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Supplemental information

Recessive *PRDM13* mutations cause fatal perinatal brainstem dysfunction with cerebellar hypoplasia and disrupt Purkinje cell differentiation

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Supplemental document S1: Detailed description of affected individuals and genetic findings

Family 1

Proband **F.1-1** was a female born from consanguineous parents of Tunisian origin. In the familial history, we can only note a complete removal of a basocellular carcinoma of the left eye in the mother. During the pregnancy, the second ultrasound screening identified bilateral foot malposition at 22 weeks of gestation (WG), confirmed by a specialized ultrasound examination at 26 WG; the latter exam also revealed mild cerebellar hemisphere hypoplasia and marked vermis hypoplasia. Fetal movements were found normal as well as amniotic fluid quantity. Fetal MRI at 30 WG identified wide pericerebellar spaces, vermis surface and cerebellar transverse diameter below the 3rd centile with abnormal shape of the fourth ventricle, not detectable primary and secondary fissures and smooth cerebellar hemisphere outline. Proband **F.1-1** was born at term (39 WG) by instrumental vaginal delivery, Apgar score was 1 and then 7, and birth weight was 3200g. She presented with marked hypotonia and major feeding difficulties with no sucking abilities, posterior cleft palate, microretrognathia, a supernumerary thumb flexion crease, an occipital angioma and normal patellar reflexes. Neurologic examination quickly evolved from a generalized hypotonia to a peripheral hypertonia and important reflex motor activity. At age 7 months, she already had numerous episodes of fainting without identified trigger probably due to either saliva inhalation or central origin. Oral feeding was not possible and nasogastric feeding was used in the first weeks of life. A scopolamine patch therapy was inefficient and led to position the baby lying on the belly to avoid complications. Head control was not acquired, she could somewhat smile and babble. Eye tracking was still intermittent. She had normal growth parameters (weight -0.8SD, median height, OFC -0.5SD). She had sleep disturbances with no more than 2-hour periods of sleep, and she required oxygen therapy. She presented round face, telecanthus, marked philtrum crease, retrognathia, marked palmar creases, tented vermilion of upper lip, mildly highly implanted columella, pes varus and genu varum. She was followed by a multidisciplinary team. At age 11 months visual interactions remained inconstant. At age 25 months, milestones have not improved significantly, she experienced additional and more severe faintness during a viral pneumopathy, oxygen needs increased and she passed away as the multidisciplinary concertation team did not opt for resuscitation. During her medical

course, she underwent an ophthalmological examination with a suspicion of papillary edema or hypoplasia and either normal or not interpretable evoked visual potentials and electroretinography. Evoked auditory potentials, cardiac, abdominal ultrasound, pelvis X rays and transfontanellar ultrasound were all normal. Brain MRI at the age of 2 months was not contributive due to the movement artefacts and was not performed thereafter due to major risk of anesthesia complications. A first electroencephalography showed numerous left hemisphere slow and wide paroxysms without status epilepticus which did not require therapy as the follow-up did not find any paroxysmal activity. Several tests were performed and obtained normal results including electromyography, electrocardiography including 24 hour-recording, thyroid function, sialotransferrin profiling and array-CGH. Sleep recording identified numerous episodes of desaturation and chronic hypercapnia.

During the fourth pregnancy (fetus **F.1-4**), similar manifestations as the first pregnancy were detected by close ultrasound follow-up, as well as a small posterior cleft palate in a female fetus (normal ultrasound at 17 WG and anomalies detected at 22 WG). The parents opted for a medical termination of the pregnancy at 24 WG. Fetal examination confirmed the posterior cleft palate and also identified a trabecular ventricular septal defect.

For the sixth pregnancy (sibling **F.1-6**), an ultrasound follow-up detected a transverse cerebellar diameter at 18th centile at 19 WG and below 5th centile at 21 WG; the second examination at 21 WG could also detect the vermis hypoplasia. At 24 WG, the same signs were found but also polyhydramnios (index = 25cm), probable right kidney duplication, but no cleft palate; it is noteworthy that examination conditions were incomplete. The parents chose to go on with the pregnancy. At 28 WG, the cerebellar biometric parameters decreased with transverse diameter below the 3rd centile, vermis hypoplasia and wide peri-cerebellar spaces as well as beginning of bilateral foot malposition but normal sucking movements and normal amniotic fluid quantity. At 33 WG, the ultrasound showed correct development of cerebellar hemispheres but marked vermis hypoplasia and normal supratentorial structures, fetal movements and amniotic fluid quantity were normal. The brain MRI at 33 WG also found the marked vermis hypoplasia with probable incomplete fissure development, wide pericerebellar spaces, brainstem hypoplasia with mildly pronounced inferior pontine sulcus, mild tapering of medulla oblongata and of cerebral peduncles; no other cerebral anomalies were detected. Similar findings were identified at the

last ultrasound examination at 37 WG. The patient had a similar clinical course in the neonatal period and the first weeks of life and passed away at age 4 months.

WES of F.1-1 and parents identified predicted deleterious variants in two genes not previously associated with Mendelian disorders. Compound variants found in *RGPD3*, NM_001144013.1:c.364G>C (p.(Ala122Pro)) and NM_001144013.1:c.2081A>T (p.(Asp694Val)) were excluded after segregation analysis. The remaining variant was in the *PRDM13* gene.

Family 2:

The first pregnancy of this healthy consanguineous couple with no familial history of neurological disorders resulted in a late miscarriage at 21 GW for a triple pregnancy obtained by *in vitro* fertilization. During the second pregnancy (case **F.2-1**), ultrasound at 26 GW showed on the infra-tentorial space cerebellar hypoplasia with abnormal foliation and brainstem dysgenesis. The pregnancy was terminated at 31 GW. Pathological examination did not observe external or visceral malformation. Brain examination showed a normal size and development of the cerebrum with normal gyration development, marked hypoplasia of the cerebellum with poor lateral expansion. Neuropathological examination revealed a lack of development of the cerebellar cortex with low cellular density in the internal granular layer. Numerous Purkinje cells heterotopia in the white matter and the superior cerebellar peduncle were observed. The dentate nucleus was pachygyric and broken up and a residual rhombic lip with attached to the nodulus. The brainstem showed hypoplasia of the inferior olivary and pontine nuclei. Cranial nerves were correctly placed and pyramidal tracts were normal. Overall, the pathological exam diagnosed a form of pontocerebellar hypoplasia. Chromosome analysis by array, *TSEN54* gene sequencing as well as custom gene panel analysis for cerebellar congenital disorders were normal. During the third pregnancy (**F.2-2**) of this couple, the mother was referred due to recurrence of cerebellar hypoplasia detected by echography at 24 WG with unusual “foliation” vermis. The MRI at 29 WG confirmed hypoplasia of the hemisphere and pons and dysplasia of the vermis. The male infant was born at 34 GW, he weighed 2624 grams and measured 44 cm. The HC was 33.5 cm. His apgars were 4-7-8. He had hyperthelorism. He had no suction reflex, was hypotonic with poor motility, and abnormal movement of the limb. EEG was normal. Brain MRI reported reduced cerebellar and pons size. He had

a very mild respiratory distress syndrome related to prematurity. He received surfactant, was intubated for 48 h and then had pressor support and oxygenotherapy 25% for 48h. After day 4, the respiratory disease related to prematurity was over and the patient had spontaneous ventilation in ambient air. In the following days the frequency of apneas bradycardia increased and the child died at 22 days of life.

Family 3

The proband **F.3-1** was born at 39+0 to a healthy Pakistani couple by normal vaginal delivery, following spontaneous onset of delivery. Her APGARs were 9-10-10. She presented with intrauterine growth retardation, with 2300g and OFC of 33.5cm. The parents were told antenatally that the baby was small for gestational age. On the second day of live, she presented with unexplained neonatal bradycardia and hypothermia, requiring admission in Neonatal Intensive Care Unit (NICU) for presumed sepsis, but with negative microbiology. During the one month that she was admitted in NICU, she had recurrent signs of respiratory distress, bradycardias and hypoglycaemia. She also had hypothyroidism and at least one clinical seizure. Her cranial ultrasound was normal. Metabolic and virological screening were normal. Brain MRI scan EEG, ECG and ECHO performed in the neonatal period were normal. At 9 months of age she presented to Emergency with altered consciousness and unwell since morning with vomiting and lethargy. She was unresponsive on arrival needing a bag valve mask and had bradycardia. She was hypertensive. EEG showed no focal epileptiform changes. Urgent brain MRI scan reported pontocerebellar hypoplasia ("Abnormal posterior fossa structures consistent with a form of pontocerebellar hypoplasia. The supratentorial brain appears normal."). Full metabolic investigation was normal. Chromosome analysis by array-CGH was normal. On follow-up, diffuse cerebral dysfunction was observed in on EEG. Renal ultrasound scan identified grade 1 hydronephrosis bilaterally. Her albumin : creatinine ratios were just mildly elevated. She had mild proteinuria in one occasion. She was put on Amlodipine for her hypertension, achieving a good control. Further kidney ultrasounds including dopplers were reported as normal. Patient **F.3-1** had further admissions to Emergency presenting with unprovoked and unexplained episodes of encephalopathy, vomiting, floppiness and autonomic instability requiring pediatric intensive care unit admission (PICU). Subsequent clinic reviews and scans have noted that the pontocerebellar hypoplasia is

apparently non-progressive. She has hypotonia, mainly central, and a possible seizure disorder, with episodes of eye rolling and fast spikes in the posterior region, and she is on levetiracetam. She also presents with global developmental delay and diffuse cerebral dysfunction as demonstrated by EEG. She is making progresses, although slow and she does have increasing learning difficulties. She presents with high myopia and has jerky pursuit eye movements, with normal fundus. Patient F.3-1 had a Holter monitor at two years and ten months, which demonstrated sinus rhythm with very occasional marked sinus arrhythmia, frequent episodes of tachycardia in keeping with dysautonomia. Her blood pressure has normalised, her kidney function normalised and she does not require anti-hypertensive treatment anymore. Her intermittent high blood pressures and dysautonomia were considered to be due to her neurological issues. Her last measurements on 04/03/2021, aged 4 years and 6 months were: Weight: 12.2Kg - <0.4th centile; her height was 90.6cm - <0.4th centile; and her OFC was 46.4cm - below the 0.4th. The clinical genetics department was contacted again when the mother was pregnant with her second child; and we were asked to provide counselling. At this stage, we offered rapid whole exome sequencing using a trio analysis to **F.3-1**, and this has been reported as negative. **F.3-2** was born at 37+1 weeks via a normal delivery with approximately 2.37 kg, in good condition, and she was admitted to NICU as planned admission for observation. Unfortunately, she presented with neonatal autonomic problems, including temperature instability, bradycardic episodes and hypoglycemia. A recurrence was likely. The infection screening was negative. She had a suspected clinical seizure on day six and electric seizures on CFM at day seven onwards, and she has also been prescribed levetiracetam. Similarly to her elder sister, she presented with hypotonia, mainly central. However, she was less floppy than her sister. Rapid whole exome sequencing was done, and a genetic diagnosis was not identified. Her MRI scan showed that the cerebellar hemispheres are slightly small; but there was no other overt pontocerebellar hypoplasia. She now presents with developmental delay and slow weight gain. Her measurements on 24/02/2021, 7 months of age were: weight: 4.9kg - Centile: <0.4th (on -4SD), length: 62cm - centile: 0.4th. Parents reported no further paroxysmal episodes after discharge from NICU, and refer to satisfactory developmental progresses. She's also doing generally better than her sister; and she has not yet had admissions to ED or PICU with crises like her sister had. She had a normal 24h Holter.

Whole-exome sequencing (WES) was performed by the Exeter Genomics Laboratory identified homozygous variants in three previously unknown disease genes: ***C6orf163*** (Chr6(GRCh37), g.88060133G>T, NM_001010868.2: c.265G>T, p.(Ala89Ser)) ; ***CAPZA1*** (Chr1(GRCh37), g.113189893A>G, NM_006135.2:c.101A>G, p.(Asn34Ser)) and ***PRDM13*** (Chr6(GRCh37), g.100062367A>T; NM_021620.3:c.1856A>T, p.(His619Leu). All three variants were shared by the two affected girls. The variant in *CAPZA1* is known in dbSNP (rs202216073) and gnomAD with MAF<0.01% and its CADD score is 25. The variant in *C6orf163* has a CADD score of 26.6 and is absent from these databases. *PRDM13* variant is known to dbSNP (rs1173266789) and gnomAD (n=2) and its CADD score is 32. The three genes were added to GeneMatcher.

Family 4

The proband **F.4-3** was the third child of first cousin parents from Oman. He was born at term (40 wk+3) after a regular pregnancy, via C-section for breech presentation and abnormal CTG monitoring. At birth, Apgar score was 3 and 6 at 1 and 5 min, respectively. His birth weight and length were on the 10th percentile and his OFC was below 3rd percentile. He developed acute respiratory distress immediately after birth and evolved subsequently with recurrent apnea and hypoventilation episodes, likely of a central etiology; as he was ventilation dependent a decision was made to a tracheostomy. His severe post-natal failure to thrive was either related to the underlying etiology and/or to reduced caloric intake. He also had chronic constipation and recurrent vomiting. This patient presented with neonatal seizures and started an anti-epileptic treatment that was not associated with additional seizures. He had severe microcephaly with OFC below the 3rd percentile and dysmorphic features including low anterior hairline, with forehead localized hypertrichosis, thick eyebrows, narrow and short upslanting palpebral fissures, epicanthal folds, long fingers. Neurological examination showed axial hypotonia with distal hypertonia, spastic quadriparesis. The patient passed away the age of 16 months. Etiologic work-up were within normal limits, including karyotype, blood aminoacids, blood lactate, blood NH4+ and creatine kinase, urine orgainc acids, urine purines and pyrimidines, transferrin isoforms (CDG screening by HPLC), and acylcarnitine profile. Opthalmological examination, ECG and heart ultrasound examination were normal. To further explore the genetic cause of the phenotype, Clinical Exome was

done at BGI Europe and reported as normal. Reanalysis of the raw data in-house (Sultan Qaboos University hospital), reveled *PRDM13* high impact mutations. Sanger sequencing was used to confirm the mutation in the affected patient. The DNA from parents and healthy siblings were not available.

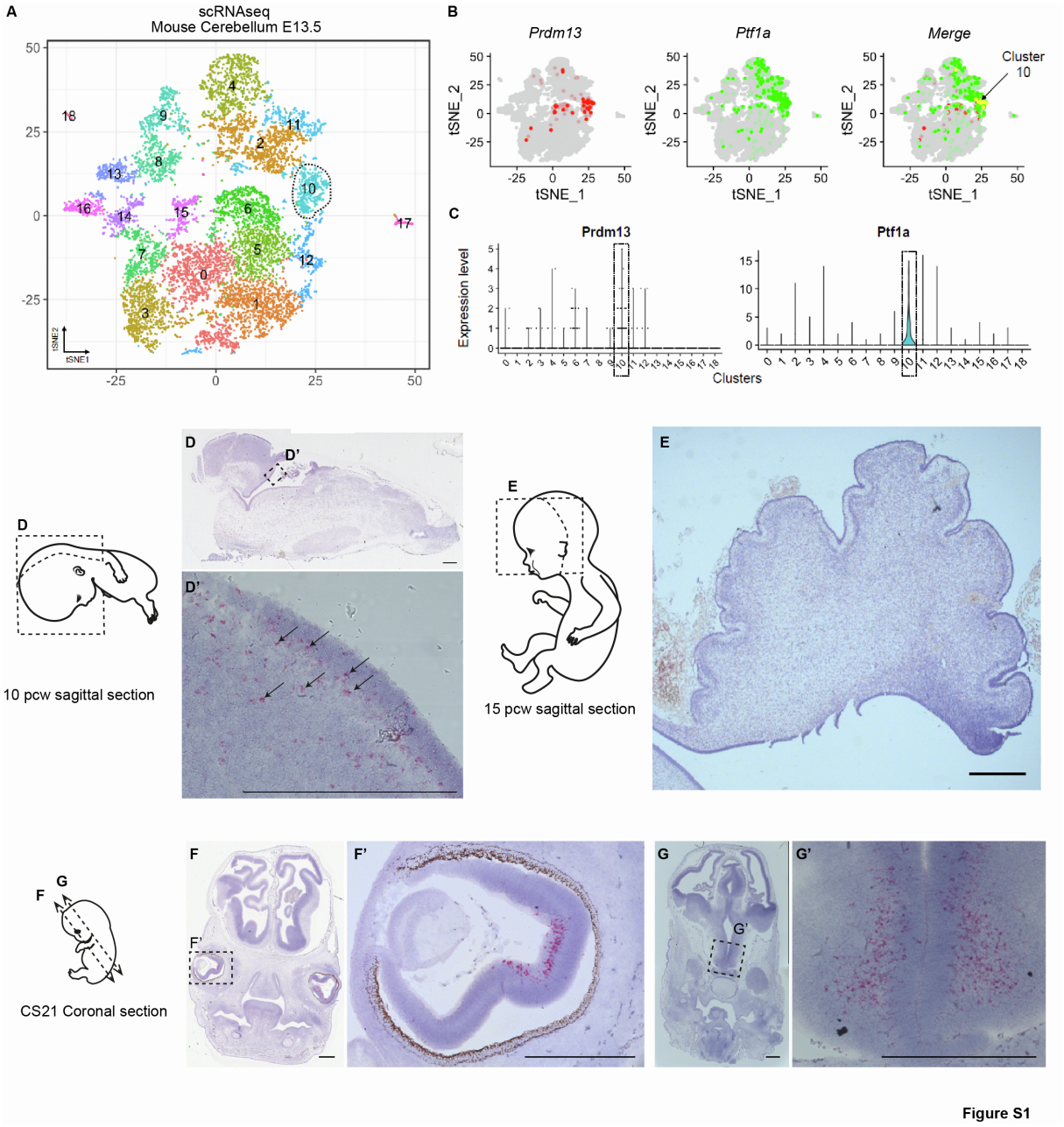

Figure S1

Figure S1 Expression of *PRDM13* during mammalian brain development

A. t-distributed stochastic neighbor embedding (t-SNE) projection plot of distinct cell populations obtained from 9,353 single cells from the mouse cerebellum at embryonic day 13.5 (E13.5). The single-cell RNA-seq dataset was retrieved from a publicly available repository stored in the GEO database under accession GSE120372[18] and re-analyzed using the Seurat package. Cells are color-coded by number of transcriptionally distinct clusters. **B.** *Prdm13* and *Ptf1a* expression levels displayed over the t-SNE projection plot (red, Prdm13-high cells; green, Ptf1a-high cells; yellow, overlap). **C**. Violin plot representation of *Prdm13* and *Ptf1a* relative expression levels across cell clusters. Cluster 10 is

characterized by high expression levels of both *Prdm13* and *Ptf1a*, which is a signature for cerebellum progenitor cells. **D-G**. Detection of *PRDM13* transcripts (red) by RNAscope in situ hybridization on sections through human embryos and fetuses. **D'**, **F'** and **G'** are higher magnifications of the region indicated by dotted lines in D, F and G respectively. **D.** Sagittal section through the brainstem at 10 PCW. *PRDM13* expression decreases and is maintained in scattered cells of the subventricular area (black arrows). **E.** Sagittal section through the cerebellum at 15PCW. *PRDM13* transcripts are no longer detected in the cerebellum at this stage. **F-G** Coronal section through a human embryo at CS21, showing the expression of *PRDM13* in the retina (**F**, **F'**) and hypothalamus (**G**, **G'**). Note the absence of *PRDM13* expression in cortical areas. Scale bars: 500 μm.

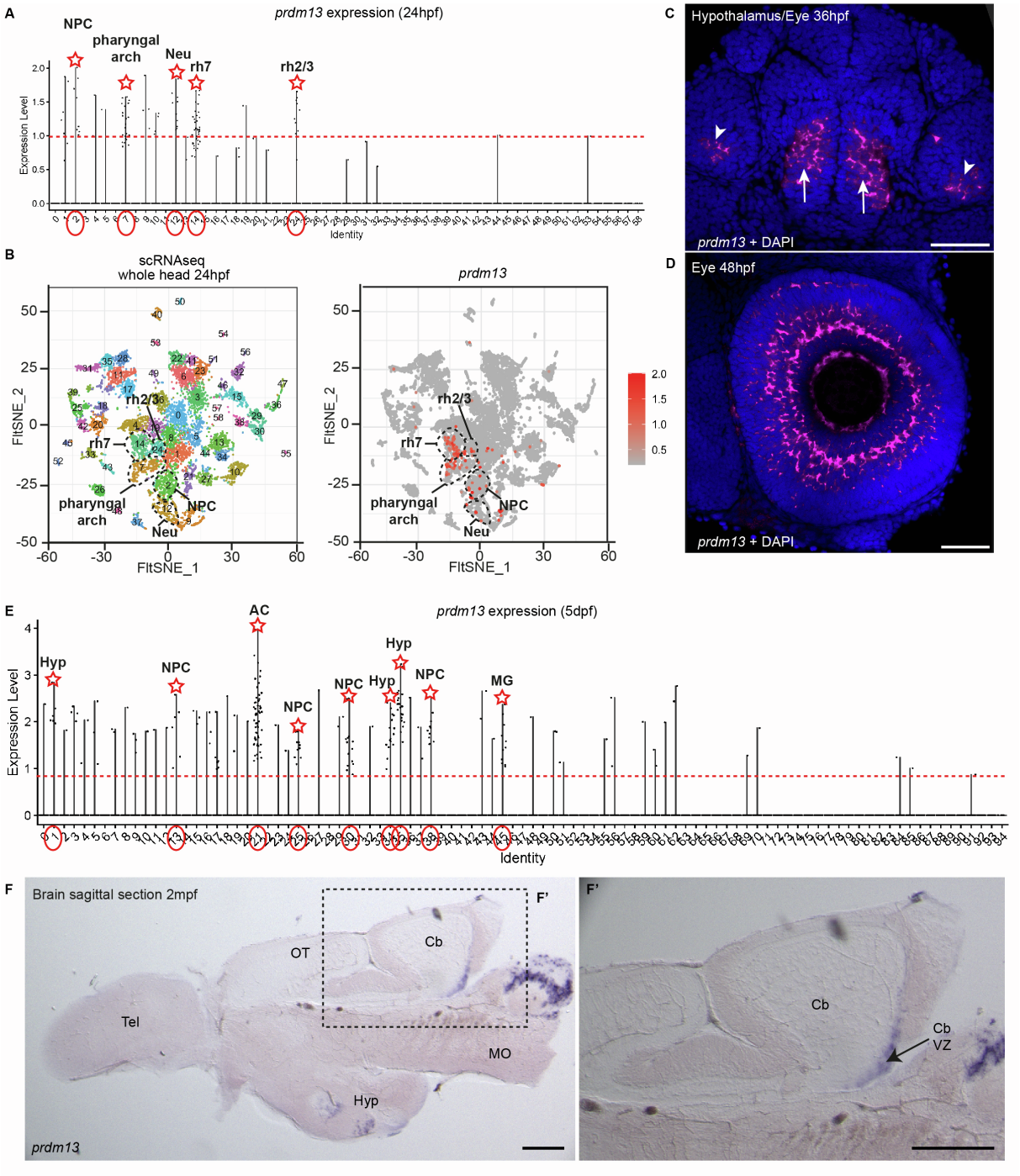

Figure S2 Expression of *prdm13* during zebrafish brain development

A. Violin plot representation of *prdm13* expression levels across single-cell clusters from the 24hpf zebrafish heads[19]. Clusters are numbered as in the original publication. Clusters in which *prdm13* expression is enriched are highlighted by red stars. They correspond to clusters 2, annotated as "committed progenitors" (NPC); 7, annotated as "pharyngeal arch"; 12, annotated as "neurons" (Neu); 14, annotated as "rhombomere 7" (rh7) and 24, annotated as "rhombomere 2/3" (rh2/3). **B**. t-distributed stochastic neighbor embedding (t-SNE) projection plots of distinct cell populations obtained from

zebrafish heads at 24hpf[19]. In the left panel, cells are color-coded according to cluster annotations from the original publication. In the right panel, cells are colored in graded intensities, reflecting the expression levels of *prdm13*. Clusters in which *prdm13* expression is enriched are circled with dotted lines. **C-D**. Optical z-plane showing *prdm13* expression (ISH, magenta) at 36hpf (**C**) in the developing hypothalamus (white arrows) and retina (white arrowheads) and at 48hpf in the inner nuclear layer of the developing retina (**D**). Cell nuclei are counterstained with DAPI (blue). Scale bar: 50µm. **E.** Violin plot representation of *prdm13* expression levels across single-cell clusters from the 5dpf zebrafish brain [19]. Clusters are numbered as in the original publication. Clusters in which *prdm13* expression is enriched are highlighted by red stars. They correspond to clusters 1, 34 and 35, annotated as "ventral forebrain" and "hypothalamus" (Hyp); clusters 13, 25, 30 and 38, annotated as "progenitors" or "radial glia" (NPC); cluster 21, annotated as retina (amacrine, GABAergic interneurons) (AC) and cluster 45, annotated as "retina (muller glia)" (MG). **F**. Sagittal section through the zebrafish brain at juvenile stage (2-month-old, 2mpf), showing the expression of *prdm13* in blue. **F'** is a higher magnification of the region outlined by dotted squares in F. *prdm13* transcripts are detected in the cerebellar VZ (Cb VZ; black arrow in F'), which maintains a neurogenic activity at this stage in zebrafish. *prdm13* expression is also detected in the hypothalamus (Hyp) and dorsal ventricular zone of the medulla oblongata (MO). Tel: telencephalon; OT: Optic tectum. Scale bar: 200µm.

Table S1. Cerebellar measurements

	24 GW		31 GW		37 GW	
	Control	**F.1-4**	**Control**	**F.2-1**	**Control**	**F.2-2**
Cerebellum plus brainstem weight (SD)	5.2 g (± 0.7)	4.0 g (-1.7 SD)	12.2 g (± 2.2)	7 g (-2.4 SD)	21.4 g (± 3.4)	13 g (-2.5 SD)
Total brain weight (SD)	111.9 g (± 17.3)	97.0 g (-0.9 SD)	229.5 g (± 29.8)	204 g (-0.8 SD)	366 g (± 50.3)	349 g (-0.3 SD)
Cerebellum plus brainstem / Brain ratio (SD)	4.6 (± 0.29)	4.1 (-1.7 SD)	5.24 (± 0.35)	3.4 (-5.2 SD)	6.1 (± 0.66)	3.7 (-3.6 SD)

Cerebellar and total brain weights for the affected cases F.1-4, F.2-1 and F.2-2 and their stage-matched controls. Control measurements are from the study of AM Guihard-Costa and JC Larroche. Early Hum Dev. 1990 Jun;23(1):27-40.