SUPPLEMENTARY MATERIAL:

Clinical reports

A:II-3

The girl is the third child of healthy non-consanguineous parents. After an uneventful pregnancy, she was born at term by cesarean section because of breech presentation, with a birth weight of 3660 g (+0.4 SD), a birth length of 53 cm (+0.6 SD) and an Occipital Frontal Circumference (OFC) of 36 cm (0.8 SD). The family history is uneventful. From the first days of life, the girl was agitated and required strict routines. Global developmental delay became evident at six months of age. All milestones of development were delayed: she was able to sit without support at 12, to crawl at 14 and to walk at 22 months, respectively. Speech development was severely impaired, with only a few words spoken at age 3 years. She hardly acquired new words and easily forgot the ones she had learnt. She reacted with jactations to unfamiliar situations and when falling asleep. At her last clinical examination at the age of 3 years and 11 months her height was 107 cm (+0.8 SD), weight 18 kg (+0.7 SD, BMI 15.7kg/m^2), and OFC 52 cm (+1.5 SD). She was a friendly but uncooperative girl, with generalised muscular hypotonia and unstable gait. She showed a hypotonic facial appearance with a long face and an open mouth with salivation. Facial dysmorphisms consisted of thin eyebrows, small palpebral fissures, hypertelorism, long nose and broad nasal tip, long and slight modulated philtrum, thin upper and lower lip vermilion, small teeth and a pointed chin (Fig. 1A). Multiple café au lait spots were noted on the left arm and the thorax. The parents reported severe behaviour problems resembling autism. The girl did not accept changes in her daily routine, did not get in contact with unknown persons, was easily frustrated and overstrained in unfamiliar situations. MRI of brain and hearing testing gave normal results. Ophthalmologic examination revealed hyperopia at age of 2 years. Chromosomal analysis revealed a normal female karyotype, and an array-CGH analysis did not show any pathogenic copy number variants (CNV).

B:II-2

This boy is the second of three children of healthy, non-consanguineous parents with an unremarkable family history. One of his sisters had a Wilms tumor and died at 13 years of age. The boy was born at 41^{+3} weeks of gestation with a weight of 3460 g (-0.6 SD), a length of 54 cm (+0.4 SD) and an OFC of 36 cm (+0.1 SD). At the age of nine months, the parents noticed developmental delay. Milestones of motor development were moderately delayed: he was sitting at 12 months and walking at 18 months. At 2 years of age he did not speak any

word and was diagnosed with global developmental delay. Brain MRI was normal. Frequent diarrhea and increased susceptibility to infections were noted by the parents. At 8 $^{11}/_{12}$ years of age he measured 140 cm (+1.3 SD), and had an OFC of 52.5 cm (- 0.4 SD). Weight was not reported. He was a friendly and cooperative boy, who presented with severe dysarthria. He had thick hair, thin eyebrows, small palpebral fissures, a long philtrum and thin upper and lower vermillion (Fig. 1B). He showed hypodontia and suspected enamel defects. He had tapering fingers. At his last clinical examination at the age of 15 $^{1}/_{12}$ years his height was 185 cm (+1.99 SD), weight was 63.5 kg (+0.53 SD, BMI 18.55 kg/m^2) and OFC was 54.6 cm (-0.56 SD). He was not prone to infections anymore. He still showed dysarthria. Facial dysmorphisms included a long face, thin eyebrows, low hanging columnella, and thin upper and lower lip vermillion. He still had four deciduous teeth. Chromosome analysis, molecular analysis for Fragile-X Syndrome and array-CGH analysis were unremarkable.

C:II-2

This boy is the second child of healthy non-consanguineous parents. A maternal uncle had Down syndrome, further family history was unremarkable. The pregnancy was complicated by polyhydramnios. He was born at 38^{+6} weeks with a weight of 4054 g (+1.55 SD), 51 cm (+0.56 SD) of length and an OFC of 36 cm (+0.99 SD). He received oxygen postnatally for a few minutes. APGAR score was 10/10/10. In the first weeks of life he suffered from tracheomalacia. In addition he had feeding problems due to swallowing difficulties. Muscular hypertonia, opisthotonos and excessive crying were reported at the age of three months. Motor development was moderately delayed: rolling at 5 months, sitting at 10 months and walking at 24 months. In addition, speech development was severely impaired, and he did not speak any word at the age of two. At the last examination at the age of one year and 8 months he had normal measurements with a weight of 13.0 kg (+0.5 SD), height 82.5 cm (-0.8 SD) and OFC 48.8 cm (-0.2 SD). Facial dysmorphisms included broad forehead, hypertelorism, thin eyebrows, small palpebral fissures, long philtrum, small mouth and retrognathia (Fig. 1C). He showed behaviour difficulties resembling autism. He was easily overstimulated and required reinforced daily routines. Further, difficulties with external tactile, vestibulary, oral-sensory, auditory and visual stimuli were observed in different tests. He also shunned unknown people. He suffered from recurrent, mostly upper airway infections and asthma, which improved over time. Gastroesophageal reflux that responded well to medications was reported. He had narrow ear canals. Brain stem evoked response audiometry (BERA) showed normal hearing at high frequencies, free field audiometry revealed hearing thresholds at 57 dBHL. He was affected by moderate hypermetropia. An ultrasound of the abdomen and heart was normal.

D:II-1

This girl is the first child of healthy, non-consanguineous parents. She was born after an uneventful pregnancy and by spontaneous delivery at term. Two maternal uncles had microcephaly, further family history was unremarkable. Her birth weight was 3600 g (+0.3 SD), birth length 52 cm (+0.1 SD), and OFC 36 cm (+0.8 SD). The postnatal period was uneventful, except reported excessive crying in the first six months and a mild delay in motor development. At 7 months, a secondary microcephaly (OFC 40.6 cm, −2.8 SD) was noted, and at 9 months she presented with episodes of tonic extension of the upper extremities and flexion of the trunk. Electroencephalogram (EEG) revealed hypsarrhythmia, suggesting the diagnosis of West syndrome. Metabolic and routine biochemical tests and brain MRI were normal. Renal ultrasound showed medullary nephrocalcinosis. She responded positively to a combined anticonvulsive therapy and could be weaned off at 16 months. Milestones of motor development were delayed: sitting without support at 12 months, crawling at 14 months and walking at 24 months. Speech development was mildly delayed: at 22 months she spoke two words. The last examination was at the age of one year and 11 months, reporting a weight of 9.0 kg (-2.0 SD), height of 84 cm (-0.3 SD), and OFC of 44 cm (-3.6 SD). Dysmorphic features consisted of hypertelorism, long philtrum, large mouth and thin upper vermilion (Fig. 1D). Array-CGH results were normal.

E:II-1

This boy is the first child of healthy non- consanguineous parents. He was born at term with a weight of 3200 g (-0.8 SD), a length of 48 cm (-1.7 SD) and an OFC of 34 cm (-1.1 SD). He had diffuse skin abnormalities which were later characterized as congenital erosive dermatitis with associated leukopenia and eosinophilia. Multiple newborn screen results revealed low T-cell receptor excisions circles (TRECS), suggestive of SCID, however, repeated T-cell count analysis over time were inconsistent with this diagnosis. Due to micrognathia, the boy suffered from severe obstructive sleep apnea, which was treated with continuous positive airway pressure (CPAP). In addition, he had a gastroesophageal reflux, pyloric stenosis, aspiration and oropharyngeal dysphagia requiring G-tube dependence. Dysmorphic features at birth included myopathic facies, bitemporal hollowing, small and up slanting palpebral fissures, upturned nose, hypoplastic midface, small mouth with downturned corners, micrognathia, and posteriorly rotated ears (Fig. 1E). Last clinical examination at the age of two years and 3 months revealed muscular hypotonia and severe global developmental delay. He was unable to sit or stand without support and he did not speak any word. His measurements were normal with a weight of 12 kg (-1.1 SD), a height of

88.9 cm (-0.7 SD) and an OFC of 47.8 cm (-0.8 SD). Facial dysmorphisms included a long face, myopathic facial appearance, bitemporal narrowing, upslanting palpebral fissures, prominent nose, short philtrum, and small mouth with downturned corners. His teeth were atypical in size, organization and colour. Non-congenital syndactyly of the fingers and toes was the result of disturbed skin wound healing. Metabolic testing, MRI of brain and muscle biopsy were inconspicuous. Echocardiogram showed a mildly dilated aorta. Microarray analysis revealed a 350kb duplication of uncertain significance at 11q14.1, which was inherited from one of the parents and therefore deemed non-contributory.

F:II-2

This boy was born as the second of three children of non-consanguineous and healthy parents after an uneventful pregnancy at term. His birth weight was 3020 g (-0.8 SD) and birth length 47 cm (-2.0 SD). At three months he suffered from bronchiolitis, and at four years of age from hemorrhagic varicella infection. In infancy and early childhood he suffered from frequent infections requiring antibiotic therapy. Milestones of development were delayed: he started walking at 3 years and spoke the first words at 4. Stuttering and difficulties in articulation impaired his speech. At the age of 7 his height was 112.3 cm (-1.9 SD), and at 17 years he measures 159 cm (-2.3 SD), despite the normal height of his parents and brothers. He is affected by moderate intellectual disability, not being able to read, write or calculate. He can perform simple daily routines, but needs help in more complex duties. At this last examination he presented as a friendly young man with long face, up-slanted palpebral fissures, prominent nose with broad nasal tip, thin upper lip vermillion and pointed chin (Fig. 1F). He had microdontia and impacted canine teeth. He had also developed a mild asthma. Metabolic work up showed mild hyperglycemia, and MODY Type 2 was diagnosed. Both he and his mother, who is also affected by MODY Type 2, carry a disease-causing variant in *GCK.* All other investigations including ECG, EEG, and abdominal ultrasound gave normal results. Karyotyping and SNP-array analysis gave normal results.

Family G

Individual G:III-1 is the first child of non-consanguineous parents. Pregnancy was complicated by severe maternal vomiting and dehydration. The child's growth parameters at birth were appropriate for her gestational age of 37 weeks. She began therapy for global developmental delays at 2 years. Speech was severely delayed, and she did not speak her first words until she was three. Dysmorphic features on her last examination (at 13 years of

age) included myopathic facies with bilateral ptosis, upslanted palpebral fissures, hypertelorism, thin and straight eyebrows, prominent nose with high bridge, thick and low columella, long philtrum, thin upper lip, and very high arched palate. The chin was small and showed a horizontal crease. The hands showed straight ulnar borders, tapered fingers and mild clinodactyly of the second and third fingers. Posture was poor. The back was flat, lacking the typical lumbar curve. She had decreased extension at both knees. Feet were flat. She has been in special education throughout her schooling. She has a poor understanding of social cues. Her behaviour is labile, displaying aggression and disruptive features. She requires assistance with personal hygiene. She has not had recurrent or unusual infections, as reported by the parents. Urinary organic acids and plasma amino acid chromatography were normal and also Fragile X studies were normal. Microarray analysis did not reveal any known pathogenic changes. Her mother, G:II-1, is similarly affected with intellectual disability and dysmorphic features. Unfortunately, the mother and her legal representatives refused to take further part in this study.

H:II-1

This boy of healthy non-consanguineous parents was born at 39 weeks of gestation after an uneventful pregnancy. Birth weight was 3070 g (-1. SD), length 51 cm (-0.4 SD), and APGAR score 10 after 1 and 5 minutes. Hypotonia was noticed in the first months of life and head holding was delayed. Sitting was reported at 10 months and walking at 13. Language was delayed, with no words spoken at three years of age. Behavioural problems were noticed at 5 years, with anxiety, poor social interaction and selective alimentation. At 7 years, his weight was 26.4 kg (+0.6 SD) and his height 124 cm (-0.1 SD). Facial dysmorphisms included a long facies, small palpebral fissures, thin nose with hypoplastic alae nasi and thin upper lip vermillion (Fig. 1G). He was able to make sentences and to understand instructions. No auditory problems were reported. Thyroid function, blood amino acids chromatography and urinary organic acids chromatography were normal. Brain MRI showed a moderate ectopia of amygdala. At 11 years, his weight was 36.2 kg (-0.2 SD), his height 142 cm (-0.6 SD) and OFC 53 cm (-0.8 SD). Physical examination showed high forehead, moderate enophthalmia, horizontal ear lobules and a short philtrum. He presented with multiple allergies. He was attending a specialized institution. He always had language difficulty, with stuttering. His anxiety seemed better controlled and he had made progress in social interactions. Fragile X study was negative. Standard karyotype showed an apparently balanced *de novo* translocation: 46,XY,t(4;14)(p15;q32.1). Array-CGH was normal.

I:II-2

This boy was the second of three children healthy non-consanguineous parents with unremarkable family history. He was born at term after an uneventful pregnancy. Birth weight was 3550 g (-0.2 SD), birth length was 51 cm (-0.7 SD) and OFC was 36 cm (+0.3 SD). Early motor development was normal, with sitting between 8 and 10 months and walking at 14 months. Language was delayed with first sentences at 3 years and 8 months. He had divergent strabismus and was operated at 6 years of age. He displayed learning difficulties at high school and was oriented towards a professional profile at 13 years. He had trouble with social interactions and was hospitalized for two acute psychiatric episodes at 23 and 24 years of age. At 29 years, his weight was 72 kg; his height was 178 cm and OFC was 59.5 cm. Physical examination showed small upslanting almond-shaped palpebral fissures, prominent nose, long philtrum, thin upper lip vermillion, high-arched palate and short stubby hands. He had borderline intellectual disability with IQ at 77. He always had difficulties with social interactions and could not keep a stable job. Fragile X study was normal. Standard karyotype showed an apparently balanced *de novo* translocation: 46,XY,t(4;14)(q31.1;q32.2). Array-CGH was normal.

J:II-1

Individual J:II-1 is the first child of non-consanguineous parents. Family history is unremarkable, except for a maternal uncle with intellectual disability who has severe speech disorder (no language) but is otherwise independent for daily activities. Pregnancy was uncomplicated. The child's growth parameters at birth were appropriate for her gestational age of 41 weeks and her Apgar score was 9-9-9. Developmental delay was first noted at 6 months of age, when she started day care. At 7 months, she was seen by a paediatrician and a pediatric neurologist, who noted severe developmental delay and spasticity. Brain CT scan at that time was normal. Brain MRI showed a mild increase in size of lateral ventricles and pericerebral space, but was otherwise normal. She had severe feeding difficulties, due to disorganized oral phase and frequent aspirations. At 3 years of age, brain MRI was repeated and showed atrophy vs hypoplasia of the globus pallidus bilaterally. These changes were stable when repeated a year later. At her last clinical examination at the age of 6 years and 6 months, she had severe motor and speech delay. She does not speak but shakes her head to say yes/no. She has severe quadriparesis, she does not stand or sit on her own. Dysmorphic features on her last examination included myopathic facies, epicanthal folds, broad nasal root, and long philtrum. She has a single palmar crease unilaterally. She has

not had recurrent or unusual infections. Extensive metabolic assessment was normal, including urinary organic acids, plasma amino acids, very long chain fatty acids, transferrin electrophoresis, urine purines/pyrimidines, and urine mucopolysaccharides. Microarray analysis did not reveal any known pathogenic changes. Huntington disease molecular testing was done because of globus pallidus involvement, and was normal.

K:II-1

Individual K:II-1 is a 9 year 11-month-old male born to healthy non-consanguineous Caucasian parents with unremarkable family history. He was born at 36 weeks of gestation after an uneventful pregnancy. His birth weight was 3.2 kg (+2SD) and birth length was 46 cm (-1SD). The patient was admitted to the Neonatal Intensive Care Unit for 29 days postnatally requiring jet ventilation and continuous positive airway pressure (CPAP), and was diagnosed with pulmonary hypertension and secondary infections. He exhibited global developmental delay. He started sitting without support 21 months, crawling at 20 months and cruising at 22 months; He is currently able to walk with assistance but uses a wheelchair for ambulating to longer distances. He started babbling at 10 months, saying single words at 22 months and using sentences at 3 years. The patient receives physical, occupational, and speech therapies and uses an augmentative device. He began finger feeding self at 5 years of age and at 8 years started using modified utensils. In infancy, he has exhibited generalized hypotonia, and two episodes of aspiration pneumonia, and was diagnosed with gastroesophageal reflux disease (GERD); his swallow study showed liquid aspiration. He had exotropia, which required 4 surgical repairs. At 8 years of age, he was diagnosed with generalized anxiety disorder, ADHD and some behavioral regulation difficulties; he was placed on Ritalin and Risperidone. Because of sleeping difficulties, he was started with melatonin. He has learning disabilities in reading and math. Severe eczema was noted in early infancy and he exhibits multiple food allergies and hyperreactive airway disease. He started exhibiting choreic movements superimposed with dystonia that interrupts his gross and fine motor skills. On his last physical exam, his weight, height and OFC were all below the 1st percentile. He had no major dysmorphic features. He had mild hypertelorism, deep set and hooded eyes, smooth philtrum and thin upper lip. Neurological exam demonstrated his abnormal body movements. Brain MRI, metabolic studies, and PWS/AS methylation methylation studies all gave normal results. Chromosomal microarray analysis revealed a duplication on chromosome 15 between coordinates 18,810,004-20,407,431 (hg18), which was reported in databases of healthy controls and therefore was considered as a benign variant.

L:II-2

Individual L:II-2 is the second of four children of healthy non-consanguineous parents. The family history is uneventful. The pregnancy was complicated by an increased risk of trisomy 21 on prenatal screening (serum marker). He was born at term with a weight of 3580g, 52.5 cm of length and an OFC of 35 cm. Milestones of motor development were moderately delayed: sitting without support 1 year and crawling 10m, he was walking at 18 months. Speech development was severely impaired, with only a few words spoken at age 3 years, short sentence at 4 years. He showed behaviour difficulties with tantrum which improved over time. He suffered from recurrent, mostly upper airway infections and asthma. He showed hypodontia and suspected enamel defects. At the last clinical examination at the age of 9 years and 9 months his height was 138 cm (+1 SD), weight 26.6 kg (-0.75 SD), and OFC 50.5 cm (-1.5 SD). Facial dysmorphisms consisted of hypertelorism, up slanting palpebral fissures, hypoplastic nostrils, thin eyebrows, low hanging columnella, long philtrum and thin upper and lower lip vermillion. He speaks with simplified language, makes well-constructed sentences, counts up to 10, does not read but recognizes letters. He has been in special education throughout her schooling. Brain MRI, Urinary organic acids, plasma amino acid chromatography and Fragile X studies all gave normal results. Microarray analysis revealed a 1.3Mb deletion at 17p12 (with PMP22), which was inherited from his mother.

A

	-1 23 6
BCL11B Homo sapiens	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Homo sapiens	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Rhesus macaque	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Rhesus macaque	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Bos taurus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Bos taurus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Canis lupus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Canis lupus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Gallus gallus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Gallus gallus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Mus musculus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Mus musculus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Rattus norvegicus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Rattus norvegicus	DTCEYCGKVFKNCSNLTVHRRSH
BCL11B Xenopus tropicalis	DTCEFCGKVFKNCSNLTVHRRSH
BCL11A Xenopus tropicalis	DTCEFCGKVFKNCSNLTVHRRSH
BCL11B Danio rerio	DTCEYCGKVFKNCSNLTVHRRSH
BCL11A Danio rerio	DTCEYCGKVFKNCSNLTVHRRSH

B

BCL11B-wt
ZnF2_C2H2 and ZnF3_C2H2

BCL11B-p.(Asn441Lys)
ZnF2_C2H2 and ZnF3_C2H2

Punwani et al.

BCL11B-wt
ZnF4_C2H2 and ZnF5_C2H2

BCL11B-p.(Asn807Lys)
ZnF4_C2H2 and ZnF5_C2H2

Individual E:II-1

<u>Figure S1.</u> p.(Asn807Lys) affects the DNA recognition site of the zinc finger domain. (A) Evolutionary conservation of the ZnF4_C2H2 domain. The sequence of BCL11B and BCL11A ZnF4_C2H2 domains is evolutionary highly conserved from humans to zebrafish. The as the four canonical 'specificity residues' −1, 2, 3 and 6, asparagine, serine, asparagine and valine, respectively, are indicated in bold. Asparagine at position 807, mutated in individual E:II, defined -1 is indicated in red. Cysteines and histidines involved in the zinc coordination are indicated in blue. Indicated in grey is the tyrosine, the only not conserved amino acid within the ZnF4_C2H2 domain. (B) Missense mutations p.(Asn441Lys) and p.(Asn807Lys) alter the DNA-binding motif of BCL11B. Comparison of the consensus sequence logos of the ZnF2_C2H2 and ZnF3_C2H2 (for p.(Asn441Lys)) and ZnF4_C2H2 and ZnF5_C2H2 (for p.(Asn807Lys)) domains for wildtype BCL11B and respectfully mutated BCL11B, as computed by the prediction algorithm "Zinc Finger Recognition Code". Wildtype BCL11B is suggested to selectively bind with genomic sequences containing a GGAAAT core, whereas the p.(Asn441Lys) should preferentially bind a GGAAGT, and p.(Asn807Lys) a GGATAG sequence.

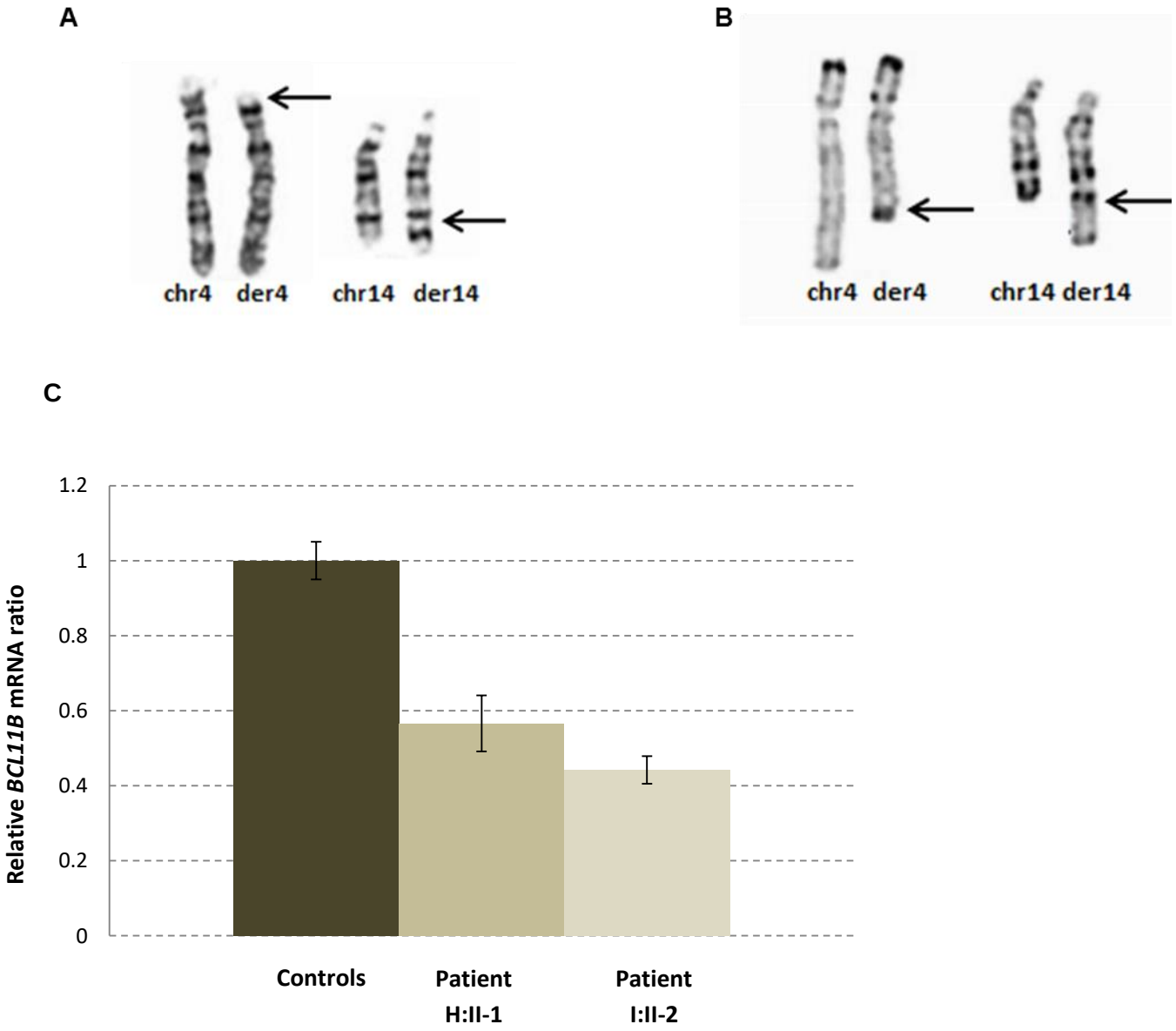
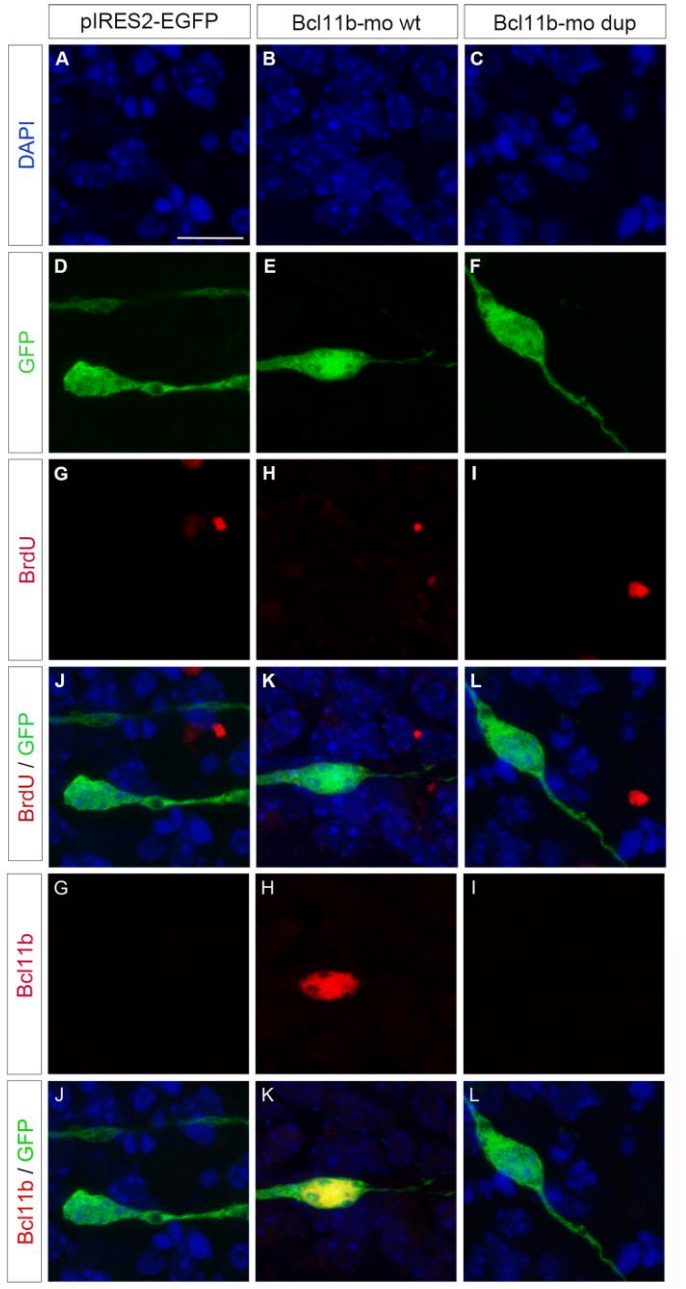


Figure S2. Cytogenetic and *BCL11B* expression analyses in patients H:II-1 and I:II-2. (A and B) partial GTG karyograms of patient H:II-2 showing t(4;14)(q31.3;q32.2) and partial RHG karyograms of patient I:II-2 showing t(4;14)(q31.3;q32.2) translocation. Black arrows denote the translocation breakpoints. (C) shows relative *BCL11B* expression in patients H:II-1 and I:II-2, mRNA level was evaluated by RT-qPCR in both patients and controls (n=3).


pIRES2-EGFP
Bcl11b-mo wt
Bcl11b-mo dup
DAPI
GFP
BrdU
BrdU / GFP
Bcl11b
Bcl11b / GFP
A
B
C
D
E
F
G
H
I
J
K
L

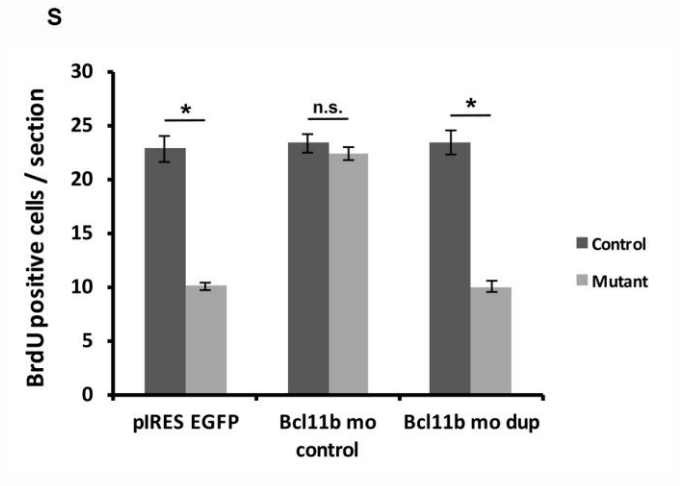

S
BrdU positive cells / section
30
25
20
15
10
5
0
*
n.s.
*
Control
Mutant
pIRES EGFP
Bcl11b mo control
Bcl11b mo dup

Figure S3. Functional analysis of the corresponding mouse p.Gly820Ala*fs27 Bcl11b mutation.** (A – R) Immunohistological analysis of Bcl11b$^{flox/flox}$;Emx1-*Cre* hippocampal slice cultures after 11 DIV electroporation. Animals were eletroporated with pIRES-EGFP (A, D, G, J, M, P), pIRES2-EGFP Bcl11b-mo wt (wildtype mouse Bcl11b cDNA) (B, E, H, K, N, Q) as well as pIRES2-EGFP Bcl11b-mo dup (corresponding duplication mutation of mouse Bcl11b cDNA (C, F, I, L, O, R). DAPI (blue) as morphological marker, GFP (green) and BrdU (red) as marker for cell proliferation as well as Bcl11b (red). Images were taken at 63X magnification, 2X zoom. (**S**) Statistical analysis of BrdU-positive cells in hippocampal slice cultures. (t-test, *; P<0.0005;, Control n=4, Mutant {Bcl11b$^{flox/flox}$;Emx1-*Cre* } n=4). Scale bar: 10μm (A).

Tables S1A-S1F. *De novo* variants identified in patients A to Lby trio whole-exome sequencing*

TABLE S1A. *De novo* variants identified in A:II-3

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.2449_2456dupAGCCACAC	p.Gly820Ala*fs**27	no
BAI3	6	c.1085G>A	p.Asp186Asn	no

TABLE S1B. *De novo* variants identified in B:II-3

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1944_1965delGGCGCGGTCAACGGGCGCGGGG	p.Gly649Ala*fs**67	no
CAMSAP1	9	c.2720_2741delGCCTGAAGCTCGGCAAGGCTGC	p.Arg907His*fs**6	no

TABLE S1C. *De novo* variants identified in C:II-2

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.2671delG	p.Ala891Pro*fs**106	no

TABLE S1D. *De novo* variants identified in D:II-1*

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1501dupC	p.Thr502His*fs**15	no
RELN	7	c.9284G>A	p.Trp3095*	no

TABLE S1E. *De novo* variants identified in E:II-1

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.2421C>G	p.Asn807Lys	no

TABLE S1F. *De novo* variants identified in F:II-2

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.242delG	p.Cys81Leu*fs**76	no
NYAP1	7	1252C>A	p.Pro418Thr	no

TABLE S1G. Maternally inherited variants identified in G:III-1

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1600delG	p.Asp534Thr*fs**29	no
CHD5	1	c.203A>C	p.Lys68Thr	no

TABLE S1H. *De novo* variants identified in J:II-1*

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1495G>T	p.Glu499*	no

TABLE S1I. *De novo* variants identified in K:II-1

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1365_1367delCAA	p.Tyr455*	no

TABLE S1J. *De novo* variants identified in L:II-2

Gene	Chromosome	Nucleotide Change	Protein Change	ExAC
BCL11B	14	c.1552delC	p.Arg518Ala*fs**45	no
DMAP1	1	c.82_84delAAG	p.Lys28del	no

* Whole-exome sequencing in individuals D:II-1 and J:II-1 was not performed in a trio setting, the *de novo* status was confirmed by Sanger sequencing.

TABLE S2. Cell blood counts and immune phenotyping of patients with alterations in BCL11B.

Individual	**A:II-3**	**B:II-2**	**C:II-2**	**D:II-1**	**E:II-1**	**F:II-2**	**G:III-1**	**H:II-1**	**J:II-1**	**K:II-1**	**L:II-2**	**Normal range**
Sex	Female	Male	Male	Female	Male	Male	Female	Male	Female	Male	Male	
Age (years)	4	15	1	4	2	18	13	13	6 6/12	9 11/12	10 5/12	
Hematocrit and cell counts												
Hematocrit (%)	35.3	43.2	47.6	31.2	39.9	40.4	40.0	43.2	36.3	37.6	39.9	32-43
WBC (10^6/mL)	4.50	15.0	15.0	6.07	-	8.7	10.8	8.1	2.4	7.5	7.2	5.5-15.5
Neutrophils (10^6/mL)	2.79	7.48	4.3	1.6	-	-	3.8	2.8	1.1	-	2.5	1.5-8
Eosinophils (10^6/mL)	0.24	1.58	0.12	0.36	0.05	0.97	0.1	0.7	0.5	-	1.16	< 0.5
Monocytes (10^6/mL)	0.27	0.92	1.05	0.73	0.22	0.59	0.46	0.7	0.2	-	0.7	0.2-0.9
Lymph. (10^6/mL)	1.51	5.34	9.45	3.22	2.4	3.52	4.5	3.4	0.5	1.95	2.9	1.5-7
Lymphocytes												
T cells (%)	71.5	70.1	65.8	37.9	42.9	79	65.4	72.3	-	77	30.7	49-83
B cells (%)	16.3	11.5	21.1	31.8	36.0	8	16.0	7.5	-	15	5.1	8-31
T-lymphocytes												
CD4+ (%)	44.9	41.8	26.3	65.5	52,5	38	59	44	28	59.7	35.4	27-53
CD8+ (%)	38.3	39.4	48.9	21.0	27,0	43	26.1	39.5	25	35.0	32.6	16-40
CD4/CD8 ratio	1.2	1.1	0.5	3.1	1.9	0,9	2.2	1.1	1.1	1.7	1.1	0.7-2.6
T-γδ (%)	13.0	13.1	18.8	8.1	18.7	19[§]	9.6	16.8	-	5.3[§]	23.9	< 6
Treg (% in CD4+)	6.8	5.1	7.3	4.9	9.4	-	4.0	5.8	-	-	3.78	4.5-10 *
Naïve CD4+ (%)	32.6	27.8	69.9	44.7	48.5	-	61.3	30.2	49	-	40.6	50-70 *
RTE in CD4+ (%)	8.2	2.4	4.9	10.7	8.8	-	27.1	10.5	16	-	13	age depend.
Naïve CD8+ (%)	20.9	27.7	48.0	43.2	73.2	-	47.1	9.8	-	-	22.1	50-90 *
DR+ CD4+ (%)	0.2	2.7	1.7	3.3	2.1	-	0.1	0.3	-	normal	1.3	0.1-1.8 *
DR+ CD8+ (%)	2.0	1.8	2.8	4.8	1.0	-	0.3	0.1	-	normal	0.6	0.3-2.6 *

TNFα+ IFNγ+ in CD4+ (%) IL17+	59.1 31.2 1.7	58 32.1 3.4	32.4 5.2 0.6	76.4 4.6 2.4	50.2 6.4 0.6	-	24.8 6.4 1.7	18.9 6.8 2.6	-	-	8.61 9.73 2.02	43-86 * 5-15 0.5-4
TNFα+ IFNγ+ in CD8+ (%)	35.7 48.8	40.8 46.6	8.4 25.9	22.3 55.3	17.2 3.6	-	8.6 17.3	7.7 12.5	-	-	4.18 12.1	10-20 * 7.5-20
Innate Lymphoid cells (% in lymphocyte gate)												
NK cells (%)	7.1	13.6	10.9	23.3	9.0	11	2.9	11.3	27.3	4	3.6	3-30
ILC (% in lymph.)	0.169	0.097	0.104	0.241	0.045	-	0.230	0.050	-	-	0.144	0.169-0.621 *
ILC1 (% in lymph.)	0.051	0.028	0.075	-	0.013	-	0.200	0.011	-	-	-	0.016-0.433 *
ILC2 (% in lymph.)	0	0.012	0.007	0.008	0.003	-	0.009	0.002	-	-	0.008	0.027-0.131 *
ILC3 (% in lymph.)	0.118	0.058	0.022	-	0.029	-	0.028	0.036	-	-	-	0.027-0.158 *
***BCL11B* alteration**	p.Gly820Ala *fs**27	p.Gly649Ala *fs**67	p.Ala891Pro *fs**106	p.Thr502His *fs**15	p.Asn807Lys	p.Cys81Leu *fs**76	p.Asp534Thr *fs**29	Positional effect	p.Glu499*	p.Tyr455*	p.Arg518Ala *fs**45	

WBC, white blood cells
Treg, regulatory T cells, defined as CD4+ CD25hiCD127lo
Naïve T cells, defined as CD45RA+CCR7+
RTE (Recent Thymic Emigrants), defined as CD31+ CD45RA+ in CD4+ lymphocytes
NK cells, defined as CD56+ CD3-
ILC (innate lymphoid cells), defined as CD45+ lin- CD127+ CD161+. ILC1 (type 1 ILC), were defined as CRTh2- c-kit- ILCs; ILC2 (type 2 ILC), defined as CRTh2+ ILCs; and ILC3 (type 3 ILC), as CRTh2- c-kit+ ILCs[13]
-, not done
Values outside the normal range are marked in red (higher than normal) and blue (lower than normal).

* according to our own reference values (n=14 healthy donors under 16)

§ For this patient, the percentage of Tγδ cells was not estimated. The given value corresponds to CD3+CD4-CD8- cells