

**Supplementary Table S1. Total Neurons distribution profile in HC**

Mean ± SD		F (DFn. DFd); p Value	Pairwise comparisons	
<b>Total Neu- rons/Area</b>  <b>CA1</b>	CON: 0.0051±0.00087 RSV:0.0042±0.00052 VPA: 0.0041±0.00048 RSV+VPA:0.0045±0.00047	Interaction:F (1, 12) = 4.036 p =0.0676# VPA: F (1, 12) = 1.210 p =0.2929 RSV: F (1, 12) = 0.6974 p =0.4200	CON vs RSV:	0.4040
			CON vs VPA:	0.2896
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	> 0.9999
			VPA vs RSV+VPA:	> 0.9999
<b>Total Neu- rons/Area</b>  <b>CA2</b>	CON: 0.0030±0.00013 RSV:0.0027±0.000037 VPA: 0.0026±0.00027 RSV+VPA:0.0031±0.00031	Interaction:F (1, 12) = 12.45 p =0.0042** VPA: F (1, 12) = 0.007008 p =0.9347 RSV: F (1, 12) = 1.116 p =0.3117	CON vs RSV:	0.6355
			CON vs VPA:	0.1516
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	0.1883
			VPA vs RSV+VPA:	0.0424*
<b>Total Neu- rons/Area</b>  <b>CA3</b>	CON: 0.0029±0.00058 RSV: 0.0027±0.00029 VPA: 0.0028±0.00087 RSV+VPA:0.0032±0.0034	Interaction:F (1, 12) = 1.494 p =0.2450 VPA: F (1, 12) = 0.9729 p =0.3434 RSV: F (1, 12) = 0.6828 p =0.4247	CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	0.8657
			VPA vs RSV+VPA:	> 0.9999

**HC:** hippocampus; **SD:** standard deviation. p <0.05 was considered significant. \*p<0.05. \*\*p<0.01, #trend. Statistical analyses: two-way ANOVA parametric test followed by Bonferroni. N<sub>CON</sub>: 4. N<sub>RSV</sub>: 4. N<sub>VPA</sub>: 4. N<sub>RSV+VPA</sub>: 4.

**Supplementary Table S2. Distribution profile of PV neurons in the HC**

Mean ± SD		F (DFn, DFd); p Value	Pairwise comparisons	
<b>PV /Area</b>	CON: 0.00022±0.000023 RSV:0.00021±0.000030 VPA:0.00023±0.000040 RSV+VPA:0.00020±0.000030	Interaction: F (1, 12) = 0.322 p =0.580 VPA: F (1, 12) = 0.03768 p =0.8493 RSV: F (1, 12) = 1.685 p =0.2186	CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	> 0.9999
<b>CA1</b>			VPA vs RSV+VPA:	> 0.9999
			CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	0.6772
<b>PV Ratio</b>	CON: 0.054±0.009 RSV:0.047±0.008 VPA: 0.057±0.005 RSV+VPA:0.050±0.011	Interaction:F (1, 12) = 0.0014 p =0.970 VPA: F (1, 12) = 0.4375 p =0.5208 RSV: F (1, 12) = 3.089 p =0.1043	RSV vs RSV+VPA:	> 0.9999
			VPA vs RSV+VPA:	> 0.9999
			CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.4516
<b>CA2</b>	CON: 0.00016±0.000036 RSV:0.00014±0.000035 VPA: 0.00016±0.000021 RSV+VPA:0.00012±0.000024	Interaction: F (1, 12) = 1.367 p =0.265 VPA: F (1, 12) = 0.7516 p =0.4030 RSV: F (1, 12) = 3.561 p =0.0836#	RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	> 0.9999
			VPA vs RSV+VPA:	0.3096
			CON vs RSV:	0.05256
			CON vs VPA:	> 0.9999
<b>PV /Area</b>	CON: 0.044±0.008 RSV:0.057±0.005 VPA: 0.050±0.013 RSV+VPA: 0.043±0.011	Interaction:F (1, 12) = 4.109 p =0.0655# VPA: F (1, 12) = 0.6387 p =0.4397 RSV: F (1, 12) = 0.3637 p =0.5577	CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	0.4130
			VPA vs RSV+VPA:	> 0.9999
			CON vs RSV:	0.3139
<b>CA3</b>	CON:0.00023±0.000043 RSV:0.00018±0.000017 VPA:0.00023± 0.000040 RSV+VPA: 0.00016±0.000019	Interaction: F (1, 12) = 0.975 p =0.342 VPA: F (1, 12) = 0.6635 p =0.4312 RSV: F (1, 12) = 16.27 p =0.0017**	CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.0300*
			RSV vs VPA:	0.2519
			RSV vs RSV+VPA:	> 0.9999
			VPA vs RSV+VPA:	0.0240*
<b>PV Ratio</b>	CON:0.072±0.013 RSV:0.063±0.004 VPA:0.059±0.013 RSV+VPA: 0.052±0.011	Interaction:F (1, 12) = 0.0289 p =0.867 VPA: F (1, 12) = 4.742 p =0.0501# RSV: F (1, 12) = 2.184 p =0.1652	CON vs RSV:	> 0.9999
			CON vs VPA:	0.7366
			CON vs RSV+VPA:	0.1433
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	> 0.9999
<b>CA3</b>			VPA vs RSV+VPA:	> 0.9999

**PV:** parvalbumin-positive interneuron; **HC:** hippocampus; **SD:** standard deviation. p <0.05 was considered significant. \*p<0.05. \*\*p<0.01, #trend. Statistical analyses: two-way ANOVA parametric test followed by Bonferroni. N<sub>CON</sub>: 4. N<sub>RSV</sub>: 4. N<sub>VPA</sub>: 4. N<sub>RSV+VPA</sub>: 4.

**Supplementary Table S3. Distribution profile of CB neurons in the HC**

Mean ± SD		F (DFn, DFd); p Value	Pairwise comparisons		
CB /Area	CON: 0.000072±0.000014	Interaction:F (1, 11) = 1.151 p =0.3062 VPA: F (1, 11) = 14.32 p =0.0030** RSV: F (1, 11) = 1.421 p =0.2583	CON vs RSV:	0.7420	
	RSV: 0.000097±0.000019		CON vs VPA:	0.4284	
	VPA: 0.000044±0.000028		CON vs RSV+VPA:	0.6306	
	CA1		RSV+VPA: 0.000045±0.000015	RSV vs VPA:	0.0224*
				RSV vs RSV+VPA:	0.0418*
			VPA vs RSV+VPA:	> 0.9999	
CB Ratio	CON: 0.017±0.003	Interaction:F (1, 12) = 0.782 p =0.3938 VPA: F (1, 12) = 1.679 p =0.2194 RSV: F (1, 12) = 1.258 p =0.2839	CON vs RSV:	0.9263	
	RSV: 0.024±0.003		CON vs VPA:	> 0.9999	
	VPA: 0.015±0.010		CON vs RSV+VPA:	> 0.9999	
	CA1		RSV+VPA: 0.016±0.008	RSV vs VPA:	0.6471
				RSV vs RSV+VPA:	> 0.9999
			VPA vs RSV+VPA:	> 0.9999	
CB /Area	CON: 0.000035±0.0000053	Interaction:F (1, 11) = 0.0014 p =0.971 VPA: F (1, 11) = 9.038 p =0.0119* RSV: F (1, 11) = 3.388 p =0.0928#	CON vs RSV:	> 0.9999	
	RSV:0.000050±0.000022		CON vs VPA:	0.2801	
	VPA:0.000010±0.0000021		CON vs RSV+VPA:	> 0.9999	
	CA2		RSV+VPA:0.000026±0.000022	RSV vs VPA:	0.0265*
				RSV vs RSV+VPA:	0.4084
			VPA vs RSV+VPA:	> 0.9999	
CB Ratio	CON: 0.015±0.004	Interaction:F (1, 11) = 0.00547 p =0.942 VPA: F (1, 11) = 10.50 p =0.0079** RSV: F (1, 11) = 1.671 p =0.2226	CON vs RSV:	> 0.9999	
	RSV:0.018±0.006		CON vs VPA:	0.1972	
	VPA: 0.006±0.003		CON vs RSV+VPA:	> 0.9999	
	CA2		RSV+VPA: 0.009±0.006	RSV vs VPA:	0.0398*
				RSV vs RSV+VPA:	0.3233
			VPA vs RSV+VPA:	> 0.9999	
CB /Area	CON:0.000069±0.000021	Interaction:F (1, 10) = 0.625 p =0.4475 VPA: F (1, 10) = 11.52 p =0.0068** RSV: F (1, 10) = 0.7661 p =0.4020	CON vs RSV:	> 0.9999	
	RSV: 0.000068±0.000014		CON vs VPA:	0.5725	
	VPA: 0.000044±0.000019		CON vs RSV+VPA:	0.1082	
	CA3		RSV+VPA: 0.000030±0.000013	RSV vs VPA:	0.4995
				RSV vs RSV+VPA:	0.0858#
			VPA vs RSV+VPA:	> 0.9999	
CB Ratio	CON: 0.022±0.005	Interaction: F (1, 10) = 0.679 p =0.429 VPA: F (1, 10) = 19.20 p =0.0014** RSV: F (1, 10) = 0.8092 p =0.3895	CON vs RSV:	> 0.9999	
	RSV: 0.022±0.006		CON vs VPA:	0.1836	
	VPA: 0.013±0.004		CON vs RSV+VPA:	0.0347*	
	CA3		RSV+VPA: 0.09±0.003	RSV vs VPA:	0.1434
				RSV vs RSV+VPA:	0.0254*
			VPA vs RSV+VPA:	> 0.9999	

**CB:** calbindin-positive interneuron; **HC:** hippocampus; **SD:** standard deviation. p <0.05 was considered significant. \*p<0.05. \*\*p<0.01, #trend. Statistical analyses: two-way ANOVA parametric test followed by Bonferroni. N<sub>CON</sub>: 4. N<sub>RSV</sub>: 4. N<sub>VPA</sub>: 4. N<sub>RSV+VPA</sub>: 3.

**Supplementary Table S4. Distribution profile of SOM neurons in the HC**

Mean ± SD		F (DFn. DFd); p Value	Pairwise comparisons	
SOM /Area	CON: 0.00017±0.000031 RSV:0.00015±0.000026 VPA: 0.000168±0.000057 RSV+VPA: 0.00021±0.000048	Interaction:F (1, 12) = 2.670 p =0.1282 VPA: F (1, 12) = 2.302 p =0.1551 RSV: F (1, 12) = 0.8441 p =0.3763	CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.6637
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	0.2745
CA1			VPA vs RSV+VPA:	0.5772
SOM Ra- tio	CON: 0.034±0.0098 RSV: 0.039±0.0047 VPA: 0.044±0.0016 RSV+VPA: 0.045±0.0052	Interaction:F (1, 12) = 0.0685 p =0.797 VPA:F (1, 12) = 2.754 p =0.1229 RSV: F (1, 12) = 0.4506 p =0.5148	CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.7516
			RSV vs VPA:	> 0.9999
			RSV vs RSV+VPA:	> 0.9999
CA1			VPA vs RSV+VPA:	> 0.9999
SOM/Are a	CON: 0.00011±0.000017 RSV: 0.00010±0.000018 VPA: 0.000084±0.000013 RSV+VPA:0.000088±0.00001	Interaction:F (1, 12) = 1.030 p =0.3301 VPA: F (1, 12) = 8.942 p =0.0113* RSV: F (1, 12) = 0.2051 p =0.6587	CON vs RSV:	> 0.9999
			CON vs VPA:	0.0907#
			CON vs RSV+VPA:	0.1888
			RSV vs VPA:	0.5879
			RSV vs RSV+VPA:	> 0.9999
CA2			VPA vs RSV+VPA:	> 0.9999
SOM Ra- tio	CON: 0.038±0.0054 RSV: 0.043±0.0059 VPA: 0.032±0.0066 RSV+VPA:0.031±0.0031	Interaction:F (1, 12) = 1.866 p =0.1970 VPA: F (1, 12) = 11.62 p =0.0052** RSV: F (1, 12) = 0.4153 p =0.531	CON vs RSV:	> 0.9999
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.4460
			RSV vs VPA:	0.0852#
			RSV vs RSV+VPA:	0.0330*
CA2			VPA vs RSV+VPA:	> 0.9999
SOM /Area	CON: 0.00010±0.000020 RSV: 0.00014±0.000016 VPA: 0.00010±0.000035 RSV+VPA: 0.00014±0.000029	Interaction:F (1, 12) = 0.0885 p =0.771 VPA: F (1, 12) = 0.04458 p =0.8363 RSV: F (1, 12) = 8.007 p =0.0152*	CON vs RSV:	0.5916
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	0.3157
			RSV vs VPA:	0.5330
			RSV vs RSV+VPA:	> 0.9999
CA3			VPA vs RSV+VPA:	0.2830
SOM Ra- tio	CON: 0.037±0.012 RSV: 0.050±0.006 VPA: 0.038±0.008 RSV+VPA: 0.044±0.013	Interaction:F (1, 12) = 0.498 p =0.4935 VPA: F (1, 12) = 0.3335 p =0.5743 RSV: F (1, 12) = 3.713 p =0.0780#	CON vs RSV:	0.5237
			CON vs VPA:	> 0.9999
			CON vs RSV+VPA:	> 0.9999
			RSV vs VPA:	0.6118
			RSV vs RSV+VPA:	> 0.9999
CA3			VPA vs RSV+VPA:	> 0.9999

**SOM:** somatostatin-positive interneuron; **HC:** hippocampus; **SD:** standard deviation. p <0.05 was considered significant. \*p<0.05. \*\*p<0.01, #trend. Statistical analyses: two-way ANOVA parametric test followed by Bonferroni. N<sub>CON</sub>: 4. N<sub>RSV</sub>: 4. N<sub>VPA</sub>: 4. N<sub>RSV+VPA</sub>: 4.

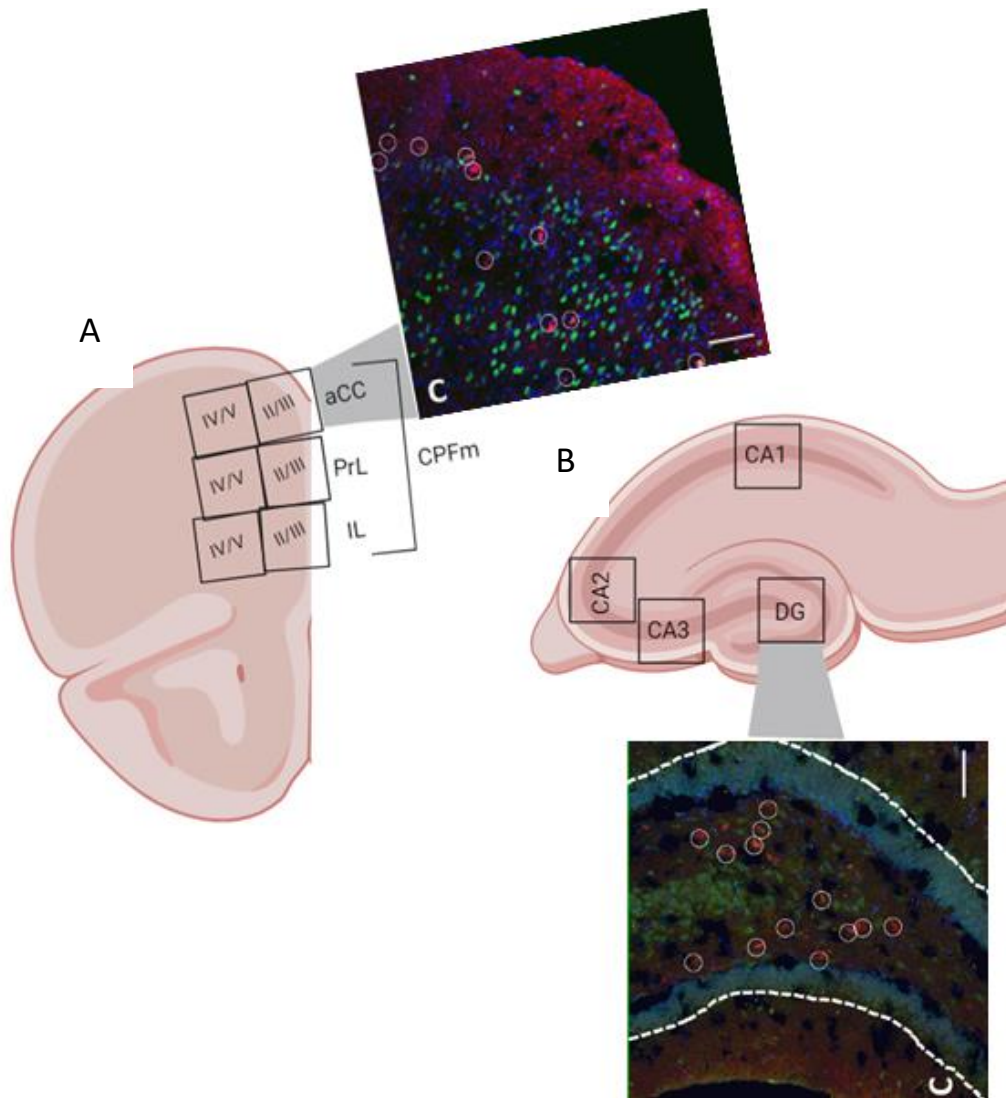
**Supplementary Table S5 – Information on key immunofluorescence and western blotting reagents**

Immunofluorescence			
Reagent	Supplier	Code	Dilution and Time
Anti-NeuN (Mouse)	Merck	MAB3771	1:500 in PBS-Triton 0.1% overnight at 4°C
Anti-NeuN	Abcam	Ab177487	1:500 in PBS-Triton 0.1% overnight at 4°C
Anti-Parvalbumin	Abcam	Ab64555	1:500 in PBS-Triton 0.1% overnight at 4°C
Anti-Calbindin	Abcam	Ab82812	1:500 in PBS-Triton 0.1% overnight at 4°C
Anti-Somatostatin	Sigma-Aldrich	SAB4502861	1:500 in PBS-Triton 0.1% overnight at 4°C
Alexa Fluor 488 Goat Anti-Mouse IgG (H+L)	Molecular Probes	Ab11029	1:2000 in PBS-Triton 0.1% in room temperature for 1h
Alexa Fluor 594 Goat Anti-Rabbit IgG (H+L)	Molecular Probes	Ab150080	1:2000* in PBS-Triton 0.1% in room temperature for 1h
DAPI Nucleic Acid Stain	Invitrogen	MP01306	30 uL per slice
Mounting Medium Fluorshield	Sigma-Aldrich	F6182-20ML	30 uL per slice

\* In SOM<sup>+</sup> assay, the concentration was 1:1000

Western blotting			
Reagent	Supplier	Code	Dilution
GABA <sub>A</sub> (goat)	Santa Cruz Biotechnology	sc-31405	1:250 in 5% BSA-0.1%TTBS
GABA <sub>B</sub> (rabbit)	Santa Cruz Biotechnology	sc-14006	1:500 in 5% BSA-0.1%TTBS
Gephyrin (rabbit)	Abcam	ab32206	1:500 in 5% BSA-0.1%TTBS
Neurologin-2 (rabbit)	Abcam	ab36602	1:500 in 5% milk -0.1%TTBS
PSD-95 (rabbit)	Abcam	ab18258	1:500 in 5% BSA-0.1%TTBS
Synaptophysin (mouse)	SIGMA Aldrich	S5768	1:1000 in 5% BSA-0.1%TTBS
Anti-β-actin (mouse)	SIGMA Aldrich	A1978	1:3000 in TTBS
IgG-HRP (goat)	Santa Cruz Biotechnology	sc-2354	1:1000 in 5% BSA-0.1%TTBS
IgG-HRP (mouse)	Santa Cruz Biotechnology	sc-2314	1:1000 in 5% milk-0.1%TTBS
IgG-HRP (rabbit)	Santa Cruz Biotechnology	sc-2004	1:1000 in 0.1%TTBS

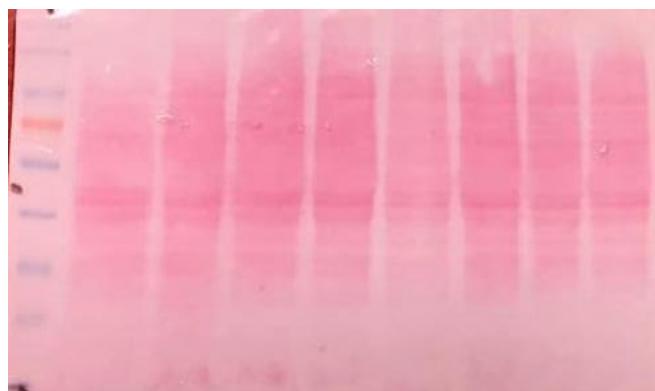
Common reagents	
PBS	0.1 M pH 7.4 Phosphate-buffered saline
SDS 10%	Sodium dodecyl sulfate
EDTA 100 mM	Ethylenediamine tetraacetic acid
TRIS/HCl buffer 500 mM pH 8	Tris(hydroxymethyl)aminomethane and chloridric acid
TBS	TRIS buffer
TTBS	0.1%Tween20-TBS



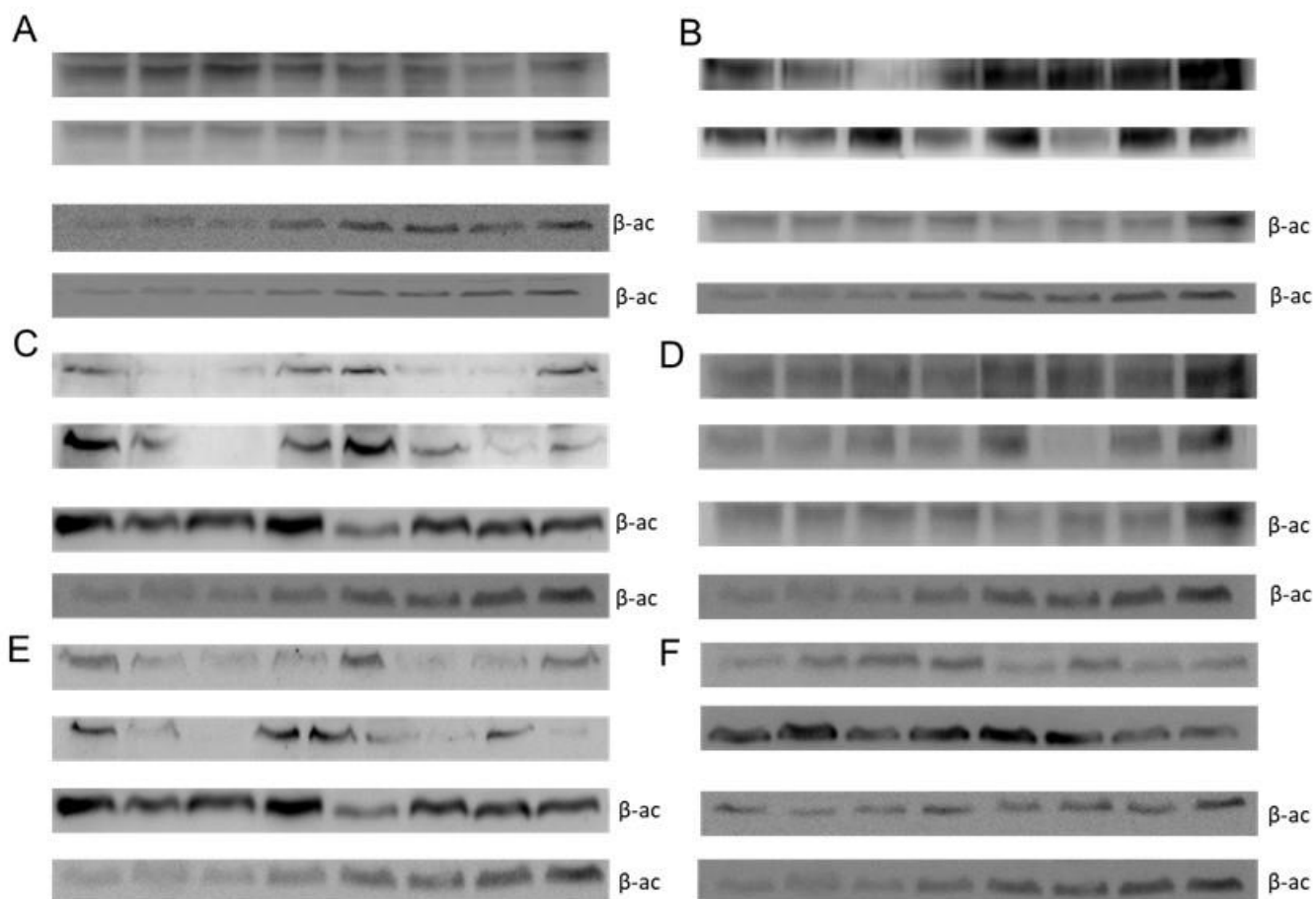
**Figure S1.** Illustrative scheme of the regions analyzed in this study. **A)** Representation of the mPFC and the three subregions evaluated (aCC, PrL, and IL), each one subdivided according to the cortical layers: upper layers (II/III) and deeper layers (IV/V). The total number of neurons, the number of each interneuron (PV+, CB+, and SOM+), and the ratio (interneuron/total neurons) were evaluated in both subdivisions of cortical layers in the three subregions. The amount observed in the deeper + the upper layers of a subregion was added to calculate the total for the whole subregion. Data for the whole mPFC was calculated as the sum of the amount observed in alle subregions. **B)** Representation of the hippocampus and the four evaluated subregions (DG, CA1, CA2, and CA3). In each of them, the total number of neurons, the number of each interneuron (PV+, CB+, and SOM+), and the ratio (interneuron/total neurons) were evaluated. **C)** Illustrative image. aCC, anterior cingulate cortex; CA, cornu ammonis; CB, calbindin-neurons; DG, dentate gyrus; IL, infralimbic cortex; mPFC, medial frontal cortex; PrL, prelimbic cortex; PV, parvalbumin-neurons; SOM, somatostatin-neurons.

Structure/Time Point	Canales		Oskvig	Canales		Kalish										Kalish							Balmer		Cui
	E12.5	E14.5	E15	E17.5	E14										E18							6h	4d	-	
	Cort	Cort	Cort	Cort	EG	CS	IN	S IN	LI	L V-VI	M	SVZ	P SVZ	RG	EG	IN	S IN	L II-IV	L V-VI	OLIG	RG	SVZ	Cells	Cells	Org
GO Terms																									
Carbohydrate Metabolism																									
Mitochondrial metabolism																									
Response to ROS																									
Response To Hypoxia																									
Macromolecule Metabolism																									
Lipid metabolism																									
Nucleic Acid Metabolism																									
Purine Metabolism																									
Pyrimidine Metabolism																									
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ncRNA metabolism/process																									
RNA processing																									
RNA splicing																									
Gene Expression																									
Aminoacid metabolism																									
Protein Metabolism																									
Translation																									
Protein Elongation																									
Post translational Modifications																									
Ubiquitination Regulation																									
Histone modification																									
Cell Cycle																									
Ion Transport																									
Eye Development																									
Cell Adhesion																									
Extracellular Matrix																									
Synapse																									
GABA metabolism / signaling																									
GLU metabolism / signaling																									
WNT pathway																									
Notch pathway																									
Hippo pathway																									
Adenylyl Cyclase/cAMP signaling																									
MAPK/ERK																									
Nervous System Development																									
GABAergic interneuron differentiation																									
Neuron development/migration																									
	1.83	5.52	5.15	7.60	5.06	6.20	4.65*	4.67*	9.93	5.41	4.45	5.32	4.52*	0	5.03*	4.88*	5.22*	6.71*	0	2.91*	7.47	5.84	7.42	6.36	

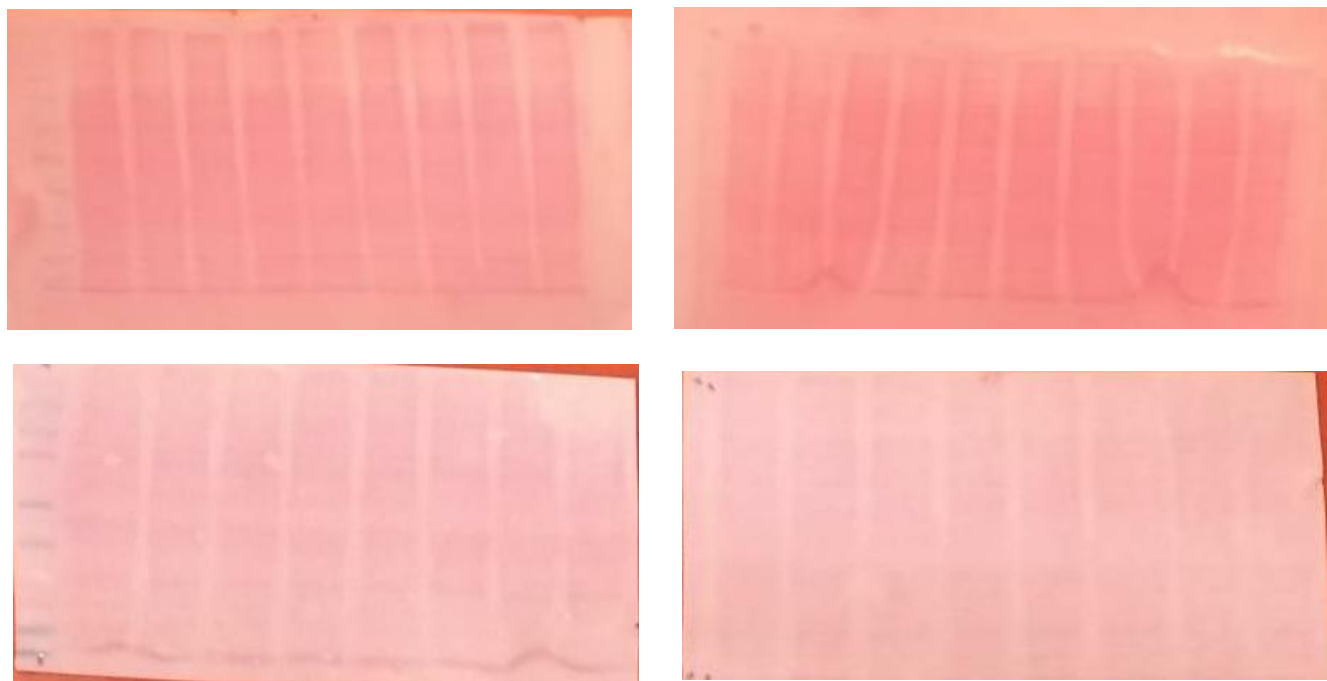
**Figure S2.** Compilation of the databases from ASD or associated models analyzed in this study. The five databases (described in Table 1) are organized according to the time-point of embryo development (where applicable) with the most relevant pathways listed in the left column. Gray squares represent pathways associated with the DGEs in general; Orange squares represent pathways specifically associated with upregulated DGEs; and blue squares represent pathways specifically associated with downregulated DGEs. The numbers in the bottom line represent the percentage of orthologs identified in the SFARI database, \*indicate that a mean was obtained from all of the cells associated with the specific region. Cort.: cortex; CS: cortical subplate; DGE: differentially expressed genes; GE: ganglionic eminence; IN: interneuron; L: layer; OLIG: oligodendrocyte; Org: organoid; RG: radial glia; S IN: striatal interneuron; SVZ: subventricular zone.



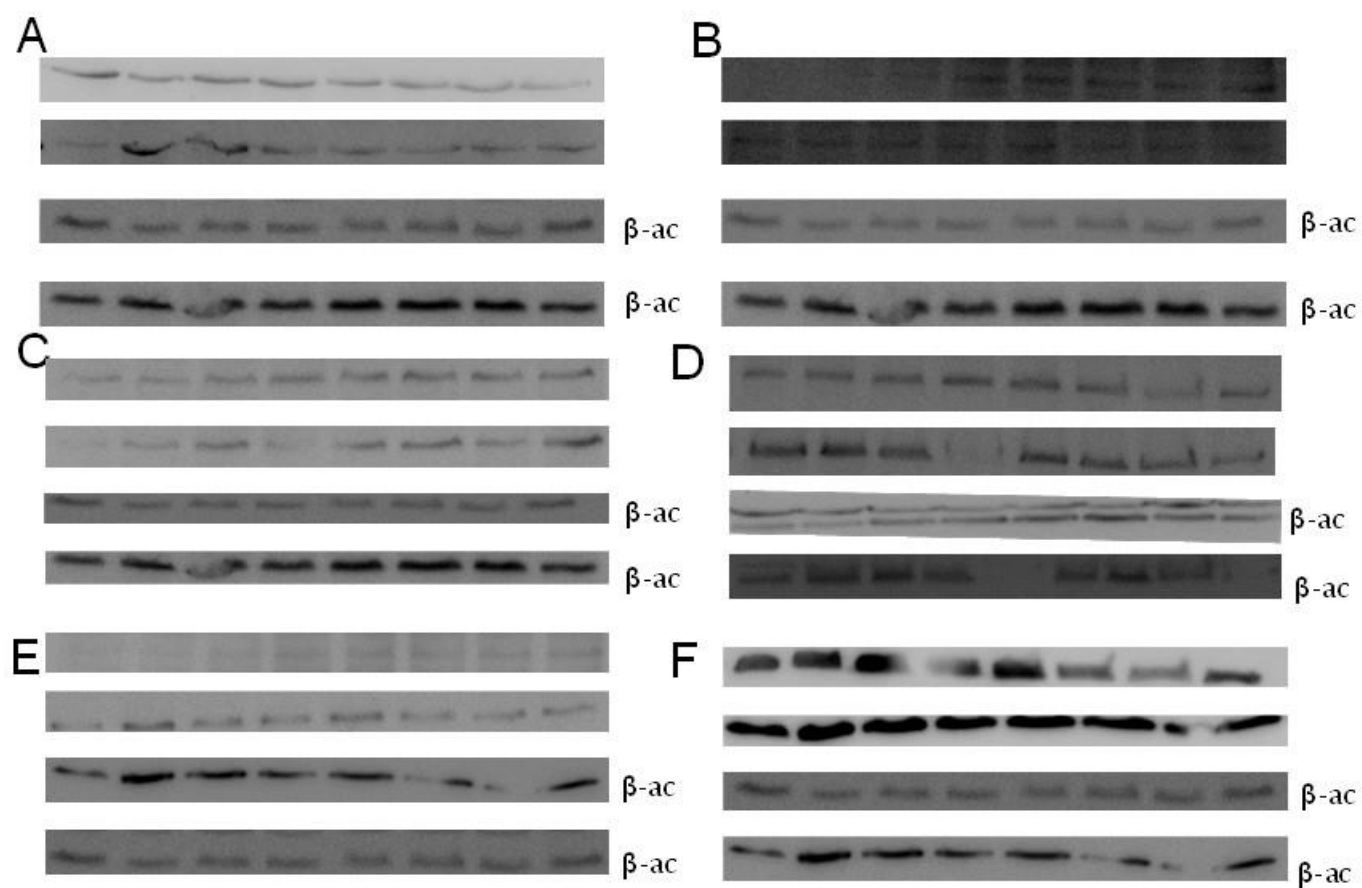
**Supplementary Figure S3. Ponceau staining of the nitrocellulose membranes – mPFC**



**Supplementary Figure S4. Nitrocellulose membranes mPFC.** A) GABA<sub>A</sub>; B) GABA<sub>B</sub>; C) Gephyrin; D) PSD-95; E) Neurolygin-2; F) Synaptophysin. In the image,  $\beta$ -ac stands for  $\beta$ -actin.



**Supplementary Figure S5. Ponceau staining of the nitrocellulose membranes – HC**



**Supplementary Figure S6. Nitrocellulose membranes HC.** A) GABA<sub>A</sub>; B) GABA<sub>B</sub>; C) Gephyrin; D) PSD-95; E) Neurolygin-2; F) Synaptophysin. In the image,  $\beta$ -ac stands for  $\beta$ -actin.