The American Journal of Human Genetics, Volume *101*

Supplemental Data

De Novo Missense Mutations in *DHX30* Impair Global Translation and Cause a Neurodevelopmental Disorder

Davor Lessel, Claudia Schob, Sébastien Küry, Margot R.F. Reinders, Tamar Harel, Mohammad K. Eldomery, Zeynep Coban-Akdemir, Jonas Denecke, Shimon Edvardson, Estelle Colin, Alexander P.A. Stegmann, Erica H. Gerkes, Marine Tessarech, Dominique Bonneau, Magalie Barth, Thomas Besnard, Benjamin Cogné, Anya Revah-Politi, Tim M. Strom, Jill A. Rosenfeld, Yaping Yang, Jennifer E. Posey, LaDonna Immken, Nelly Oundjian, Katherine L. Helbig, Naomi Meeks, Kelsey Zegar, Jenny Morton, the DDD study, Jolanda H. Schieving, Ana Claasen, Matthew Huentelman, Vinodh Narayanan, Keri Ramsey, C4RCD Research Group, Han G. Brunner, Orly Elpeleg, Sandra Mercier, Stéphane Bézieau, Christian Kubisch, Tjitske Kleefstra, Stefan Kindler, James R. Lupski, and Hans-Jürgen Kreienkamp

Supplemental Note: Case Reports

Individual A is a 5-year-old girl, the only child of unaffected, non-consanguineous French parents of Caucasian European descent. Regarding family history, her mother was diagnosed with multiple sclerosis 13 years ago, her father's twin brother had a history of epilepsy and one of her father's sisters had absence seizures, while one of her maternal uncle presents a intellectual deficiency of unknown etiology. The pregnancy was uncomplicated with normal screening ultrasounds. She had normal birth length (51 cm, +0.7 SD), weight (3100 gram, -0.7 SD) and Occipito-Frontal Circumference (OFC; 33.5 cm, -0.9 SD). She had myoclonic seizures since the age of one month, accompanied by repeated absence seizures. Her milestones of motor development were delayed: she was able to sit independently at 12 months and to walk at 32 months of age. At last thorough clinical assessment, at 3 years and 9 months old, her height was 97 cm (-0.5 SD), her weight was 16 kg (-1.4 SD) and her OFC was 47 cm (-2 SD). Her walking was unsteady with frequent slips and falls. She spoke 20 words. She displayed muscular hypotonia and poor fine motor skills. Toilet training was delayed, and she was unable to feed herself. She experienced constipation and sleep disturbance which required melatonin (5 mg/d) with nonetheless persistent night awakening. She showed attention deficit and had auto-aggressive and hetero-aggressive behaviors, low frustration tolerance and showed hand flapping motor stereotypies. Physical examination revealed no obvious dysmorphic features, save convergent strabismus and relatively broad hands and feet, together with a mild distal joint hyperlaxity, a café-au-lait spot in the left basi-thoracic region, two one-centimeter large hypochromic patches respectively next to the left shoulder blade and at the right side. Left cutaneous plantar reflex response was indifferent, whereas the right one was uncertain. Normal control of voluntary purposeful eye movement ruled out a possible oculomotor apraxia. Brain MRI and metabolic investigations, including serum creatine kinase (SCK) were unremarkable. Electroencephalogram (EEG) was normal, as well as neurotransmitter levels in cerebrospinal fluid. *FMR1*, *RAI1*, *CDKL5*, *MECP2* and *FOXG1* genes sequencing and array-CGH gave normal results. Trio whole exome sequencing (trio-WES), with DNA samples of both unaffected parents and the proband, revealed a *de novo* variant c.1478G>A, p.Arg493His, in *DHX30* (NM_138615.2).

Individual B is a 13-year-old boy, the only child of unaffected, non-consanguineous parents of Dutch ancestry. Family history was non-contributory. He was born after 37 weeks of gestation

with a birth weight of 2660 grams (-0.5 SD). As a baby, he had poor feeding due to hypotonia. Unilateral cryptorchidism was present. Psychomotor development was severely delayed. He was able to walk unsupported at the age of 4 years and spoke his first words at the age of 6 years. Automutilation had been reported at younger age. Esophoria of the right eye and mild hypermetropia were observed. A brain MRI at the age of 8.5 years was normal. At the last examination at the age of 13 years, he was able to speak 4 single words and to walk short distances unsupported, however he required a wheelchair for longer distances. His had an ataxic and dystonic gait and severe hypotonia of the trunk. He has a cheerful behavior, but needs structure and has stereotypies. Food has to be minced because of swallowing difficulties. Physical examination at the age of 13 years showed a low-normal OFC of 52.5 cm (-1.5 SD). His height was normal (0 SD) and his weight was at -1 SD He has facial dysmorphisms including mild facial hypoplasia, prominent crus helix, thin eyebrows, full upper eyelids, full and squared nasal tip and eversion of the lower lip. Examination of the extremities showed broad hands and tapering fingers, broad first digits of the feet, pedes planus and decreased reflexes. His skin is remarkably thin. Previous genetic investigations, consisting of standard metabolic screening, screening for Pitt-Hopkins syndrome (*TCF4*), West syndrome (*ARX*), X-linked alpha-thalassemia with mental retardation (*ATRX*), Rett syndrome (*MECP2*) and a 250k SNP array all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant c.1478G>A, p.Arg493His, in DHX30 (NM_138615.2).

Individual C is a 6-year-old girl, the first child of unaffected, non-consanguineous parents of Turkish origin. She has a 2.5-year-old brother whose development was uneventful. She was born after an uneventful pregnancy at 40 weeks of gestation with a birth weight of 3190g (-0.67 SD) length of 50cm (-0.77 SD), and a borderline OFC of 34 cm (-0.69 SD). She could roll over at four months and mother reports an unremarkable development until the age of six months, when the girl received a vaccination. Developmental delay, muscular hypotonia, and strabismus first became evident at the age of six months. At the age of 6 years and 3 months she had severe developmental delay with a secondary microcephaly, OFC 49 cm (-1.8 SD), truncal and orofacial hypotonia, poor fine motor skills and joint hyperlaxity. Weight is 25.8kg (+0.9 SD) and length 111cm (-1.7 SD). She cannot walk and is wheelchair-bound. Language development is significantly delayed and limited to a single word: no. She showed attention deficit and had auto-aggressive and hetero-aggressive behaviors and low frustration tolerance. She has strabismus of the right eye, hyperopia and bilateral hearing loss (Brainstem Auditory Evoked

Responses-performed). Facial dysmorphisms including synophrys and epicanthus were observed. Further, she has severe sleep disturbances that did not improve with melatonin treatment. Brain MRI, EEG, extensive metabolic investigations, including serum creatine kinase (SCK), blood lactate and pyruvate levels, as well cerebrospinal fluid (CSF) analysis were unremarkable. Conventional chromosome analysis of lymphocytes, array-CGH, direct sequencing of the *MECP2* and *UBE3A* genes including the methylation of the *UBE3A* locus all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.1685A->G, p.His562Arg, in *DHX30* (NM_138615.2).

Individual D is a 8-year-old girl, the first child of unaffected, non-consanguineous parents of Yemenite/Tripolitan Jewish descent. She has a younger brother whose development is uneventful. She was last examined at 8 years of age. Pregnancy followed *in-vitro* fertilization, and was otherwise uncomplicated. She was born at 40 weeks gestation with birth weight of 3030 grams (-0.75 SD). All milestones of motor development were delayed: she started to roll over at 12 months of age, and could not yet sit independently nor walk at 8 years. In terms of fine motor development, she tried to feed herself with a spoon and could reach for objects. She had difficulties chewing. She did not speak any words at 8 years, but could choose between 'yes' and 'no' on a touch screen computer (iPad). She is described as a pleasant, quiet, smiling child with no behavioral concerns. Sleep difficulties were apparent; she would lie awake quietly for hours at night. Ocular exam including funduscopic examination was normal other than difficulty with accommodation, for which she required eyeglasses. Hearing was reported as normal, but Brainstem Evoked Response Audiometry (BERA) showed slow conduction in the right brainstem. She did not have outright seizures, although episodes of deep inspirium followed by laughter were suspected as epilepsy-equivalent symptoms. EEG showed multifocal abnormalities. Brain MRI obtained at 15 months of age showed delayed myelination, enlarged ventricles, and cerebellar atrophy. Growth parameters at 8 years of age were height 116 cm (-2.1 SD) and weight 17.2 kg (-2.6 SD). Physical examination revealed a high palate, generalized hypotonia, bruxism, choreiform movements, bilateral single palmar crease, and head lag. Prior work-up included abdominal US, echocardiography, thyroid function tests, lactate, ammonia, plasma amino acids, homocysteine, transferrin isoforms analysis, very long chain fatty acids, urine organic acids, and urine for creatinine and guanidinoacetate were unremarkable. Chromosome analysis, array-CGH, *MECP2* sequencing, and methylation of the *UBE3A* locus all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the

proband, revealed a *de novo* heterozygous variant, c.2342G>A, p.Gly781Asp, in *DHX30* (NM_138615.2).

Individual E is a 17-year-old boy, third child of unaffected, non-consanguineous parents of Caucasian European ancestry. He has two unaffected older sisters. The pregnancy was uncomplicated with normal screening ultrasounds. He was born after 40 weeks of gestation with normal birth length (51 cm, 0 SD), weight (3830 g, +1 SD) and OFC (34.5 cm, 0 SD). He had neonatal hypotonia with poor feeding. Unilateral cryptorchidism and left post-axial hexadactyly of the foot were present. All milestones of motor development were severely delayed: he was able to sit independently at 3 years and to walk at 6 years of age. A brain MRI at the age of 4 years showed cortical atrophy and enlarged ventricles. At the age of 17 years, his height was 165 cm (-1,5 SD), his weight was 55 kg (- 0,75 SD) and her OFC was 54 cm (-1,5 SD). He is still unable to speak and he can walk only short distances. His has an ataxic gait. He has facial dysmorphisms including broad and large eyebrows, eversion of lower eyelids, and orofacial hypotonia. Examination of the extremities showed persistence of fingerpads. He had bruxism. He is described as a pleasant, quiet, smiling boy. However, he displays hetero-aggressive behavior when he is tired or during periods of nervousness. Standard metabolic screening was unremarkable. Previous genetic investigations, including screening for Fragile X Syndrome (*FMR1*), Bardet Biedl syndrome (BBS1-10), intellectual disabilities genes panel (275 genes), analysis of mtDNA common mutations and a 15k SNP array all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2342G>A, p.Gly781Asp, in *DHX30* (NM_138615.2).

Individual F is a 14-year-old girl, of unaffected, non-consanguineous parents of Hispanic ancestry. She has one full sibling and three maternal half siblings, all of whom have demonstrated normal development. She was born after 40 weeks of gestation with birth length of 51 cm (-0.65 SD), and weight of 2892 g, (-1.7 SD), further birth history was unremarkable. Developmental delays were noted in the first year of life and no period of normal development occurred. She has had no period of regression. Last physical exam, at the age of 14 years, revealed a small for age young lady who is below the 3rd percentile for all growth parameters. She is still unable to speak. She is non-dysmorphic, hypotonic and hyper-extensible. She requires assistance to walk and her gait is significant for ataxia and dystonia. She also has chorea at rest, and demonstrates persistent hand-wringing. She requires gastrostomy feeding to get adequate calories. Biochemical studies have included the following:

creatine/guanidinoacetate (plasma), urine creatine/guanidinoacetate, alpha aminoadipic semialdehyde (plasma), creatine kinase, serum homocysteine, uric acid, CSF glycine, serum glycine, CSF 5-methyltetrahydrofolate, CSF BH4/neopterin, CSF 5-hydroxyindoleaceticacid/homovanillic acid/3-O-methyldopa, copper (serum), ceruloplasmin, carbohydrate deficient transferrin (mono/di and asialo/di ratio), urine amino screen, serum amino acids , urine organic acids, urine mucopolysaccharides, urine oligosaccharides, very long chain fatty acids, phytanic acid, urine purines/pyrimidines, CSF lactate, serum lactate, all of which were unremarkable. Brain MRI revealed a diffuse volume loss, worsening over time. Her last EEG was moderately abnormal due to the presence of vertex spikes, left posterior temporal spikes and generalized spike and wave bursts. Extended genetic investigations, including *COG8* del/dup analysis, PWS/AS méthylation analysis, *UBE3A* sequencing, *SURF1* sequencing, *CDKL5*, *SLC9A6* sequencing and del/dup analysis, *FOXG1* sequencing, oligoarray testing, *TCF4* sequencing, *BOLA3* sequencing, *FMR1* CGG repeat analysis, analysis of mtDNA common mutations all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2344C>T, p.(Arg782Trp), in *DHX30* (NM_138615.2).

Individual G is a 14-year-old girl, the second twin of the second pregnancy of unaffected, non-consanguineous parents. During the pregnancy decreased fetal movement was noted. She has a twin brother and an older brother, both of whom have had normal development. She was born after 35+5 weeks of gestation with a normal birth weight, length and OFC. Directly after birth, she had breathing problems that required resuscitation, and spent the first 10 days of life in a neonatal intensive care unit for feeding difficulties. She received surgery for pressure equalizer (PE) tubes at 8 months. Developmental delays were noted in the first year of life. She learned to walk with 3 years, and is still non-verbal at the age of 14 years. A brain MRI at the age of 5 years revealed delayed myelination. Attention deficit hyperactivity disorder (ADHD) was documented. At the examination at 14 years of age she had an ataxic gait, chronic constipation, moderate anxiety, non-purposeful use of hands, tantrums, screams and vocalizations but no sentences. Facial dysmorphisms included long facies, mandibular prognathism, low set ears, open mouth appearance, and large and wide-spaced teeth. Further, long tapering fingers, flat feet with ankle pronation, and mild scoliosis were noted. Cardiac evaluation was unremarkable. Multifocal epileptiform activity was observed on EEG, however she had no history of seizures. Singleton-WES revealed a heterozygous variant, c.2344C>T, p.Arg782Trp, in *DHX30* (NM_138615.2), shown to be *de novo* through Sanger sequencing in both parents.

Individual H is a 8-year-old girl, the second of two children of unaffected, non-consanguineous parents of Dutch ancestry. Family history was non-contributory. She was born after 41+6 weeks of gestation with a birth weight of 3880 gram (+0.5 SD). As a baby, she had a mild esophageal reflux and was hypotonic. Psychomotor development was severely delayed. A brain MRI at the age of 6 months revealed wide ventricles, frontotemporal cerebral atrophy and delayed myelination. One year later, at the age of 1.5 years, a second brain MRI showed no progression of cerebral atrophy. Myelination had slightly progressed, but was still delayed. Physical examination at the age of 5 years showed a secondary microcephaly, with an OFC of 46 cm (-3 SD). Her height was 104 cm (-1.5 SD) and her weight was 14.5 kg (-2 SD). She has facial dysmorphisms including mild facial hypoplasia, mild synophrys, protrusion of the tongue and eversion of her lower lip. At the last examination at the age of 8 years, she was still not able to speak or to walk. Her development was severely delayed. Her hearing and vision were normal. There was no constipation. She was known to have a high pain threshold. No automutilation had been reported. No epileptic episodes have been noticed. Previous genetic investigations, consisting of standard metabolic screening, screening for Prader-Willi syndrome (methylation 15q11-q13), Rett syndrome (*MECP2*, *CDKL5*, *FOXG1*), Kleefstra syndrome (*EHMT1*), Pitt-Hopkins syndrome (*TCF4*), mitochondrial DNA depletion syndrome (*SUCLA2*, *TK2*, *POLG*), optic atrophy (*OPA1*) and a 250k SNP array all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2344C>T, p.Arg782Trp, in *DHX30* (NM_138615.2).

Individual I is 6-year-old girl, the second of two children of unaffected, non-consanguineous parents of Northern/Western European/Honduran descent. Family history was non-contributory. She was born at 37 weeks of gestation with a birth weight of 3175 gram (-0.7 SD). She was noted to have muscular hypotonia and developmental delay since 3 months of age. She had frequent upper respiratory infections. Her eyes used to move to the left or to the right aimlessly. At 2-3 years of age, she developed episodes of rapid breathing, loss of consciousness and jerking movements of the eyes and upper extremities. EEG showed posterior sharp waves bilaterally (probably epileptiform) and generalized slowing. She was started on antiepileptic medicine at 3 years of age. Currently, at the age of 6 years, she has severe hypotonia, severe global developmental delay and generalized joint laxity. She does not walk. She cannot sit without support. She is nonverbal. She does not understand any commands. She smiles all the time and has unprovoked prolonged laughing episodes. She has no purposeful movements of

her hands. She has midline play and hand-wringing stereotypy. She has uneven gaze and strabismus. Her left pupil is more dilated than the right. She has poor eye contact and cannot focus on objects. She has high tolerance to pain and she rarely cries. She eats only pureed food from a bottle. She tolerates only thick-consistency liquids, requiring milk to be with pureed vegetables and food. She choeks on her saliva. A brain MRI at the age of 10 months revealed benign communicating hydrocephalus. Another brain MRI at 3 years of age revealed generalized prominence of the CSF spaces and cerebral atrophy. Physical examination at 6 years of age showed relative macrocephaly, with an OFC of 52.25 cm (+1.3 SD). Her height was 100 cm (-3 SD) and her weight was 15 kg (-2 SD). She has facial dysmorphic features that include a high forehead, thin eyebrows, flat nasal bridge, posteriorly rotated ears, smooth philtrum and thin bow shaped upper lip. She has generalized joint laxity and persistent fetal fingertip pads. Standard metabolic screening including urine organic acids, and transferrin isoform analysis for congenital glycosylation disorder were unremarkable. Previous genetic investigations, including blood chromosomal analysis, SNP whole genome microarray, screening for Prader-Willi syndrome/Angelman syndrome (methylation 15q11-q13), *UBE3A* sequence analysis and deletion duplication study, Rett syndrome (*MECP2*, *CDKL5*, *FOXG1*) including sequencing and deletion duplication studies, Pitt-Hopkins syndrome (*TCF4*), Courtagen's epiSEEK® panel that includes sequencing of 327 genes implicated in seizure disorder, and sequence analysis and deletion testing of the mitochondrial genome all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2353C>T, p.Arg785Cys, in *DHX30* (NM_138615.2), and a *de novo* heterozygous alteration c.2969A>C, p.D990A in *SPEN*.

Individual J is a 4 8/12-year-old boy, the fourth of four children of unaffected, non-consanguineous parents of Mexican ancestry. There is no noted family history of neurodevelopmental disorders, and no other siblings in the family are symptomatic. Pregnancy was uncomplicated, however mother noted diminished fetal movement. He was born at 37 weeks of gestation via repeat caesarian section with a birth weight of 3150 grams. He had early feeding difficulties, which were ascribed to a cleft palate, for which he underwent surgery at 9 months of age. He developed a social smile at 2 months and rolled over at 6 months. At 6 months of age, he was referred to a neurologist because of hypotonia. He was found to have strabismus and partial syndactyly of the right toes. Brain MRI at 8 months showed cavum of the septum pellucidum, prominent ventricles and extra-axial spaces, thin corpus callosum, and delayed myelination. A subsequent MRI at 4 years revealed shortened corpus callosum with a

thin body and absent splenium; cavum septum pellucidum et vergae; decreased white matter volume, prominent extra-axial spaces and enlarged third ventricle. On examination, at the age of 4 8/12 years, he was a small, thin, well-appearing boy; his weight was 11.3 kg (-3.5 SD) and head circumference was 47.25 cm (-2.5 SD). He was slightly hirsute, with bushy eyebrows, and had less prominent palmar creases than expected. There was partial cutaneous syndactyly of the 2nd and 3rd toes on the right foot. Heart and lung sounds were normal, and there was no organomegaly. He made eye contact, smiled, was alert and attentive, and followed objects. There was a decrease in facial expression, but he did not have myopathic facies. He was vocal, but did not say any words. His muscle tone was diminished throughout (trunk and limbs) and he slipped through on vertical suspension. He was able to pull to stand, and could reach up with his arms. He was able to crawl; he walked with assistance, but did so on a wide base and was unsteady. He did not have a tremor on reaching for objects. Tendon reflexes were normal at the biceps, triceps, knees and ankles. EEG at 4 years was normal, as was renal ultrasound and echo cardiogram. Expanded newborn screen, karyotype (46XY), chromosomal microarray, test for Smith-Lemli-Opitz syndrome (7-dehydrocholesterol), methylation PCR for Prader-Willi syndrome, plasma amino acids, ammonia, acylcarnitine profile thyroid screen, were all unremarkable. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2353C>T, p.Arg785Cys, in *DHX30* (NM_138615.2).

Individual K is a 6-year-old girl, the second child of unaffected, non-consanguineous parents of Dutch ancestry. Family history was non-contributory. She was born at home after 40 weeks of gestation with a birth weight of 2970 grams (-1 SD). She fed slowly but gained enough weight. She was a quiet baby. She roll over at a normal age. At twelve month of age she could not sit independently and a delay in motor development and hypotonia were noted. She was able to walk independently at 5 years of age. By six years of age her walk was wide based and unsteady with her arms flexed and raised. She has no verbal words except for some intonated sounds, but and uses some gestures and sounds to communicate. She can have temper tantrums and frustration intolerance. She has stereotypic movements of her head and hands. She is usually in a good mood and likes to hug. There is no auto mutilation. Eye contact is short. She has hypermobile joints and flat feet. An intermittent esotropia of the left eye was detected. Physical examination at the age of 6 years showed normal biometry. She has mild facial dysmorphisms including mild facial hypoplasia, full upper eyelids, and mild epicanthus. Examination of the extremities showed hypermobile joints and pedes planus. A brain MRI at

the age of 1.5 years showed delayed myelinisation and wide ventricles, probably due to cerebral atrophy. Routine biochemical and metabolic screening were unremarkable. Previous genetic investigations, including screening for SMA (*SMN1*), Fragile X syndrome (*FMR1*), (atypical) Rett syndrome (*MECP2*), Angelman syndrome and a 250k SNP array all gave normal results. The SNP array did show a large copy neutral homozygous region (arr[hg19] 5q31.2q32(137,946,508-149,314,425)x2 hmz (2837 SNPs; 11,4 Mb) possibly caused by a UPD or distant consanguinity. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2353C>T, p.Arg785Cys, in *DHX30* (NM_138615.2).

Individual L is a 8-year-old boy, the second child of unaffected, non-consanguineous Caucasian parents. There is no family history of relevance, and there are no concerns about his elder sister. The pregnancy was uncomplicated and he was born at home by normal vaginal delivery at 40 weeks gestation. He was noted to be a floppy, sleepy baby. All milestones of motor development were delayed: sitting at 18 months of age and crawling at the age of 3 years. He could pull to stand and walk with hands held at the age of 6 years. At 8 years of age he was able to take 10 to 15 independent unsteady steps. He remained non-verbal and doubly incontinent at the age of 13 years. He still requires help with feeding. He has always made gradual progress and has never regressed. He is a generally happy child and hand flaps when excited. He has delayed visual maturation, has had surgery for a squint and has been registered as partially sighted. On examination, at the age of 6 years, he is hypotonic with no focal neurological signs. He was microcephalic with a head circumference of 48cm (-3.2 SD). He has some subtle dysmorphic features: short, broad thumbs, relatively large ears, pencilled eyebrows, downslanting palpebral fissures and widow's peak anterior hairline. His facial appearance is hypotonic. Two EEG's have been normal. MRI scan of the brain at the age of 12 months showed prominent ventricles and reduction in the volume of the white matter posteriorly. There was diffuse delay in myelination with particular involvement of the temporal lobes and optic radiation. The optic nerves, chiasm and tracts were extremely thin. The posterior fossa appeared normal. Routine baseline biochemical and metabolic investigations, thyroid function tests, purines and pyrimidines were all unremarkable. Previous genetic investigations, including array-CGH, and direct sequencing of *MEF2C* and *FOXG1* all gave normal results. Trio-WES, with DNA samples of both unaffected parents and the proband, revealed a *de novo* heterozygous variant, c.2354G>A, p.Arg785His, in *DHX30* (NM_138615.2).

Supplemental Figures

Figure S1. Facial phenotype of individuals with *DHX30* associated disorder. Facial images of individual B at the ages of 8.5 and 13 years, individual C at the age of 6 years, individual E from the age of 8 till 16 years, individual F at age of 14 years, individual H at the ages 4 and 8 years, individual I at age 6 years, individual J at the age of 4 years, individual K at age of 4.5 years, and individual L at the age of 6 years.

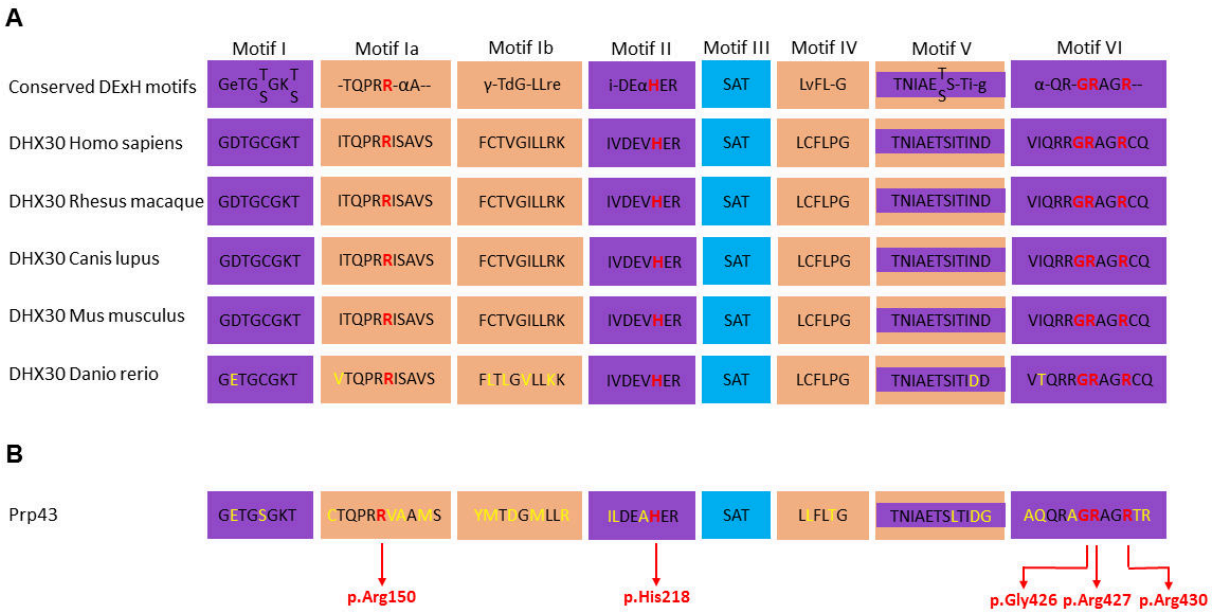

Figure S2. Identified *de novo* missense mutations affect highly conserved residues within helicase sequence motifs. (a) Evolutionary conservation of the motifs within helicase core region. Comparison to other superfamily2 helicases (based on ref.[1]) and DHX30 homologs. The position of the *de novo* mutations identified in this study are shown in red. Non conserved amino acid are shown in yellow. Nucleotide-interacting motifs (I, II and VI) are shown in purple, nucleic acid-binding motifs (Ia, Ib and IV) in orange, motif V, which binds nucleic acid and interacts with nucleotides, in purple and orange, and motif III, which couples ATP hydrolysis to RNA unwinding, in blue. Note that the motifs are evolutionary highly conserved from humans to zebrafish. **(b)** Conserved sequence motifs within helicase core region of the Prp43 in *Saccharomyces cerevisiae*. Note that the residues corresponding to positions of the *de novo* mutations identified in this study, marked with vertical red arrows and shown in red, are highly conserved.

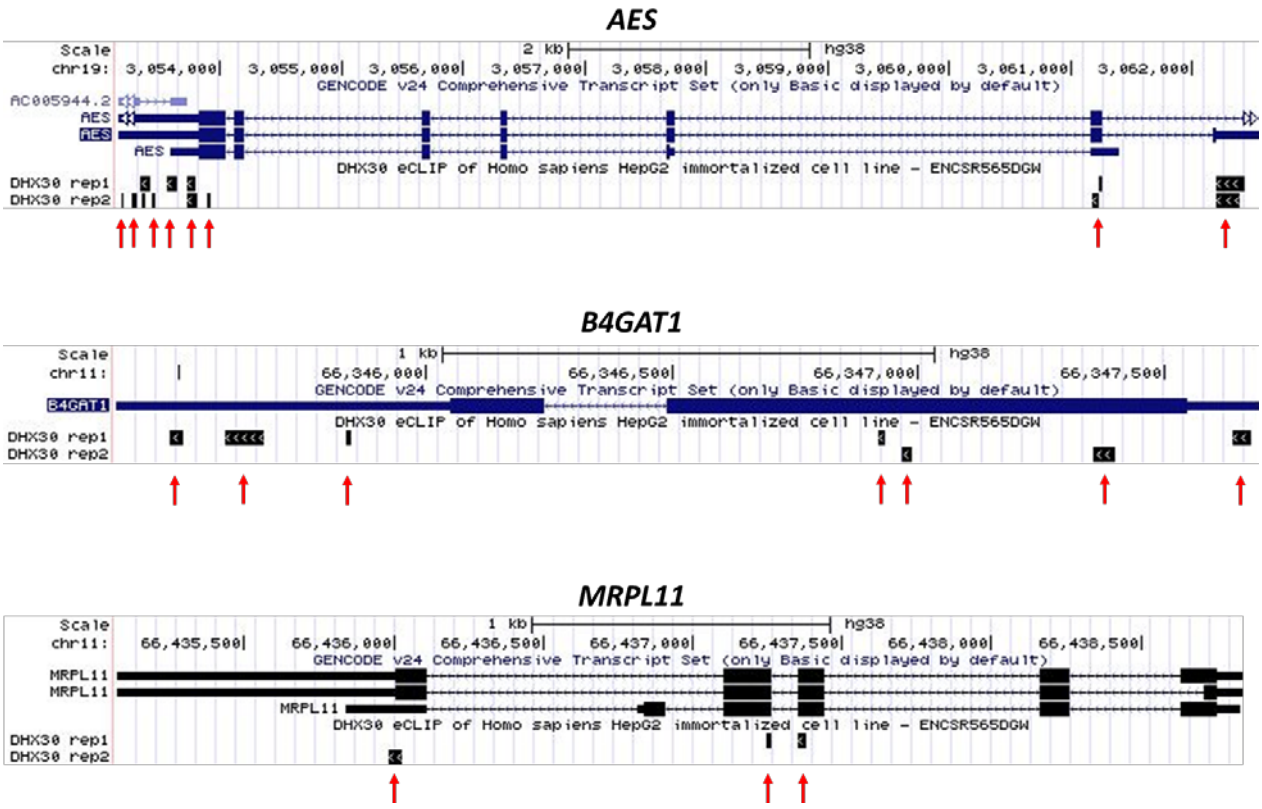

Figure S3. UCSC genome browser view of the DHX30 eCLIP binding sites of *AES*, *B4GAT1* and *MRPL11*. The position of DHX30 binding sites is marked with vertical red arrows (GRCh38/hg38).

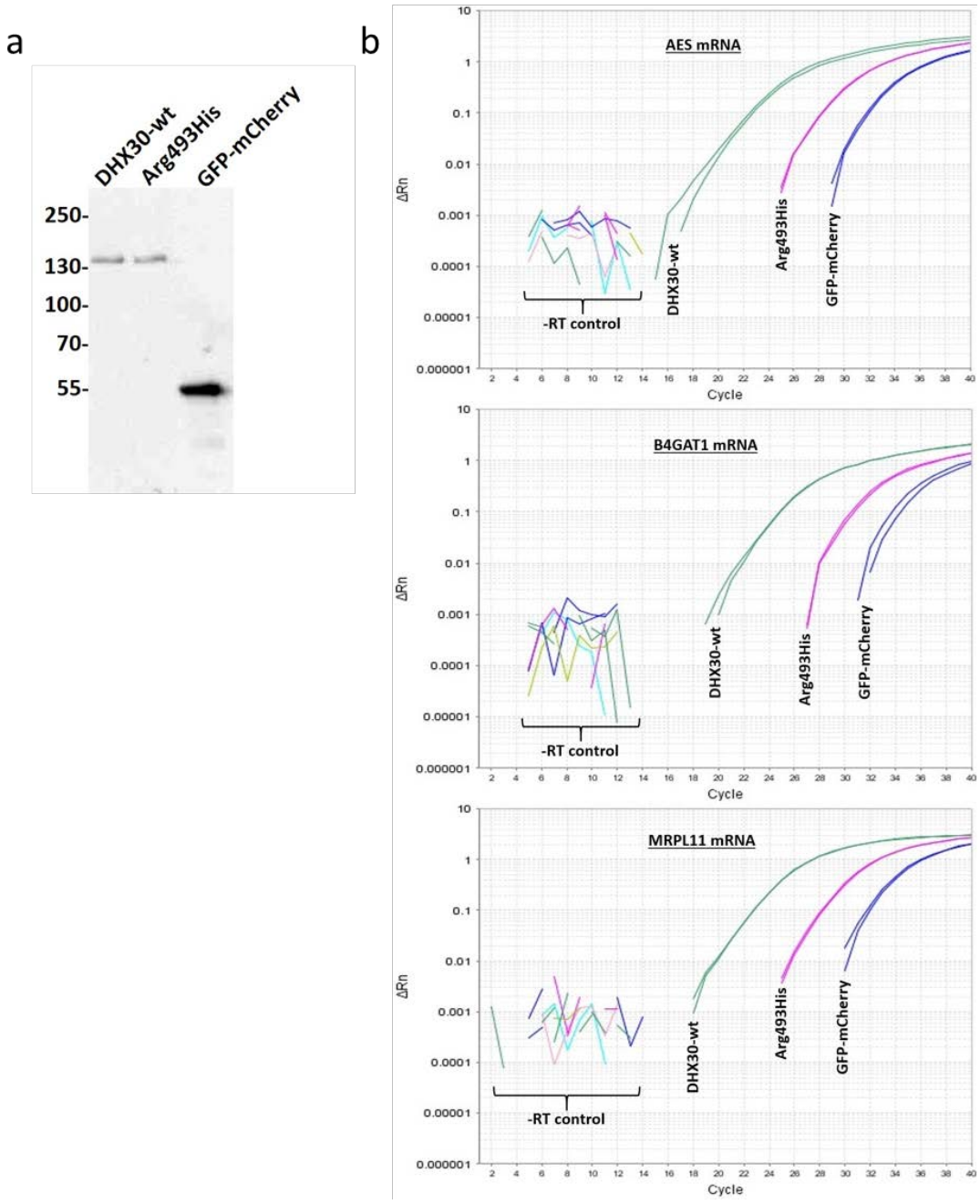

Figure S4. Reduced target mRNA association of p.Arg493His. (a) In a Western blot analysis, anti-GFP antibody detects DHX30-wt, p.Arg493His and GFP-mCherry, respectively, in GFP-Trap_A precipitates obtained from transfected HEK293T cells. Positions of molecular size markers are indicated on the left (in kDa). **(b)** RNAs extracted from GFP-Trap_A precipitates were subjected to gene expression analysis utilizing TaqMan probes specific for human mRNAs. The panel shows representative fluorescence curves for *AES*, *B4GAT1* and *MRPL11* transcripts associated with DHX30-wt (green curves), p.Arg493His (pink) and GFP-mCherry (blue), respectively. Same colour curves represent duplicates. Note that no detectable signal is generated when no reverse transcriptase is included during cDNA synthesis (-RT control).

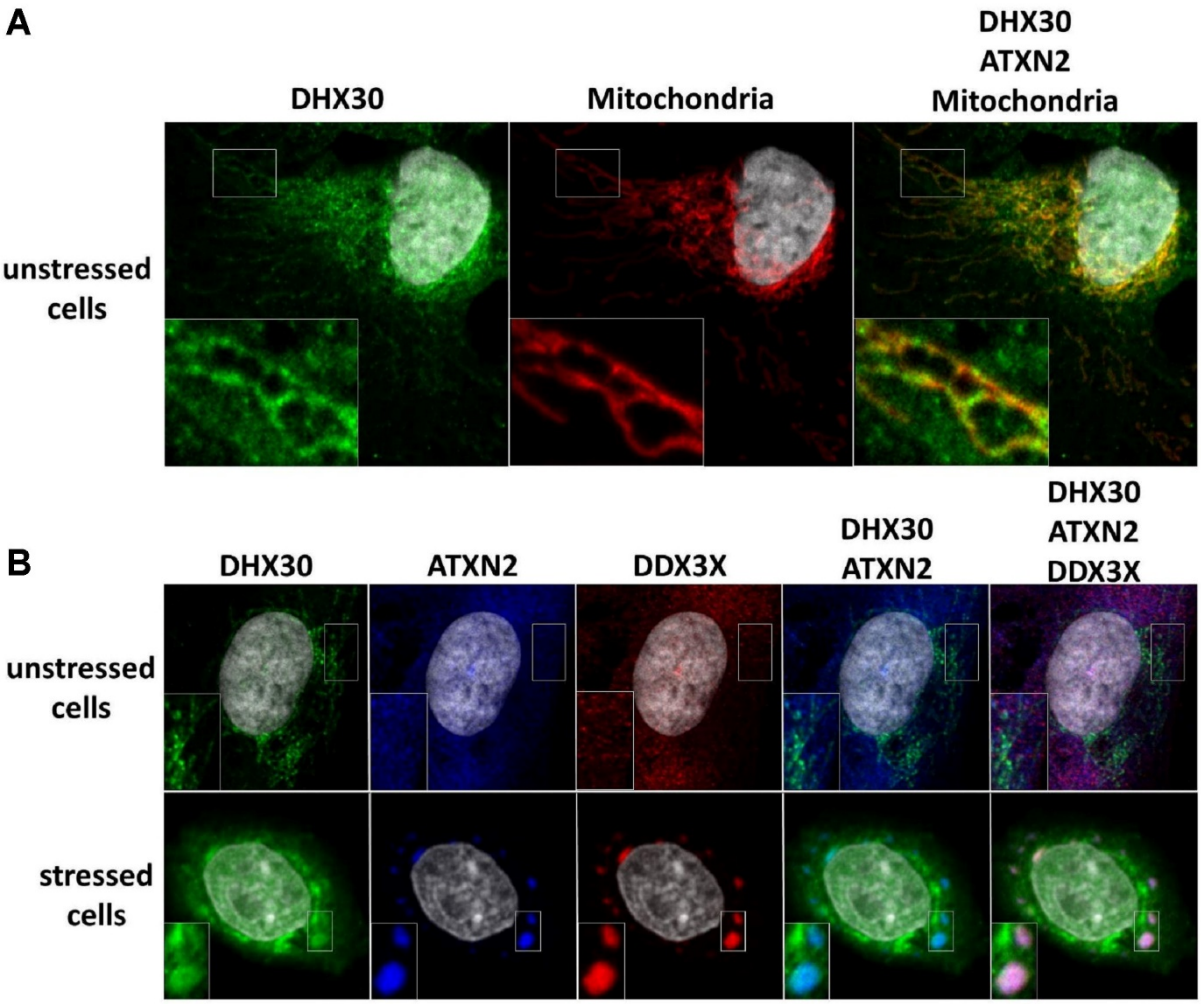

Figure S5. Endogenous DHX30 co-localizes with mitochondria and partially redistributes into SGs in stressed cells. (a) Immunocytochemical detection of endogenous DHX30 (green), mitochondria (red) and ATXN2 (blue). Note that DHX30 strongly co-localizes with mitochondria in unstressed U2OS cells. **(b)** Immunocytochemical detection of endogenous DHX30 (green), DDX3X (red) and ATXN2 (blue). DHX30 partially accumulates in ATXN2- and DDX3X-labelled SGs after stress induction (lower row). Insets inserted in the lower left corner of each panel are magnifications of regions boxed in the same panel. Nuclei are identified via DAPI staining (gray).

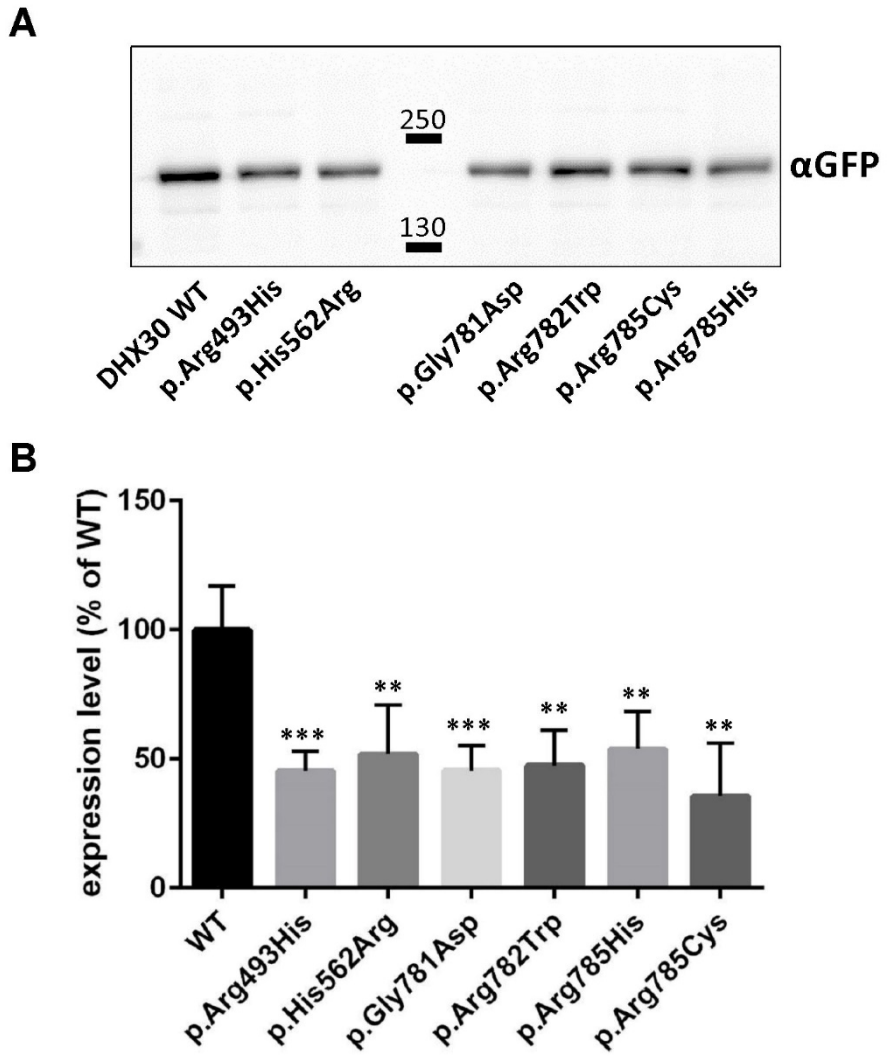

Figure S6. Expression levels of DHX30 variants upon transient transfection in 293 cells. A. Cells expressing WT or variant forms of GFP-tagged DHX30 were lysed and subjected to Western Blot analysis using anti-GFP. B. quantitative analysis, with the expression of DHX30 WT set to 100 %. **, ***, significantly different from WT ($p<0.01$, 0.001, respectively; ANOVA, followed by Dunnett's multiple comparisons test; n=4).

Supplemental Acknowledgments

The DDD study presents independent research commissioned by the Health Innovation Challenge Fund (grant number HICF-1009-003), a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute (grant number WT098051). The views expressed in this publication are those of the author(s) and not necessarily those of the Wellcome Trust or the Department of Health. The DDD study has UK Research Ethics Committee approval (10/H0305/83 granted by the Cambridge South REC, and GEN/284/12granted by the Republic of Ireland REC). The research team acknowledges the support of the National Institute for Health Research through the Comprehensive Clinical Research Network.

C4RCD Research Group:

Data analysis and interpretation: Newell Belnap[1], David Craig[1], Matt De Both[1], Matthew Huentelman[1], Madison LaFleur[1], Marcus Naymik[1], Vinodh Narayanan[1], Ryan Richholt[1], Isabelle Schrauwen[1], Ashley Siniard[1], Szabolcs Szelinger[1]

Data analysis and interpretation/wet lab: Chris Balak, Ana Claasen[1], Sampath Rangasamy[1]

Enrollment and Clinical Data: Keri Ramsey[1]

[1]Center for Rare Childhood Disorders, Translational Genomics Research Institute (TGen)

445 N 5th Street, Phoenix, AZ, U.S. 85004

Supplemental references:

1. Tanner, N.K., and Linder, P. (2001). DExD/H box RNA helicases: from generic motors to specific dissociation functions. Mol Cell 8, 251-262.