ncam</i>	<i>Crmp1</i>
<i>Smad1/2</i>	<i>Sall3</i>	<i>Trib2</i>	<i>Tubb4a</i>	<i>Stmn2</i>
<i>Sox2</i>	<i>Nr2e1</i>	<i>Kcnj10</i>	<i>Tubb3</i>	<i>Srrm3</i>
<i>Sox11</i>	<i>Rlbp1</i>	<i>Ttyh1</i>	<i>Map6</i>	<i>Mllt11</i>
<i>Vim</i>	<i>Fjx1</i>	<i>Rgma</i>	<i>Dbn1</i>	<i>Ctnn</i>
<i>Sox8</i>	<i>Gramd4</i>	<i>Cspg5</i>	<i>Tagln3</i>	<i>Kctd13</i>
<i>Sox9</i>	<i>Car2</i>	<i>Slc1a2</i>		<i>Map1b</i>
<i>Tst</i>	<i>Mt3</i>	<i>Atp1a2</i>		<i>Nsg2</i>
<i>Creb5</i>	<i>Tmem98</i>	<i>Plpp3</i>		
<i>Cbr3</i>	<i>Eepd1</i>	<i>Fbln2</i>		
<i>Bcar3</i>	<i>Hes5</i>	<i>Ednrb</i>		
<i>Rgcc</i>	<i>Abcb9</i>	<i>Lrig1</i>		
<i>Palld</i>	<i>Thbs4</i>	<i>Bcan</i>		
	<i>Trim47</i>	<i>Ddah1</i>		
		<i>Aldh1l1</i>		
		<i>Nsg1</i>		

Table S1 – LIST OF GENES RELATED TO NEURAL STEM CELLS, NEURONAL PROGENITORS AND MATURE NEURONS PHENOTYPE USED FOR GSVA. RELATED TO FIGURE 1.

Table S2

Gene	padj	log2FoldChange
<i>Ctnn</i>	5,49E-05	0,604238035
<i>Nsg2</i>	7,35E-49	1,727314216
<i>Syt1</i>	7,61E-13	2,227795404
<i>Snap25</i>	3,64E-16	2,029859858
<i>Srrm3</i>	4,64E-09	2,730721037
<i>Stmn2</i>	3,77E-09	5,992616238
<i>Trim67</i>	3,04E-24	1,812663846
<i>Vamp1</i>	0,000220385	1,924336274
<i>Map1b</i>	2,15E-07	0,794654091
<i>Nsg1</i>	0,297850458	0,209721151
<i>Sdc2</i>	4,11E-52	1,808401762
<i>Mllt11</i>	0,195099757	0,202658043
<i>Rab3a</i>	0,638534197	0,112657704
<i>Gdap1</i>	1,42E-09	1,1904085
<i>Scn2a</i>	0,000377824	0,873899441

Table S2 – SUMMARY OF GENES RELATED TO NEURONAL DIFFERENTIATION. RELATED TO FIGURE 2. The table reports the up-regulated genes related to neuronal differentiation obtained by RNAseq analysis. Padj: adjusted p-values for multiple comparisons using Benjamini-Hochberg method; log2FoldChange: transcript expression fold-changes estimated for each expressed transcript comparing early and mature organoids.

Table S3

Gene	padj	log2FoldChange
<i>Slc1a2</i>	4,94E-05	0,658058139
<i>Aqp4</i>	1,71E-159	4,837930849
<i>Gfap</i>	3,21E-90	8,579001665
<i>Slc1a3</i>	0,423719767	0,105205933
<i>Slc4a4</i>	0,076303712	0,23595097
<i>Bmp6</i>	2,44E-25	5,425047152
<i>Paqr6</i>	0,000495747	6,123786905
<i>Ccdc80</i>	1,94E-26	3,708314486
<i>Itih3</i>	1,13E-31	4,280860113
<i>S100b</i>	6,30E-93	3,332617766
<i>Fgfr3</i>	0,37888097	0,095574269
<i>Bmpr1b</i>	1,94E-07	0,723439631
<i>Mlc1</i>	8,69E-49	1,804347648

Table S3 – SUMMARY OF GENES RELATED TO ASTROCYTES MATURATION. RELATED TO FIGURE 2.

The table reports the up-regulated genes related to astrocytes specification and maturation obtained by RNAseq analysis. Padj: adjusted p-values for multiple comparisons using Benjamini-Hochberg method; log2FoldChange: transcript expression fold-changes estimated for each expressed transcript comparing early and mature organoids.

Table S4

Gene	padj	log2FoldChange
<i>Ndufa13</i>	1,96E-09	0,579612369
<i>Ndufa1</i>	9,21E-05	0,485793112
<i>Ndufa3</i>	0,389560593	0,000146566
<i>Ndufb7</i>	0,000584419	0,337754699
<i>Ndufv1</i>	0,00058828	0,340111982
<i>Cox7a1</i>	0,00070363	1,967445617
<i>Ndufa6</i>	0,001096148	0,456180369
<i>Cox7b</i>	0,007337685	0,527524247
<i>Ndufa2</i>	0,010621595	0,342780332
<i>Ndufa7</i>	0,012868154	0,316526053
<i>Sdhaf2</i>	0,02248562	0,343630353
<i>Atp5k</i>	0,024527403	0,326084878
<i>Ndufb10</i>	0,02526666	0,283391207
<i>Sdhc</i>	0,046640279	0,321295658
<i>Sdha</i>	0,081350186	0,200270153
<i>Ndufaf7</i>	0,087155821	0,264375515
<i>Pparg</i>	7,56E-08	3,680015045
<i>Acox2</i>	0,02919019	3,068473817
<i>Acox1</i>	2,81E-41	0,986916873
<i>Lbp</i>	4,65E-20	7,488102566
<i>Dbp</i>	9,33E-71	2,4342719
<i>Ech1</i>	7,99E-22	0,977763635
<i>Acad11</i>	1,39E-12	0,945714818
<i>Acad12</i>	1,33E-05	1,283203566
<i>Acadsb</i>	2,67E-10	1,421285689
<i>Slc25a20</i>	5,51E-07	0,653743564
<i>Cpt1c</i>	2,05E-14	0,772569274
<i>Echdc2</i>	4,85E-11	1,952724424
<i>Hadhb</i>	0,000356887	0,405122041

Table S4 – SUMMARY OF GENES RELATED TO MITOCHONDRIAL METABOLISM, OXPHOS AND BETA-OXIDATION. RELATED TO FIGURE 3. The table reports the up-regulated genes related to mitochondrial metabolism, OXPHOS and beta-oxidation pathways obtained by RNAseq analysis. Padj: adjusted p-values for multiple comparisons using Benjamini-Hochberg method;

log2FoldChange: transcript expression fold-changes estimated for each expressed transcript comparing early and mature organoids.

Table S5

Gene	padj	log2FoldChange
<i>Chrm1</i>	0,136379601	0,617163002
<i>Chrm4</i>	0,217145943	0,563989234
<i>Grin2b</i>	NA	0,162510622
<i>Grin1</i>	0,087245674	0,58285698
<i>Grm4</i>	0,024267454	0,881524425
<i>Grik1</i>	5,42E-06	1,652355096
<i>Gabra4</i>	3,05E-22	5,054167025
<i>Gabrb1</i>	8,47E-15	5,002966532
<i>Camk4</i>	0,37045951	0,452115506
<i>Gabra1</i>	2,56E-06	5,404443403
<i>Slc32a1</i>	2,27E-09	8,089790215
<i>Gabra5</i>	9,04E-10	3,736376851
<i>Gabrg2</i>	3,89E-21	6,425753483
<i>Gad2</i>	1,49E-12	5,63162592
<i>Kcc2</i>	5,84E-13	3,108971239

Table S5 – SUMMARY OF GENES RELATED TO EXCITATORY AND INHIBITORY SYNAPSES. RELATED TO FIGURES 4. The table reports the up-regulated genes related to excitatory and inhibitory synaptic components obtained by RNAseq analysis. Padj: adjusted p-values for multiple comparisons using Benjamini-Hochberg method; log2FoldChange: transcript expression fold-changes estimated for each expressed transcript comparing early and mature organoids.

Table S6

Gene	padj	log2FoldChange
<i>Ywhae</i>	0,005424201	3,069025528
<i>Bcan</i>	5,10E-25	10,57776201
<i>Ptpns</i>	1,88E-08	5,897610719
<i>Usp9x</i>	0,424854451	1,001908583
<i>Nr2e1</i>	0,094932196	1,935368493
<i>Uba6</i>	0,252404414	1,381840478
<i>Gli3</i>	0,062419944	2,135685348
<i>Dcx</i>	3,96E-39	13,31853782
<i>Plxna3</i>	4,07E-12	7,200959936
<i>Btg2</i>	1,86E-08	5,898859373
<i>Kdm6b</i>	0,004022945	3,164903452
<i>Hdac1</i>	6,86E-12	7,126560033
<i>Cdk5r1</i>	5,01E-109	-22,38809832
<i>Srf</i>	1,13E-12	-7,379351954
<i>Nr4a3</i>	7,98E-18	-8,857228097
<i>Prox1</i>	6,31E-16	-8,342593401
<i>Lef1</i>	0,022549229	-2,565965341
<i>Drd1</i>	0,051259239	-2,225130449
<i>Dlx2</i>	0,009572507	-2,878319862
<i>Neurod6</i>	2,64E-145	-25,86811259
<i>Alk</i>	0,718873648	-0,485848463
<i>Dlx1</i>	3,81E-08	-5,774644064
<i>Fgf13</i>	1,18E-145	-25,90031399
<i>WNT3a</i>	0,113906933	-1,84109367

<i>Cdk5r2</i>	2,14E-120	-23,5363099
<i>Trp73</i>	1,42E-07	-5,540736952
<i>Kcna1</i>	5,76E-36	-12,75493809
<i>Fezf2</i>	3,18E-45	-14,34023151
<i>Mdk</i>	1,49E-50	-15,17601342
<i>Lmx1a</i>	0,001758385	-3,417015204
<i>Lhx5</i>	0,501319578	-0,857810381

Table S6 – SUMMARY OF GENES RELATED TO HIPPOCAMPAL SPECIFICATION AND DIFFERENTIATION. RELATED TO FIGURE 7. The table reports the down-regulated and up-regulated genes related to hippocampal specification and differentiation obtained by RNAseq analysis. Padj: adjusted p-values for multiple comparisons using Benjamini-Hochberg method; log2FoldChange: transcript expression fold-changes estimated for each expressed transcript comparing mature organoids and adult cerebral tissue.

Table S7

Gene	Forward	reverse
<i>mNeurod1</i>	GAGGAACACGAGGCAGACAAG	TCTCCCCGTTTCTCAGAGA
<i>mTgfb2</i>	GCCCCTGCTGTACCTTCGT	GGTGCCATCAATACCTGCAA
<i>mGria1</i>	GGGCCAGATTGTGAAGCTAGA	GGCTGGGATCGTGTCTGTGT
<i>mNrp2</i>	GCATGGCAAAAACCACAAGA	AGCAGTGGCATGTGGAGCTT
<i>mFzd9</i>	CTCCTTGGCCTTCCTGATCA	CAGGGTGCAGCCTGTGTTT
<i>mNectin3</i>	CAATGGCCTGATGGTTTATTGG	TCTGCGTAAGCGGGATGTCT
<i>mSpock1</i>	CAGCCCGCACAAAGTATGTG	CTTCTTCTGCCTGGGCAACA
<i>mDkk3</i>	ACTGCCAGTTCTCCAGCTTCA	TCTCGGGTGCATAGCATCTG
<i>mElavl2</i>	GGCAGCATTGGCGAGATAGA	CCCAAGCTCTGCCCTGTTATT
<i>mElavl4</i>	AGTCCCCAACAGGCGATAC	GCAAATTGTCCAGCCTGAATC
<i>mKif14</i>	TGAGGCCTTTTCAGCAAAAGAG	CATATCGGGATGCTCCACAGT
<i>mProx1</i>	CTGAAGACCTACTTCTCGGACG	GATGGCTTGACGCGCATACTTC
<i>mSct</i>	GCAGCGAGCAGGACACAGA	GTGTTTCGACCACAGCAAGCA
<i>mAlk</i>	ATCTTCGGGACGGGACACA	TCATCGTCCAGGTCAGATTCC
<i>mPou3f1</i>	TTGGGCTAGAGAAGGATGTGGTT	GGAAAAGGGACTGAGTAGAGTGTGG
<i>mZbtb20</i>	CCTCATCCACTCGACACATTCAC	GAAGGTTGATGCTGTGAATGCGC
<i>mGrik4</i>	TCTCCAGGAGTCTGGCTCTTCA	CGCACAAGGATGTGGACTGTAC
<i>mSox5</i>	TACAAGGCTGGGTCCAGTGA	TGGGCCTAAGCCTGGTGTT
<i>mSox1</i>	GCCGAGTGGAAGGTCATGTC	TGTAATCCGGGTGTTCTTCA
<i>mSlc1a3</i>	ACCCCTGGGTTTTTATTGGA	GGGTAGGGTGGCAGAACTTG
<i>mDach1</i>	CCATGGCAATGAGTCAGATGA	TCGGGAACACGCTCCTTAAT
<i>mStat3</i>	GGGCACGAACACAAAAGTGA	CTCCCTAAGGGTCAGGTGCTT
<i>mCdh1</i>	TCCTGGGCAGAGTGAGATTTG	TCTGTCGCCACTTTGAATCG
<i>mKlf4</i>	CTATGCAGGCTGTGGCAAAA	GGTTTCTCGCCTGTGTGAGTTC
<i>mOct3/4</i>	CTGGGCGTTCTCTTTGGAAA	TGTTCTTAAGGCTGAGCTGCAA
<i>mNanog</i>	AAGCGGTGGCAGAAAAACC	GTGCTGAGCCCTTCTGAATCA
<i>mMusashi</i>	GTTTGAGAGCGAGGACATCGT	CTTGCAATCCACCATTTTGT
<i>mNes</i>	TGTCCCTTAGTCTGGAAGTGG	GGTGTCTGCAAGCGAGAGTT
<i>mDcx</i>	TTGGACATTTTGACGAACGA	CCCTTCTCCAGTTCATCCA
<i>mSox2</i>	GCACATGAACGGCTGGAGCAACG	TGCTGCGAGTAGGACATGCTGTAGG
<i>mVim</i>	AGGAGGCCGAGGAATGGT	CATCGTTGTTCCGGTTGG
<i>mPax6</i>	CAGCCCACCACACCTGTCT	ATGGGTGGCAAAGCACTGTAC

Table S7 – LIST OF PRIMERS USED FOR SGZ-DERIVED NSCs AND HIPPOCAMPAL CHARACTERIZATION. RELATED TO FIGURES 2A AND 7A.