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Supplemental Data

Deficiency for the Ubiquitin Ligase UBE3B in a Blepharophimosis-Ptosis-Intellectual Disability Syndrome

Lina Basel-Vanagaite, Bruno Dallapiccola, Ramiro Ramirez-Solis, Alexandra Segref, Holger Thiele, Andrew Edwards, Mark J. Arends, Xavier Miró, Jacqueline K. White, Julie Désir, Marc Abramowicz, Maria Lisa Dentici, Francesca Lepri, Kay Hofmann, Adi Har-Zahav, Edward Ryder, Natasha A. Karp, Jeanne Estabel, Anna-Karin B. Gerdin, Christine Podrini, Neil J. Ingham, Janine Altmüller, Gudrun Nürnberg, Peter Frommolt, Sonia Abdelhak, Metsada Pasmanik-Chor, Osnat Konen, Richard I. Kelley, Mordechai Shohat, Peter Nürnberg, Jonathan Flint, Karen P. Steel, Thorsten Hoppe, Christian Kubisch, David J. Adams, and Guntram Borck

Supplementary Note

Clinical description of the three individuals affected by the *UBE3B*-deficient BPID syndrome

Individual 1 (family 1) was the first child of healthy first-cousin parents of Israeli Arab origin. The family history was unremarkable. She was born after an uneventful pregnancy at 42 weeks of gestation by vaginal delivery. Birth weight was 2625 g (-2 SD) and occipito-frontal circumference (OFC) 31.5 cm (-2 SD). The Apgar score was 6 at 1 minute and 9 at 5 minutes. Shortly after birth she was diagnosed with atrial septal defect (ASD), ventricular septal defect (VSD) and aortic coarctation which required surgical intervention. At the age of 6 months she underwent balloon dilatation of the aorta because of aortic restenosis. Following recurrent vomiting, she was diagnosed with bowel malrotation with absent ligament of Treitz and at the age of 3 months a Ladd's operation was performed. She had frequent episodes of stridor due to severe laryngomalacia observed on bronchoscopy. Due to congenital dislocation of the hip she underwent a right hip reduction operation.

The patient was evaluated for the first time in the genetics clinic at the age of 1 year. She had severe failure to thrive and severe psychomotor developmental delay. Her weight was 6.35 kg (-3.5 SD), height 67.5 cm (-2.2 SD) and OFC 39.8 cm (-3 SD). Physical examination revealed dysmorphic features including prominent forehead, arched eyebrows with medial flaring, telecanthus, blepharophimosis (palpebral fissure length -3 SD), left ptosis, anteverted nares, long philtrum, small mouth and mild micrognathia. The ears were low set. She had thin skin and sparse scalp hair. At the last examination at the age of 3 years and 3 months her weight was 11.4 kg (-3 SD), height 92 cm (-1.5 SD) and OFC 44 cm (-4 SD). The teeth were of normal size and position. Global psychomotor skills were severely delayed. She sat unsupported at the age of 2 years 8 months. At the age of 3 years 3 months she cannot stand unsupported. She produces only several babbling sounds and

cannot say any words. She has good eye contact, is alert and smiles frequently, and actively looks for objects.

Routine laboratory analysis results at the ages of 10 months and 3 years 3 months were normal (except for low HDL levels), including normal thyroid function. Amino acids in blood, organic acids in urine, blood lactate, pyruvate and ammonia were all normal. Cytogenetics Whole-Genome 2.7M Array (Affymetrix) revealed no pathogenic copy-number variant. Audiological evaluation and tympanometry revealed mild conductive hearing loss (40-45 dB) and ventilation tubes were inserted. Ophthalmologic evaluation showed left ptosis and astigmatism. Eye fundus examination was normal. A renal ultrasound scan revealed minimal left pyelectasis. At the age of 22 months, brain MRI showed a Chiari malformation type 1, ventriculomegaly and a hypoplastic corpus callosum. She had no seizures and no EEG recording was performed.

Detailed clinical evaluations of individuals 2 and 3 (family 2) have been reported previously[19]. We here provide a clinical follow-up.

Individual 2. At the last examination at the age of 7 years and 7 months her weight was 17.2 kg (3^{rd}-10^{th} percentile), height 110 cm (3^{rd}-10^{th} percentile) and OFC 49 cm (3^{rd} -10^{th} percentile). Inner canthal distance (ICD) was 3.5 cm (+2 SD). At the age of 5 years, feeding behavior improved. At the age of 6 years, she still had gastro-esophageal reflux and constipation and bone age was delayed (corresponding to 5 years). Language was still absent with no babbling sounds. At 3 years of age, TSH was elevated (4.5 mIU/L, normal 0.3–3.6), with normal serum levels of thyroid hormones. Thyroid ultrasound showed a reduced gland volume. At 5 years of age, TSH levels had normalized.

Physical examination remained unchanged throughout the years. At the last examination the girl had a quite distinctive facial gestalt with prominent forehead, sparse eyebrows, blepharophimosis, upward slanted palpebral fissures, unilateral ptosis, epicanthal folds, hypertelorism and dystopia canthorum, mid-face hypoplasia, long philtrum and mild

micrognathia. The ears were low set with over-folded helices, prominent anti-helix and wide antitragus. There was clinodactyly of the fifth fingers.

Individual 3. At the last examination at the age of 3 years 1 month, his height was 86 cm (-2 SD), weight 10.3 kg (-2 SD) and OFC 44.5 cm (-2 SD). Inner canthal distance was 3.3 cm (+2 SD). He had recurrent bronchitis until one year of age. Therapy with growth hormone was started at 18 months of age and did not result in any significant improvement in height.

Craniofacial characteristics included microcephaly, blepharophimosis, upward slanting palpebral fissures, hypertelorism, telecanthus, mid-face hypoplasia, posteriorly rotated ears with over-folded helices (notched in the superior helical portion), prominent antihelices and wide antitragus, triangular nose tip with anteverted nares, long philtrum, high palate and micrognathia. Ectodermal abnormalities included fine sparse hair and eyebrows, thin pale skin and fragility of blood vessels. At 3 years of age, he is not able to walk. At 2 years and 10 months he started to produce babbling sounds but stopped shortly after and language was completely absent.

Individuals 2 and 3 had no history of seizures and no EEG recordings were performed. Array-CGH with a resolution of 75 kb (44k array, Agilent) was performed in Individual 2; no pathogenic copy number variants were detected.

Individual 4. At the last examination at the age of 1 year, her height was 69 cm (5^{th} percentile), weight 7,270 kg (-3 SD) and OFC 41.2 cm (-3 SD). She had recurrent bronchitis and otitis. She has right hip dislocation, laryngomalacia, and cervico-thoracic and thoraco-lumbar scoliosis. She had complex epilepsy starting at the age of 8 moths. EEG showed left temporal spikes and she was treated with levetiracetam.

Craniofacial characteristics included microcephaly, blepharophimosis, hypertelorism, telecanthus, mid-face hypoplasia, posteriorly rotated and low-set ears, triangular nose tip with anteverted nares, long philtrum, thin upper lip, and micrognathia. Ectodermal abnormalities included fine sparse hair and eyebrows. At 18 months of age, she was not able to sit alone and language was completely absent. Array-CGH with a mean resolution of 200

kb was performed (Cytochip Oligo ISCA 4x44K Bluegnome array) and yielded a normal result. Smith-Lemli-Opitz syndrome had been considered a differential diagnosis in Individuals 3 and 4 but ruled out by a normal 7-dehydrocholesterol level.[19]

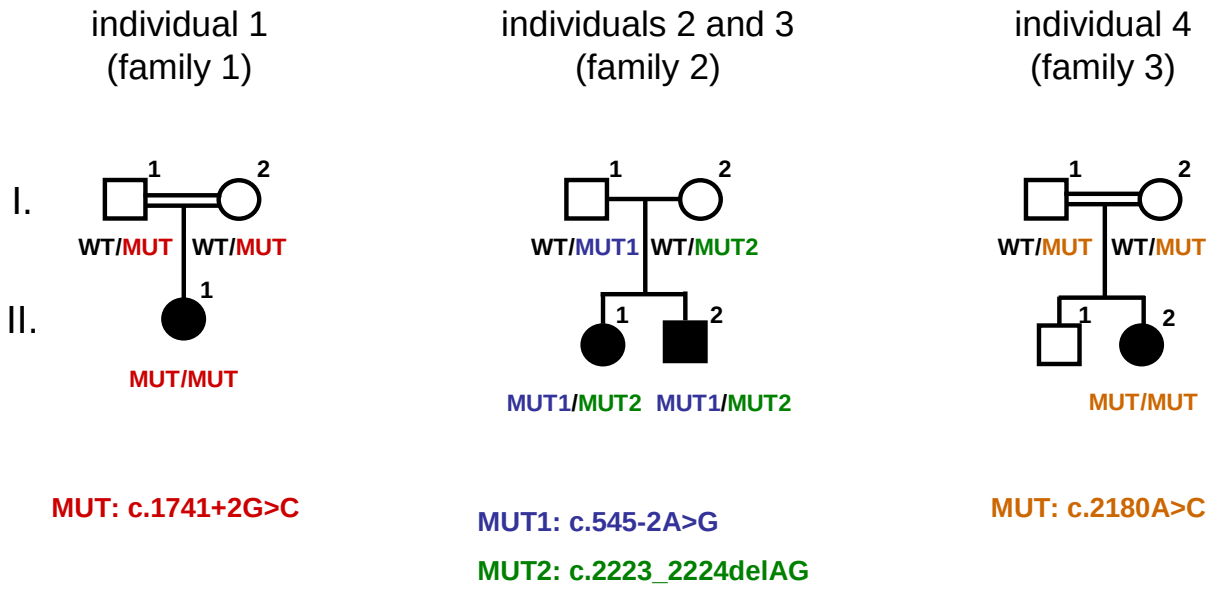


Figure S1. Pedigrees of the three families

Individuals affected by the BPID syndrome caused by biallelic *UBE3B* mutations are represented by black symbols. Parents of the affected individuals in family 1 and family 3 are first cousins.

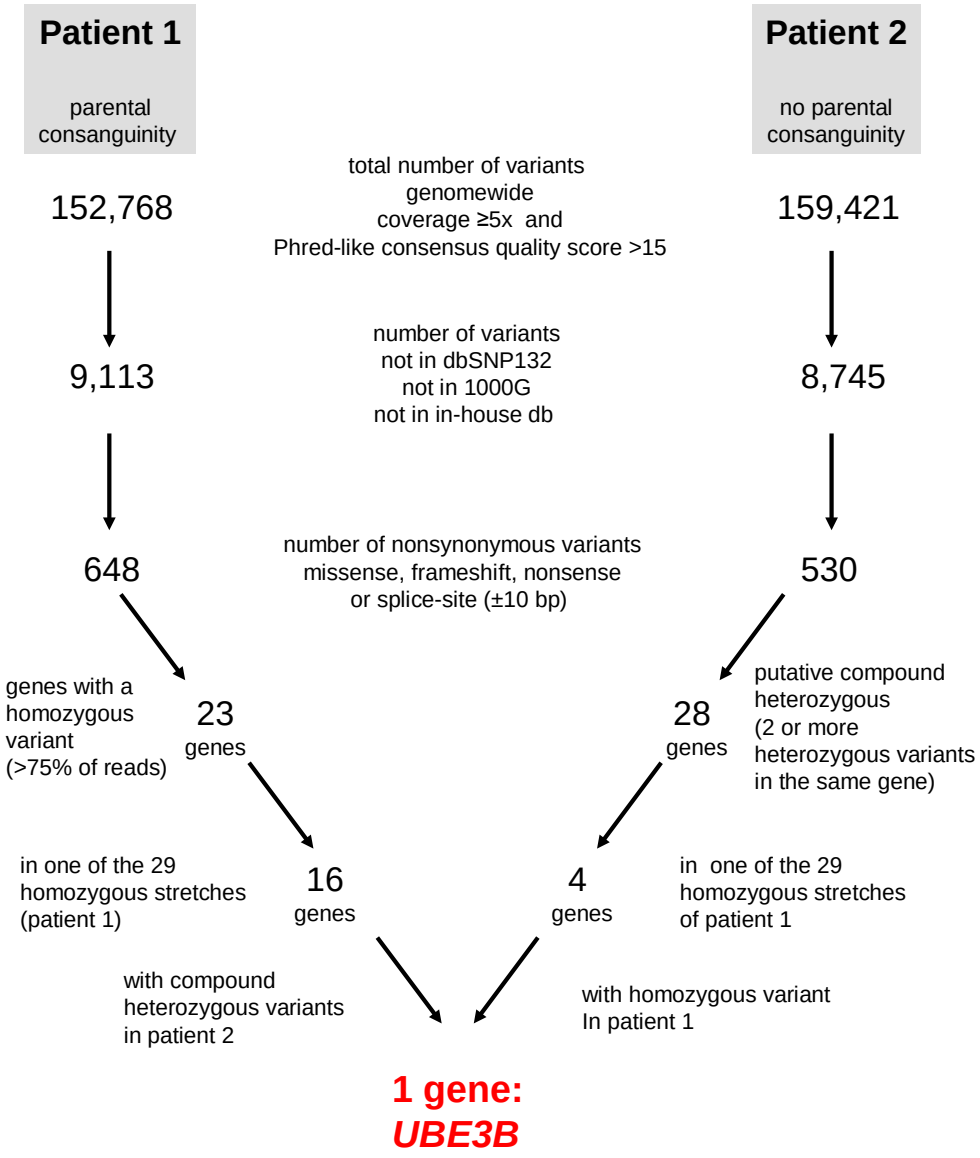


Figure S2. Flow Chart for Filtering of Sequence Variants Called in the Exomes of Patients 1 and 2

1000G, 1000 Genomes data.

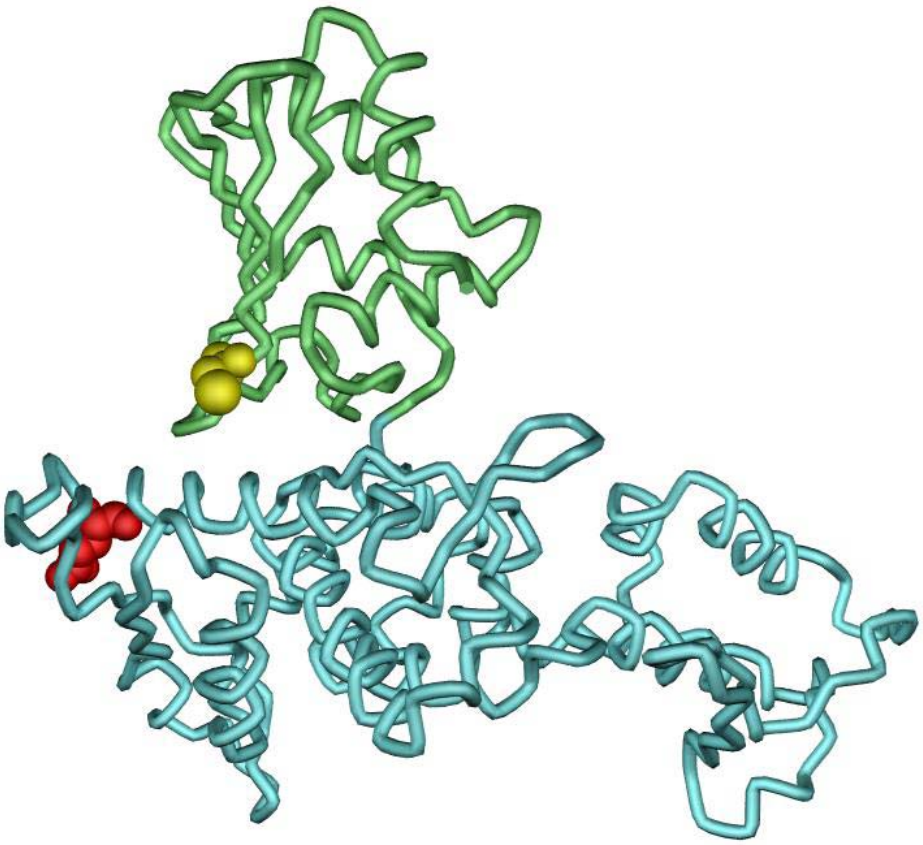
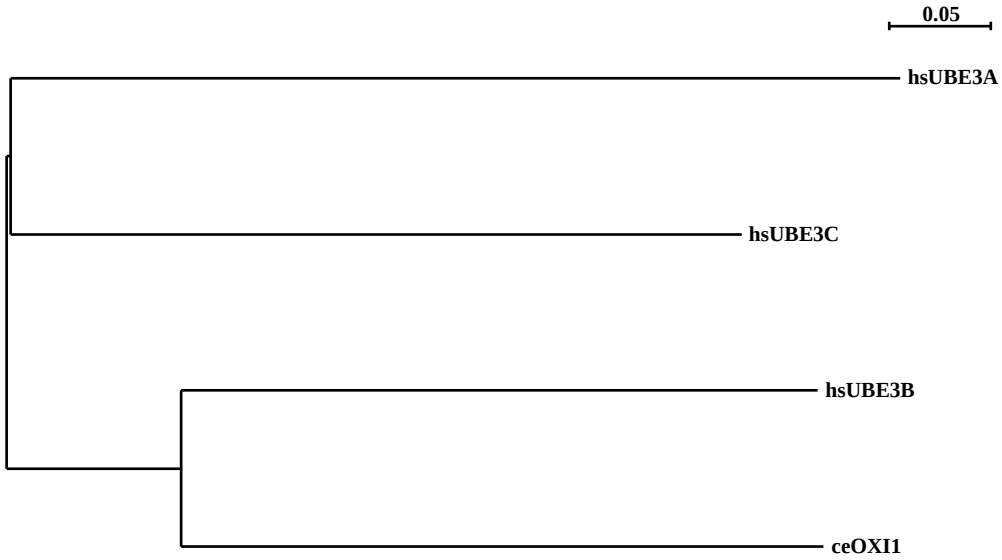


Figure S3. Three Dimensional Modeling of the UBE3B HECT Domain with the Catalytic Cysteine and the p.Gln727Pro Substitution

All HECT domains consist of two subdomains that are connected by a flexible linker. The large N-terminal subdomain (shown in cyan) contains the E2 binding site, while the small C-terminal subdomain (green) harbours the catalytic Cys residue (yellow) required for ubiquitin transfer to the substrate. The mutated Gln residue (shown in red) and the E2 binding site locate to opposite ends of the large subdomain. Thus, an influence of the mutation on the E2-mediated loading of the HECT domain is unlikely. Various published HECT domain structures differ in the rotation angle of the two subdomains relative to the flexible linker. In the SMURF2 structure (PDB:1ZVD), the catalytic cystein is pointing away from the E2-site, suggesting that it is poised for substrate ubiquitination. While the mutated Gln is not within reach of the catalytic site, it is on the same face of the HECT domain and might be involved in substrate recognition and binding. Thus, a possible consequence of the Gln-Pro mutation might be an inefficient substrate ubiquitination.

A



B

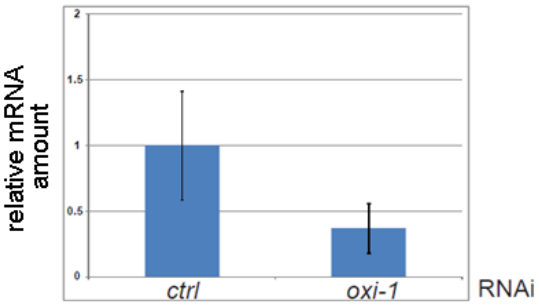


Figure S4. *oxi-1*, a *C. Elegans* Ortholog of UBE3B

(**A**) Phylogenetic tree of *C. elegans* OXI-1 (ceOXI1) and *Homo sapiens* (hs) UBE3A, UBE3B and UBE3C.

(**B**) Real-time PCR shows a reduction of *oxi-1* mRNA levels after *oxi-1(RNAi)* as compared to *control(RNAi) (ctrl).* Error bars, s.d.

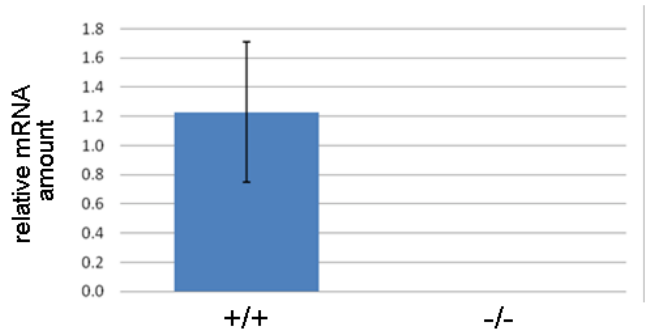


Figure S5. Absence of *Ube3b* mRNA in *Ube3b*$^{-/-}$ Mice

RT-PCR shows absence of *Ube3b* mRNA in *Ube3b*$^{-/-}$ mice. Relative quantity (± s.d.) of *Ube3b vs B2m* mRNA in WT (+/+) and *Ube3b*$^{-/-}$ mice (n=3 and n=5 mice, respectively).

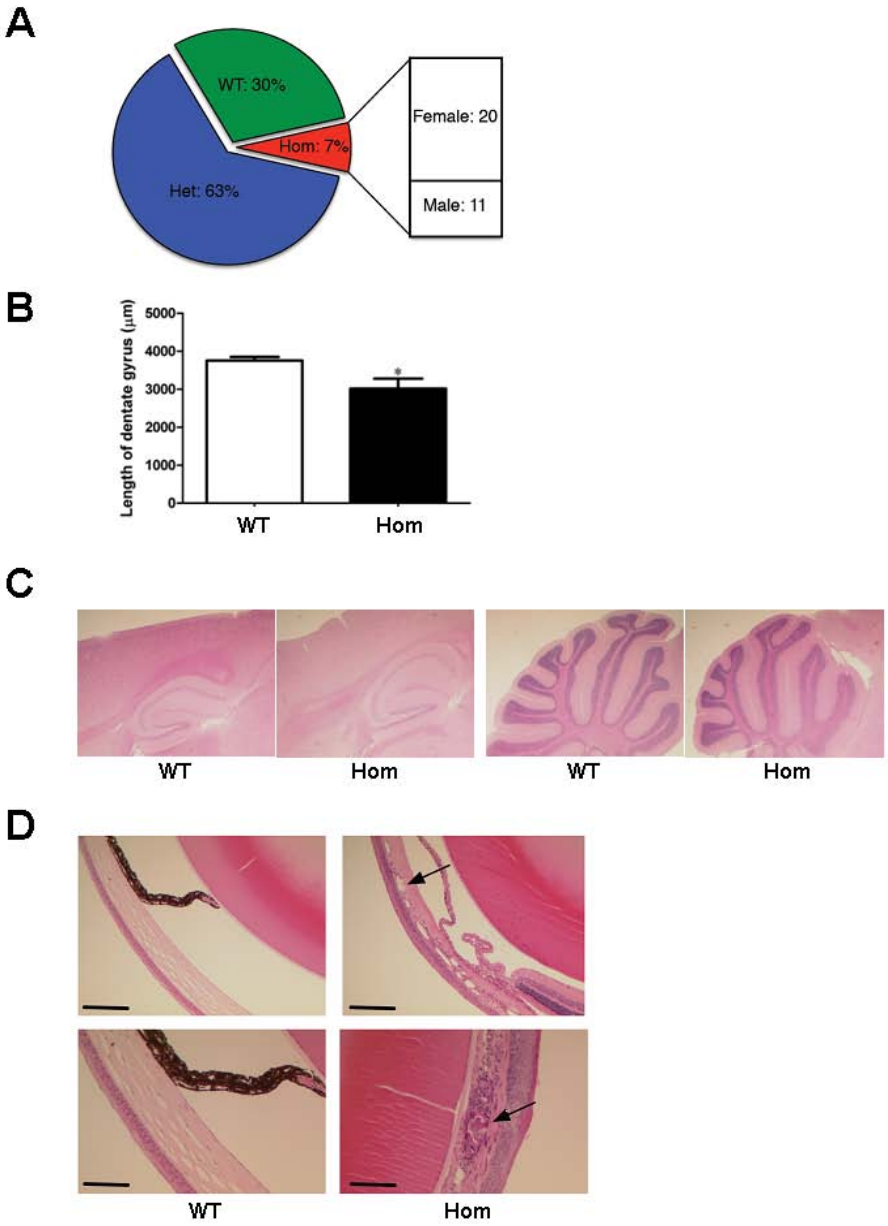


Figure S6. Additional Phenotypic Analyses of *Ube3b*$^{-/-}$ Mice

(**A**) Homozygous *Ube3b*$^{-/-}$ mice (Hom; red) show significantly reduced viability at 2 weeks of age compared to both wildtype (WT; green) and heterozygous (Het; blue) littermates (χ^2 test, *P*<0.0001). Total n=418 mice (124 WT, 263 Het, 31 Hom).

(**B**) Reduced length of the dentate gyrus is evident in homozygotes (* P<0.05). Error bars represent SEM.

(**C**) Histological examination (H&E stained sections at 25x magnification) shows normal brain structures in the *Ube3b*$^{-/-}$ mice with no obvious differences compared with the wildtype mice, including cerebral hemisphere with hippocampus (left panels) and cerebellum (right panels).

(**D**) *Ube3b*$^{-/-}$ mice show evidence of dilated lymphovascular channels (arrow in top panel) as well as acute inflammation with calcification (arrow in bottom panel) of the cornea. Scale bars indicate 300 μm (top) and 150 μm (bottom).

Primer	Primer sequence 5'→3'	Length of PCR product (bp)
UBE3B-1F	TTAGCGCTTTGGAGGGTGTTG	564
UBE3B-1R	GGAACAGCAGTTTCCTGGCAAT	
UBE3B-2F	CGAGAGCATGGTTCATCGATAG	646
UBE3B-2R	CTTCCTGACTCTTGCATGCATC	
UBE3B-3F	CTGTAGGAAGGAACTGCATGTG	502
UBE3B-3R	AAAGCAGTGGACCTGAATGGAC	
UBE3B-4F	GTAGCTACGTTCTGCTCTTGC	525
UBE3B-4R	AGGGTCCAACACACACCAAAG	
UBE3B-5F	TAACTCCTGGAGTGGTCATTGC	552
UBE3B-5R	GTAATCACTTGGAGGCTAAGGC	
UBE3B-6F	TTCCAGGTACGCATTGCCTTTC	444
UBE3B-6R	CTTTACCTGACCCACAAGTGAG	
UBE3B-7F	ATGCCTGGCTCACAATACAGT	363
UBE3B-7R	GCAGTCACAAATGTGCTCACTT	
UBE3B-8F	TGTCCCCAATTTCAGCCTTCAG	484
UBE3B-8R	TTGCAGATCACCCGTGGTTATG	
UBE3B-9F	ATTGTTCTTTGGGACCAGTGGC	569
UBE3B-9R	CAGGAAGCTAAAGGCTGACATC	
UBE3B-10F	TTGGCCCGTTTCTTACCTTTG	577
UBE3B-10R	AGGAAAACTGACAGGATGTTCAC	
UBE3B-11F	AGAGTGTGACTCATGGAGCTTG	597
UBE3B-11R	TGATCATGCCACTGCACTGTAG	
UBE3B-12F	TGGCCTGGCTGTGCATTTAG	452
UBE3B-12R	GTGAGCATGAGCAATGTAACTG	
UBE3B-13F	ATGGGGATAACACTGGGCTTCA	613
UBE3B-13R	TTACTGCACACTGCTCACTTGG	
UBE3B-14F	CCCGTCATCTAGGTTTTAAGC	635
UBE3B-14R	TCAACAGGAGATTCAGGGCA	
UBE3B-15F	GCAGACACTTAGCACTTACCCA	464
UBE3B-15R	TAACAGAAGCGTCCTGACAGGT	
UBE3B-16F	TGGACCAGACAGTCTCTGTTG	485
UBE3B-16R	CAGCACTGGGTGTAACCTCA	
UBE3B-17F	AGGATTTTCTCGTGGGCCATTC	562
UBE3B-17R	GCAGTCAACAGGACAAATGACC	
UBE3B-18F	TCCTGTCATGCATCCACGTTTC	445
UBE3B-18R	TCCCCACTGGTTGTCTGCAAG	
UBE3B-19F	TGACCCAGCACTCAATCTGTTC	437
UBE3B-19R	AGATGAGATGCCACAGGCAAAG	
UBE3B-20F	CCCTCCGGATTGGAAACTTAG	574
UBE3B-20R	GTTAGGGCCATGGAGCACTAT	
UBE3B-21F	CGAAGTCCCAGAAGGAGATG	537
UBE3B-21R	GCTTCAAAGGCCAGGTCAAC	
UBE3B-22F	CCCTCCATGACATCCCGAAGT	539
UBE3B-22R	GGCCTAACAAAGTGCTGGGATT	
UBE3B-23F	TGCCCTCAAAGGCTTGATGTG	545
UBE3B-23R	CAAAGCAGTCTAGCCAGTCCA	
UBE3B-24F	CAGGGCCAGCTCTCAAATTC	608
UBE3B-24R	CAAGGCCAGCATAGGCAAAG	
UBE3B-25F	TGGGCATTTGTGCTGTTCCTAC	622
UBE3B-25R	CTGAGGAAGGAAAGAGAAAGGG	
UBE3B-26F	GGCCCCATTTCCAAAAGCTTAC	641
UBE3B-26R	TTAGCGCTTTGGAGGGTGTTG	

Table S1. Sequences of oligonucleotide primers used to PCR amplify and sequence the *UBE3B* coding exons and exon-intron boundaries

bp, base pairs.

Patient	Number of reads	Number of reads on target	Mean target coverage (fold)	Coverage 1x or more (%)	Coverage 10x or more (%)	Coverage 30x or more (%)
1 (Fam. 1)	101,262,749	21,249,936	124.5	97.5	89.8	80.2
2 (Fam. 2)	101,035,928	21,225,224	124.2	98.0	91.1	82.2

Table S2. Statistics of exome sequencing in patients 1 and 2

Chromosome	From (position hg19)	To (position hg19)	Size (bp)	Number of genes
1	101,722,147	104,116,413	2,394,267	9
2	83,084,389	85,143,835	2,059,447	10
2	116,066,984	119,695,176	3,628,193	10
2	198,012,407	201,354,935	3,342,529	24
2	237,489,904	242,694,399	5,204,496	69
3	403,288	3,887,093	3,483,806	10
3	15,311,464	21,179,734	5,868,271	35
3	26,057,822	28,381,887	2,324,066	10
4	57,686,821	61,529,532	3,842,712	13
4	84,519,076	90,743,331	6,224,256	55
4	96,127,869	99,337,881	3,210,013	8
6	37,502,110	40,347,258	2,845,149	21
6	73,102,442	78,171,941	5,069,500	42
6	91,260,116	95,054,582	3,794,467	6
7	81,667,468	86,542,455	4,874,988	8
7	90,584,944	93,515,993	2,931,050	25
10	16,562,600	18,789,724	2,227,125	16
10	30,978,206	38,939,345	7,961,140	61
10	42,775,414	46,963,951	4,188,538	55
10	49,239,507	73,822,507	24,583,001	171
11	68,675,497	71,306,127	2,630,631	29
11	71,507,311	98,436,527	26,929,217	248
11	120,490,713	133,714,543	13,223,831	190
12	108,035,903	113,348,870	5,312,968	86
13	40,325,310	46,550,138	6,224,829	87
13	113,053,470	115,090,193	2,036,724	37
15	31,369,258	42,005,772	10,636,515	113
19	1,507,710	7,529,415	6,021,706	169
19	43,290,773	53,418,611	10,127,839	377
Total:			183,201,219	1,994

Table S3. Regions of Homozygosity in the Exome of Patient 1

Twenty-nine regions of homozygosity >2 Mb as determined by analysis of SNPs in the exome sequence data from patient 1. The *UBE3B* gene is located in the 5.3 Mb chromosome 12 region highlighted in red.