Supplemental Data

	Hamanaka et al.,2020				Li et al., 2021	
Individual	1	2	3	4	5	
Gender	Male	Female	Male	Female	NA	Female
Age (y)	22	28	14	11	NA	NA
Origin	Japanese	Japanese	Israeli	Malaysian	NA	Chinese
cDNA variant	c.1950_1969dup	c.1976_1982del	c.1991del	c.1991del	c.1982_1991del	c.1960_1978del
Protein variant	p.(Arg657Profs*35)	p.(Ala659Valfs*24)	p.(Gly664Alafs*21)	p.(Gly664Alafs*21)	p.(Gly661Alafs*21)	p.(Leu654Argfs*25)
Exon	17	17	17	17	17	17
Protein domain	Intracellular	Intracellular	Intracellular	Intracellular	Intracellular	Intracellular
Status	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Inheritance	De novo	De novo	De novo	De novo	DNA of parents unavailable	De novo
Final diagnosis	PME	PME	PME	PME	NA	PME
Medical history and symptoms	<ul> <li>Global developmental delay (rolling over: 1 y; first words: 2-3 y).</li> <li>Intractable seizures: GTCS (6 y) absences (9 y), atonic seizures (11 y). Ataxia, intention tremor, rigidity, myoclonus. Motor and verbal regression (wheelchair, few words, dysarthria). Severe ID (IQ = 25).</li> <li>Spasticity, pyramidal reflexes. Normal HC. No dysmorphic features. Normal brain MRI.</li> </ul>	Motor delay (walking unaided at 28 mo). Intractable seizures: GTCS (11 mo), atypical absence (5 y), complex partial seizures and atonic seizures (10 y). Ataxia, intention tremor, rigidity, myoclonus. Motor regression (wheelchair). Severe ID (few words; IQ = 25). On examination, spasticity, increased tendon reflexes, HC at -2 SD, no dysmorphic features. Mild cerebellar atrophy on brain MRI.	Motor delay (walking: 2 y). Absence seizures (2 y) responsive to treatment. Ataxia, intention tremor. Motor and verbal skills regression (wheelchair). Severe ID (no words). HC: -2.5 SD. No dysmorphic features. Small vermis on brain MRI.	Global developmental delay (walking: 2 y; first words: 30 mo). Intractable atonic seizures (4 y). Ataxia, intention tremor, myoclonus. Regression (walking with support). Severe ID (few words). HC: 2nd percentile. No dysmorphic features. Normal brain MRI.	NA	Global developmental delay and ID (no more than 3 words, walks with difficulty). Low frequency atonic seizures at 4 y. Recurrence of seizures since 6 y. Ataxia and tremor. Brain MRI: cerebellar tonsillar hernia. Growth retardation noted at 10 mo.

## Supplementary Table 1 Variants previously described in the literature

Courage et al.,2021		Xiaozhen et al., 2021		Herzog et al., 2021	Shu <i>et al.</i> , 2021	
Individual	1	2	1	2		
Gender	Male	Female	Female	Male	Female	Male
Age (y)	38	39	4	3	20	6
Origin	Australian	Canadian	Chinese	Chinese	Caucasian	Chinese
cDNA variant	c.1993del	c.2032del	c.2056C>T	c.1483G>T	c.2067G>A	c.1934del
Protein variant	p.(Arg665Glyfs*20)	p.(Glu678Argfs*7)	p.(Gln686*)	p.(Gly495Trp)	p.(Trp689*)	p.(Gly645Alafs*5)
Exon	17	17	17	14	17	17
Protein domain	Intracellular	Intracellular	Intracellular	extracellular	Intracellular	Intracellular
Status	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Inheritance	De novo	DNA of the parents unavailable	De novo	De novo	Not inherited from the mother	De novo
Final diagnosis	PME	PME	PME	Epilepsy without ID	PME	Severe ID & seizures
Medical history and symptoms	Developmental delay. Ataxia, tremor (2.5 y), drop-attacks and absence seizures (4 y); GTCS (11 y); severe generalised and multifocal myoclonus (15 y); slow cognitive and motor regression (wheelchair at 11 y); severe ID. No pyramidal signs. Normal HC.	Developmental delay. Ataxia (2.5 y), dysarthria (16 y), tremor (14 y), resting and action myoclonus (10 y). Generalized epileptiform discharges (2.5 y); GTCS (5 y); recurrent convulsions with possible absences and focal component (14 y). Increasing difficulty with gait from 11y (wheelchair 14y). Moderate ID. No pyramidal signs. Normal HC.	Mildly delayed development (head control: 6 mo, sitting: 10 mo, standing alone: 13 mo, walking with support: 18 mo, first words: 18 mo). Epilepsy at 2 y: atonic seizures, then partial complex crises and atypical absences. Motor and intellectual regression (loss of ambulation and language). Normal brain MRI.	Epilepsy at 2 y with good response to treatment. Normal developmental milestones. Normal brain MRI.	Global developmental delay (first words: 3–3.5 y, walking: 5 y). Refractory status epilepticus, ataxia and myoclonus at 10 y. Absence seizures. Progression of ataxia and myoclonus (wheelchair at 13 y). Gradual cognitive decline. Cerebellar signs (ataxia, dysarthria, dysmetria ), oculomotor apraxia and resting action-exacerbated myoclonus. Normal brain MRI.	Global developmental delay; severe ID (cannot stand or walk at 6 y; no words). Early-onset recurrent febrile seizures (GTCS): 6 mo to 2 y. Normal brain MRI.

Supplementary Table 1 Variants previously described in the literature (continued)

GTCS: generalized tonic-clonic seizures; HC: head circumference; ID: intellectual disability; mo: month; NA: not available; NM\_032108.3; y: year; PME: progressive myoclonic epilepsy

Supplementary Table 2 In Silico	prediction of the pathogenio	c effect of SEMA6B missense variants

Position	Nucleotide change	Predicted amino acid change	SIFT4G	MutationTaster	Polyphen 2	CADD score
Exon 7	c.481C>G	p.(Leu161Val)	Damaging (0.041)	Polymorphism (0.523)	Probably damaging (0.999)	23.8
Exon 7	c.547G>C	p.(Val183Leu)	Tolerated (0.07)	Disease Causing (0.983)	Possibly damaging (0.774)	24.9
Exon 14	c.1589C>T	p.(Ser530Leu)	Damaging, Tolerated (0.527-0.012)	Disease Causing (0.784)	Probably damaging (0.936)	26.6
Exon 17	c.2138C>T	p.(Thr713Met)	Damaging (0.001)	Disease Causing (0.996)	Probably damaging (0.993)	25.2

Numbering is according to the cDNA sequence (GenBank entry NM\_032108.3)



**Supplementary Figure 1 Full-length gels showing** *Sema6b* **expression at the RNA level (A) or at the protein level (B) in HEK293T cells.** Expected size of the PCR product is 346 bp. Expected size of the SEMA6B main isoform are 98.5 kDa for WT and p.(Val183Leu), 42.5 kDa for p.(Arg372\*) and 77.3 kDa for p.(Trp689\*). The actual size of the proteins is bigger due to glycosylation. Three independent experiments were performed. (bp: base pairs, kDa: kilodalton, WT: wild-type, RT-: no reverse transcriptase control, GFP: green fluorescent protein), MW: molecular weight.



Supplementary Figure 2 Subcellular localisation of wild type or mutant forms of SEMA6B protein overexpressed in neuronal cells. 3D-images were obtained using a Leica Confocal microscope (objectives x63, scale bar =  $10 \mu$ m). White arrows show a different localisation of the SEMA6B variant compared to the wild-type form.



**Supplementary Figure 3 Distribution of axon guidance phenotypes.** The co-injection of the Sema6B variants together with dsRNA to silence endogenous SEMA6B did not change the distribution of aberrant axon guidance phenotypes in qualitative or quantitative manner compared to what we found in the absence of Sema6B. The reduction of DiI injection sites with normal axon guidance seen in the absence of SEMA6B (dsS6B 3'UTR) was rescued by concomitant electroporation of wild-type mouse *Sema6B*. However, none of the variants was able to rescue axon guidance.