SUPPORTING INFORMATION

Variants in *CUL4B* are associated with cerebral malformations

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**Supp. Figure S1.** Effect of the novel amino acid altering variants p.(Leu785del), p.(Ala621dup), and p.(Pro50Leu) on the subcellular localization of CUL-4B and its interaction with RBX1, DDB1, and SGN5.

**A.** Immunostaining of U-2 OS cells using variable exposure times for WT, p.(Pro50Leu), p.(Ala621dup), and p.(Leu785del) FLAG-tagged CUL-4B, showing normal nuclear localization of CUL4B for all three variants.

**B.** Western blot showing the RBX1, DDB1, and SGN5 protein levels in HEK293T cells expressing WT, p.(Pro50Leu), p.(Ala621dup), and p.(Leu785del) FLAG-tagged CUL-4B (left panel; Western blot with anti-FLAG is the same as in Figure 4). The interaction of WT and mutant CUL-4B to RBX1, DDB1, and SGN5 was assessed by immunoprecipitation (IP) (right panel). No consistent changes in binding of CUL-4B with its interacting partners were observed for the different mutants.
**Supp. Figure S2.** CUL-4B does not interact with LIS-1.

A. LIS-1 and WDR62 both contain a DWD box sequence, which is highly predictive for binding to DDB1 and CUL-4B. The DWD box is a 16 amino acid sequence with hydrophobic amino acids at position 1, 2, 10, and 12 (blue); acidic amino acids at position 7 and 14 (red); an aromatic amino acid at position 13 (green); and a basic amino acid at position 16 (yellow).

B. Co-transfection of HEK293T cells with FLAG-tagged CUL-4B and LIS-1. IP with antibodies against FLAG-tagged CUL-4B with subsequent immunoblot of precipitates shows no binding of FLAG-tagged CUL-4B to LIS-1. DDB1 is shown as control.

C. Knock-down of CUL-4B with a short hairpin RNA in HEK293T cells results in normal levels of WDR62 and LIS-1, suggesting that these are not substrates of CUL-4B. WDR5 levels are increased. shGFP is shown as control.
**Supp. Tables**

**Supp. Table S1. Clinical guideline for testing for CUL4B variants**

<table>
<thead>
<tr>
<th>Required</th>
<th>- Male sex</th>
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<tr>
<td></td>
<td>- Intellectual disability/developmental delay</td>
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<tr>
<td>Clinical criteria (at least 5, 4 if neuroimaging not performed)</td>
<td>- Cerebral MRI abnormalities, especially malformations of cortical development, ventriculomegaly or decrease in white matter volume</td>
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<td>- Short stature and/or macrocephaly and/or obesity</td>
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<td>- 2 or more of the following neurological problems: behavioral problems, tremor, seizures, gait disturbances</td>
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<td>- 3 or more of the following facial dysmorphisms: high/prominent forehead, malformed abnormally positioned ears, hyperplastic supraorbital ridges/deep-set eyes/narrow palpebral fissures, low nasal bridge with rounded tip, prominent lower lip, prognathia</td>
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<td>- 2 or more of the following hand/feet abnormalities: sandal gap, brachydactyly/small hands/feet, wasted lower leg muscles, pes cavus</td>
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<td>- 1 or more of the following: genital abnormalities, gynaecomastia, kyphosis</td>
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<tr>
<td>Exclusion</td>
<td>Family history not in accordance with XL inheritance pattern</td>
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**Supp. Clinical Reports**

**Family 1 (N151)**
This family comprises three affected members (Figure 3). The proband (IV:3, Figure 3) was the first child of healthy unrelated parents. He was born after an uneventful pregnancy and delivery at 34+1 weeks of gestation with a birth weight of 1735 grams (-1.5 SD) and a head circumference (HC) of 34 cm (+2.5 SD). His Apgar scores were 2, 5 and 8 after 1, 5 and 10 minutes respectively. Because of the relative macrocephaly cerebral Magnetic Resonance Imaging (MRI) was performed. This showed a severe ventriculomegaly with remnants of a haemorrhage in both occipital horns (left grade III, right grade II), a persistent cavum septum pellucidum, and bilateral polymicrogyria of the posterior perisylvian cortex (Table 1). Because of progressive ventriculomegaly, a ventriculoperitoneal drain was placed. In addition, he had bilateral short humeri, coronary hypospadias, bilateral cryptorchism, and bilateral inguinal herniae. Investigations of eyes, kidneys, and coagulation were normal. At the time of last examination the proband was 10 years old. He had a severe intellectual disability (ID) without speech and was in a wheelchair. He had developed seizures for which he was treated with valproic acid. Physical examination showed a height of 111 cm (-5 SD) and a weight of 18.3 kg (-0.3 SD). He had a high, prominent forehead, downslanting palpebral fissures, a depressed nasal bridge with a full tip of the nose. His ears were small, simple and prominent. He had a pronounced philtrum with a tented upper lip, an everted lower lip and mild prognathism. His hands and feet showed short and broad hands, 5th finger clinodactyly bilateral, widely spaced toes and syndactyly 2-3 of toes (Figure 2).

The medical data of his maternal uncle (III:3, Figure 3) and a brother of his maternal grandmother (II:3, Figure 3) have been published previously (Sengers, et al., 1985). They both had a congenital ventriculomegaly, severe ID without speech, short stature, obesity and hypogenitalism. The maternal uncle also had a diaphragmatic hernia. His facial dysmorphisms consisted of a prominent forehead, low set ears with an overfolded helix, a short philtrum and a tented upper lip (Figure 2).

250K Affymetrix SNP array analysis and sanger sequencing of *L1CAM* in the proband gave normal results. *AP1S2*, the other known X-linked hydrocephalus gene, was unlikely involved because it was outside the linkage interval in this family.
Family 2 (D112)

This family comprises three affected members (Figure 3). The proband (III:1, Figure 3) was delivered in the 40th week of gestation after an uneventful pregnancy. He was born with a length of 54 cm (+2 SD), a weight of 3230 grams (-0.6 SD) and a HC of 36 cm (+1.2 SD). His Apgar scores were 10 and 10 after one and five minutes, respectively. He had pronounced muscular hypotonia and cryptorchidism. He started to walk in his second year of life and speech development was delayed. At the age of 4 years psychological testing revealed an IQ of 63. At the time of the last examination he was 8 years old. He spoke 2-3 word sentences and had an overfriendly personality. His HC was 55.5 cm (+1.8 SD), his height 122 cm (-2 SD), and his weight 21.5 kg (-0.8 SD). He had elongated and narrow palpebral fissures, a broad and low nasal base with thick alae nasi and a rounded nasal tip, a thin upper lip and a slightly prominent lower lip, a very prominent chin, and low set ears with thick helices. He had small hands and feet, gaps between all toes, syndactyly of 2nd-4th toes, and bilateral pes adductus requiring foot orthotics (Figure 2). Reevaluation of a brain MRI performed at the age of 2 years showed predominantly posterior ventriculomegaly, mildly diminished white matter volume with hyperintensity of deep white matter, and a thin corpus callosum (Table 1, Figure 1).

His maternal uncle (II:3, Figure 3) was born at term after an uneventful pregnancy with a length of 47 cm (-1.5 SD), a weight of 2740 grams (-2 SD) and a HC of 33 cm (-1.2 SD). His psychomotor development was delayed. In childhood he was agitated, excitable and aggressive. As an adult he was able speak 2-3 word sentences and lived in a residential setting. His height was 185 cm (0 SD) and his weight 100 kg (+2.5 SD). His palpebral fissures were similar to those of his nephew, the nose was slightly smaller but also had a rounded tip, he had an everted lower lip, and his chin was very prominent (Figure 2).

The maternal half-brother of the proband’s mother (II:4, Figure 3) was born at term with a length of 50 cm (0 SD), a weight of 3500 grams (0 SD) and a HC of 35 cm (+0.2 SD). His psychomotor development was delayed and he was an excitable child like his half-brother. His HC at the age of 14.5 years was 57.5 cm (+1 SD). As an adult he spoke single words but no sentences and lived in a residential setting. His height was 185 cm (0 SD) and his weight 100 kg (+2.5 SD). His facial appearance resembled that of his half-sister’s son with long and narrow palpebral fissures, a broad and bulbous nose with thick alae nasi, and a very prominent chin (Figure 2).

Karyotyping of GTG-banded chromosomes from lymphocytes revealed a normal male karyotype in all three affected males. Fragile-X-Syndrome was excluded by PCR and Southern blot.
Family 3 (P142)
This family comprises three affected members (Figure 3). The proband of this family had moderate ID. He was not able to read or write and worked in a sheltered environment. There were no behavioral disturbances. On physical examination in adulthood he had a head circumference of 58.5 cm (+0.3 SD) and obesity with a weight of 115 kg (>+3 SD). He had a long face with large ears, hypotelorism, thick and everted lips, and gynecomastia. Brain MRI performed in adulthood was normal (Table 1).

Family 4 (N146)
This family comprises two affected members (Figure 3). The proband (III:1, Figure 3) was born by cesarean section at 41+1 weeks of gestation with a birth weight of 3400 grams (0 SD). His Apgar scores were 7 and 8 after one and five minutes, respectively. In the first years of life he had recurrent airway infections. His development was delayed (developmental age of 4 years at the age of 14 years) and he was diagnosed with attention deficit hyperactivity disorder. At the age of 14 years his HC was 54 cm (-0.8 SD) and his height 143.3 cm (-3.5 SD). He spoke 1-2 word sentences and had a friendly personality. He had several dysmorphisms, consisting of epicanthal folds, periorbital fullness, narrow palpebral fissures, low nasal bridge with rounded nasal tip, prominent lower lip, small ears, short hands, bilateral 5th finger clinodactyly, small feet with a sandal gap, flexion contractures of the knees, joint laxity, torticollis (probably of ocular origin), and a hyperpigmentation on his lower back (Figure 2). An X-ray of his lumbar spine showed no abnormalities. Cerebral MRI at the age of 2 years showed unilateral cortical dysplasia of the right hemisphere parietal with an enlarged fissure of Sylvius and a cavum veli interpositi (Table 1). Cardiac ultrasound demonstrated no cardiac malformations. Metabolic investigations (in urine and blood) showed no abnormalities. Chromosomal investigations (including Agilent 44K array analysis), and DNA-analysis of FMR1, MECP2 and RSK2 gave normal results.

His maternal uncle (II:5, Figure 3) was also developmentally delayed. He was born after an emergency cesarean section with probably neonatal hypoxia. At the age of 36 years he was living in a residential setting. He had a diminished attention span, but was not hyperactive. His facial appearance resembled that of this nephew with periorbital fullness, narrow palpebral fissures, a rounded nasal tip, prominent lower lip, small feet, and flexion contractures of the knees.
Family 5 (D173)
This family comprises four males with ID, three of whom carrying the *CUL4B* variant (Figure 3). The proband (III:5, Figure 3) is a 30-year-old man with a severe learning disability. He was born from a pregnancy complicated by pre-eclampsia by cesarean section. His birth weight was 2920 grams (-1.3 SD) and length 49 cm (-0.5 SD). His Apgar scores were 7, 8, and 9 after one, five, and 10 minutes, respectively. His development was delayed with walking at 2 years of age. He had hypotonia, ataxia, epilepsy, spasticity of his hands, and a small penis. Brain Computed Tomography (CT), electroretinography, and visual evoked potential test were normal at 22 months. At 30 years of age he had a height of 165 cm (-2.5 SD), a weight of 66 kg (+1 SD), and a HC of 59 cm (+0.8 SD). He had aggressive bursts and speech was absent. He walked in a slightly forward-flexed walking posture. He had several dysmorphisms, including a high, prominent forehead, a prominent supraorbital ridge, a bulbous nose, a prominent chin, small cupped auricles with thickened helices and uplifted ear lobules (Figure 2)

The eldest maternal cousin (III:1, Figure 3) was born after an uneventful pregnancy and delivery at term with a birth weight of 2650 grams (-2 SD), and a length of 46 cm (-2.5 SD). His Apgar score was 10 after one minute. After birth he had feeding difficulties. His psychomotor development was delayed with walking at the age of 26 months. He was diagnosed with an intention tremor, strabismus, delayed bone age, and generalized slowing and disorganization of the EEG was seen, though no seizures have been noted. At the age of 45 years he his height was 157 cm (-4 SD), his weight was 63 kg (+1.3 SD) and his HC was 58 cm (0 SD). He had a moderate learning disability with a pleasant personality. He used two-word sentences, and he was able to walk. Dysmorphic features were noted consisting of a high forehead, deep set eyes, hyperplastic supra-orbital ridges, up-slanting of palpebral fissures, abnormal and low-set ears, a broad base to nose with a full tip, and a prominent chin (Figure 2). He also had hypotonia and pes planus. Cerebral MRI showed a diminished white matter volume and cerebellar mid-vermian atrophy (Table 1).

The younger maternal cousin (III:2, Figure 3) was born after an uneventful pregnancy and delivery at 37 weeks of gestation with a birth weight of 3080 grams (0 SD) and a length of 49 cm (+0.5 SD). His Apgar score was 9 after one minute. He started to walk normally at the age of 13 months. At 2 years of age speech delay was diagnosed. The EEG analysis revealed a generalized slowing and disorganization, but no epileptic discharges were seen. At 39 years his height was 163 cm (-3 SD), his weight 75 kg (+2 SD), and his HC 58 cm (0 SD). He had a moderate learning disability with an easy-going personality. He used single words, and walked without support. Dysmorphic features were detected consisting of a high forehead, down-
slanting palpebral fissures, a broad base to nose with a bulbous tip, a prominent chin, and abnormal and low-set ears (Figure 2). Also brachydactyly, syndactyly II-III of toes, pes planus and hypotonia were noted. Cerebral MRI showed mild ventriculomegaly with mildly diminished white matter volume (Table 1).

The proband also had a 41-year old severely intellectually disabled half-brother (III:4, Figure 3). He did not carry the familial CUL4B variant. He had ataxia, behavioral problems, spasticity, epilepsy and absent speech. Dysmorphic features consisted of a high forehead, deep set eyes, low nasal bridge, and a broad base to nose. He had a sandal gap in both feet. His height was 174 cm (-1.5 SD).

Conventional karyotype and cytogenetic fragile-X analysis in all affecteds gave normal results.

Family 6 (D203)
The family comprises three affected members (Figure 3). The proband (III:1, Figure 3) was the first child of healthy and non-consanguineous parents. He was born after an uneventful pregnancy at 39 weeks of gestation with a birth weight of 3450 grams (0 SD), body length 56 cm (+3 SD), and HC 34 cm (0 SD). Apgar scores were 9 and 9 after one and five minutes, respectively. Muscle hypotonia during infancy, bilateral cryptorchidism and unilateral mild/moderate hypoacusis were diagnosed. His psychomotor development was delayed, with unsupportive sitting at 8 months and walking at 26 months. Few episodes of “absence” attacks were observed since the first year of life. At the age of 14 years he had moderate ID with behavioral problems especially hyperactivity and irritability, and speech delay (short sentences, pronunciation defect, dysarthria?). His height was 146 cm (-2.8 SD), weight 40 kg (+1 SD), and HC 55 cm (0 SD). He had mild facial dysmorphism with macrostomia, large protruding tongue with open-bite appearance, prominent lower lip, prognathia, anteverted nares, low nasal bridge, with rounded tip, prominent and posteriorly rotated ears and sloping shoulders (Figure 2). A CT scan of the brain showed no evident abnormalities (Table 1).

His maternal uncle (II:5, Figure 3) was the 3rd child of non-consanguineous parents. After delivery bilateral cryptorchidism was noted. His psychomotor development was delayed with talking at the age of 4 years. Until 4 years, recurrent generalized grand mal seizures were noted. At the age of 38 years he demonstrated moderate ID, a spectrum of behavioral abnormalities with hyperactivity, irritability, aggressiveness and a tendency to overeating. Tremor of the upper limbs and gait abnormalities were also observed. He had short stature (166
cm, -2.5 SD) with overweight (84 kg; +2.6 SD) and a HC of 59 cm (+0.8 SD). At physical evaluation mild facial dysmorphism with a prominent lower lip, rounded nasal tip, deep set eyes, prognathism and small feet were noted (Figure 2).

The proband’s cousin (III:2, Figure 3) was born as a second child of healthy and non-consanguineous parents. Delivery was at 38 weeks of gestation after an uncomplicated pregnancy. His birth weight was 2300 grams (-2 SD), HC 33 cm (-0.5 SD), body length 48 cm (-0.2 SD). Apgar scores were 9 and 9 after 1 and 5 minutes, respectively. Bilateral cryptorchidism and unilateral talipes equinovarus were recognized. During infancy he frequently suffered from bronchitis infections. His psychomotor development was delayed with walking at the end of 2 years of age, although gait abnormalities were present. At the age of 4-5 years of life epileptic seizures with absence attacks started. Psychological evaluation at 9 year of life showed severe ID with severely impaired expressive language ability. Gabble speech of only few isolated words, with slow progression of speech development was observed. Poor attention span, hyperactivity, irritability, and tendency to aggressiveness were also evident. On physical evaluation he had a height of 135 cm (-0.5 SD), a weight of 29 kg (0 SD), and a HC of 53 cm (0 SD). The following dysmorphic facial features were noted: high forehead, abnormally positioned ears, macrostomia with prominent lower lip, prognathism and bilateral astigmatism (Figure 2). Results of renal ultrasound, electroencephalogram, and brain MRI were normal, although the resolution of the MRI images was diminished (Table 1).

**Family 7 (D102)**

This family comprises four males with ID, two brothers and two maternal half-brothers (Figure 3). The proband (II:2, Figure 3) had delayed psychomotor and particularly speech development. He had moderate to severe ID with absent speech and lived in a residential setting. Furthermore, he had some behavioral problems, often scratching himself with his finger nails. His adult height at the age of 39 years was 161 cm (-3 SD) with a weight of 54 kg (0 SD) and a HC of 56 cm (-1 SD). His face was long with a pointed chin and prominent lower lip, the protruding and rather small auricles had thick helices and widened lobes (Figure 2). Noteworthy were also a small penis and small testicles with a hypogonadotrophic hypogonadism that required testosterone substitution.

His elder brother (II:1, Figure 3) was 41 years old and showed moderate to severe ID since early childhood. Both maternal half-brothers were deceased.
Fragile X syndrome was investigated by PCR and Southern blot analysis with a normal result and array-CGH analysis (resolution of 200 kb) was performed and revealed no abnormalities. These investigations were followed by an unsuccessful variant search (resequencing chip) in 17 X-chromosomal genes known to cause ID (Jensen, et al., 2011). Neuroimaging has not been performed in this family.

Family 8 (D287)
This family comprises three affected members (Figure 3). The proband (III:1, Figure 3) was a 20-year-old man. He was born at 34 weeks of gestation with a birth weight of 1670 grams (-1.5 SD). He was born with hypospadias which was corrected surgically. Psychomotor development was delayed with sitting at the age of 12 months and walking at age 3 years. He had moderate ID with an IQ of 35-46 (SON-R and CFT1 tests at the age of 12 years) and motor clumsiness. At the age of 17 10/12 years he showed short stature (height 154 cm (-4 SD), weight 65 kg (+1.5 SD), HC 57 cm (0 SD)). Minor anomalies were synophrys, deep set eyes, mild hypertelorism, flat nasal bridge with broad, flat nose with triangular tip, small ears (5.5 cm length, -2 SD), somewhat high palate, small mouth, small hands, and feet, brachydaactyly (-2.4 SD) and mild cutaneous syndactyly of toes II-IV.

His brother (III:2, Figure 3) was born at 37 weeks of gestation via cesarean section with a weight of 3 kg (0 SD) and a history of polyhydramnios. He was able to walk at the age of 4 years and developmental testing (SON-R) at the age of 4 4/12 years revealed an IQ of 55 and muscular hypotonia. At the age of 15 years he showed short stature like his brother and despite lack of hypertelorism a similar pattern of minor anomalies (height 136 cm (-5 SD), weight 49.5 kg (+3 SD), head circumference 55 cm (-0.6 SD)). In addition, he had a frontal sweep and laterally sparse eyebrows and relatively large ear lobes. Genitalia were infantile with testis volume of <2 ml.

Both parents were healthy but the mother had a brother with ID (II:4, Figure 3). He had limited speech and walking abilities and mild obesity. He died at the age of 53 from pancreas cancer. Minor anomalies were strabism divergence and relatively small ears with large lobes.

Molecular X-inactivation studies at the FMR1 locus revealed complete skewing in the mother of the proband. MLPA screening for X-chromosomal copy number changes using the MRC-Holland kit P106 revealed no abnormality in the proband. Neuroimaging has not been performed in this family.
Family 9 (GOLD506)
The proband (IV-1) was the first child of a mother from a family with XLID (Whibley, et al., 2010). The proband was born after a pregnancy complicated by maternal hypertension requiring delivery to be induced at 38 weeks. Birth weight was 2550 grams (-1.5 SD). Poor feeding initially required nasogastric feeding. At 2 years grand mal seizures began for which he was treated with Sodium valproate. Development was delayed with walking at the age of 3½ - 4 years. Language was especially delayed. At 11 years of age he was unable to talk in sentences and attended a special school. He had an essential tremor more noticeable when concentrating or when angry. He was friendly but easily upset and was angry and aggressive to others. He also self harmed by biting when distressed. He did appear to feel pain. At 11 years, he had a slender build with a HC of 52.5 cm (-0.8 SD). He had prominent front teeth, a narrow long face with low set posterior rotated ears (Figure 2). He had bilateral cryptorchidism. His hands and feet were small with bilateral 2-3 syndactyly of toes. His ankle reflexes were absent. He had a CT scan aged 8 following a head injury which was reported as normal.

The mother had 3 affected brothers and 3 affected maternal uncles. One brother died at 52 years of age. He had severe ID. He was looked after in a residential setting. He attended special school when young and acquired little or no speech. He was reported to have small hands and feet. He had a longstanding seizure disorder and was maintained on medication. He died at 52 years of age secondary to epilepsy which worsened later in life. Another brother died at 14 years of age due to heart failure. He was also intellectually disabled with epilepsy and failure to thrive. No further details are available. Only one affected brother of the mother was alive. He was now 37 years old (III-11). He lived in a residential setting and attended sheltered employment as a gardener. He had little speech although understanding of language was good. He could wash and dress and self care although not able to live independently. His HC was 62.5 cms (+2.5 SD), his height was approximately 165-170 cm (-2/-2.5 SD). He had mild gynaecomastia and central truncal obesity. Genital development was reduced with bilaterally cryptorchidism and micropenis. Hands and feet were small (feet size 39 shoe) with bilateral toes 2-3 syndactyly. Bilateral pes cavus and reduced muscle bulk in lower legs was marked (Figure 2).

A deletion of 5’ promoter of CUL4B was noted by customized high resolution array on the X chromosome 119,578,701-119,584,448 (Hg18) (Whibley, et al., 2010). Analysis of transcript in lymphocytes revealed absence of transcript and protein was absent by Western blot analysis.
**Family 10 (P307)**

This family comprises two affected members (Figure 3). The proband (II:1, Figure 3) was the first child of healthy consanguineous parents. He had moderate DD with a developmental quotient (according to Brunet Lezine scale) at 66, macrocephaly and autistic behavior with repetitive behaviors and outburst. At 2.5 years of age, his HC was 54 cm (+2.5 SD) and he showed dysmorphic features with high prominent forehead, narrow palpebral fissures, prominent lower lip and large, non malformed ears (Figure 2). Cerebral MRI performed at the age of 2.5 years showed massive ventriculomegaly with a slightly thick and undersulcated cortex (simplified gyral pattern). The white matter volume was very diminished with dilated perivascular spaces in the high frontal white matter. The corpus callosum was thin and arched by the enlarged ventricles. The thalami, hippocampi and ventral pons were small (Table 1, Figure 1).

Subsequently, the mother had dizygotic twins, one affected boy and an unaffected girl (II:2 and II:3, Figure 3). He was born at 36+2 weeks of gestation with a birth weight of 2240 grams (-1.3 SD) and a HC of 33 cm (0 SD). Prenatal ultrasound showed ventricular dilatation. Neurological examination after birth showed truncal hypotonia. No dysmorphic features were noted. Brain MRI performed at the age of 6 months, showed massive ventriculomegaly associated with very simplified gyral pattern. The white matter volume was very diminished and the corpus callosum was thin and arched by the enlarged ventricles. The pons and the cerebral peduncles were small (Table 1, Figure 1). At 6 months of age, intracranial hypertension was suspected and he was treated with a VP drain. His head circumference was 52 cm (+5 SD). On examination, he was able to hold his head and to turn from ventral to dorsal decubitus. He also showed high prominent forehead, prominent lower lip, and low implanted ears (Figure 2).

**Family 11**

The proband (II:2, Figure 3) was the second child of healthy parents. He was born after a uneventful pregnancy at 38 weeks of gestation. Birth weight was 3340 grams (0 SD), length was 49 cm (+0.3 SD). He was referred in the neonatal period for hypotonia and feeding difficulties that initially required nasogastric feeding. An unusual eye motility disorder, called Brown syndrome, and macrocephaly (+3 SD) were noted. Development was delayed. At 3 years of age, he walked with aid, he babble and developed autistic features. His weight was 13.9 kg (0 SD), his height 92 cm (-1.8 SD) and his HC 55 cm (+2.5 SD). Dysmorphic features included dysplastic and malpositioned ears, small chin, upturned nose and bilateral 2-3 syndactyly of
toes. Genital development was abnormal with bilateral cryptorchidism and micropenis. He had a kyphoscoliosis and ankle reflexes were absent. On an MRI performed at 13 days of age, bilateral perisylvian polymicrogyria and a gaminolytic cyst bilaterally in the ganglionic eminence region were noted in combination with mild ventriculomegaly, diminished white matter volume, and hyperintensity of the corticospinal tracts, which were significantly improved at a follow-up MRI at the age of 11 months (Table 1, Figure 1).

**Supp. Methods**

**Massive parallel sequencing**

In patients with XLID, targeted enrichment and sequencing of the X chromosomal exons was performed using the Agilent SureSelect Human X Chromosome Kit on the Solexa Genome Analyzer GAIIx or exome sequencing was performed using the Agilent SureSelect Exome Kit (v2, 50Mb) on the SOLiD 4 platform (Life Technologies). In patients with MCD, exome sequencing was performed at the Centre National de Genotypage, Evry, France. For the XLID families only X chromosome data were evaluated. Reads were mapped to the reference genome using RazerS/BWA (Weese, et al., 2009), SplazerS or Life technologies bioscope software version 1.3. Sequence variations were detected using the DiBayes algorithm using high call stringency settings or the snpStore with the Maq consensus statistics (Li, et al., 2008). We excluded variants present in dbSNPv135 (www.ncbi.nlm.nih.gov/SNP/), the 1000 genomes Pilot Project (www.1000genomes.org), the Exome Variant Server (evs.gs.washington.edu), and in the in-house variant database containing data from 672 exomes analyzed by the Department of Human Genetics in Nijmegen, The Netherlands.

**Plasmid construction**

Human CUL-4B cDNA was cloned into pENTR-D-TOPO (Invitrogen, Grand Island, NY, USA). The resulting pENTR-CUL-4B plasmid was recombined with p3XFLAG-CMV-puro destination plasmid by LR clonase II (Invitrogen) to create the p3XFLAG-*CUL4B* expression vector. The p3XFLAG-CMV-puro destination plasmid was made by introduction of a puromycin resistant gene cassette into *Xhol* site of the p3XFLAG-CMV destination vector that was kindly provided by K.I. Nakayama (Kyushu University, Fukuoka, Japan). Expression construct of myc-tagged CUL-4B was described previously (Hu, et al., 2008; Nakagawa and Xiong, 2011). Patient-
derived variants were introduced by PCR-based site-directed mutagenesis. pcDNA3-Lis1, which was constructed by Li-Huei Tsai and her colleagues (Shu, et al., 2004) was purchased from Addgene (Addgene plasmid 12574; Addgene, Cambridge, UK). The shRNA construct for human CUL4B was described previously (Nakagawa and Xiong, 2011).

Generation of stable cell lines
To generate FLAG-CUL-4B stably expressing cells, HEK293T cells were seeded at 8 x 10^5 cells per 10 cm^2 well (one well of 6-wells plate). On the next day, expression constructs were introduced using polyethylenimine (PEI) (Polysciences, Warrington, USA). Three µg of plasmid DNA was diluted in 300 µl Opti-MEM (Invitrogen) and then 15 µg of PEI was added to the mixture, which was incubated for 15 min at room temperature. After incubation, the mixture was added onto cells. Twenty-four hours later, puromycin (1 µg/ml) was treated for 7 days. CUL-4B deficient cells were generated by retroviral infection and puromycin selection, detailed procedure of which was described previously (Kotake, et al., 2007; Nakagawa and Xiong, 2011).

Supp. References

