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1 **Patterned expression of Pannexin 1 channels in the**
2 **adult cerebellar Purkinje cells**

3
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15

16

17

18 **Abstract**

19

20 Pannexin1 (PanX1) are recently discovered proteins that can form large pore
21 channels at the cell surface. They have been implicated in ATP-dependent cell-to-cell
22 communication and in several pathophysiological processes such as inflammation,
23 cell death and epilepsy. Using immunohistochemistry in the adult mouse, we
24 describe the presence of PanX1 in the deep cerebellar nuclei, in large cells of the
25 granular layer, presumably Golgi interneurons, in some Bergmann glia radial
26 processes as well as in the soma and dendrites of Purkinje cells. In the latter, PanX1,
27 like many other proteins, distribute heterogeneously. Only Zebrin II-positive Purkinje
28 cells express PanX1, in accordance with the so-called "zebra-striped" modular
29 architecture of the cerebellum. This distribution in zebra-stripes suggest that PanX1
30 may contribute to the control of ensemble activity within cerebellar microdomains or
31 to the response of Purkinje cell to excitotoxicity and cell-death messages.

32

33 Introduction

34 Pannexins are membrane proteins ubiquitously present in vertebrate
35 organisms. They were discovered in 2000 by sequence homology with the
36 invertebrate innexins [1]. They show structural similarities with connexins [1] but do
37 not seem to form gap-junctions in native cells ([2] and refs in [3]. Among the three
38 types of pannexins described to date, pannexin 1 (PanX1) is by far the most studied.
39 It is heavily expressed in the central nervous system, in both neurons and glial cells.
40 Its mRNA has been detected in the retina, the cerebellum, the neocortex and
41 hippocampus, in the amygdala, *substantia nigra* and the olfactory bulb, [4-6, 7 , 8, 9,
42 10 , 11]. PanX1 can be activated by cytoplasmic Ca^{2+} , membrane depolarization,
43 extracellular ATP and K^+ , by mechanical stretch and caspase cleavage ([12, 13 , 14]
44 and refs in [3]). They can form large conductance channels of up to 550 pS [13] that
45 carry non-selective ion fluxes and are permeable to molecules up to 1 kDa, including
46 ATP itself [14, 15],[16]. Once released, ATP can act on purinergic receptors or can
47 be further degraded into adenosine by ectonucleotidases. Thus pannexins mediate
48 auto- and paracrine communication, making them possible actors of retrograde
49 neuronal communication and modulators of neuronal activity, for example through
50 presynaptic adenosine receptors [17]. However, their exact physiological role
51 remains unclear. Evidence shows that they can initiate Ca^{2+} waves, for instance after
52 mechanical stretch [13], control the vascular tonus, regulate differentiation, cell death
53 and immune function [3]. In pathological conditions, they may activate the
54 inflammasome ([12] reviewed in [18]), contribute to caspase-mediated apoptosis [19]
55 and ischemic cell-death [20], and enhance epileptic seizures. They could also play a
56 role as tumor suppressors (refs in [3]).

57

58 In the cerebellum, PanX1 proteins are heavily expressed, particularly in
59 Purkinje cells [7 , 10], but their contribution to cerebellar physiology or pathology
60 remains totally unknown. Despite its apparent homogeneous crystal-like organization,
61 the cerebellum is, in fact, highly compartmentalized. It displays genetically
62 determined and reproducible topographic domains: the transverse zones and
63 parasagittal stripes, including the cortical so-called zebra-stripes [21]. The latter can
64 be revealed by the patterned expression of numerous molecules, particularly in
65 Purkinje cells. Zebrin II, now known to be the respiratory enzyme Aldolase C, was the
66 first example of such a protein [22]. It is expressed in parasagittal bands of Purkinje
67 cells that extend throughout the cerebellar cortex, separated by bands of Zebrin II-
68 negative Purkinje cells. Many other proteins match Zebrin II Purkinje cells patterns.
69 For example, phospholipase C β 4 (PLC β 4; [23]), the metabotropic glutamate
70 receptor 1b (mGluR1b; [24]) and the early B-cell factor 2 (Ebf2; [25]) are expressed
71 in Zebrin II-negative Purkinje cell stripes. On the other hand, PLC β 3 [26], the
72 excitatory amino-acid transporter 4 (EAAT4; [27]), and the GABA-B 1b receptor [28]
73 are expressed in Zebrin II-positive Purkinje cell. These striped distributions of
74 neurotransmitter receptors and their effectors indicate that Zebrin II-negative and -
75 positive Purkinje cells respond differentially to their inputs. For instance, the
76 glutamatergic climbing fibers that form synapses onto Zebrin II-positive Purkinje cells
77 release more glutamate per action potential than those terminating on Zebrin II-
78 negative ones [29]. Zebrin II-positive Purkinje cells seem to be adapted to respond to
79 increased glutamate inputs and to better resist death and neurodegeneration of
80 various origins [30, 31 , 32 , 33]. Sphingosine kinase, a lipid metabolism protein that
81 plays an important role in apoptosis, is also found selectively in Zebrin II-positive
82 Purkinje cells [30]. Overall, the proteins expressed in Zebrin II-positive Purkinje cells,

83 including Zebrin II itself, seem to confer these cells capacities to respond to sustained
84 neuronal activity and to the associated increase in metabolic demand.

85 In the present study, we use a type of antibodies directed against the extreme
86 C-terminal domain of PanX1 to investigate the distribution of PanX1 in the
87 cerebellum. These antibodies that had never been used in this structure reveal a
88 zebra-striped pattern of PanX1 in Purkinje cells. This differential rostro-caudal
89 expression, typical of the cerebellum, had never been described before for PanX1. It
90 suggests that PanX1s contribute to the adaptation of Purkinje cells to the increased
91 activity observed in the zebra stripes, and thus that pannexins may play important
92 functions in the physiology and pathophysiology of the cerebellum.

93

94 **Materials and methods**

95 **Animals**

96 The animals used were all adult (n=25) C57BL/6JRj males and females
97 C57BL/6JRj mice (from 8 to 12 weeks of age), purchased at Janvier Laboratory (Le
98 Genest-St-Isle, France). Mice were housed at the central animal facility of Les Saints-
99 Pères (Paris, France). Housing and all procedures were performed in accordance
100 with the guidelines of the French Ministry of Agriculture and the European
101 Community and have been approved by the ethical committee of Paris Descartes
102 University (agreement: Paris Descartes # 14-013). A minimal number of animals was
103 used and they were handled with maximum care in order to minimize their suffering.

104

105 **Tissue preparation**

106 The animals were deeply anaesthetized with sodium pentobarbital (175 mg/Kg

107 i.p.). The intracardiac perfusion consisted of 50 ml 0.01 M phosphate-buffered saline
108 (PBS, pH: 7.4), followed by 50 ml of 4% paraformaldehyde in 0.1 M PBS. The
109 cerebellum was removed, postfixed for 1 day in 4% paraformaldehyde solution and
110 cryoprotected in a 30% sucrose phosphate-buffered solution at 4°C for 2 days before
111 cutting. The cerebellum was cut into longitudinal or sagittal 80 µm-thick sections
112 using a freezing sliding microtome (Frigomobil Reichert-Jung) and collected in
113 plastic-wells containing PBS. Serial sections over the entire rostrocaudal length of the
114 cerebellum were treated and analyzed.

115

116 **Antibody Characterization**

117 **Table 1. Primary antibodies used in this study.**

Antigen	Immunogen	Source, host species, RRID	Dilution
Pannexin 1	18 kDa C-terminal portion of human Pannexin 1 protein	Chemicon, Millipore, USA ; rabbit polyclonal ; affinity purified. RRID : AB_2236635	1:750
Pannexin 1	18 kDa C-terminal portion of human Pannexin 1 protein, affigel-conjugated	Dr. V. Schestopalov, Miami, USA ; rabbit polyclonal ; affinity purified. RRID : AB_2315054	1:750
Calbindin	Calbindin D-28k protein	Swant, Switzerland ; mouse monoclonal. RRID : AB_10000347	1:7500
Zebrin II (Aldolase C)	Recognizes a single polypeptide band of apparent molecular weight 36 kDa on cerebellum homogenates from all vertebrate classes studied with this Ab.	Dr. R. Hawkes, Calgary, Canada ; mouse monoclonal. RRID : AB_2315622	1:50

118

119 The two types of PanX1 antibodies that we used were rabbit polyclonal

120 antibody raised against the C-terminus of the human PanX1. The first one came from
121 Chemicon (USA). The second one was made by V. Schestopalov [11] as follows. A
122 rabbit polyclonal antibody against the carboxyl terminus of human PanX1 cDNA
123 encompassing the entire coding region was synthesized by PCR amplification of the
124 cDNA insert from clone IMAGE: 4390851 using two gene-specific primers (Px1F: 5'-
125 TCTGGATCCTACACGCTGTTTGTCCA-3'; Px1R: 5'-
126 TCTAAGCTTGCAAGAAGAATCCAGAAG-3'). It was then inserted into the BamHI
127 and HindIII sites of pET-23a to yield the pETPx1 plasmid. The resultant 18 kDa
128 pETPx1 protein fused to the His-tagged C-terminus was purified from *E. coli* and
129 used for immunization of the rabbit. The affinity purified rabbit serum containing
130 specific anti-human PanX1 activity and lacking any significant non-specific activities
131 was used for the immunohistochemistry. In the present study, we verified the
132 specificity of the two PanX1 antibodies we used with their immunogenic peptide in
133 western-blot and immunohistochemistry experiments (see results). The sequence of
134 the peptide corresponds to the 135 last amino-acids (C-ter) of the human PanX1
135 protein. Blasting this peptide sequence shows its high specificity for PanX1. The
136 specificity of PanX1 antibody was tested by peptide pre-adsorption: 1/2000 anti-
137 PanX1 was pre-incubated for 2 h at room temperature with 1/400 of immunizing
138 peptide and then used for western-blot of 30 µg of protein extract from adult mice
139 cerebellum. The same peptide was used in immunohistochemistry with the same pre-
140 incubating procedure except that the PanX1 antibody was used at 1/750 and
141 blocking peptide dilutions were 1/750, 1/150 and 1/75. Both anti-PanX1 antibodies
142 gave similar results. Therefore, data obtained with these two different antibodies were
143 pooled.

144

145 **Immunostaining for light microscopic analysis**

146 Cerebellar sections were processed for PanX1, calbindin (CaBP), Zebrin II
147 expression analysis. Sections were first incubated in blocking buffer containing PBS-
148 T-G (PBS 0.1%, Triton X-100 0.25%, Glycine 0.75% and gelatine from porcine skin
149 0.25%, Sigma, UK) for 1 hour at room temperature. Following three washes in PBS-
150 G (PBS-T-G, without Triton), sections were incubated for 72 hours at room
151 temperature in a mixture of the primary antibodies. Following three washes in PBS-G,
152 the slices were incubated at room temperature for 2 hours with a mixture of
153 secondary antibodies containing: anti-mouse Alexa fluor (AF) 546 (1:250) and anti-
154 rabbit AF488 (1:250), both from Molecular Probes (USA). Following a final wash in
155 PBS-G, the sections were air-dried and mounted in a homemade Mowiol-based
156 medium. Images were acquired with a LSM510 or LSM710 microscope (Zeiss) and
157 processed with the ImageJ software (public domain: <http://rsbweb.nih.gov/ij/>). Final
158 montages were made with Adobe Photoshop.

159

160 **Results**

161 **Distribution of PanX1 immunoreactivity: rostrocaudal** 162 **gradient and parasagittal stripes.**

163 We first verified the specificity of the PanX1 antibodies using western blot and
164 immunohistochemistry. In western blots of mouse cerebellar extracts, the PanX1
165 antibodies recognize a single ~ 43 kDa band as expected for PanX1 in brain extracts
166 [11]. This band disappeared after incubating the antibodies with a saturating
167 concentration of their immunogenic peptide (Fig 1A). In the immunohistochemistry
168 experiments, the pattern of PanX1 labeling observed in sagittal cerebellar sections

169 vanished with increasing concentrations of the blocking peptide in a dose-dependent
170 manner (Fig 1B-E). We also verified that the blocking peptide did not interfere with
171 the immunolabeling procedure by testing it with an antibody directed against
172 parvalbumin (Fig 1F). As expected, the peptide did not affect the parvalbumin
173 labeling.

174 We next used the anti-PanX1 antibodies to assess the distribution of PanX1 in
175 the cerebellum. As a marker of Purkinje cells, we used the calcium binding protein
176 calbindin-D28k (CaBP). CaBP labeling also allowed us to verify the aspect of these
177 cells and the quality of intracardial fixation. Confocal images of sagittal cerebellar
178 slices made in the vermis revealed that PanX1 antibodies strongly label the soma
179 and dendrites of Purkinje cells (Fig 2A), as previously observed [10, 34]. The
180 labeling was spotty and more concentrated in proximal dendrites (Fig 2A,B). In distal
181 branches, it was also detectable in the vicinity of spines at the level of the thin spiny
182 branchlets (Fig 2B top insets). Remarkably, numerous PanX1 spots seemed to be
183 located outside Purkinje cells themselves, in close apposition to their dendrites. This
184 is suggestive of a possible PanX1-mediated communication between Purkinje cells
185 spines and their afferences (Fig 2B top insets). Molecular layer interneurons were
186 negative for PanX1 labeling (Fig 2B). The radial processes of some - but not all -
187 Bergman glial cells were immunoreactive for PanX1 (Fig 2B). Granule cells were
188 devoid of labeling, but longitudinal sections revealed the presence of PanX1 in round
189 cell bodies of the granular layer, presumably from Golgi cells (Fig 2C). In the deep
190 cerebellar nuclei (DCN), Purkinje cell axons were strongly immunoreactive for
191 PanX1, and some large cells immunopositive for CaBP were also reactive for PanX1
192 antibodies (Fig 2D). All these observations are in agreement with previous literature
193 [7, 10, 35, 36].

194 Intriguingly, and in contrast with previous data, PanX1 labeling of Purkinje
195 cells was almost absent in the anterior cerebellar lobules, although well-shaped
196 Purkinje cells were present, as attested by CaBP labeling (Fig 3A). Mosaic
197 parasagittal images of entire cerebellar sections (Fig 3B) revealed a clear rostro-
198 caudal gradient of PanX1 labeling, in contrast to the homogeneous CaBP labeling.
199 This was particularly obvious in the vermis, when comparing the rostral lobule III (Fig
200 3A) with the caudal lobule IX (Fig 3C). In the central and posterior lobules, PanX1
201 immunofluorescence was discontinuous, as PanX1-immunopositive zones were
202 intermingled with regions of PanX1-immunonegative Purkinje cells. Staining in the
203 lobule X was constant among slices and more pronounced than in the other lobules.

204

205 **Distribution of Pannexin1 labeling matches that of Zebrin II.**

206 The rostro-caudal heterogeneous expression pattern of PanX1 that we
207 observed in sagittal sections is reminiscent of that of the several tens of proteins
208 expressed in zebra stripes in the cerebellum (reviewed in [21]), the most studied
209 being Zebrin II [22], identified as the Aldolase C enzyme [37]. The very name "Zebrin"
210 comes from the zebra-like pattern that clearly appears in longitudinal sections.,

211 Labelings of PanX1 in longitudinal sections displayed a striking zebra-type
212 distribution, while that of CaBP appears homogeneous attesting the actual presence
213 of Purkinje cells in the overall slice (Fig 4). In those longitudinal sections, the flocculi,
214 paraflocculi and Crus II regions consistently appeared PanX1 positive (Fig 4A,D),
215 while lobules I to V displayed several little stripes of few immunopositive Purkinje
216 cells (Fig 4 B,C).

217 The profile of PanX1 distribution in rostral longitudinal section being typical of
218 that of Zebrin II, we made double labelings of these two proteins to compare their

219 respective distributions using large scale mosaic imaging (Fig 5).

220 In parasagittal sections, the overall distribution of PanX1 matched that of
221 Zebrin II (Fig 5A). This was clear from lobule VIb to the posterior tip of lobule VII, and
222 in lobules VIII to X, where Purkinje cells were almost uniformly labeled. In other
223 lobules, there was sometimes a difference in the intensity of staining, which was
224 likely due to different subcellular distributions of Zebrin II and PanX1. However,
225 examination at higher magnification showed that the boundaries of strongly
226 immunoreactive bands and weakly immunoreactive (or unreactive) bands were very
227 similar for these two markers.

228 We made longitudinal sections in the rostral, medial and caudal parts of the
229 cerebellum to verify if PanX1 and Zebrin II distributions matched over the whole
230 antero-posterior structure (Fig 5B-D). In rostral regions, most Purkinje cells were
231 Zebrin II-negative and PanX1-negative (Fig 5B). In the vermis, the midline and the
232 two lateral Zebrin II-positive bands on each side (presumably P1+, P2 +, P3+
233 according to [38]) were PanX1-positive and were separated by broad PanX1-
234 negative bands (lobule III or IV). In medial and caudal sections, staining in lobules
235 VIb, VII and X was uniform and dense across Purkinje cells (Fig 5C and D). In lobule
236 IX, the midline and the two lateral Zebrin II-positive bands were also PanX1-positive
237 (Fig 5D). Sections through the posterior and the nodular cerebellum showed perfect
238 match between PanX1- and Zebrin II-positive Purkinje cells (Fig 5D).

239 In conclusion, the distribution of PanX1-positive Purkinje cells matches that of
240 Zebrin II-positive cells either in rostral, medial or caudal parts of the cerebellar cortex.

241

242 **Discussion**

243 In the present study, we detect PanX1 in Purkinje cells, DCN cells, Bergmann

244 glia processes and in scattered cells in the granular layer (likely Golgi interneurons)
245 [7, 9, 10, 35, 36]. Our results are in agreement with previous literature for all cell
246 types expressing PanX1. However, we evidence for the first time a rostro-caudal
247 gradient in the distribution of PanX1 in cerebellar Purkinje cells. In our experiments,
248 PanX1 were almost undetectable in the anterior lobules but strongly labeled in the
249 caudal lobules, particularly in the posterior zone. This distribution matches that of the
250 standard in the subject, the Zebrin II protein, which is expressed according to a
251 striped pattern, highly reproducible and highly conserved across evolution [21]. To
252 our knowledge, the antibodies that we used here had only been tested before in the
253 retina [11], but never in the cerebellum. In our western-blot, made on adult
254 cerebellum protein extracts, the anti-PanX1 antibodies detect a 43 kDa band as
255 expected for PanX1. In addition, we used the corresponding immunizing PanX1
256 peptide pre-adsorbed and incubated with the PanX1 antibody to verify specificity,
257 both in western-blot and in immunohistochemistry. In this latter set of experiments,
258 the dose-dependent blocking effect of the immunizing peptide further confirmed the
259 validity of our data.

260 Why the specific localization of PanX1 in Zebrin II-positive Purkinje cells has
261 never been reported before is an intriguing question. It may be that our antibodies
262 recognize a different subset of PanX1 isoform(s) compared to previous studies.
263 Several protein modifications explain the various banding patterns observed in
264 western-blot, such as pre- and post-translational modifications (glycosylation, S-
265 nitrosylation or caspase-mediated cleavage). PanX1 seems to be subjected to
266 numerous splicing modifications. Besides the full-length variant named PanX1a (48
267 kDa), alternative splicing of the human or rat PanX1 encoding genes generates at
268 least four variants (PanX1a-d), of predicted molecular weights of 48, 40, and 34 kDa

269 for PanX1a, 1c, and 1d respectively [39]. PanX1 can also be glycosylated and S-
270 nitrosylated, which modulates their surface expression and activity [34, 40]. This
271 could explain discrepancies between our study and the others published so far. Cone
272 *et al.* [34] made an elegant comparative analysis of labelings obtained with four
273 different antibodies in the adult mouse cerebellum, in which they show that different
274 polyclonal antibodies actually detect distinct species of PanX1. These antibodies
275 revealed various sub-cellular and cellular localization of PanX1, depending on the
276 epitope design. Our antibodies likely target a specific species that is restricted to
277 Zebrin II-positive Purkinje cells. PanX1 isoforms with molecular weight around 43
278 kDa, like ours, likely corresponds to non - or weakly - glycosylated forms that tend to
279 be more confined to intracellular compartments [34] but can also reach the
280 membrane in some conditions [34, 41]. The species that we detect here seems to
281 reach the membrane because dendrites and spines were heavily stained in our
282 immunohistochemistry experiments.

283 The distribution of PanX1 proteins in zebra stripes is intriguing,. PanX1 have
284 been so far essentially implicated in pathologies. They have been shown to be
285 activated by pathological conditions such as epileptic seizures or infection [42, 43]. In
286 turn, once activated, they contribute to worsen these conditions. Their activation can
287 engage the inflammasome and trigger apoptotic processes [44, 45] (see also [46]).
288 Zebrin II-positive Purkinje cells better resist to infectious and chemical injuries than
289 their Zebrin II-negative sisters and resist better to cell-death. Therefore, the
290 preferential localization of PanX1 in Zebrin II bands seems rather paradoxical, unless
291 the specific species of PanX1 we have evidenced there display a higher threshold for
292 activation. In this case, they would be less activated in pathological condition than
293 their Zebrin-II negative counterparts, thus rendering the Zebrin-II positive cells more

294 resistant to insults. However, this remains an unsubstantiated hypothesis.

295 Another picture emerges if we consider instead the role of pannexins in normal
296 conditions [3], rather than focus on their deleterious effects. PanX1 exert some of
297 their paracrine functions as ATP channels. ATP, which in turn activates PanX1, can
298 be hydrolyzed into ADP, AMP, and eventually in adenosine by ectonucleotidases.
299 These molecules are agonists of purinergic and adenosine receptors, known to
300 modulate neuronal activity in the cerebellar cortex [47, 48]. For example, adenosine
301 receptors A1R, expressed at both pre- and post-synaptic sides glutamatergic
302 afferences on Purkinje cells, decrease glutamate release [49] and inhibit long-term
303 depression [50]. Thus pannexins could trigger the activation of presynaptic A1R by
304 releasing ATP - further converted into adenosine - and reduce glutamate release. In
305 this scheme, PanX1 would participate to a negative feedback control of synaptic
306 transmission. Such a mechanism would provide a homeostatic regulation of neuronal
307 transmission in the cerebellum, as has recently been suggested elsewhere [51]. The
308 presence of PanX1 in Zebrin II-positive regions could thus help neurons adapt to the
309 increased activity observed in these microzones. It is striking that the majority of the
310 molecules present in parasagittal bands are directly involved in synaptic
311 transmission, control of neuronal activity, or cell responses to the associated
312 metabolic demand. The specific species of PanX1 present in zebrin II-bands may
313 well contribute to this adaptation.

314 In conclusion, we can hypothesize that the PanX1 species we have evidence
315 here provide Zebrin II-positive Purkinje cells with additional tools to adapt to the
316 specific physiology of this zone characterized by increased glutamate release,
317 enhanced metabolic demand and better resistance to cell-death. All these
318 possibilities remain hypotheses for the moment, but our data open new perspectives

319 that call for further investigation.

320

321 **Figure Legends**

322

323 **Fig 1. Specificity of the anti-PanX1 antibody.**

324 Western blot of mouse cerebellum extracts labeled with the PanX1-antibody.

325 **A:** The two left lanes correspond to PanX1 antibody alone (- BP); the two right lanes
326 correspond to PanX1 antibody + blocking peptide (+ BP). **B:** Confocal images of
327 immunohistochemical labelings of PanX1 without blocking peptide. **C-E:** Confocal
328 images with the same antibody incubated in the presence of increasing
329 concentrations of the blocking peptide (dilutions as indicated). **Images B to E** were
330 taken from Lobule X. **F:** Immunolabeling of parvalbumin (PV) protein made in the
331 presence of the PanX1-blocking peptide (1/150). This control indicates that the
332 blocking peptide does not inhibit any immunohistochemistry reaction but that directed
333 against PanX1. Scale bar: 50 μ m.

334

335 **Fig 2. Cellular distribution of PanX1 in the cerebellar cortex.**

336 **A:** Confocal images acquired in a PanX1 positive zone, PanX1 in green (top),
337 CaBP in magenta (bottom). **B:** At high magnification, Purkinje cell dendrites show
338 dense punctiform PanX1 staining, notably in the spiny branchlets (top inserts). Notice
339 the numerous PanX1 positive radial processes that are likely Bergman glial
340 processes (white arrows in merged image, top center and bottom). **C:** Labeling in
341 longitudinal cerebellar sections confirms Purkinje cell staining and reveals round
342 middle size cells immunopositive for PanX1 (white arrows heads), presumably Golgi
343 cells. **D:** Fastigial deep cerebellar nucleus. Some large cells are immunopositive for
344 PanX1 (in green) and for CaBP in magenta (center). Smaller cells are also positive
345 for PanX1 but negative for CaBP (merged image, right). Notice the intense labeling of

346 Purkinje cells axons. Scale bars in A : 50 μm ; in B : 20 μm and 10 μm (inserts); C
347 and : 50 μm .

348

349 **Fig 3. Localization of PanX1 in the cerebellum: dense labeling in Purkinje cells**
350 **of the posterior lobules.**

351 **A:** In lobule III, molecular layer displays very low PanX1 staining (green),
352 although Purkinje cells are well present (CaBP, in magenta, was used as a Purkinje
353 cell marker). **B:** Automated mosaic confocal images of sagittal slices from the
354 cerebellar vermis region. White squares indicate the location of the high
355 magnification confocal images shown in A (lobule III) and C (lobule IX). **C:** Purkinje
356 cells soma and dendrites in lobule IX display very strong PanX1 labeling. Scale bars
357 in A and B: 20 μm B; in C: 500 μm .

358

359 **Fig 4. Longitudinal cerebellar sections reveal striped PanX1 labeling.**

360 **A:** Mosaic reconstructions of CaBP (magenta) and PanX1 (green) labeling
361 show that PanX1 are distributed in subsets of several tens of Purkinje cells drawing
362 stripes in lobules I-V. **B, C** and **D** are confocal images made in the regions indicated
363 by white squares in A. Note the intense PanX1 labeling in Crus I/II and in the
364 paraflocculi (PFI). DCN, deep cerebellar nucleus, Roman numbers indicate to
365 corresponding lobules. Scale bars in A: 500 μm , B: 100 μm , C and D: 50 μm

366

367 **Fig 5. PanX1 and Zebrin II distribution in Purkinje cells match throughout the**
368 **cerebellum.**

369 Images are mosaic reconstruction made from confocal images. **A:** Zebrin II
370 and PanX1 immunolabeling colocalize in Purkinje cells in a sagittal cerebellar slice

371 taken in the vermis. **B**: Same colocalization observed in a longitudinal slice made in
372 the rostral part of the cerebellum. **C**, **D**: Same as in B but in the medial and caudal
373 parts of the cerebellum respectively. Lobules are indicated as Roman numbers; PFI,
374 paraflocculus; Sim, *lobulus simplex*; Cop, *copula pyramidis*; PM, *lobulus*
375 *paramedianum*; FN, fastigial nucleus. Scale bars in B to F: 500 μ m.

376

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383

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