MINPP1 prevents intracellular accumulation of the chelator inositol hexakisphosphate and is mutated in Pontocerebellar Hypoplasia

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Inositol polyphosphates are vital metabolic and secondary messengers, involved in diverse cellular functions. Therefore, tight regulation of inositol polyphosphate metabolism is essential for proper cell physiology. Here, we describe an early-onset neurodegenerative syndrome caused by loss-of-function mutations in the multiple inositol-polyphosphate phosphatase 1 gene (MINPP1). Patients are found to have a distinct type of Pontocerebellar Hypoplasia with typical basal ganglia involvement on neuroimaging. We find that patient-derived and genome edited MINPP1−/− induced stem cells exhibit an inefficient neuronal differentiation combined with an increased cell death. MINPP1 deficiency results in an intracellular imbalance of the inositol polyphosphate metabolism. This metabolic defect is characterized by an accumulation of highly phosphorylated inositols, mostly inositol hexakisphosphate (IP₆), detected in HEK293 cells, fibroblasts, iPSCs and differentiating neurons lacking MINPP1. In mutant cells, higher IP₆ level is expected to be associated with an increased chelation of intracellular cations, such as iron or calcium, resulting in decreased levels of available ions. These data suggest the involvement of IP₆-mediated chelation on Pontocerebellar Hypoplasia disease pathology and thereby highlight the critical role of MINPP1 in the regulation of human brain development and homeostasis.

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inositol polyphosphate (IPs) comprise an ubiquitous family of small molecule messengers controlling every aspect of cell physiology. The best characterized is the calcium release factor inositol trisphosphate (I(1,4,5)P3 or simply IP3), a classical example of a second messenger, generated after receptor activation by the action of phospholipase C on the lipid phosphoinositide PIP2. Each of the six hydroxyl groups of the inositol ring can be phosphorylated, and the combination of these phosphorylations generates multiple derivatives. Among them, inositol hexakisphosphate (IP6, or phytic acid) is the most abundant in nature. In plants, IP6 is the most abundant inositol-polyphosphate species, reaching cellular concentrations of ~15–100 μM. IP6 is synthesized from inositol monophosphate (IP) or from IP3 by the action of several inositol phosphate kinases: IPMK (inositol-polyphosphate multikinase, also known as IPK2), IP3-3K (inositol -1,4,5-trisphosphate 3-kinase), ITPK1 (inositol tetrakisphosphate 1-kinase), and IPKK (inositol pentakisphosphate 2-kinase, also known as IPK1). Subsequently, the fully phosphorylated ring of IP6 can be further phosphorylated to generate the more polar inositol pyrophosphates such as IP7. While IP6, anabolism is well studied, its catabolism has been less characterized. Mammalian cells dephosphorylate IP6 through the action of the MINPP1 (multiple inositol-polyphosphate phosphatase 1) enzyme that is able to degrade IP6 to IP3.9. The analysis of mouse knockouts for the inositol kinases responsible for IP6 synthesis have highlighted an important role for this pathway in controlling central nervous system development, since knockout of Itpk1 or Ipmk is embryonically lethal due to improper neural tube development.10,11 In mammals, IP6 has been directly associated with a pleiotropy of functions, including iron homeostasis, control of mRNA export, DNA repair, and membrane dynamics.1 Moreover, IP6 is considered as a natural antioxidant since its iron-chelating property enables it to inhibit iron-catalyzed radical formation.12 Although not yet thoroughly studied, some of the physiological roles of IP6 could be related to its high affinity for polyvalent cations.13,14

To investigate the role of IP6 in mammalian physiology, many studies use IP6 exogenously added to cell lines in culture, often observing antiproliferative properties.15 These studies give little attention to the chelating property of IP6: cations-IP6 precipitation depletes the medium of essential ions such as calcium or iron. In addition, the physiological relevance of extracellular IP6 in mammals is not established. Extracellular pools of IP6 have only been demonstrated in a cestode intestinal parasite and several studies suggest that dietary IP6 cannot be absorbed as such through the digestive system and is absent from body fluids.16,17 Instead, de novo synthesis of IP6 occurs in all mammalian cells, including in the brain with high levels in regions such as the brainstem and striatum.18,19 The existence of several cellular pools of IP6 has been suggested.16,19,20 However, the dynamic regulation of the endogenous intracellular pools of IP6 is not fully understood, since its high cellular concentration precludes the determination of IP6 pool-specific fluctuations. Therefore, the exact function(s) of IP6 in cell homeostasis and mammalian development remain an area of intense investigation.

Several human diseases have been genetically associated with alterations in phosphoinositide (the lipid derivatives of inositol) metabolism.21 However, so far, no Mendelian disorder has been shown to be caused by an imbalance in the cytosolic inositol-polyphosphate pathway, with the exception of two variants in a gene involved in the conversion of the pyrophosphates forms of inositol, associated with hearing or vision impairment.22,23

Pontocerebellar hypoplasia (PCH) is a group of early-onset neurodegenerative disorders that includes at least 13 subtypes, based on neuropathological, clinical, and MRI criteria.24,25 PCH is usually associated with a combination of degeneration and lack of development of the pons and the cerebellum, suggesting a prenatal onset. The genetic basis is not known for all of the cases, and preliminary data from different PCH cohorts suggest that many subtypes remain to be identified. Based on the known molecular causes, PCH often results from a defect in apparently ubiquitous cellular processes such as RNA metabolism regulation and especially tRNA synthesis (i.e., mutations in EXOSC3, TSEN54, TSEN2, TSEN34, CLP1, and RARS2). Multiple additional conditions show neurological symptoms and imaging comparable to typical PCH syndromes and are caused by defects in diverse pathways involved in mitochondrial, glycosylation or purine nucleotide metabolisms. This observation further supports the disruption of ubiquitous pathways as the unexplained basis of these neurological conditions.25

In this study, we demonstrate that loss-of-function (LOF) mutations of the MINPP1 gene cause a specific PCH syndrome. We also show that the absence of MINPP1 leads to an abnormal accumulation of intracellular IP6. Using patient-derived cells, we observe that this increase in IP6 is associated with impairments in neuronal differentiation and survival. In addition, we find a deregulation of cytosolic cation (e.g., Ca2+, Fe3+) homeostasis when IP6 accumulates inside the cells. These observations suggest that the regulation of IP6 by MINPP1 is critical to preserve neuronal cation homeostasis.

Results

Loss-of-function mutations of the MINPP1 gene are associated with a distinct subtype of Pontocerebellar hypoplasia. To identify additional etiological diagnoses of patients with PCH, we explored a group of 15 probands previously screened negative with a custom gene panel approach.26 Whole-exome sequencing (WES) was then performed through trio sequencing (i.e., both parents and the proband). Among the candidate genes that were identified, the MINPP1 gene was recurrent and the most obvious candidate (Table 1 and Supplementary Note). The MINPP1 gene has not been previously associated with any Mendelian disorders. To assess how frequently MINPP1 mutations could be involved in PCH, we explored two other cohorts of pediatric cases with neurological disorders. The presence of MINPP1 mutations was investigated using a custom gene panel or WES (see “Methods”). Three additional families with MINPP1 biallelic variants were identified, all the affected being diagnosed with PCH

In total, biallelic variants in MINPP1 were identified in eight affected children from six unrelated families (Fig. 1, Table 1, Supplementary Fig. 1). These variants include homozygous early-truncating mutations in the families CerID-30 and PCH-2712, compound heterozygous missense and frameshift variants in the family CerID-11, a homozygous missense variant in the endoplasmic reticulum (ER) retention domain of the protein in the family CerID-09 and homozygous missense variants in the histidine phosphatase domain of the protein in the families TR-PCH-01 and PCH-2456 (Fig. 1b, d). These four missense variants are predicted to be disease-causing using MutationTaster and SIFT27, and involve amino acids fully conserved across evolution (Table 1, Fig. 1c). To predict the impact of the variants on protein structure, we utilized a phytase crystal structure of D. castellii and evaluated the consequences of the missense variants involving amino acids included in the model (Supplementary Fig. 1b). Tyr53Asp variant introduces a buried charge and disrupts a hydrogen bond with the donor amino-acid Ser299. The Phe228Leu substitution breaks a buried hydrogen bond with Lys241, both amino-acid positions are close to the IP6 binding site. The Arg401Gln substitution replaces a buried charged
The eight patients presented with almost complete absence of motor and cognitive development, progressive or congenital microcephaly, spastic tetraplegia or dystonia, and vision impairments (Table 2). For most of the patients, the first symptoms included neonatal severe axial hypotonia and epilepsy that started during the first months or years of life. Prenatal symptoms of microcephaly associated with increased thalami echogenicity were detected for the individual CerID-11, while the seven other patients presented with progressive microcephaly. For patients from the families CerID-09 and 11, the phenotype appeared to be rapidly progressive and the affected children died in their infancy or middle-childhood. Strikingly, all the affected children harbor a unique brain MRI showing a mild to severe PCH, fluid-filled posterior fossa, with dilated lateral ventricles. In addition, severe atrophy at the level of the basal ganglia or thalami often associated with typical T2 hypopersignal were identified in all the patients MRI (Table 2, Fig. 1a lower panel). This imaging is distinct from other PCH syndromes and thereby defines a subtype that we propose as PCH type 14.

**MINPP1 missense PCH mutations are deleterious for protein function.** The MINPP1 enzyme is predominantly localized in the ER lumen\(^{28}\). It removes phosphate groups from inositol-polypolyphosphate substrates starting at position \(329^{29,30}\), with high affinity for IP\(_3\) and IP\(_6^{31}\). Indeed, it has been described as the main mammalian phytase, or enzyme involved in IP\(_6^{32}\) degradation (Fig. 2a). Despite its name, this gene does not have any paralog in the human or mouse genome. In order to determine the effect of the patient mutations on the endogenous enzyme, we obtained skin fibroblasts from patients of the CerID-30 family. MINPP1 protein was undetectable in patients’ cells (Fig. 2b), supporting a complete loss of function of MINPP1 as the cause of this PCH subtype. The MINPP1 gene is widely expressed in the developing and adult mouse\(^{31,33}\), and rat\(^3\), as well as in human tissues (Supplementary Fig. 2a). This broad expression pattern suggests a general role for this enzyme in regulating inositol-polypolyphosphate metabolism. To investigate this role, we generated a HEK293T cell model KO for the MINPP1 gene (MINPP1\(^{-/-}\) HEK293) using genome editing (Fig. 2c). MINPP1\(^{-/-}\) HEK293 cells showed a 30% decrease in their growth rate after 48 h of culture, likely resulting from a proliferation defect, in the absence of significant difference in the binding of the apoptosis marker Annexin-V (Fig. 2d, Supplementary Fig. 2b, c). This defect was partially rescued by transient overexpression of WT MINPP1 (\(p=0.0029\); Fig. 2e, Supplementary Fig. 2d). Contrastingly, overexpression of two of the MINPP1 missense variants (i.e., Y53D and E486K) did not rescue growth, suggesting that these variants have a major impact on the protein function. MINPP1 has two predicted N-glycosylation sites (Fig. 1d). In order to evaluate the glycosylation status of the endogenous and over-expressed WT MINPP1 as well as the Y53D and E486K missense variants, we treated the protein extract with the PNGase enzyme\(^33\). A shift in the molecular weight after treatment, in all the samples, indicated that MINPP1 is indeed glycosylated without major impact of the missense variants (Supplementary Fig. 2e).

Patient and genome-edited MINPP1 mutant neural progenitors show an impaired neuronal differentiation with increased apoptosis. To investigate the mechanism at the origin of the neurological symptoms of MINPP1 patients, we derived induced pluripotent stem cells (iPSCs) from patient CerID-30-2...
(Supplementary Fig. 3a–e). In order to assess a contribution of the genetic background or other factors to the phenotype, we also generated MINPP1−/− iPSCs in isogenic background (Supplementary Fig. 3a–e). Surprisingly, a dual SMAD inhibition-based neural induction protocol33,34, did not allow the generation of viable neural progenitor cells for both MINPP1 mutant lines. Differentiation of patient-derived iPSCs systematically generated mixed cell populations with undefined HNK1 negative cells (Supplementary Fig. 3h). These observations suggest a critical role for MINPP1 during neuroectodermal induction in vitro, and led us to use a different protocol that preserved neural rosette environment, using only noggin as a SMAD inhibitor35 (Supplementary Fig. 3g). In these conditions, control cells efficiently differentiated toward TUJ1+ neurons after 14 days (Fig. 3a). In contrast, MINPP1 mutant lines showed a significant 53% decrease in TUJ1+ post-mitotic cells mirrored by a significant 2.9-fold increase in the number of PAX6+ neuronal progenitors (Fig. 3a, b). To test the presence of a neurodegenerative phenotype in our MINPP1 mutant cells, we assessed the apoptosis level using a TUNEL assay. We observed an increase in apoptosis at day 10 of neuronal differentiation that became more significant at day 14 with a 1.9-fold increase in the number of TUNEL+ cells among the MINPP1 mutant differentiating neurons, respectively (see Fig. 3c, d). Interestingly, we observed no significant difference in apoptosis levels among the undifferentiated iPSC lines (Fig. 3d). These results suggest that the differentiation of neural progenitors into neurons is specifically vulnerable to this inositol phosphate metabolism defect.

**Inositol-polyposphate metabolism is altered in HEK293 cells, iPSCs, and differentiating neurons mutated for MINPP1.** The localization of human MINPP1 into the ER, and the demonstration that its drosophila homolog (i.e., mipp1) is anchored to the plasma membrane outside of the cell36, prompted us to investigate the presence of phytase activity in conditioned media from control and MINPP1 mutant HEK293 cells. In conditioned medium from control cells, exogenously added IP6 was substantially processed after 2 h, and completely degraded after four hours (Supplementary Fig. 4a). Conversely, although partially degraded, IP6 is still detectable after six hours of incubation in MINPP1 mutant conditioned media. This result suggests that...
| Family | CerID-30 | CerID-30 | CerID-09 | CerID-11 | TR-PCH-01 | PCH-2712 | PCH-2456 | PCH-2456 |
|--------|----------|----------|----------|----------|-----------|----------|----------|----------|
| Subject | CerID-30-1 | CerID-30-2 | CerID-09-2 | CerID-11-1 | TR-PCH-01-1 | 2712-2-1 | 2456-5-2 | 2456-5-3 |
| Gender F/M | F | F | M | M | F | F | M | F |
| Origin (country) | Tunisia | Tunisia | Tunisia | France | Turkey | Egypt | Egypt | Egypt |
| Documented consanguinity | No | No | Yes | No | Yes | Yes | Yes | Yes |
| Age at last follow-up | 12 years | 9 years | 11 years | 18 months | 12 years | 2y10m | 4.5 years | 1.5 years |
| Weight at birth (kg) | 3.2 (M) | 4.2 (+2 SD) | 3.2 (M) | 2.4 (~2SD) | 3.710 | 3.0 (M) | 3.4 (M) | 3 (M) |
| Weight at last examination (kg) | 30 (~1.5 SD) | 23.4 (~0.5 SD) | 16.9 (~4SD) | 10 (~1SD) | 36 | 10.5 (~2SD) | 10.5 (~3SD) | 8.5 (~3SD) |
| Length at birth (cm) | 50 (M) | 51 (M) | 50.5 (M) | 43.5 (~3SD) | 50 | NA | 49.2 (M) | 47.5 (~1SD) |
| HC at birth (cm) | 34 (M) | 35 (+1SD) | 35 (M) | 30.6 (~3SD) | 36 | 33 (~1SD) | 34 (M) | 32 (~1SD) |
| HC at last examination (cm, age) | 48 cm (8 years; ~2SD) | 47 cm (4 years; ~2SD) | 47 cm (11 years; ~5SD) | 42 cm (~2,55) | 41 cm (~5SD) | 44.5 (~5SD) | 42 (~3SD) |
| Onset | Perinatal | Perinatal | Perinatal | Perinatal | Perinatal | Perinatal | Perinatal | Perinatal |
| Progressive encephalopathy | No | No | Yes | Yes | No | Yes | Yes | Yes |
| Posture: bedridden (B), sitting (S), walking (W) | B | B | B | B | B | B | B | B |
| Development | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Gross motor (normal/delayed/absent) | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Language (normal/delayed/absent) | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Social (normal/delayed/absent) | Absent | Absent | Absent | Absent | Delayed | Absent | Absent | Absent |
| Seizures | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Epileptic seizures | + | + | + | + | + | + | + | + |
| Onset | 7 years | 4 years | 5 years | 1 day | 2 months | 3 months | 5 months | |
| Neurological Findings | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Axial hypotonia | + | + | + | + | + | + | + | + |
| Distal hypertonia | + | + | + | + | + | + | + | + |
| Pyramidal signs | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia | Spastic tetraplegia |
| Extra-pyramidal signs | + | + | + | + | + | + | + | + |
| Ophthalmological findings | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Nystagmus | + | + | + | + | + | + | + | + |
| Abnormal ocular movement | + | + | + | + | + | NA | + | + |
| Optic atrophy | + | + | + | + | + | + | + | + |
| VEP/ERG | NA | ERG | VEP | ERG/VEP | NA | abnormal VEP, left eye | abnormal VEP | – |
| Others | Ptosis/ cataract | Ptosis/ cataract | Blindness | Ptosis | Cataract | Ptosis | Cataract | couldn’t follow object |
| Investigations | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Metabolic | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| EEG | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal | Abnormal |
| MRI | Age at last investigation (years (y); months (m); days (d)) | 2y7m | 8m2d | 1y11m | 8m23d | 4 m | 6y8m | 2.5 y | 1.5 y |
| Cerebellum | Hypoplasia | Hypoplasia | Hypoplasia | Hypoplasia | Hypoplasia | Hypoplasia | Hypoplasia | Hypoplasia |
| Pons | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy |
| Cerebral cortex atrophy | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy | Atrophy | atrophy | atrophy |
| Ventricles | Enlarged | Enlarged | Enlarged | Enlarged | Enlarged | Enlarged | enlarged | enlarged |
| Family                                      | CerID-30 | CerID-30 | CerID-09 | CerID-11 | TR-PCH-01 | PCH-2712 | PCH-2456 | PCH-2456 |
|--------------------------------------------|----------|----------|----------|----------|-----------|----------|----------|----------|
| Corpus callosum                            | −        | −        | Thin     | NA       | −         | Thin     | Thin     | Thin     |
| Basal Ganglia hypoplasia/atrophy           | +        | +        | +        | +        | thalamic atrophy | +       | +        | +        |
| Basal ganglia T2 hypersignal Spectroscopy  | +        | NA       | +        | +        | thalami   | +       | −        | −        |
| Others (WM defect)                         | WM       | WM       | NA       | NA       | WM        | −−       | −−       | −−       |
| Respiratory tract congestion               | No       | No       | Yes      | No       | Yes       | No       | Yes      | No       |
| Apnoea                                     | No       | No       | No       | No       | No        | No       | Yes      | No       |
| Impaired swallowing                        | Yes      | Yes      | Yes      | Yes      | Yes       | Yes      | Yes      | Yes      |
| Joint stiffness                            | Yes      | Yes      | Yes      | Yes      | No        | Yes      | Yes      | No       |
| Skeletal deformities                       | Scoliosis| Scoliosis| Scoliosis| Scoliosis| Scoliosis  | Scoliosis| scoliosis| No       |
| Cardiovascular findings                    | No       | No       | NA       | NA       | No        | recurrent urinary infection | Normal | Normal |
| Urine screening                            | Normal   | Normal   | NA       | Normal   | NA        | Normal   | Normal   | Normal   |
| Peripheral blood smear                     | NA       | NA       | NA       | Thrombocytosis | Normal | NA       | NA       | NA       |
| Muscle wasting                             | Yes      | Yes      | Yes      | Yes      | Yes       | Yes      | Yes      | Yes      |
| Facial dysmorphism                         | Yes      | Yes      | No       | No       | No        | Yes      | Micronathia| Non-specific small head, prominent nose, low set ear |
| Other                                      | NA       | NA       | Mild Normochromic, normocytic anaemia | Abnormal fat distribution | NA | BAEP: no response | −− | −− |

*EEG: electroencephalogram, ERG: electroretinography, HC: head circumference, M: median, NA: not available, VEP: visual evoked potential.*
MINPP1 accounts for the main secreted phytase activity of HEK293 cells.

To explore precisely a disruption in inositol phosphate metabolism, and to better address the role of MINPP1 in this metabolic pathway, we used tritium inositol (myo-[³H]-inositol) metabolic labeling of cultured cells, and analyzed inositol derivatives with SAX-HPLC (strong anion-exchange high-performance liquid chromatography) as previously described. We applied this method to HEK293 cells, skin fibroblasts, and iPSCs before or during neuronal differentiation at day 10.

Fig. 2 PCH-associated mutations of MINPP1 are deleterious for protein function. a Schematic representation of inositol phosphate cycle. myo-inositol (inositol); phosphatidylinositol (PI); phosphatidylinositol phosphate (PIP); phosphatidylinositol 4,5-bisphosphate (PIP₂); diacylglycerol (DAG); phospholipase C (PLC); inositol phosphate (IP); inositol 1,4,5-trisphosphate (IP₃); inositol 1,3,4,5-tetrakisphosphate (IP₄); inositol pentakisphosphate (IP₅); inositol hexakisphosphate (IP₆); inositol-polyphosphate multikinase (IPMK); I(1,4,5)P₃ 3-Kinase (IP₃-3K); inositol-phospholipase C (PLC); inositol phosphate (IP); inositol bisphosphate (IP₂); inositol 1,4,5-trisphosphate (IP₃); inositol 1,3,4,5-tetrakisphosphate (IP₄); inositol pentakisphosphate 2-kinase (IPPK); multiple inositol-polyphosphate phosphatase 1 (MINPP1).

b Western blot analysis of MINPP1 level in patient fibroblasts and HEK293 cells with β-Actin shown as loading control. Patient fibroblasts CerID-30-1 and CerID-30-2 (b) and MINPP1−/− HEK293 clones (c) show absent MINPP1. The uncropped blots are provided as a source data file and are representative of two independent experiments.  

c Assessment of cell proliferation rate. MTT assay was performed 48 h post-nucleofection. The data are presented as mean percentage relative to control (Ct) ± s.d., with triplicate determinations from four replicates. The normalization was done with 3 h MTT assay data (n = 4, one-way ANOVA, Tukey’s post hoc test). At 48 h Ctrl vs. MINPP1−/−: p = 0.0018; at 48 h Ctrl vs. MINPP1−/−; p = 0.0009). e MINPP1−/− HEK293 cells were transiently transfected with plasmids encoding empty vector, wild type, Y53D or E486K variant MINPP1. To assess the cell proliferation rate, MTT assay was performed 48 h post-nucleofection. The data are presented as mean percentage relative to control (Ct) ± s.d., with triplicate determinations from four replicates. The normalization was done with 3 h MTT assay data (n = 4, one-way ANOVA, Tukey’s post hoc test). At 48 h Ctrl vs. MINPP1−/−: p = 0.0018; at 48 h Ctrl vs. MINPP1−/−; p = 0.0009).

MINPP1 (referred to as day-10 differentiating neurons) from control and MINPP1 mutant cell lines. Exogenously added [³H]-inositol is imported into the cytosol and converted into phosphoinositide lipids before processing into inositol phosphates (IPs) (Fig. 2a). As expected, after 3 days of [³H]-inositol labeling, IP₆ was detected as the most, or the second most abundant intracellular inositol derivative in control cell lines (black hollow bars in Fig. 4), but was absent in the cell culture media (Fig. 4, Supplementary Fig. 4b). In all the cell models studied, the disruption of MINPP1 enzyme activity had a strong impact on...
intracellular IPs profile when compared with their respective controls. The investigation of MINPP1−/− HEK293 cells revealed a ~3-fold increase in IP$_6$ level, as well as an increase in IP$_4$ levels, and surprisingly a severe decrease in IP$_1$ and IP$_2$ levels (Fig. 4a).

Differences in the same direction, although to a lesser extent, were detected in patient fibroblasts (Fig. 4b) suggesting cell-type-specific differences. Indeed, in iPSCs, IP$_6$ levels showed a significant 1.6-fold increase in patient-derived MINPP1 KO iPSCs, also associated with an increase in IP$_4$ levels (Fig. 4c).

Finally, we studied differentiating neurons at day 10, a time point when differences in the TUJ1/PAX6 cell populations are not detected (Supplementary Fig. 3f). These data revealed significant 1.64-fold increases in IP$_6$ levels in MINPP1 mutant differentiating cells, respectively, with a trend or significant decrease in IP$_2$ levels (Fig. 4d). Interestingly, the defects in IP$_6$ and IP$_2$ levels in MINPP1−/− HEK293 cells were fully rescued, considering a ~70% transfection efficiency, by the transient overexpression of WT MINPP1 (Supplementary Fig. 4c, d). We also performed radiolabeling experiments on MINPP1−/− HEK cells with Y53D and E486K MINPP1 variants. The Y53D variant had no impact on the IP$_6$ and IP$_2$ levels, indicating the clear loss of enzyme activity (Supplementary Fig. 4c, d). However, the E486K variant did not affect the enzyme activity in this overexpression system, with apparently unchanged levels of IP$_6$ and IP$_2$. This result is in line with the previously demonstrated preserved enzyme activity in the absence of the ER retention signal.

In all the cell models tested, including HEK293 cells, patients’ fibroblasts, and undifferentiated and differentiated iPSCs, a
comparable imbalance of IPs levels were observed, where increase
in the amounts of higher inositol-polyphosphate derivatives IP5
and IP6 were associated with a decrease in lower-phosphorylated
IP2 and IP species (Fig. 4). Differences observed between the
various cell models tested are likely to be caused by cell-type
differences and potentially also by the genetic background.
Nevertheless, these observations clearly demonstrate the critical
role played by MINPP1 in cellular inositol-polyphosphate
homeostasis, with the conversion of higher to lower IPs. The
most robust finding was that IP6 is systematically increased in
MINPP1 mutant cells compared to controls. Altogether, these
observations exclude a major contribution of extracellular higher-
phosphorylated IPs to this metabolic defect, but highlight an
unappreciated role for MINPP1 in the regulation of the
intracellular pool of de novo synthesized IP6, the most abundant
inositol derivative.

**Fig. 4 MINPP1 absence leads to disruption in inositol phosphates metabolism.** a–d SAX-HPLC analysis of inositol phosphate levels in MINPP1−/−
HEK293 cells (a), patient fibroblasts (CerID-30-1 and CerID-30-2) (b), MINPP1 LOF (patient-derived (CerID-30-2 (blank circle) and MINPP1−/− (blank triangle)) iPSCs (c), and their day-10 differentiating neuron counterparts (d). The peaks ([3H]-IPn) were identified based on comparison to standards.

[3H]-IPn levels are presented as percentage of total radioactivity in the inositol-lipid fraction ([3H]-PIPn). All error bars represent standard deviation (s.d.).

(For (a), n = 2 and both experiments are represented. For (b–d), n = 4, two-tailed student’s t-test, *p ≤ 0.05 and **p ≤ 0.01). IPn, inositol phosphates; PIPn, phosphatidylinositol phosphates. Source data are provided as a source data file.

**IP6 accumulation can deplete free iron in presence of high iron condition.** Considering the strong impact of MINPP1 mutations on cellular IP6 levels, and the known chelator properties of this molecule, we hypothesized that MINPP1 defects can have consequences for intracellular cations homeostasis. An intracellular accumulation of IP6 could theoretically lead to the accumulation
of chelated cations inside the cell, potentially reducing the pool of free cations. At physiological pH, IP₆ has a strong binding affinity to iron⁴⁴, therefore we evaluated the ability of HEK293 cells to store iron, in low iron (−FAC) or high iron (provided with ferric ammonium citrate; +FAC) conditions. Then, we used a colorimetric ferrozine-based assay with a HCl/KmnO₄ pretreatment step that separates iron from its binding molecules to measure total intracellular iron⁴⁰,⁴¹. After 2 days of incubation with FAC, we observed a significant 1.5-fold increase in the total iron content in MINPP1−/− HEK293 cells, under high iron conditions compared to control (Fig. 5a). Although based on non-physiological iron conditions, this observation suggests that IP₆ could play a role in the regulation of metal ion cellular storage such as iron. To investigate a potential increase of iron chelation affecting the free iron cellular pool, we measured the cellular free Fe²⁺/³⁺ content using standard cell lysis and colorimetric assay. We detected a 58% depletion in total free iron levels in MINPP1 mutant cells under high iron conditions (Fig. 5b). Interestingly, this depletion was mainly contributed by a decrease in Fe³⁺ levels (Fig. 5c–d). IP₆, the major IPs accumulating in mutant HEK293 cells, is known to have higher affinity for Fe³⁺ versus Fe²⁺.⁴²,⁴₃ These data are consistent with a massive accumulation of complexed iron in the absence of the MINPP1 enzyme, in the presence of high iron, and suggest the potential involvement of IP₆-mediated abnormal cation homeostasis as the underlying disease mechanism.

**Discussion**

The direct physiological role(s) played by IP₆ in mammals has been difficult to define, due to the technical challenges associated with its measurement, and its complex anabolism. Furthermore, the well-described role of IP₆ (phytate) and phytase activity in plants and bacteria had led to the thinking that extracellular IP₆ degradation also supplies mammalian cells with phosphate and cations. On the contrary, we demonstrate that intracellular, not extracellular, IP₆ influences cation homeostasis. An imbalance of IPs derivatives has not been so far directly involved in disease, and the previously investigated Minpp1 KO mouse did not reveal any obvious phenotype.⁴³ Surprisingly, we discovered that the absence of MINPP1 in humans results in a very severe early-onset neurodegenerative disorder with specific features. Patients with loss-of-function mutations in the MINPP1 gene present with PCH associated with typical basal ganglia or thalami involvement identified by MRI. The prenatal onset of the phenotype is obvious for patient CerID-11, which supports a critical and early role for MINPP1 in neuronal development and survival. In agreement with this, we observed that patient-derived and genome-edited iPSCs mutant for MINPP1 cannot be differentiated toward neural progenitors that efficiently give rise to neurons. This defect is associated with a significant increase in apoptosis levels among the MINPP1 mutant cells. Interestingly, this cell survival defect could recapitulate the neurodegenerative phenotype observed in the patients. Although the exact mechanisms underlying the inefficient differentiation and the apoptosis have not been identified yet, the sensitivity of human differentiating neurons to the disruption of IPs metabolism is likely to be involved.⁴⁶

While a key role for MINPP1 in the regulation of IP₆ cellular levels has been investigated before⁴¹, we provide the first evidence for its critical importance on cellular physiology and human development. Our analysis of IPs profiles using metabolic labeling unambiguously identified a typical imbalance resulting from MINPP1 defect. The increase in IP₆ and IP₇ levels is consistent with a previous mouse model study⁴¹, but our more complete assessment of the IPs metabolism imbalance also revealed alterations in lower-phosphorylated IPs. Furthermore, the consequences of this metabolic block are associated with cell-type-dependent differences in the IPs profile, such as the IP₆ depletion in mutant iPSCs and robust IP₆ accumulation in day-10 differentiating neurons mutated for MINPP1.

The discrepancy related to the supposed mostly cytosolic localization of IP₆ and the ER localization of MINPP1 remains an unsolved problem.⁹,²⁹,³¹ Hypothetically, the specific subcellular localization of MINPP1 prevents IP₆ accumulation in a specific compartment (e.g., the ER) that would have primary consequences on local cation homeostasis. We identified that MINPP1-mediated IP₆ regulation impacts free cations availability, as
illustrated with the altered iron content of MINPP1−/− HEK293 cells as well as the severe depletion of cytosolic calcium identified in Minpp1−/− mouse primary neural progenitors and HEK293 cells. Interestingly, the absence of MINPP1 also severely disrupts signaling based on ER calcium that could potentially be the place of the primary defect. Calcium signaling has broad functions in neural cell physiology and brain development. Basal calcium levels influence neuronal physiology and cell survival47-49, and calcium signaling plays a role in neural induction and differentiation50-53. Consequently, a disruption in calcium homeostasis
could be involved in PCH disease pathogenesis. A link between MINPP1 and calcium regulation has been suggested previously but it was through the synthesis of I(1,4,5)P3. Hypothetically, coupling the limitation of IP_{3}-mediated chelation of calcium with the promotion of IP_{3} synthesis could be an efficient way for MINPP1 to regulate calcium signaling dynamics and homeostasis.

A mild or absent structural brain defect was also observed in other PCH mouse models. AMPD2 null mutations cause PCH9 but the Ampd2 single KO mouse is not associated with any obvious histological brain defect. CLP1 is involved in tRNA processing and mutated in PCH10, however, the Clp1 mutant mouse showed only a mild decrease in the brain weight and volume, a phenotype overlooked before the identification of patients with a brain phenotype. Differences in the phenotype of human and mouse with MINPP1 loss-of-function mutations could be related to an increased sensitivity of the human brain development to metabolic defects, although the impact of the genetic background cannot be excluded at this point.

Disrupted cation homeostasis, including metal accumulation, is central to multiple degenerative diseases such as neurogeneration with brain iron accumulation. Parkinson’s disease (manganese accumulation) and Wilson’s disease (copper accumulation) are usually suspected in PCH, however, the severe defects identified in MINPP1 patient MRIs suggest major neurodegeneration at the level of these subcortical nuclei, a feature not typically associated with other PCH subtypes. These structures are well known to be primarily affected by metal ions accumulation and further investigation will be needed to determine how cation chelation could contribute to the disease pathogenesis. Nevertheless, our results reveal an unappreciated basic role for highly phosphorylated IPs in cellular homeostasis which is critical during neurodevelopment.

Methods

Patients recruitment and investigation. The patients from families CerID-09, CerID-11, and CerID-30 included in this project were referred to the Departments of Pediatric Neurology, Genetics, Metabolism or Ophthalmology of the Necker Enfants Malades Hospital. Family TR-PCH-01 was recruited by the French Centre for Cerebellar Malformations and Congenital Diseases at Trousseau Hospital. Patients from families PCH-2456 and PCH-2712 were referred for neurological or genetic assessment at the National Research Center in Cairo and Hacettepe University Medical Faculty Department of Pediatric Neurology in Turkey. Written informed consents have been obtained both from the participants and the legal representatives of the children. Details regarding sequencing, filtering, and prioritization protocols for each family are outlined in Supplementary Note.

Protein multiple-sequence alignment. The following protein sequences are aligned through COBALT: Multiple Alignment tool: NP_004888.2 (H. sapiens MINPP1), NP_034929.1 (M. musculus Mmp11), NP_062136.1 (R. norvegicus Mmp11), NP_957394.1 (D. Reiro Mmp11), XP_02925472.2 (X. tropicalis Mmp11), XP_313302.4 (A. gambiae Mmp11), NP_957394.1 (D. Reiro Mmp11b), NP_989975.1 (G. gallus Mmp11), NP_034929.1 (M. musculus Mmp11), NP_062136.1 (R. norvegicus Mmp11), NP_368356.1 (A. thaliana histidine acid phosphatase family protein), NP_813655.1 (B. thalidomiamorcin Bmp11).

Maintenance and culture of human-induced pluripotent stem cells (iPSCs), fibroblasts, and HEK293 cells. All human cell culture and storage protocols were performed with approval from French Research Ministry (DC 2015-2595, 09/05/2016) and all participants provided written consent. iPSC lines Ctrl-D10 and CerID-30-2 were generated by Duke iPS Cell Share Resource Facility, using RNA-based reprogramming method. CerID-30-2 and Ctrl-D10 were derived from unrelated individuals. Ctrl-D10 iPSC line was derived from BJ fibroblasts of a non-disease affected male new born (ATCC cell line, CRL-2522). The control iPSC line (IMAGIN004, referred to as Ctrl-004 in this study and described at https://hpscreg.eu/cell-line/IMAGIN004-A) was generated by Imagine Institute iPSC Platform, using non-integrating Sendai virus approach (CytoTune-2.0). The ability to make iPSC clones during reprogramming (i.e., reprogramming efficiency) was assessed for the Ctrl-004 line, from which the KO is also derived. This showed a standard ~0.3% efficacy to generate clones on vitronectin. The cloning efficiency attained during the generation of MINPP1−/− iPSC lines is ~0.05% following nucleofection and sorting. All iPSC lines were assessed for embryonic pluripotency markers OCT4, SOX2, SSEA-4, and TRA-1-81 (Supplementary Figs. 3a, b and 6a, b). The absence of obvious chromosomal abnormalities was verified by CGH array 60 K (data not shown). The differentiation into mesoderm and endoderm lineage following embryoid body formation was assessed. In addition, no obvious differences in proliferation and apoptosis were observed among all the iPSC lines. iPSCs were maintained in vitronectin-coated (10 μg/ml; 07180, STEMCELL) 3.5-cm dishes (335001, Falcon) in complete mTeSR-1 medium (STEMCELL, 88580) supplemented with 1% Penicillin-Streptomycin (PS) (15140122, Gibco) in a humidified incubator (5% CO_{2}, 37 °C). The media were replaced daily and iPSCs were mechanically passed with ROCK inhibitor Y-27632 (10 μM; 72304, STEMCELL) every 7-8 days, with a 1:3 split ratio.

Human primary fibroblasts and HEK293T (HEK293) cells were cultured in Dulbecco’s modified Eagle medium (DMEM) (11960092, Gibco) supplemented with 10% Fetal Bovine Serum (FBS) (16000044, Gibco) and 1% PS in a humidified incubator (5% CO_{2}, 37 °C).

Generation of genome-edited MINPP1−/−. HEK293 and iPSCs. MINPP1−/− HEK293 and iPSC lines were generated via a CRISPR-Cas9 genome editing strategy, as previously described. Briefly, sgRNAs targeting the first exon of MINPP1 transcript variant 1 (NM_004897.5) were designed on CRISPRSite website (http://crisprsite.tor.es) and further cloned into the pSPcass(BB)-2A-GFP plasmid (PX658, 48138, Addgene). For the generation of MINPP1−/− HEK293 clones, transfection of pSPcass(sgRNA)-2A-GFP into HEK293 cells was performed with Lipofectamine 2000 (20166019, Invitrogen), as per manufacturer’s instructions (2.5 μg of DNA per well of a 6-well plate). Two days post transfection, single GFP+ HEK293 cells were sorted into 96-well plates by Fluorescence-activated cell sorting (FACS) (BD FACSAria II SORP, BD Biosciences). Indel mutations of clones were detected by Sanger sequencing, and target editing efficiency was assessed by TIDE analysis (https://tide-calculator.nki.nl; data not shown).

For the generation of MINPP1−/− iPSC clones, transfection of pSPcass(sgRNA)-2A-GFP into iPSC line (Ctrl-H104) was performed on AssayA 4D-Nucleofector X-Unit (AAL-100K, Lonza) according to manufacturer’s instructions. Briefly, iPSCs were pre-treated with Y-27632 (10 μM) for 1 h and further dissociated into single-cell suspension with Accutase (STEMCELL, 07920). 4×10^5 iPSCs were nucleofected per 20 μl nucleocuvette strip, using Primary Cell 4D X Kit S (V4XP-3032, Lonza), 1 μg DNA per reaction, and the program CA-127. After nucleofection, iPSCs were plated on vitronectin prep-coated 6-well plates and cultured in complete mTeSR-1 medium supplemented with Y-27632 (10 μM). Two days post transfection, GFP+ iPSCs were sorted by FACS and plated at low densities of 1–2×10^4 cells per 6 cm dish (Falcon, 30307) in mTeSR-1 medium supplemented with Y-27632 (10 μM). After 12–16 days, a subculture of individual colonies was performed using Quick-DNA/RNA Miniprep Plus Kit (D7003, Zymo) and Sanger sequenced. Target editing efficiency was assessed by TIDE analysis (https://tide-calculator.nki.nl; data not shown).

After clonal selection and expansion, no unusual chromosomal abnormalities were detected by CGH array (60 K, data not shown). Validation of MINPP1−/− iPSC clones were further assessed by the absence of MINPP1 using western blot (Supplementary Fig. 3c).

Generation of Minpp1−/− mice. Minpp1−/− mice were generated with the aid of LEAT platform of Imagine Institute by using a CRISPR/Cas9 system. In this study, animals were used in compliance with the French Animal Care and Use Committee from the Paris Descartes University (APAFIS#961-2015062317361). Guide RNAs (sgRNAs) targeting the first exon of the gene were designed via the CRISP-Targeting Interface (https://crisprtargeting.net/) and introduced into individual colonies was processed for DNA extraction using Quick-DNA/RNA Miniprep Plus Kit (D7003, Zymo) and Sanger sequenced. Target editing efficiency was assessed by TIDE analysis (https://tide-calculator.nki.nl; data not shown).

After clonal selection and expansion, no unusual chromosomal abnormalities were detected by CGH array (60 K, data not shown). Validation of MINPP1−/− iPSC clones were further assessed by the absence of MINPP1 using western blot (Supplementary Fig. 3c).

Generation of stable HEK293 cell lines. HEK293 cells were transfected with either FLAG-HA-empty or FLAG-HA-MINPP1 plasmids by performing lipofection with the Lipofectamine 3000 reagent (Invitrogen, L30000015) as per manufacturer’s instructions (2.5 μg of DNA per well of a 6-well plate und) under serum-free media. Six hours post transfection, the media were replaced with normal DMEM. For the culture growth above the 70% confluence, the media were fed every 2 days by serum-free media. Two weeks post transfection, the cell culture media were replaced with the media supplemented with the selection antibiotic geneticin (0.6 mg/ml, Life, 10131027) and continued to be replaced twice.
a week until the cells in the 6-well plate reach high confluence. At high confluence, the cells were passaged and continued to be cultured for 2–3 weeks with the cell culture media supplemented with the generation of clones was assessed by checking the presence of MNPPI1 with western blot.

**E14.5 mouse neural progenitor cell culture.** Cerebral cortices (both halves) from E14.5 mice were dissected in Dulbecco Phosphate Balanced Salt Solution (DPBS, 14190250, Gibco). All procedures were done at room temperature, unless otherwise stated. Cortices were then incubated for 15 min at 37 °C in neurocult medium basal medium (STEMCELL, 05700) with Trypsin (1/100, 25300062, Gibco) and Dnasel (1/1000, 79254, Qiagen). Next, neurocult basal medium with 10% FBS was added to the mixture to deactivate the Trypsin. The mixture was then triturated with fire polished Pasteur pipettes to obtain a homogenous mixture of cells. The cells were then centrifuged for 5 min at 300 × g, washed twice and finally resuspended in neurocult medium basal medium supplemented with 1% PS. One-quarter of the media was replaced with fresh medium containing 5000 units of penicillin and 1%PS. The cells were plated on poly-L-lysine (0.01 mg/ml, P6282, Sigma) and laminin (10 µg/ml; Gibco, 23017015) pre-coated dishes. Cells were passaged post 5 days of culture. All experiments were performed before passage 3.

**Determination of iPSCs into neural lineage.** iPSCs were differentiated into neural lineage by following a published protocol35. In brief, iPSCs were dissociated into single clumps with Accutase and seeded on polyorhmyline (0.1 mg/ml, P4957, Sigma) and laminin (4 µg/ml) pre-coated 4-well-plates (179820, Nunc) and cultured in mTSER supplemented with Y-27632 (10 µM). The day after, cell culture medium was changed to N2SF medium (STEMCELL, 05701), endothelial growth factor (EGF) (20 ng/ml, PHG0314, Gibco) and 1% PS. Cells were then plated on poly-L-lysine (0.01 mg/ml, P6282, Sigma) and laminin (10 µg/ml; Gibco, 23017015) pre-coated dishes. Cells were passaged post 5 days of culture. All experiments were performed before passage 3.

**Inositol labeling and SAX-HPLC analysis.** HEK293 cells and fibroblasts were plated at 10,000 cells/well on 6-well plates and radiolabeled for 5 days with 5 µCi/ml myo-[3H]-inositol (1 mCi, NET14A001, Perkin Elmer) in inositol-free DMEM medium (DML13-500, Caisson) supplemented with dialyzed FBS (Gibco, 26400-044) and 1%PS. One-quarter of the media was replaced with fresh medium containing 5 µCi/ml [3H]-inositol on day 2. For iPSCs and their day 7 neurally-differentiating counterparts, the inositol metabolic radiolabeling was performed for 3 days and inositol-free DME media were supplemented with either bFGF (10 ng/ml, PHG0264, Invitrogen) or Noggin (100 ng/ml, 78060.1, STEMCELL), Y-27632 (10 µM), and 1%PS. The media were replaced every 3 days.

**MTT proliferation assay.** Cells were cultured with methylthiazolyltetrazolium bromide (MTT) (0.5 mg/ml, M5653, Sigma) for 1.5 h. Then, MTT and the cell culture medium were removed and 100% dimethyl sulfoxide (100/µl/well) was added to dissolve the formazan crystals. Next, the cell culture plate was left to shake for 15–30 min in the dark at room temperature, followed by transfer of the samples into flat-bottom 96-well microtiter plate (655101, Greiner). The optical density was read on a microplate reader at 560 nm (1681135, Biorad).

**Apoptosis assay.** The apoptosis is measured using the Click-it tUNEL Alexa Fluor 594 Imaging Assay (C10246, Thermofisher) according to manufacturer’s instructions. For a positive control, cells are treated with tunicamycin (10 µg/ml, 3516/10, Biotechne) for 6 h to induce apoptosis (data not shown). For quantification analysis, a minimum of 5 fields per replicate was analyzed in ImageJ. The percentage of TUNEL+ cells was counted relative to the total number of Hoechst+ cells counted which was comparable (i.e., Hoechst+) between replicates and conditions.

**Quantification of free intracellular Ca2+ levels.** Cells were plated at 25,000 cells/well on flat-bottom 96-well plates (Greiner, 653088). After 24–48 h in culture, the cells were loaded with Fluor-4 AM (5 µM, F14201, Invitrogen) diluted in assay buffer (NaCl 140 mM, Glucose 11.5 mM, KCl 3.9 mM, MgCl2, 1.4 mM, NaHPO4 1.2 mM, NaHCO3 5 mM, HEPES 10 mM, pH 7.4) with or without CaCl2 (1.8 mM) to a final concentration of 5 µM. After 30 min of incubation in a humidified incubator (5% CO2, 37 °C), the loading medium was removed and the cells were washed twice with the assay buffer. Then, the cells were excited at 485 nm, and the fluorescence emission was recorded at 535 nm via a fluorometers (200ProTecan and Tristar LB941) in the presence of assay buffer. The measured fluorescence intensities were then normalized to cell number via MTT proliferation assay. For Ca2+ stimulation experiments, ionomycin (10 µM, I0634-1MG, Sigma) or caffeine (10 mM, C0750, Sigma) were quickly added to the wells in the presence of assay buffer and fluorescence intensities were recorded every 30 s for a minimum of 10 min. All the Ca2+ stimulation experiments were performed with early batch HEK293 cells36.

**Quantification of intracellular total and free iron levels.** The total iron content was measured with a colorimetric ferrozine-based assay according to previous studies37,38. Briefly, cells were cultured on 6-well plate (92006, TPP) and incubated with iron (ferri, ferric, and ferrous) stocks (FeSO4) (100 µM, RES0490-A702X, Sigma) in serum-free DMEM for 48 h. Then, the cells were lysed with NaOH (50 mM, 200 µl/well) and 50 µl of the lysate was kept apart for protein quantification. The remaining cell lysate was mixed with equal volumes of 10 mM HCl and iron-releasing reagent (HCl 1.4 M, KMnO4, 4.5%) and incubated for 2 h at 60 °C. Once the samples reached room temperature, 30 µl of iron-detection reagent (6.5 mM ferrozine, 6.5 mM neocuproine, 2.5 M ammonium acetate, and 1 M ascorbic acid) was added to each sample. After 30 min of incubation at room temperature, the samples were transferred to flat-bottom 96-well microtiter plate (Greiner, 655101). The optical density was read on a microplate reader at 560 nm (Biorad, 1681135). For iron-detection experiments, the data are normalized against the protein levels, quantified via Pierce BCA Protein assay kit (32225, Thermo Scientific).

**Immunofluorescence.** Cells were fixed in cold 4% paraformaldehyde for 10 min followed by three washes of PBS and permeabilization in 0.25% PBS-Triton-X-100 for 10 min. Cells were blocked for 1 h in 1% BSA and 22.52 mg/ml glycine diluted in 0.1% PBS-Tween-20 (PBST). Cells were then incubated overnight at 4 °C with the following primary antibodies diluted in 1% BSA/0.1% PBST: anti-PAx6 (1:4000, 901301, BioLegend), anti-TUBULIN β3 (1:1000, 801201, BioLegend), anti-OCT4 (1:100, sc-5279, Santa-Cruz), anti-SOX2 (1:1000, AB5063, Millipore), anti-NESTIN (1:200, NS5143, Sigma). After washing with 0.1% PBST, cells were incubated for 1 h with Alexa Fluor-coupled secondary antibodies (A12424, A-11043, Invitrogen) diluted 1:500 in 0.1% PBST. Washing three times with 0.1% PBST, cells were then incubated with Hoechst 33342 (H3576, Invitrogen) diluted 1:1000 in 0.1% PBST. After 10 min of incubation, cells were washed twice with 0.1% PBST and either kept in PBS or mounted with ProLong Diamond Antifade Mountant (P36965, Invitrogen). Images were acquired with CELENA-S Digital Imaging System (CS20001, Logos) and Zeiss AxioPlan-2. For quantification analysis, a minimum of 10 fields per replicate were analyzed in ImageJ 1.8.0. The percentage of PAx6+ TUNEL+ cells were counted standard conditions. The total number of Hoechst+ cells per field which was comparable (i.e., Hoechst+) between conditions and treatments.

**Protein extraction and western blot analysis.** Protein extraction, quantification, separation in gel electrophoresis, and transfer were performed using standard procedure37. Nitrocellulose membranes were blocked either in Odyssey-TM Blocking Buffer (927–0003, LCOR) or in 5% dry milk diluted in 0.2% PBST for 1 h and further incubated overnight at 4 °C with the following primary antibodies diluted in either Odyssey-TM Blocking Buffer or 2.5% dry milk diluted in 0.2%
PBST: anti-MINPP1 (1:2000, sc-10399, Santa-Cruz), anti-β Actin (1:5000, AM4302, Invitrogen). After three washes with 0.2% PBST, the membranes were then incubated for 1 h at room temperature with HRP (1:10,000, sc-2314, sc-2020, Santa-Cruz) or IRDye-coupled (1:10,000, 925-68070, 925-32211, LICOR) secondary antibodies. After three washes with 0.2% PBST, the membranes were developed either with HRP (Chemidoc XRS, Biorad) or Odyssey CLx imaging system (LICOR) and analysed with ImageJ Software Free 5.2. Uncropped scan blots are available in the Source data file.

**Statistics.** Data were analyzed where appropriate with one-way analysis of variance (ANOVA), two-tailed student’s t-test, and two-way ANOVA with GraphPad Prism 8 and Microsoft Excel 2016. N and ± values are detailed in figure legends. All the data are presented as mean ± the standard deviation (s.d.). Statistically significant differences are indicated on the figures by *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Patient’s consent precluded the deposition of WES data in public database. These sequence data are available from the corresponding author upon reasonable request. All the other source data and uncropped blots are provided within the source data file.

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