

## Electronic Supplementary Material

**Article title:** Cartography of hevin-expressing cells in the adult brain reveals prominent expression in astrocytes and parvalbumin neurons

**Journal:** Brain Structure and Function

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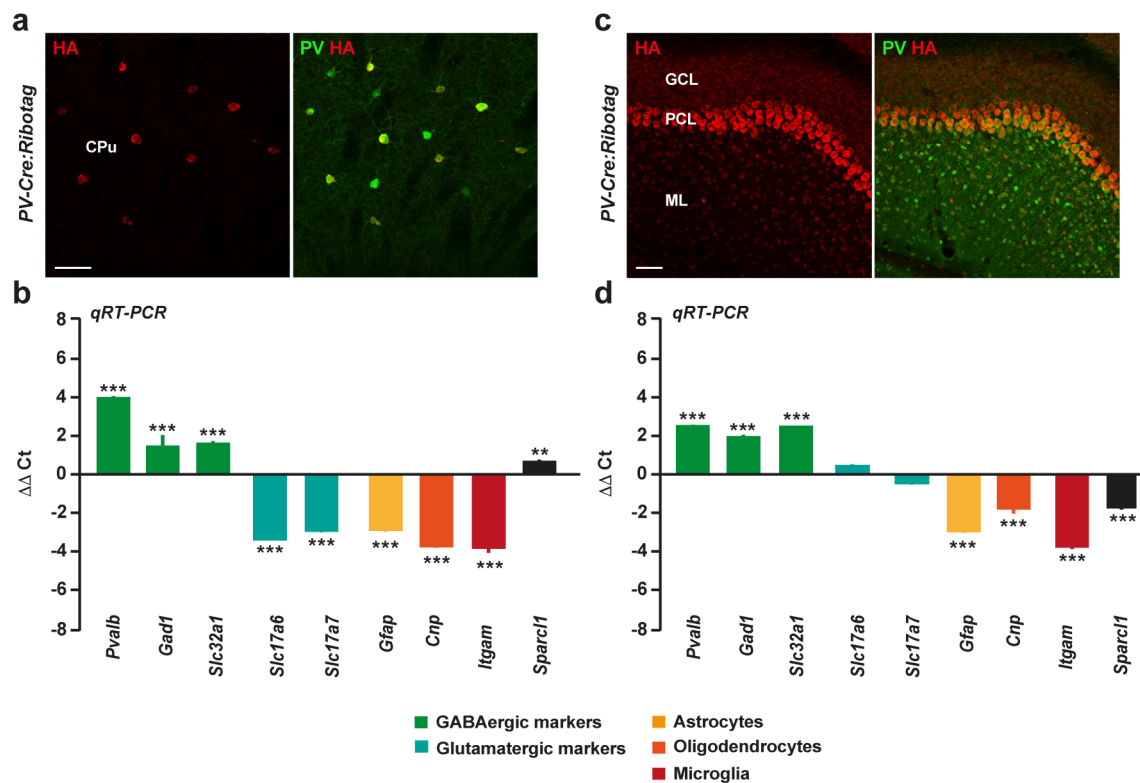
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**Supplementary Data**

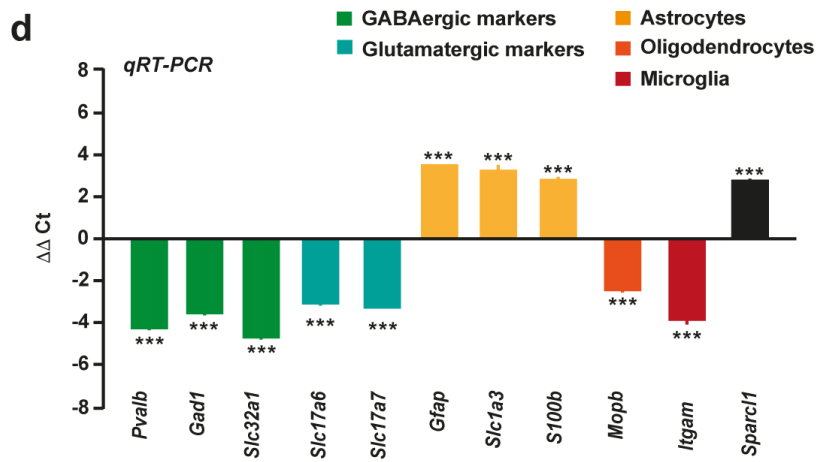
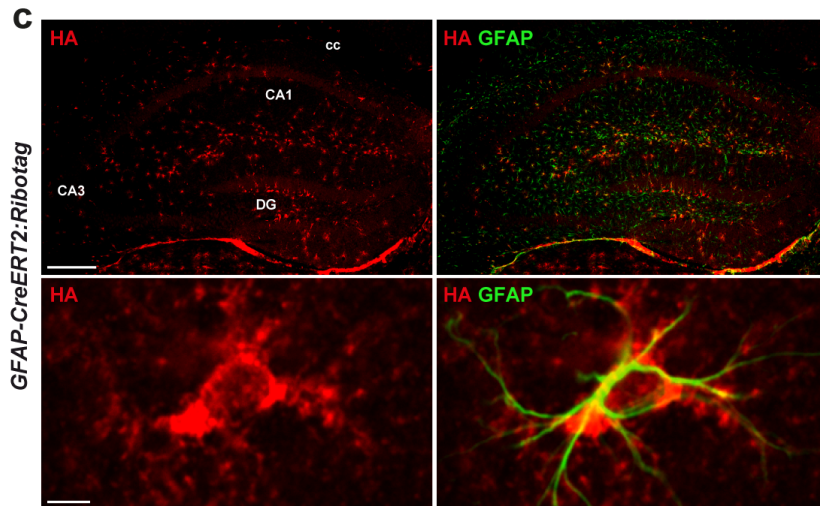
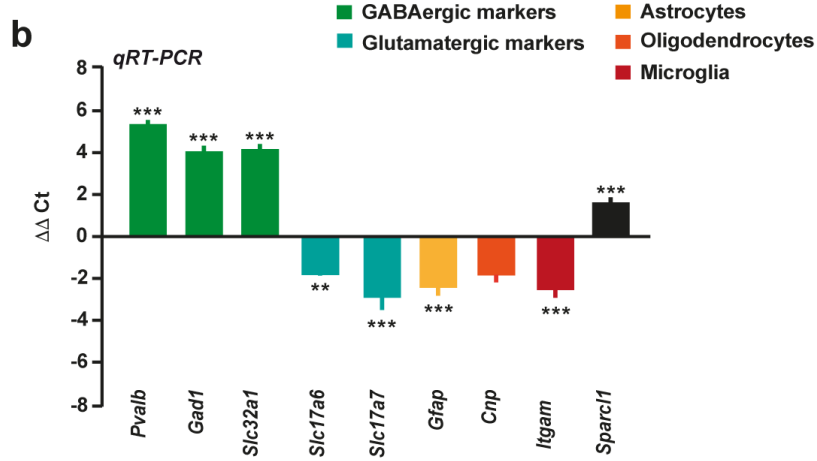
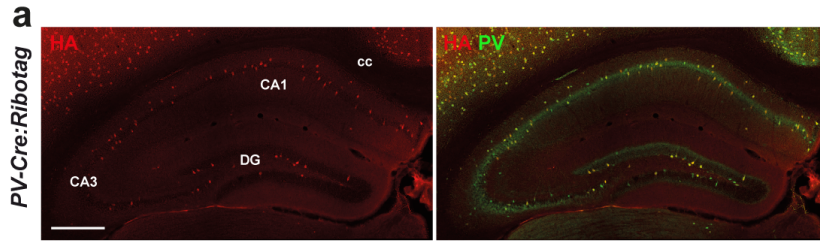
**Supplementary Materials and Methods**

**Supplementary References**

## Supplementary Data



**Supplementary Figure 1.** *Sparcl1* gene is expressed in striatal PV interneurons but not in cerebellar PV-positive cells. **(a)** HA (red) and PV (green) immunofluorescence in the striatum of PV-Cre:Ribotag mice. Scale bar 40  $\mu\text{m}$ . **(b)** Quantitative RT-PCR analysis of mRNAs isolated following HA immunoprecipitation from striatal of PV-Cre:Ribotag mice. All genes were normalized to *Hrpt2*. Data are expressed as the fold change comparing the pellet fraction versus the input. Positive control genes including GABAergic markers (*Pvalb*, *Gad1*, *Slc32a1*; green bars) were enriched in the pellet compared to the input fraction. Glutamatergic neuronal markers (*Slc17a6* and *Slc17a7*; blue bars) and glial markers including *Gfap* for astrocytes (yellow bar), *Cnp* for oligodendrocytes (orange bar), and *Itgam* for microglia (red bars) were de-enriched in the pellet fraction. *Sparcl1* gene was enriched in the pellet samples ( $n = 8$ ). **(c)** HA (red) and PV (green) immunofluorescence in the cerebellum of PV-Cre:Ribotag mice. Scale bar 50  $\mu\text{m}$ . **(d)** Quantitative RT-PCR analysis of mRNAs isolated in the PV-Cre:Ribotag mice after cerebellar HA immunoprecipitation. Positive control genes, including the GABAergic markers *Pvalb*, *Gad1*, and *Slc32a1* (green bars), were significantly enriched in the pellet fraction. mRNAs for glial cell markers (*Gfap*, *Cnp*, *Itgam*; yellow, orange, and red bars), for glutamatergic neurons (*Slc17a6* and *Slc17a7*; blue bars) and for *Sparcl1* gene were found de-enriched in the pellet fraction compared with the input fraction ( $n = 8$ ). Data are analyzed by two-tailed Student t test. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  pellet vs. input.



**Supplementary Figure 2.** Hippocampal PV-expressing neurons and astrocytes are enriched in *Sparcl1* mRNA. **(a)** HA (red) and PV (green) immunofluorescence in the dorsal hippocampus of PV-Cre:Ribotag mice. Scale bar 300  $\mu\text{m}$ . **(b)** Quantitative RT-PCR analysis of mRNAs isolated following HA immunoprecipitation from hippocampi of PV-Cre:Ribotag mice. All genes were normalized to  $\beta$ -actin. Data are expressed as the fold change comparing the pellet fraction versus the input. Glial markers including *Gfap* for astrocytes (yellow bar), *Cnp* for oligodendrocytes (orange bar), and *Itgam* for microglia (red bars) as well as markers for glutamatergic neurons (*Slc17a6* and *Slc17a7*; blue bars) were de-enriched in the pellet samples. Positive control genes including GABAergic markers (*Pvalb*, *Gad1*, *Slc32a1*; green bars) were enriched in the pellet compared to the input fraction. mRNA of *Sparcl1* gene was found significantly enriched in PV-expressing neurons (n = 8). **(c)** HA (red) and GFAP (green) immunofluorescence in the dorsal hippocampus in GFAP-CreER<sup>T2</sup>:Ribotag mice. Scale bar 300  $\mu\text{m}$ . Note that HA-expressing astrocytes are also GFAP-positive. Scale bar 10  $\mu\text{m}$ . **(d)** Quantitative RT-PCR analysis of mRNAs isolated in the GFAP-CreER<sup>T2</sup>:Ribotag mice after hippocampal HA immunoprecipitation. Positive control genes, including the glial markers *Gfap*, *Slc1a3*, and *S100b* (yellow bars), were enriched in the pellet fraction compared with the input fraction. Markers for GABAergic neurons (*Pvalb*, *Gad1*, *Slc32a1*; green bars), glutamatergic neurons (*Slc17a6* and *Slc17a7*; blue bars), oligodendrocytes (*Mobp*; orange bar), and microglia (*Itgam*; red bar) were de-enriched in the pellet samples. A significant enrichment in *Sparcl1* mRNA was found in astrocytes (n = 5). Data are analyzed by two-tailed Student t test. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  pellet vs. input.

## Supplementary Materials and methods

### Animals

Male and female mice, 8–12-week old, Parvalbumin-Cre (PV-Cre) [The Jackson Laboratory, Hippenmeyer et al., 2005], GFAP-CreER<sup>T2</sup> [The Jackson Laboratory, Hirrlinger et al., 2006] (C57BL/ 6J background) and Ribotag:loxP mice [The Jackson Laboratory, (Sanz et al., 2009)] were used in this study. Homozygous Ribotag:loxP female mice were crossed with homozygous PV-Cre male mice to generate PV-Cre:Ribotag mice. Similarly, GFAP-CreER<sup>T2</sup>:Ribotag mice were produced by crossing heterozygous tamoxifen-inducible GFAP-CreER<sup>T2</sup> male mice with homozygous Ribotag:loxP female mice. Animals were maintained in a 12-hour light/dark cycle, in stable conditions of temperature and humidity, with food and water *ad libitum*. All experiments were in accordance with the guidelines of the French Agriculture and Forestry Ministry for handling animals (authorization number/license D34-172-13).

### Tamoxifen administration

Tamoxifen (Sigma, T5648) was dissolved in sunflower oil/ethanol (10:1) to a final concentration of 10 mg/ml and injected intraperitoneally at 100 mg/kg on 3 consecutive days to induce recombination in GFAP-CreER<sup>T2</sup>:Ribotag mice. Two weeks after the last tamoxifen administration, mice were sacrificed for imaging and targeted qRT-PCR studies.

### Tissue preparation and immunofluorescence

Mice were rapidly anaesthetized with euthasol (800 mg/kg, i.p., Med Vet, France) and transcardially perfused with 4 % (weight/vol) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.5) (Puighermanal et al., 2015). Brains were post-fixed overnight in the same solution and stored at 4° C. 30- $\mu$ m thick sections were cut with a vibratome (Leica, France) and stored at -20° C in a solution containing 30 % (vol/vol) ethylene glycol, 30 % (vol/vol) glycerol, and 0.1 M sodium phosphate buffer, until they were processed for immunofluorescence. Hippocampal, striatal, and cerebellar sections were identified using a mouse brain atlas (Franklin and Paxinos 2007). Sections were processed as follows: free-floating sections were rinsed three times 10 minutes in Tris-buffered saline (50 mM Tris-HCL, 150 mM NaCl, pH 7.5). After 15 minutes incubation in 0.15 % (vol/vol) Triton X-100 in TBS, sections were rinsed in TBS again and blocked for 1 hour in a solution of 3 % BSA in TBS. Finally, they were incubated 24 hours at 4 °C in 1 % BSA, 0.15 % Triton X-100 with

the primary antibodies (Table 1). Sections were rinsed three times for 10 minutes in TBS and incubated for 45–60 minutes with Cy3- goat anti-rabbit (1:400, Theermo Fischer Scientific, A10520) and Alexa fluor 488- goat anti-mouse (1:400, Life Technologies, A11001). Sections were rinsed for 10 minutes twice in TBS and twice in Tris-buffer (1 M, pH 7.5) before mounting in 1,4-diazabicyclo-[2. 2. 2]-octane(DABCO, Sigma-Aldrich).

Confocal microscopy and image analysis were carried out at the Montpellier RIO Imaging Facility. Images covering the entire dorsal hippocampus, striatum and cerebellum were single confocal sections acquired using sequential laser scanning confocal microscopy (Zeiss LSM780). Double-labeled images from each region of interest were single section obtained using sequential laser scanning confocal microscopy (Zeiss LSM780). Photomicrographs were obtained with the following band-pass and long-pass filter setting: Alexa fluor 488/Cy2 (band pass filter: 505–530), Cy3 (band pass filter: 560–615).

**Table 1:**

<b>Antigen</b>	<b>Host</b>	<b>Dilution</b>	<b>Supplier</b>	<b>Catalog Number</b>
<i>HA</i> (Hemagglutinin)	Mouse	1:1000	Biologend	901502
<i>PV</i> (Parvalbumin)	Rabbit	1:1000	Swant	PV25
GFAP	Chicken	1:1000	Abcam	Ab4674

### **Polyribosome Immunoprecipitation**

HA-tagged-ribosome immunoprecipitation was performed as described previously (Puighermanal et al., 2017) in the striatum, hippocampus, and cerebellum of PV-Cre:Ribotag and GFAP-CreER<sup>T2</sup>:Ribotag mice. Briefly, each brain region were homogenized by bouncing in 1-ml polysome buffer (50 mM Tris, pH 7.4, 100 mM KCl, 12 mM MgCl<sub>2</sub>, and 1 % NP-40 supplemented with 1 mM DTT, 1 mg/ml heparin, 100 µg/ml cycloheximide, 200 U/ml RNaseOUT, and protease inhibitor mixture). Samples were then centrifuged at 10,000xg for 10 m to collect the post-mitochondrial supernatant. Then, 100 µl of each supernatant was transferred to a new tube serving as input fraction for validation. Anti-HA antibody (5 µl/sample; Covance, #MMS-101R) was added to the remaining supernatant and incubated overnight at 4 °C with constant gently rotation. The following day, samples were added to protein G magnetic beads (Invitrogen, #100.04D) and incubated overnight at 4 °C with constant gently rotation. On the third day, magnetic beads were washed twice in a magnetic rack for 10 m each in high-salt buffer (50 mM Tris, pH 7.4, 300 mM KCl, 12 mM MgCl<sub>2</sub>, 1

% NP-40, 1 mM DTT, and 100 µg/ml cycloheximide). After washing, 350 µl of Qiagen RLT buffer (supplemented with β-Mercaptoethanol) were added to the pellets and to the input samples. Total RNA was extracted according to manufacturer's instructions using a Qiagen RNeasy Micro kit and quantified using Nanodrop 1000 spectrophotometer. Eight biological replicates, each one composed of a pool of 2 mice, were used for qRT-PCR analysis.

### **cDNA Synthesis and Quantitative Real-Time PCR**

After HA-tagged-ribosome immunoprecipitation, synthesis of cDNA and qRT-PCR were performed as previously described (Puighermanal et al., 2017) using SYBR Green PCR master mix on the LC480 Real-Time PCR System (Roche) (n = 8 for the PV-Cre:Ribotag mice and n = 5 for the GFAP-CreER<sup>T2</sup>:Ribotag). Analysis was performed using LightCycler 480 Software (Roche). Results are presented as linearized Cp-values normalized to housekeeping genes *β-actin* (for the hippocampus), *Hprt2* (for the striatum), and *Tbp2a* (for the cerebellum) and the ΔCP method was used to give the fold change. Data are expressed as the fold change comparing the pellet fraction versus the input (3 biological replicates per set of primers). The immunoprecipitated RNA samples (pellet) were compared to the input sample in each case. Primer sequences are indicated in Table 2.

**Table 2:**

<b>Name of the gene</b>	<b>Primer forward</b>	<b>Primer Reverse</b>
<i>Pvalb</i>	TCGATGACAGACGTGCTCAG	CTTCACCTCATCCGGGTTCT
<i>Gad1</i>	TTGTGCTTTGCTGTGTTTTAGAGA	CCCCCTGCCCAAAGATAGAC
<i>Slc32a1</i>	TCACGACAAACCCAAGATCAC	GTCTTCGTTCTCCTCGTACAG
<i>Slc17a6</i>	CGCTGTTCGGGGATGGTTTGC	GTGGACGAGTGCAGCAATGAGG
<i>Slc17a7</i>	TGTGCCCATCATCGTGGGT	CGTCATCTCCTCCGGCTCT
<i>Gfap</i>	AGCGAGCGTGCAGAGATGA	AGGAAGCGGACCTTCTCGAT
<i>Slc1a3</i>	CATCGTGCTGACATCTGTGG	TCACCGAGTTCCCCATTTCA
<i>S100b</i>	TTCCACCAGTACTCCGGGCG	GCGACGAAGGCCATGAACTCC
<i>Mobp</i>	AGGACTGGATCTGCTGTGCCT	GGGGACTTTGGCTTGGCTGG
<i>Cnp</i>	GCTGCACTGTACAACCAAATTCTG	ACCTCCTGCTGGGCGTATT
<i>Itgam</i>	ATGGTCACCTCCTGCTTGTGAG	CCAGCAGTGATGAGAGCCAAGA
<i>Sparcl1</i>	CCTCTGCGAGCTTCCCTGGT	GGCTGCTCGTATTTCTGAAGCGG

<i><math>\beta</math>-actin</i>	CGTGAAAAGATGACCCAGATCA	CACAGCCTGGATGGCTACGT
<i>Hprt2</i>	GCAGTACAGCCCCAAAATGG	GGTCCTTTTCACCAGCAAGCT
<i>Tbp2<math>\alpha</math></i>	ATCGAGTCCGGTAGCCGGTG	GAAACCTAGCCAAACCGCC

### **Statistical analysis**

Unpaired Student's t-test was used to compare changes in gene expression between inputs and pellets. Significance threshold was set at  $p < 0.05$ . Prism 6.0 software was used to perform statistical analyses.



## Supplementary References

Hippenmeyer S; Vrieseling E; Sigrist M; Portmann T; Laengle C; Ladle DR; Arber S. 2005. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol* 3(5):e159

Hirrlinger PG, Scheller A, Braun C, Hirrlinger J, Kirchhoff F. Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. *Glia*. 2006;54:11–20.

Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS (2009) Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proc Natl Acad Sci USA* 106(33):13939–13944

Puighermanal E, Cutando L, Boubaker-Vitre J, Honoré E, Longueville S, Hervé D, Valjent E (2017). Anatomical and molecular characterization of dopamine D1 receptor-expressing neurons of the mouse CA1 dorsal hippocampus. *Brain Struct Funct* 222:1897-1911

Puighermanal E, Biever A, Espallergues J, Gangarossa G, De BundelD, Valjent E (2015) drd2-cre:ribotag mouse line unravels the possible diversity of dopamine d2 receptor-expressing cells of the dorsal mouse hippocampus. *Hippocampus* 25(7):858–875