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Supplemental Data

Aberrant Function of the C-Terminal Tail of HIST1H1E Accelerates Cellular Senescence and Causes Premature Aging

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SUPPLEMENTAL FIGURES

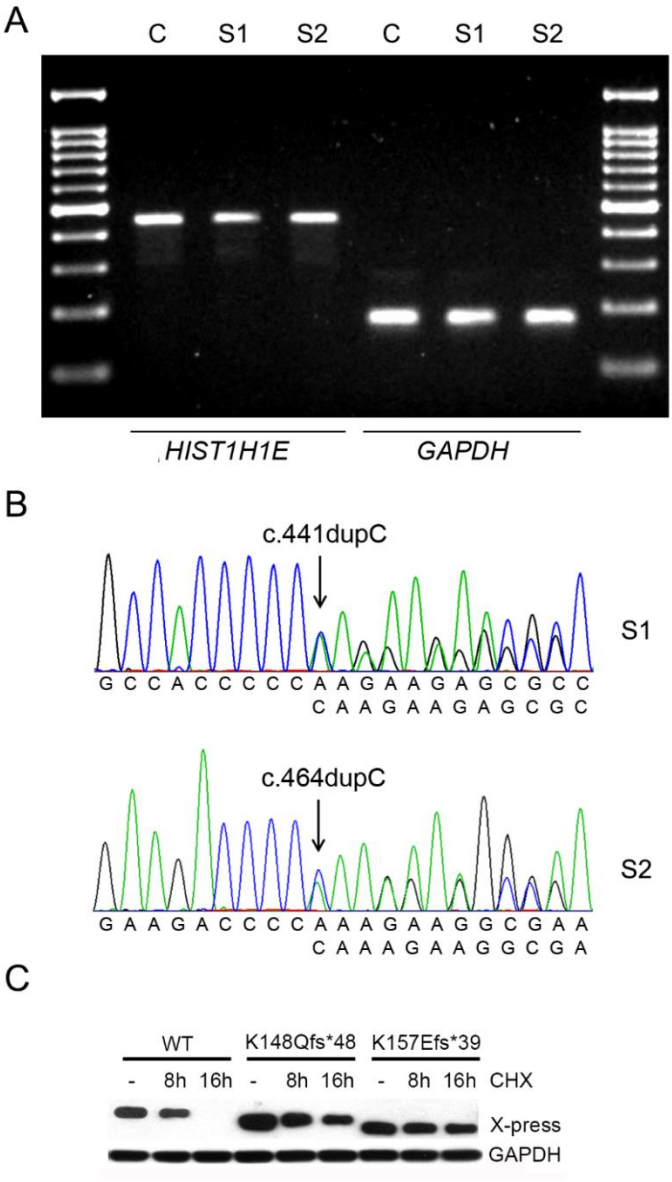

Figure S1. Disease-causing *HIST1H1E* frameshift mutations do not affect RNA and protein stability. (**A**) RT-PCR products obtained from total RNA from fibroblasts of affected subjects S1 and S2 show equal amount of *HIST1H1E* cDNA, indicating stability of the mutant transcripts, which is consistent with the notion that intronless genes generally evade nonsense-mediated RNA decay. GAPDH is reported as internal control documenting the same use of template cDNA. (**B**) Chromatograms showing the heterozygous state of the two studied mutations as assayed on cDNA obtained from total RNA of fibroblasts from affected subjects S1 and S2. (**C**) Protein stability was assessed in COS-1 cells transiently transfected with wild-type Xpress-tagged *HIST1H1E* and mutants carrying the c.441dupC (p.Lys148Glnfs*48 [K148Qfs*48]) and c.464dupC (p.Lys157Glufs*39 [K157Efs*39]) mutations. After transfection (48 h), cells were treated with cycloheximide (CHX, 20 μg/ml) for the indicated time or left untreated. Protein levels was assessed by immunoblotting, using an anti-Xpress monoclonal antibody. GAPDH levels are shown to document equal loading of total proteins from cell lysates. Western blot from a representative experiment of three performed is shown.

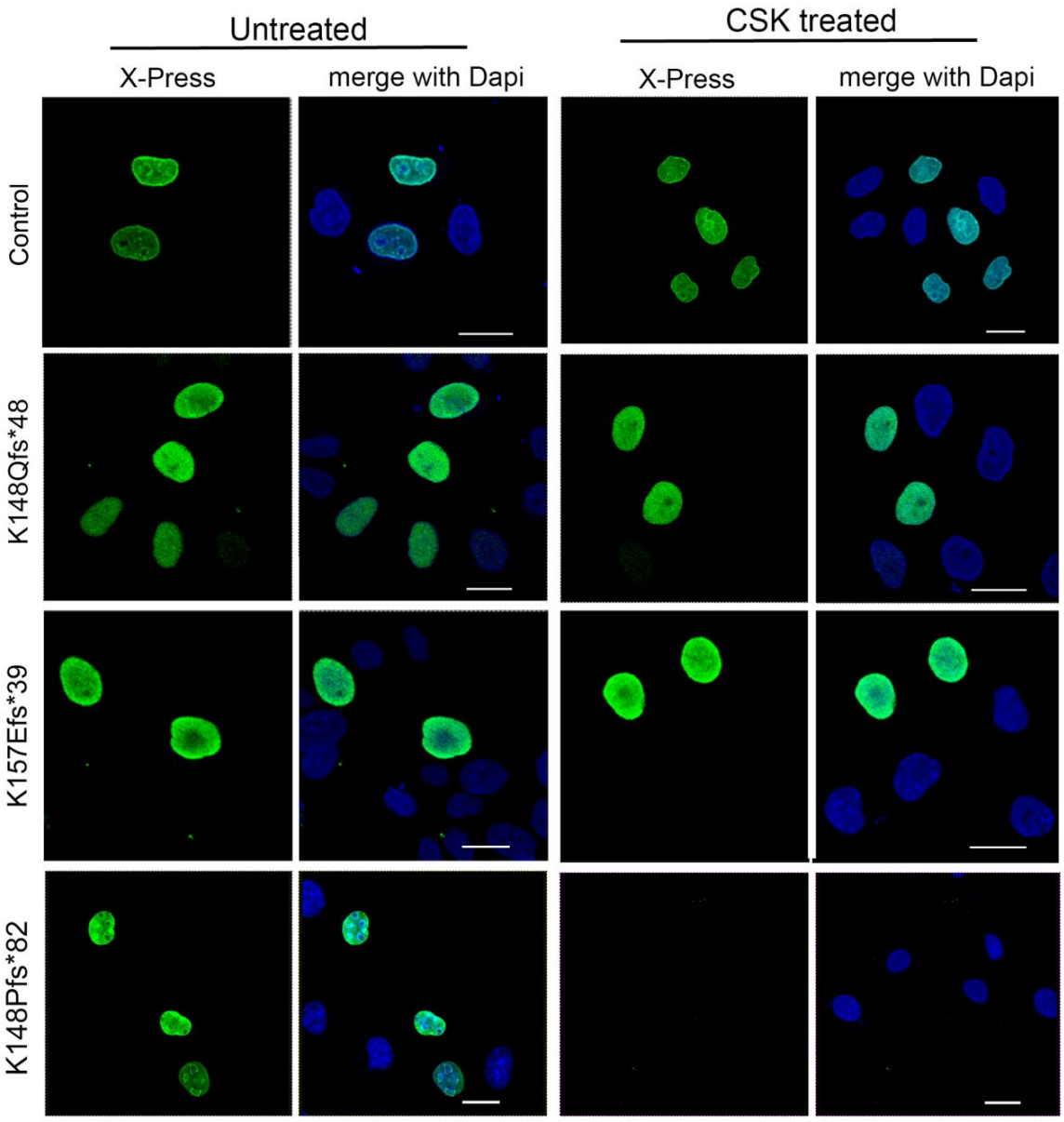

Figure S2. Disease-causing HIST1H1E mutants display proper subcellular localization and stably bind to chromatin. CLSM analyses were performed on HeLa cells transfected with vectors expressing Xpress-tagged wild-type HIST1H1E, two selected disease-causing mutants (p.Lys148Glnfs*48 [K148Qfs*48] and p.Lys157Glufs*39 [K157Efs*39]), and a HIST1H1E protein generated to express the third open reading frame at an equivalent position of the *C*-terminus (p.Lys148Profs*82 [K148Pfs*82]; not occurring in affected subjects). After 48 h from transfection, cells were treated with CSK (right) or left untreated (left), fixed, and stained (anti-X-press antibody). Nuclei were stained with DAPI (blue). Images show that, similarly to the wild-type protein, the disease-causing HIST1H1E mutants stably bind to chromatin. By contrast, the mutant carrying the alternative open reading frame was characterized by compromised chromatin binding, as shown by its loss of nuclear localization after CSK treatment. Bars correspond to 20 μm. Images are representative of > 200 analyzed cells.

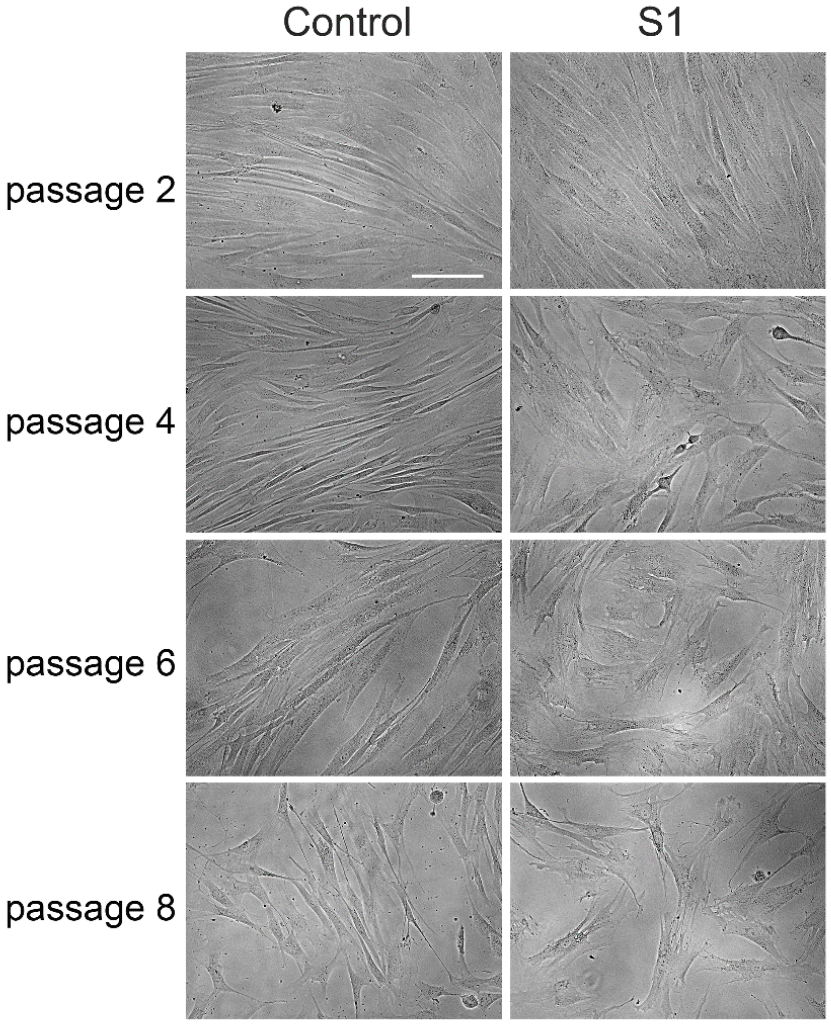

Figure S3. Accelerated cellular senescence in fibroblasts from affected subject S1. Images show morphological changes in cells endogenously expressing the heterozygous c.441dupC frameshift (p.Lys148Glnfs*48) in *HIST1H1E*. Morphology of cells rapidly progress from a thin and spindle shape to a large, flattened and irregular shape, which is visible since early passages. Photographs are at the same magnification (75 µm).

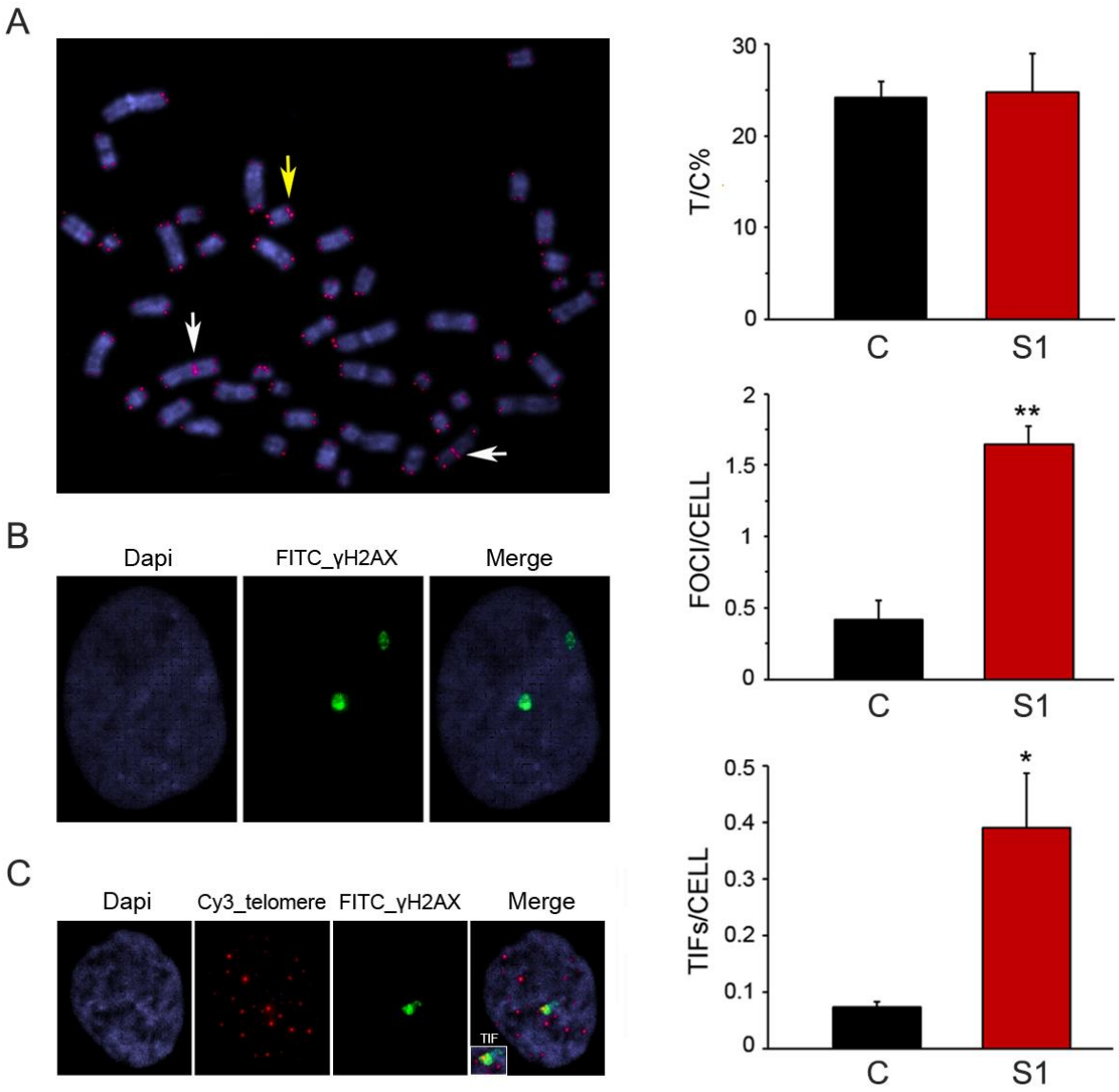

Figure S4. Telomere status and DNA damage sensitivity in fibroblasts from affected subject S1. (**A**) Q-FISH analysis. Representative image of a metaphase staining by Q-FISH (left). The yellow and white arrows indicate the telomeric signal and centromeres of chromosomes 2, respectively, which are used as internal reference in each metaphase. The telomere length was measured as the ratio between total telomeres fluorescence (T) and fluorescence of centromeres of chromosomes 2. The graph (mean ± SEM) shows no differences between control and S1 samples ($p < 0.05$, Mann-Whitney U test) (right). (**B**) Representative image of nuclei (DAPI) positive for γH2AX (green), a marker of DSBs (left). The graph shows the frequency of γH2AX foci per cell (mean ± SEM) (right). A significant increase in the frequency of foci was observed in S1 cells with respect to control cells (**$p < 0.001$; two-tailed Student's t-test). (**C**) Representative image of nuclei (DAPI) stained for γH2AX (green) and Cy3 (telomere-specific probe, red) (left). A magnification of telomere-γH2AX co-localisation (telomere dysfunction-induced foci, TIF) is also shown. The graph (mean ± SEM) shows the frequency of TIFs per cell (right), which occur with higher frequency in cells from subject S1 (*$p < 0.05$).

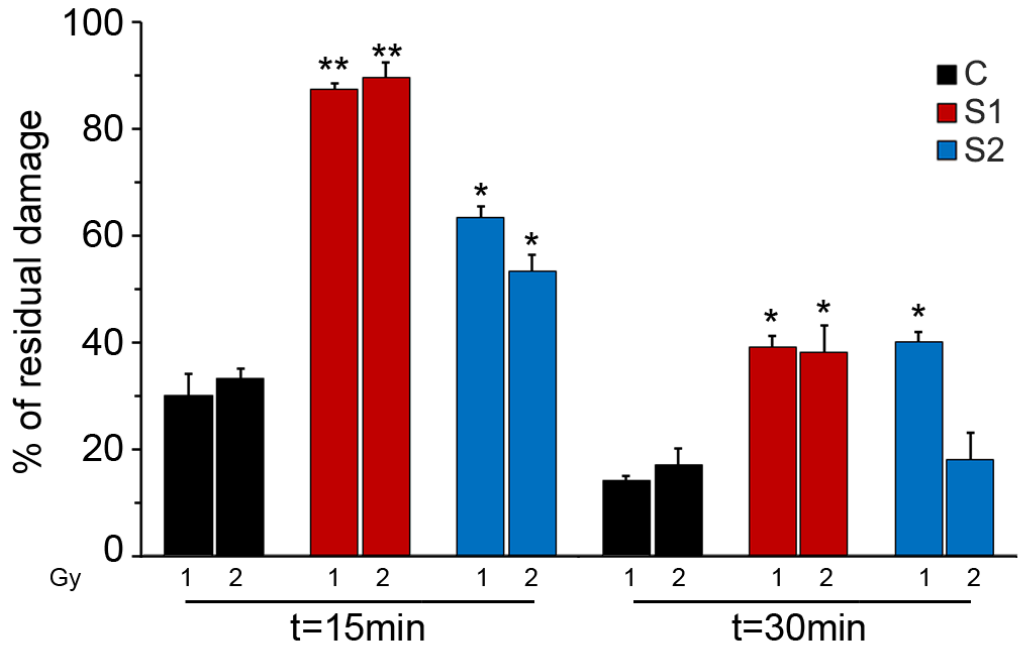

Figure S5. Defective HIST1H1E function is associated with defective/delayed DNA repair. DNA damage was induced by 1 or 2 Gy γ-ray irradiation. Following treatment, cells were incubated at 37 °C for 15 or 30 min to allow DNA repair. The percentage of residual DNA damage was calculated as follows: [(tail moment at time t after irradiation – basal tail moment)/(tail moment at t=0 – basal tail moment) × 100]. A lower/delayed capability to repair single/double strand breaks was observed in fibroblasts from affected subjects S1 and S2 compared to control cells (*p <0.02, **p < 0.001; two-tailed Student's t-test). For each experimental point, at least 75 cells were analyzed. Values are mean ± SEM of three independent experiments.

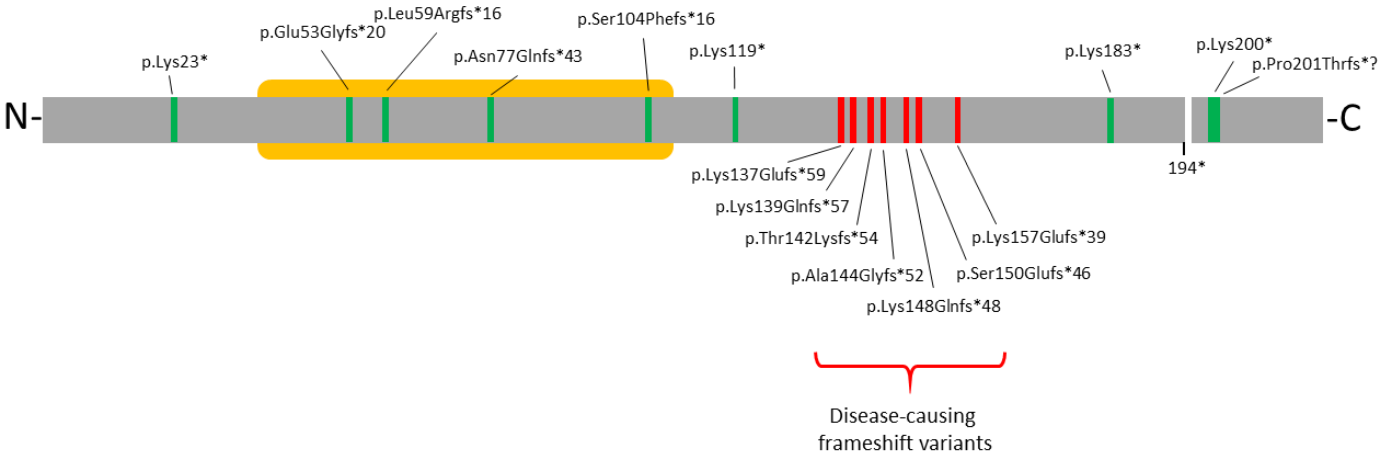

Figure S6. Truncating variants in *HIST1H1E*. Schematic diagram representing the HIST1H1E structure, and the position of the disease-causing frameshift mutations identified in this and previous studies (below the cartoon, red) and those reported in gnomAD (above the cartoon, green). The globular domain is shown in yellow. All disease-causing mutations result in a shorter protein with an identical divergent C-terminal tail (the new stop codon is shown below the cartoon, 194*). Differently, truncating mutations annotated in gnomAD either affect regions of the protein much more proximal to the N-terminus (the majority clustering within the globular domain) or are close to the C-terminus, downstream the regulatory serine/threonine residues. While the former are predicted to result in highly unstable proteins that likely undergo accelerated degradation and/or proteins unable to bind chromatin, the latter are expected to be loss-of-function mutants or behave as the wild-type protein, but are not expected to have a dominant negative effect.

SUPPLEMENTAL TABLES

Table S1. Predicted amino acid sequence of disease-causing frameshifts affecting the *C*-terminal region of HIST1H1E.

AA Change	AA Lenght	AA Sequence
Ref. Seq. (NP_005312.1)	219	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPKKSAKKTPKKAKKPAAAAGAKKAKSPKKAKAAKPKKAPKSPAKAKAVKPKAAKPKTAKPKAAKPKKAAAKKK*
p.Lys137Glufs*59 (S12)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKEAQEGDGGGHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Lys139Glnfs*57 (S4, S6)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPQEGDGGGHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Thr142Lysfs*54 (S3, S9)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKAKGVGHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Ala144Glyfs*52 (S8, S13, S16, S20)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGGGHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Ala145Glyfs*51 (S15)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAGHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Thr146Hisfs*50 (S14)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAAHPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Thr146Aspfs*42 (S19)	186	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAADPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Lys148Glnfs*48 (S1, S5, S10, S11, S17, S18)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPQEERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Ser150Glufs*46 (S7)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPKKERQEDPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Lys157Glufs*39 (S2)	194	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPKKSAKKTPKEGEEAGCSCWSQKSEKPEKGESSQAKKGAQEPSEGQSS*
p.Lys148Profs*82 (frameshift not occurring in affected subjects)	228	MSETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASKERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKA ASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPPRRAPRRPQRRRRSRLQLLEPKKRKARKRRKQPSQKRRPRAQRRPKQLNPRRLNQRPPSPRQPSQRRRQPRK SRKFLWPTA*

Table S2. Clinical features of the affected subjects with frameshift *HIST1H1E* mutations.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Previously reported	-	-	-	-	-	-
Mutation						
cDNA (NM_005321.2)	c.441dupC	c.464dupC	c.425_431delinsAGGGGGTT	c.414dupC	c.441dupC	c.414dupC
Protein change *(www.mutalyzer.nl)*	p.Lys148Glnfs*48	p.Lys157Glufs*39	p.Thr142Lysfs*54	p.Lys139Glnfs*57	p.Lys148Glnfs*48	p.Lys139Glnfs*57
De novo inheritance	+	+	+	+	+	U
Epidemiology						
Origin	caucasian	caucasian	caucasian	caucasian	caucasian	african american
Gender	male	female	male	female	female	female
Age (years) at last observation	49y	4y 6m	30y 9m	14m	12y	3y
Duration gestation (weeks)	U	40w	38w	38w	41w	39w
Pregnancy	uneventful	late hyperemesis	decreased fetal movement	wide ventricles seen on ultrasound	uneventful, amniocentesis was performed	uneventful
Neonatal problems	U	-	fetal heart decelerations. Apgar 4 and 6, cyanosis at birth, intubation for ventilation (NICU)	primary cesarean (1st pregnancy breech position)	-	congenital hypotonia, feeding difficulties, FTT
Birth Weight, g (SD)	U	3714g (+0.73SD)	3713.8g (+0,4SD)	4470g (+2.75SD)	3150g (-0.41SD)	3573g (+0.45SD)
Birth Length, cm (SD)	U	53.3cm (+1.8SD)	52.1cm (+1SD)	U	49.5cm (-0.2SD)	53.3 cm (+1.8SD)
Birth OFC, cm (SD)	U	U	35 cm (+0.1 SD)	U	33cm (-1.32SD)	35 cm (+0.1SD)
Weight at last observation, kg (SD)	U	21 kg (+1.93SD)	59 kg (+0.09SD)	10.3 kg (+1.54SD)	41.6 kg (-0.02SD)	14.1 kg (0.14SD)
Height at last observation, cm (SD)	U	111 cm (+1.83SD)	167.2 cm (+0.62SD)	74 cm (+0.56SD)	146.2 cm (-0.74SD)	91.4 cm (-0.96SD)
OFC at last observation, cm (SD)	U	53.8 cm (+2.1SD)	60cm (+3.4SD)	50 cm (+3.48SD)	54.9 cm (+1.14SD) (+2SD at age 4, while length -0,5SD)	49.2 cm (-0.07SD)
Growth						
Delay/ ID : mild=1; moderate=2; severe=3, unspecified	2	1	1	0	1	2
Sitting unsupported (months)	U	10	8	8.5	12	6
Walking independently (months)	U	16	24	not yet at age 14m, but makes steps holding table since age 1 year	30	24
Motor delay	+	+	+	seems late but still within range	+	+
Speech delay	+	+	+	U	+	+
Hypotonia	U	-	+	+	-	+
Seizures, type and treatment	U	childhood focal seizures	febrile seizures	-	-	-
Hearing loss	+	-	+	-	-	+
Frequent otitis media	U	-	+	-	-	+
Eye defects	U	U	+	-	+	+
Hypermetropia (H)/ myopia (M)	U	-	M	-	H	-
Astigmatism	U	-	-	-	+	+ - simple hyperopic
Strabismus	U	-	+	-	+	-

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Craniofacial features						
Coarse face	U	-	-	-	-	-
High hairline	+	+	+	+	+	+
Abnormal hair	sparse hair, generalized hypopigmentation of hair, hypotrichosis	frontal upsweep, thin, sparse hair	alopecia totalis (sparse frontotemporal hair) and sparse eyelashes since age 10y; delayed hair growth as child; no eyebrows until about age 4.	sparse hair	thin hair, Widow's peak (like father)	-
Prominent forehead	+	+	+	+	+	+
Bitemporal narrowing	+	U	+	+	U	U
Epicanthus/telecanthus	epicanthus	U	U	U	U	U
Downward slant palpebral fissures	U (small)	+	+	- (narrow)	+ (narrow)	+
Deepset eyes	U	U	+	U	U	U
Ptosis	+	-	+	-	-	-
Hypertelorism	+	-	U	+	+++	+
Small/pointed chin	U	+	+	+	-	-
Nasal bridge anomalies	narrow nasal bridge	wide, low and flat	U	wide	-	wide
Upturned nasal tip	U	+	-	-	-	-
Full nasal tip	U	+	+	+	-	+
Thick alae nasi	U	+	+	+	-	+
Short nose	U	-	-	-	-	-
Prominent cheek bones	U	U	+	U	U	U
Smooth philtrum	U	+	U	-	-	-
Broad philtrum	U	-	U	-	-	-
Long philtrum	U	+	U	-	-	+
Mouth abnormalities	U	thin upper vermillion	U	thin upper vermillion	accentuated Cupid's bow, high palate	-
Micrognathia	+	U	+	U	U	U
Widely spaced teeth	U	-	U	U	widely spaced central incisors	U
Enlarged tongue	U	U	+	-	U	U
Ear abnormalities	low set	thickening of superior scaphohelices	low set, upturned ear lobes	-	low set, simple helix, upturned ear lobes	-
Hand abnormalities	brachydactyly, broad thumbs, deep palmar crease	-	bilaterally brachydactyly (shortening of distal phalangeal area), unilateral palmar crease	-	tapering fingers, slightly shorter 4th metacarpals. Camptodactyly. Palmar erythema, low-set thumbs	-
Joint hyperlaxity/stiffness	U	-	U	U	very stiff fingers	-
Nail anomalies	nail dysplasia	-	middle ridges on his two thumbnails	-	thin and short nails	-
Feet abnormalities (flat feet, sandal gap,...)	U	-	orthopedic insoles, flat feet	-	orthopedic insoles, stiff feet, plantar erythema	-
Toe abnormalities	broad hallux, deviation of hallux	broad toes	U	-	long halluces	-
RX abnormalities	U	-	-	U	osteopenia, advanced bone age, multiple small stress fractures	-
Other limb abnormalities	U	-	U	-	lower limb pitting edema, genua valga, slight leg lenght difference	-

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Other						
Skull abnormalities	dolichocephaly (scaphocephaly), abnormal skull ossification	dolichocephaly	macrocephaly	mild turricephaly	scaphocephaly	-
Cryptorchidism	U	-	unilateral	-	-	-
Renal abnormalities	U	U	kidney cysts bilaterally	U	U	U
Skin	multiple nevi, skin hyperpigmentation, cutis laxa	1 hypopigmented lesion on the left knee	cutis marmorata when young.	-	mild cutis marmorata, dry skin, multiple lentigines solaris in face	-
Scoliosis (mild, severe)	U	-	U	-	mild	mild
Widely spaced/inverted nipples	U	-	U	-	+	-
Cardiac abnormalities	-	-	-	small 'atrial septum defect	-	-
Pectus excavatum/carinatum	excavatum, scapular wings	-	U	-	excavatum	-
dentition (normal; pointed; delayed prim / delayed permanent/missing permanent)	U	early loss of primary teeth/delayed permanent teeth	small, poorly enameled teeth	pointed teeth	small, pointed teeth, some permanent teeth missing, short roots	U
sleep problems	U	+ (restless sleeper and early awake)	U	-	'+ (frequently awake during night, early awake)	-
Laboratory abnormalities	pancytopenia (possible autoimmune origin), autoimmune disorder (systemic lupus erythematosus), congenital hypothyroidism	mildly elevated creatine (age 2 1/2)	low hemoglobin (iron supplementation ongoing)	-	Hypophosphatasia (zinc deficiency)	-
Neuroradiology				cerebral ultrasound at 6m: mild ventricular enlargement		
MRI brain (at age in months)	-	approx 2y 6m	CT scan at 4 m of age and 6 y normal	-	U	9m
MRI brain abnormality	U	mild ventricular enlargement	U	U	-	mild inferior vermian hypoplasia
Autism/Behavioral problems	abnormal behavior, psychotic episodes	autism spectrum disorder	U	U	diminished eye contact in childhood and socialization anomalies	-
Clinical diagnosis/tested for:	U	Clinical suspicion of Sotos syndrome NSD1 on WES normal, CMA normal	Clinical suspicion of Sotos syndrome NSD1 gene normal. FXS, Pallister-Killian syndrome, Prader Willi syndrome, Smith Magenis syndrome screening normal	SNP array normal; WES panel overgrowth syndromes	Clinical suspicion of Sotos syndrome NSD1 gene normal	U

	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13
Previously reported	-	-	-	-	-	-	-
Mutation							
cDNA (NM_005321.2)	c.447dupG	c.430dupG	c.425delinsAG	c.441dupC	c.441dupC	c.408dupG	c.430dupG
Protein change *(www.mutalyzer.nl)*	p.Ser150Glufs*46	p.Ala144Glyfs*52	p.Thr142Lysfs*54	p.Lys148Glnfs*48	p.Lys148Glnfs*48	p.Lys137Glufs*59	p.A144Gfs*53
De novo inheritance	+	U	U	+	+	+	+
Epidemiology							
Origin	caucasian	asian	caucasian	caucasian	caucasian	caucasian	caucasian
Gender	male	male	female	male	female	male	female
Age (years) at last observation	11y 11m	4y 9m	2y	1y 7m	6y	4y	17m
Duration gestation (weeks)	34w	at term	at term	39w	40+2w	39w	39w
Pregnancy	ultrasound diagnosis of macrocephaly (32 weeks)	U	U	uneventful	uneventful	uneventful	uneventful
Neonatal problems	3 weeks in NICU	U	U	-	Apgar 6-9-9, insufflation breaths and PEEP. Ventilatory assistance 4 days after birth	-	primary cesarean (1st pregnancy breech position)
Birth Weight, g (SD)	2600g (-1.75SD)	3400g (0SD)	U	4100g (+1.4SD)	3482g (+0.27SD)	3886g (+1.05SD)	3780g (SD +0.81SD)
Birth Length, cm (SD)	51 cm (0SD)	U	U	55 cm (+2.7SD)	U	55 cm (+2.7SD)	50.8 cm (+0.59SD)
Birth OFC, cm (SD)	35 cm (+0.1 SD)	U	U	39cm (+3.0SD)	U	35 cm (+0.1SD)	38 cm (SD +1.82SD)
Weight at last observation, kg (SD)	37.7 kg (-0.44SD)	21.5 kg (+1.29SD)	13.3 kg (+1.16SD)	15 kg (+2.8SD)	26.2 kg (+1.65SD)	21.5 kg (+2.11SD)	9.995 kg (-0.88SD)
Height at last observation, cm (SD)	139.7 cm (-1.62SD)	109.8 cm (+0.43SD)	86 cm (+0.08SD)	92.5 cm (+3.65SD)	120.2 cm (+0.99SD)	106 cm (+0.64SD)	77.1 cm (-0.93SD)
OFC at last observation, cm (SD)	58.8 cm (+3.98SD)	55 cm (+3.53SD)	49.4 cm (+1.07SD)	47.3 cm (+0.41SD)	56 cm (+2.94SD) at age 6y 9m	56 cm (+3.68SD)	50.7 cm (+3.19SD)
Growth							
Delay/ ID : mild=1; moderate=2; severe=3, unspecified	2	0	unspecified	2	2	1	2
Sitting unsupported (months)	12	11	U	8	14	9	9
Walking independently (months)	30	30	U	not yet at 2y 9m, can make steps with support	66	15	not yet but crawling
Motor delay	+	+	U	+	+	+	+
Speech delay	+	+	U	+	+	+	+
Hypotonia	+	+	U	-	+	+	+
Seizures, type and treatment	single childhood seizure	U	febrile seizures	febrile	recurrent status epilepticus, valproate treatment	-	-
Hearing loss	-	U	+	-	-	-	bilateral mild to moderate sensorineural hearing loss
Frequent otitis media	+	U	U	-	-	- (two times)	-
Eye defects	+	+	U	U	+	lacrimal duct stenosis (operation at 1.5y)	+
Hypermetropia (H)/ myopia (M)	M	U	U	-	H	-	M
Astigmatism	+	U	U	-	-	-	+
Strabismus	-	+	U	-	+ (exotropia)	-	-

	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13
Craniofacial features							
Coarse face	-	U	U	+	+	+	+
High hairline	+	+	U	+	+	+	+
Abnormal hair	-	U	U	-	thin hair	-	-
Prominent forehead	+	+	+	+	+	+	+
Bitemporal narrowing	U	U	U	U	U	U	+
Epicanthus/telecanthus	U	epicanthus	epicanthus	U	U	-	+
Downward slant palpebral fissures	-	+	U	+ (accentuated by puffy eyelids)	-	-	+
Deepset eyes	U	U	+	U	U	+	-
Ptosis	-	U	U	-	-	-	+ (mild)
Hypertelorism	+	U	U	+	+	+	+
Small/pointed chin	-	U	U	-	-	-	+
Nasal bridge anomalies	-	wide	wide	wide	wide	wide	-
Upturned nasal tip	-	+	U	-	+	-	+
Full nasal tip	+	U	+	+	+	+	+
Thick alae nasi	-	U	U	-	-	+	+
Short nose	-	U	U	-	-	-	+
Prominent cheek bones	U	U	U	U	U	-	-
Smooth philtrum	-	U	U	-	-	-	-
Broad philtrum	-	U	U	-	-	-	-
Long philtrum	-	U	U	-	-	-	+
Mouth abnormalities	thin upper vermillion	U	U	-	-	thin upper vermillion, thick lower vermillion, drooping lower lip	thin upper vermillion
Micrognathia	U	U	U	U	U	-	-
Widely spaced teeth	-	U	U	+	U	+	-
Enlarged tongue	U	U	U	U	U	-	-
Ear abnormalities	low set	U	low set	low set, protruding	-	low set, rotated ears	low set, posteriorly rotated
Hand abnormalities	-	tapering fingers, 5th finger clinodactyly, bilateral single palmar crease	U	tapering fingers, broad thumbs	small hands	relatively large hands	single transverse palmer creases
Joint hyperlaxity/stiffness	-	U	U	-	-	-	+
Nail anomalies	-	U	U	-	-	flaky nails	-
Feet abnormalities (flat feet, sandal gap,...)	-	bilateral pes planus	U	-	orthopedic insoles	-	-
Toe abnormalities	-	5th toe overlaps 4th toe	U	-	U	relatively large feet	overlapping toes
RX abnormalities	advanced bone age	-	U	U	-	advanced bone age	scoliosis
Other limb abnormalities	-	U	U	-	valgus hips	-	hip dysplasia

	Subject 7	Subject 8	Subject 9	Subject 10	Subject 11	Subject 12	Subject 13
Other					hemangioma leg		gastroesophageal reflux disease
Skull abnormalities	-	U	U	scaphocephaly	scaphocephaly	U	dolichocephaly with patent sagittal suture
Cryptorchidism	bilateral	U	-	bilateral	-	+	-
Renal abnormalities	U	U	U	U	U	U	U
Skin	-	flat hyperpigmented patches on abdomen and thighs (dad attributes to post viral infection)	U	-	-	mild cutis laxa in abdominal area	-
Scoliosis (mild, severe)	-	U	U	-	-	-	mild
Widely spaced/inverted nipples	-	U	U	-	-	+	
Cardiac abnormalities	-	U	U	-	-	atrial septum defect	small atrial septum defect
Pectus excavatum/carinatum	-	U	U	-	-	-	-
dentition (normal; pointed; delayed prim / delayed permanent/missing permanent)	crowded teeth	U	U	small teeth, widely spaced	tooth dysgenesia with extreme short radices of milk molars with 4 elementes missing	early eruption of teeth	high arched palate
sleep problems	-	U	U	-	U	-	-
Laboratory abnormalities	-	U	U	-	-	-	-
Neuroradiology						-	
MRI brain (at age in months)	3 y; repeat 5y	U	U	12 m	5m	U	14m
MRI brain abnormality	partial agenesis of corpus callosum	periventricular white matter abnormality	U	arachnoid cyst	cavum septum pellucidum	U	Mild prominence of the subarachnoid fluid spaces
Autism/Behavioral problems	ADHD	self-stimulatory behaviors	U	stereotypic movements with hands, rolls with eyes when tired	-	-	none at 17m
Clinical diagnosis/tested for:	U	U	U	CMA and metabolic screening normal	Clinical suspicion of Sotos syndrome NSD1, PTEN and EZH2 genes normal, FXS normal, CMA normal	Clinical suspicion of Simpson-Golabi-Behmel syndrome; CMA, metabolic screening, FXS and gene panel normal	Rasopthay panel normal; SNP array normal; WES

	Subject 14	**Subject 15**	**Subject 16**	**Subject 17**	**Subject 18**	**Subject 19**	**Subject 20**
Previously reported	*Duffney et al. 2018*	*Takenouchi et al. 2018*	*Tatton-Brown et al. 2017*	*Tatton-Brown et al. 2017*	*Tatton-Brown et al. 2017*	*Tatton-Brown et al. 2017*	*Tatton-Brown et al. 2017*
Mutation							
cDNA (NM_005321.2)	c.435dupC	c.433dup	c.430dupG	c.441dupC	c.441dupC	c.436_458del23	c.430dupG
Protein change *(www.mutalyzer.nl)*	p.Thr146Hisfs*50	p.Ala145Glyfs*51	p.Ala144Glyfs*52	p.Lys148Glnfs*48	p.Lys148Glnfs*48	p.Thr146Aspfs*42	p.Ala144Glyfs*52
De novo inheritance	+	+	+	+	U	+	+
Epidemiology							
Origin	caucasian	asian	caucasian	caucasian	U	U	caucasian
Gender	male	female	female	male	female	female	male
Age (years) at last observation	10y	21y	13y	15y 6m	4y 3m	1y 10m	8y 6m
Duration gestation (weeks)	38w	36w	at term	41w	at term	37w	41w
Pregnancy	early delivery (maternal car accident at 38 weeks)	U	U	uneventful	U	uneventful	complicated by exposure to chicken pox
Neonatal problems	2 weeks in NICU, jaundice, micrognathia increased muscle tone and feeding difficulties	U	congenital hypotonia	congenital hypotonia, feeding difficulties	congenital hypotonia, feeding difficulties	hypoglycemia and increased muscle tone	U
Birth Weight, g (SD)	3200g (-0.3SD)	2876g (+1.6SD)	3580g (+0.47SD)	4750g (+2.4SD)	4790g (+2.62SD)	3250g (+0.8SD)	3740g (+0.78SD)
Birth Length, cm (SD)	49.5cm (-0.2SD)	49cm (+1.4SD)	53cm (+1.65SD)	U	57cm (+3.76SD)	49cm (+0.7SD)	U
Birth OFC, cm (SD)	U	33.4 cm (+0.9SD)	U	U	U	37cm (+3.3SD)	U
Weight at last observation, kg (SD)	54.5 kg (+2.66SD)	U	48.8 kg (+0.4SD)	U	24 kg (+2.45SD)	12 kg (+0.65SD)	33 kg (+1.34SD)
Height at last observation, cm (SD)	144.8 cm (+0.96SD)	151.8 cm (-1.74SD)	150.8 cm (-0.8SD)	166.5 cm (-0.6SD)	108 cm (+0.8SD)	85 cm (+0.12SD)	133.2 cm (+0.56SD)
OFC at last observation, cm (SD)	53 cm (+0.25SD)	54.4 cm (-0.15SD; relative macrocephaly)	55.8 cm (+1.57SD)	58.7 cm (+2.03SD)	55 cm (+3.78SD)	51 cm (+2.65SD)	59 cm (+4.92SD) at age 6,3
Growth							
Delay/ ID : mild=1; moderate=2; severe=3, unspecified	2	3	1	2	unspecified	2	3
Sitting unsupported (months)	9	U	U	U	U	U	U
Walking independently (months)	24	30	U	U	U	U	U
Motor delay	+	U	U	U	U	U	U
Speech delay	+	+	U	U	U	U	+
Hypotonia	-	U	+	+	+	U	U
Seizures, type and treatment	single childhood seizure	U	U	U	U	U	U
Hearing loss	-	U	U	U	U	U	-
Frequent otitis media	-	U	U	U	U	U	U
Eye defects	U	cataracts at age 21y	U	U	U	U	delayed visual maturation
Hypermetropia (H)/ myopia (M)	-	U	U	U	U	U	U
Astigmatism	+	U	U	U	U	U	+
Strabismus	+	+	+	U	U	U	left amblyopia

	Subject 14	Subject 15	Subject 16	Subject 17	Subject 18	Subject 19	Subject 20
Craniofacial features							
Coarse face	-	U	U	U	U	U	U
High hairline	-	+	+	U	U	U	U
Abnormal hair	-	U	U	U	U	U	U
Prominent forehead	-	U	U	U	U	U	U
Bitemporal narrowing	U	U	U	U	U	U	U
Epicanthus/telecanthus	U	epicanthus	telecanthus	U	U	U	U
Downward slant palpebral fissures	+	U (short)	U	U	U	U	U
Deepset eyes	U	U	U	U	U	U	U
Ptosis	-	U	U	U	U	U	U
Hypertelorism	+	U	U	U	U	U	U
Small/pointed chin	+	U	U	U	U	U	U
Nasal bridge anomalies	low	wide	U	U	U	U	U
Upturned nasal tip	-	U	U	U	U	U	U
Full nasal tip	-	U	U	U	U	U	U
Thick alae nasi	-	U	U	U	U	U	U
Short nose	-	U	U	U	U	U	U
Prominent cheek bones	U	+	U	U	U	U	U
Smooth philtrum	+	U	U	U	U	U	U
Broad philtrum	+	U	U	U	U	U	U
Long philtrum	+	+	U	U	U	U	U
Mouth abnormalities	accentuated Cupid's bow	high-arched, wide uvula	U	U	U	U	U
Micrognathia	+	U	U	U	U	U	U
Widely spaced teeth	-	U	U	U	U	U	U
Enlarged tongue	U	U	U	U	U	U	U
Ear abnormalities	-	simple auricles	U	U	U	U	U
Hand abnormalities	clinodactyly for 5th fingers	bilateral 5th finger clinodactyly	U	U	U	camptodactyly	U
Joint hyperlaxity/stiffness	+	U	U	U	U	U	U
Nail anomalies	+, nail hypoplasia	U	U	dry, flaky nails	U	U	U
Feet abnormalities (flat feet, sandal gap,...)	pes planus	U	U	U	U	U	talipes equi-varus
Toe abnormalities	long halluces	U	U	U	U	U	U
RX abnormalities	multiple small stress fractures	U	advanced bone age	U	U	U	U
Other limb abnormalities	-	U	U	U	U	U	U

	Subject 14	Subject 15	Subject 16	Subject 17	Subject 18	Subject 19	Subject 20
Other							
Skull abnormalities	-	U	U	U	U	U	U
Cryptorchidism	-	U	-	bilateral	U	U	U
Renal abnormalities	U	U	U	U	U	U	U
Skin	-	hyperkeratosis, multiple lentigines	U	multiple nevi, redundant skin on palm of hands	U	U	U
Scoliosis (mild, severe)	severe	U	severe	U	U	U	U
Widely spaced/inverted nipples	+	inverted nipples	U	U	U	U	U
Cardiac abnormalities	-	U	U	U	U	U	U
Pectus excavatum/carinatum	-	U	U	U	U	U	U
dentition (normal; pointed; delayed prim / delayed permanent/missing permanent)	multiple caries	U	U	major dental problems with crumbling teeth	U	U	U
sleep problems	'+ (staying asleep, gets up at night and can't go back to sleep)	U	U	U	U	U	U
Laboratory abnormalities	U	diabetes mellitus	U	U	U	U	U
Neuroradiology							
MRI brain (at age in months)	24m	U	4m	U	U	U	U
MRI brain abnormality	arachnoid cyst and mild hydrocephalus	U	mild ventricular enlargement	U	U	U	thin corpus callosum and periventricular leukomalacia
Autism/Behavioral problems	lack of eye contact, ADHD, obsessive behaviors with fixated interest	auditory hypersensitivity, high pitched voice	U	anxiety disorder refractory to medical treatment, developed phobias	U	U	challenging behavior
Clinical diagnosis/tested for:	SHANK3 related disorder and FXS screening normal, CMA normal	U	U	U	U	Clinical suspicion of Weaver syndrome	U

Table S3. Aging signs/features of the affected subjects with frameshift *HIST1H1E* mutations. The table includes the subjects included in the present study (S1 to S13) and those previously reported in the literature (S14 to S20).

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**N**
Age (years)	49	4.5	30.7	1.2	12	3	11.9	4.7	2	1.6	6	4	1.4	10	21	13	15.5	4.2	1.9	8.5	20
Hypotrichosis	Y	Y	Y	Y	Y						Y										6
Skin hyperpigmentation	Y				Y			Y							Y		Y				5
Hyperkeratosis															Y						1
Dry skin					Y										Y						2
Cutis laxa	Y											Y					Y				3
Cutis marmorata		Y	Y		Y																3
Nail abnormalities			Y		Y							Y					Y				4
Dental problems		Y	Y		Y							Y		Y			Y				6
Osteopenia					Y																1
Limb fractures					Y									Y							2
Advanced bone age					Y		Y					Y				Y					4
Joint stiffness					Y								Y	Y							3
Lower limb pitting edema					Y																1
Hearing loss	Y		Y										Y								3
Cataracts															Y						1
Ptosis	Y		Y										Y								3
Diabetes Mellitus															Y						1
Pancytopenia/autoimmune disorder (systemic lupus erythematosus)	Y																				1

Table S4. List of the CpG sites located in the promoter regions (200-1500 bp from transcription start sites, TSS) of genes found to be differentially methylated in affected individuals with *HIST1H1E* mutations and controls.

IlmnID	CHR	MAPINFO	UCSC RefGene Name	UCSC RefGene Group	mean controls	mean Pt1	mean other patients
cg25880954	1	47900630	MGC12982;FOXD2	TSS1500;TSS1500	0.78	0.91	0.81
cg04863005	1	59043208	TACSTD2	TSS200	0.39	0.09	0.31
cg15100762	1	66516476	LOC101927139;PDE4B;PDE4B; PDE4B;PDE4B;PDE4B	TSS200;Body;Body;Body;Body; Body	0.85	0.93	0.95
cg09408571	1	101003634	GPR88	TSS200	0.63	0.79	0.72
cg06223162	1	101003688	GPR88	TSS200	0.46	0.54	0.43
cg16180556	1	110230269	GSTM1;GSTM1	TSS200;TSS200	0.29	0.28	0.52
cg24506221	1	110230401	GSTM1;GSTM1	TSS200;TSS200	0.24	0.38	0.48
cg20803293	1	110254709	GSTM5	TSS200	0.35	0.27	0.51
cg16810724	1	110752159	KCNC4-AS1;KCNC4;KCNC4;KCNC4	Body;TSS1500;TSS1500;TSS1500	0.62	0.64	0.49
cg06205333	1	112161618	RAP1A;RAP1A	TSS1500;TSS1500	0.61	0.82	0.48
cg10185505	1	150335496	RPRD2;RPRD2;RPRD2	TSS1500;TSS1500;TSS1500	0.72	0.54	0.56
cg12650227	1	152572930	LCE3C	TSS1500	0.61	0.19	0.56
cg08477332	1	153590243	S100A14	TSS1500	0.35	0.43	0.45
cg23216745	1	154929762	PBXIP1;PYGO2	TSS1500;3'UTR	0.82	0.89	0.90
cg27003165	1	162381929	SH2D1B	TSS200	0.52	0.40	0.67
cg13124890	1	162382662	SH2D1B	TSS1500	0.49	0.28	0.67
cg01062020	1	162382848	SH2D1B	TSS1500	0.32	0.08	0.47
cg19368440	1	164744070	LOC100505795;PBX1;PBX1;PBX1	TSS200;Body;Body;Body	0.32	0.55	0.79
cg07533224	1	205819345	PM20D1	TSS200	0.43	0.52	0.48
cg12898220	1	205819356	PM20D1	TSS200	0.48	0.57	0.51
cg05841700	1	205819383	PM20D1	TSS200	0.36	0.30	0.37
cg11965913	1	205819406	PM20D1	TSS200	0.26	0.25	0.28
cg07167872	1	205819463	PM20D1	TSS200	0.36	0.42	0.34
cg24503407	1	205819492	PM20D1	TSS1500	0.37	0.46	0.40
cg16334093	1	205819600	PM20D1	TSS1500	0.49	0.51	0.53
cg07157834	1	205819609	PM20D1	TSS1500	0.53	0.57	0.62
cg00541777	2	3652840	COLEC11;COLEC11;COLEC11;COLEC11; COLEC11;COLEC11;COLEC11;COLEC11; COLEC11;COLEC11;COLEC11	TSS1500;TSS1500;TSS1500;TSS1500; Body;Body;Body;Body;Body;Body; Body	0.73	0.66	0.81
cg10326673	2	30669757	LCLAT1;LCLAT1;LCLAT1;LCLAT1	TSS1500;TSS1500;TSS1500;TSS1500	0.23	0.50	0.42
cg15652532	2	30669759	LCLAT1;LCLAT1	TSS1500;TSS1500	0.23	0.52	0.39
cg24521141	2	38744309	LOC101929596	TSS1500	0.78	0.95	0.89
cg05043910	2	99872119	LYG2	TSS1500	0.52	0.53	0.60
cg23122642	2	113992694	PAX8-AS1;PAX8-AS1;PAX8;PAX8; PAX8;PAX8	TSS1500;TSS1500;Body;Body;Body; Body	0.61	0.52	0.58
cg21482265	2	113992762	PAX8;PAX8;PAX8;PAX8;PAX8;LOC440 839;LOC654433	Body;Body;Body;Body;Body;Body; TSS1500	0.67	0.61	0.68
cg19083407	2	113993142	PAX8;PAX8;PAX8;PAX8;PAX8; LOC440839;LOC654433	Body;Body;Body;Body;Body;Body; TSS1500	0.59	0.57	0.57
cg08010094	2	139539001	NXPH2	TSS1500	0.52	0.18	0.38
cg19840088	2	149894678	LYPD6B	TSS1500	0.60	0.93	0.64
cg20351137	2	177133606	MTX2;MTX2	TSS1500;TSS1500	0.63	0.67	0.49
cg16955800	2	183981465	NUP35	TSS1500	0.34	0.45	0.21
cg20517941	2	201600636	LOC100507140;AOX2P	TSS1500;Body	0.22	0.39	0.33
cg21893210	2	220108407	GLB1L;GLB1L;GLB1L	5'UTR;5'UTR;TSS200	0.39	0.49	0.20
cg24061197	2	220108496	GLB1L;GLB1L;GLB1L	5'UTR;5'UTR;TSS200	0.42	0.59	0.27
cg01588581	2	241832900	C2orf54;C2orf54	TSS1500;Body	0.50	0.41	0.55
cg01904194	2	241832904	C2orf54;C2orf54;C2orf54	TSS1500;TSS1500;Body	0.60	0.45	0.62
cg08144588	3	3080327	CNTN4;CNTN4;CNTN4	Body;TSS1500;Body	0.81	0.94	0.75
cg00457450	3	15107267	MRPS25	TSS1500	0.74	0.30	0.38
cg08033130	3	45983597	CXCR6;FYCO1	TSS1500;Body	0.43	0.34	0.56
cg20540428	3	73045686	PPP4R2	TSS1500	0.35	0.20	0.51
cg06085042	3	195425033	MIR570	TSS1500	0.59	0.50	0.66
cg15727583	3	196757701	MFI2;MFI2	TSS1500;TSS1500	0.77	0.97	0.85
cg01132407	4	645781	PDE6B;PDE6B;PDE6B	TSS1500;Body;Body	0.65	0.85	0.50
cg19247841	4	48485301	SLC10A4	TSS200	0.11	0.53	0.08
cg19978674	4	57523826	HOPX;HOPX;HOPX;HOPX;HOPX	5'UTR;Body;Body;TSS1500;TSS1500	0.35	0.21	0.64

cg07952421	4	69435601	UGT2B15;UGT2B17	TSS1500;TSS1500	0.70	0.86	0.82
cg12011299	4	100065546	ADH4	TSS200	0.40	0.29	0.62
cg05635388	4	122721892	EXOSC9;EXOSC9	TSS1500;TSS1500	0.62	0.60	0.39
cg04096619	5	9547595	SNORD123;SEMA5A	TSS1500;TSS1500	0.81	0.93	0.86
cg06961054	5	56204405	SETD9;SETD9	TSS1500;TSS1500	0.53	0.24	0.33
cg06795995	5	56204613	SETD9;SETD9	TSS1500;TSS1500	0.42	0.19	0.24
cg25340688	5	135416398	MIR886	TSS200	0.49	0.53	0.45
cg03395511	6	291903	DUSP22	TSS200	0.32	0.52	0.26
cg13824270	6	4020946	PRPF4B	TSS1500	0.53	0.24	0.26
cg00944873	6	24646780	KIAA0319;KIAA0319;KIAA0319; KIAA0319;KIAA0319	TSS1500;TSS1500;TSS1500;TSS1500; TSS1500	0.33	0.40	0.36
cg07792871	6	29942706	HCG9	TSS200	0.31	0.26	0.35
cg17857094	6	30907280	DPCR1	TSS1500	0.69	0.42	0.66
cg05030953	6	31241000	HLA-C	TSS1500	0.31	0.11	0.57
cg03849834	6	41195891	TREML4	TSS200	0.81	0.40	0.79
cg03558010	6	46890220	GPR116;GPR116	TSS1500;5'UTR	0.77	0.79	0.93
cg02872426	6	110736772	DDO;DDO	TSS200;TSS200	0.33	0.25	0.88
cg07164639	6	110736958	DDO;DDO	TSS1500;TSS1500	0.24	0.27	0.76
cg21309351	6	138540608	KIAA1244;PBOV1	Body;TSS1500	0.78	0.81	0.89
cg14593639	6	142622028	ADGRG6;ADGRG6;ADGRG6;ADGRG6	TSS1500;TSS1500;TSS1500;TSS1500	0.47	0.54	0.71
cg05155812	7	855012	SUN1;SUN1;SUN1;SUN1	TSS1500;TSS1500;TSS1500;TSS200	0.59	0.66	0.59
cg08776296	7	134856544	C7orf49;C7orf49;C7orf49;C7orf49; C7orf49;C7orf49;C7orf49	TSS1500;TSS1500;TSS1500;TSS1500; TSS1500;TSS1500;TSS1500	0.61	0.42	0.77
cg00795791	7	135346062	PL-5283	TSS1500	0.63	0.98	0.78
cg17960959	7	135346502	C7orf73	TSS1500	0.53	0.75	0.57
cg21537187	7	135662562	MTPN;LUZP6	TSS1500;TSS1500	0.68	0.19	0.71
cg09293560	7	150068240	REPIN1;REPIN1;REPIN1;REPIN1	TSS200;5'UTR;Body;5'UTR	0.59	0.77	0.64
cg07547279	7	151433873	PRKAG2;PRKAG2;PRKAG2	TSS1500;Body;Body	0.61	0.93	0.50
cg20877230	8	6876684	DEFA3;DEFA1;DEFA1B	TSS1500;TSS1500;TSS1500	0.48	0.57	0.49
cg20223677	8	7332846	DEFB104B;DEFB104A	TSS1500;TSS1500	0.73	0.90	0.89
cg20934259	8	11997366	USP17L2;FAM66D	TSS1500;Body	0.69	0.88	0.78
cg03983883	8	79577618	ZC2HC1A	TSS1500	0.32	0.25	0.23
cg04046119	8	107460025	OXR1;OXR1;OXR1	TSS200;Body;Body	0.88	0.51	0.39
cg10596483	8	143751796	JRK;JRK	TSS1500;TSS1500	0.21	0.19	0.22
cg03249723	9	98880057	LOC158434	TSS1500	0.35	0.49	0.38
cg21717724	9	123604514	PSMD5;LOC253039	Body;TSS1500	0.66	0.70	0.40
cg04622888	9	124990010	LHX6;LHX6;LHX6;LHX6	TSS200;Body;Body;Body	0.57	0.94	0.75
cg13523132	9	139638566	LCN6;LCN10	3'UTR;TSS1500	0.85	0.82	0.77
cg08713344	10	3183772	PITRM1-AS1;PITRM1;PITRM1;PITRM1	TSS200;Body;Body;Body	0.66	0.91	0.79
cg10171609	10	5405573	UCN3	TSS1500	0.87	0.94	0.96
cg10379346	10	123355239	FGFR2;FGFR2;FGFR2;FGFR2;FGFR2; FGFR2;FGFR2;FGFR2	5'UTR;5'UTR;5'UTR;5'UTR;5'UTR; 5'UTR;TSS1500;Body	0.46	0.90	0.51
cg06791446	10	123355268	FGFR2;FGFR2;FGFR2;FGFR2;FGFR2; FGFR2;FGFR2	5'UTR;5'UTR;5'UTR;5'UTR;TSS1500; 5'UTR;5'UTR	0.51	0.87	0.54
cg25460273	10	129704427	PTPRE	TSS1500	0.50	0.78	0.62
cg18493115	11	1643842	HCCA2;KRTAP5-4	Body;TSS1500	0.82	0.97	0.95
cg07243930	11	3647365	TRPC2	TSS1500	0.68	0.32	0.68
cg15570860	11	8986840	TMEM9B;TMEM9B;TMEM9B;TMEM9B-AS1	TSS1500;TSS1500;TSS1500;Body	0.70	1.00	0.74
cg23722437	11	13983009	SPON1	TSS1500	0.65	0.89	0.73
cg23284931	11	13983273	SPON1	TSS1500	0.51	0.88	0.69
cg07093428	11	18433500	LDHC;LDHC	TSS1500;TSS1500	0.76	0.89	0.73
cg19767548	11	18433554	LDHC;LDHC	TSS1500;TSS1500	0.84	0.93	0.74
cg14332815	11	18433564	LDHC;LDHC	TSS1500;TSS1500	0.78	0.86	0.63
cg11821245	11	18433683	LDHC;LDHC	TSS200;TSS200	0.56	0.54	0.41
cg07469075	11	35548139	PAMR1;PAMR1;PAMR1;PAMR1	TSS1500;TSS1500;TSS1500;5'UTR	0.36	0.36	0.54
cg01578633	11	57159066	PRG2;PRG2;PRG2;PRG2	TSS1500;TSS1500;TSS1500;TSS1500	0.39	0.43	0.64
cg15971518	11	57159174	PRG2	TSS1500	0.28	0.14	0.51
cg23304078	12	739312	LOC100049716;NINJ2;NINJ2	TSS1500;Body;5'UTR	0.59	0.42	0.56
cg05578102	12	739986	LOC100049716;NINJ2;NINJ2	TSS200;Body;5'UTR	0.39	0.07	0.37
cg20927656	12	7863229	DPPA3	TSS1500	0.52	0.35	0.64
cg01120761	12	7903170	CLEC4C;CLEC4C	TSS1500;TSS1500	0.79	0.47	0.95
cg15411736	12	9886905	CLECL1	TSS1500	0.80	0.93	0.76
cg04531182	12	10563981	KLRC4-KLRK1	TSS1500	0.36	0.87	0.20
cg08041188	12	10564015	KLRC4-KLRK1	TSS1500	0.40	0.92	0.25
cg22221831	12	15039397	MGP	TSS1500	0.41	0.62	0.83

cg11180750	12	21283013	SLCO1B1	TSS1500	0.34	0.39	0.84
cg02656474	12	22198837	CMAS	TSS1500	0.29	0.09	0.35
cg03842440	12	41831532	PDZRN4;PDZRN4	TSS200;Body	0.60	0.30	0.80
cg05788368	12	110506138	C12orf76	TSS1500	0.62	0.62	0.81
cg13861644	12	130822286	PIWIL1	TSS1500	0.66	0.76	0.44
cg27630820	12	130822294	PIWIL1;PIWIL1	TSS200;TSS200	0.58	0.82	0.45
cg19272349	13	37681255	CSNK1A1L	TSS1500	0.66	0.71	0.61
cg04306507	14	55594613	LGALS3	TSS1500	0.42	0.45	0.70
cg26251192	14	74003199	ACOT1;HEATR4;HEATR4	TSS1500;5'UTR;5'UTR	0.57	0.59	0.58
cg18561199	14	95027379	SERPINA4;SERPINA4;SERPINA4	TSS1500;TSS1500;TSS1500	0.32	0.37	0.45
cg03012280	15	41098255	ZFYVE19;DNAJC17	TSS1500;Body	0.61	0.57	0.70
cg17395184	15	42750462	ZFP106	TSS1500	0.81	0.82	0.64
cg03433313	16	819064	MIR662	TSS1500	0.78	0.91	0.62
cg08624915	16	31538718	AHSP	TSS1500	0.73	0.40	0.72
cg26624021	16	56995739	CETP	TSS200	0.40	0.68	0.88
cg09889350	16	56995813	CETP	TSS200	0.33	0.55	0.84
cg17107388	16	58533766	NDRG4;NDRG4;NDRG4;NDRG4; NDRG4;NDRG4;NDRG4;NDRG4	TSS1500;TSS1500;TSS1500;TSS1500; TSS1500;Body;Body;Body	0.66	0.38	0.30
cg22273830	17	1508471	SLC43A2;SLC43A2;SLC43A2	TSS200;Body;Body	0.30	0.56	0.26
cg24587835	17	5674234	LOC339166	TSS1500	0.29	0.10	0.35
cg13377102	17	7832764	KCNAB3	TSS200	0.88	0.66	0.31
cg13407335	17	7832852	KCNAB3	TSS200	0.86	0.63	0.22
cg16513459	17	7832932	KCNAB3	TSS200	0.83	0.57	0.20
cg01323777	17	7832943	KCNAB3	TSS200	0.88	0.64	0.19
cg27162435	17	7833163	KCNAB3	TSS1500	0.73	0.43	0.16
cg14918082	17	7833237	KCNAB3	TSS1500	0.75	0.46	0.14
cg05513408	17	39166655	KRTAP3-1	TSS1500	0.62	0.36	0.69
cg11440486	17	48585216	MYCBPAP	TSS1500	0.55	0.42	0.54
cg20111217	17	48585264	MYCBPAP	TSS1500	0.53	0.44	0.58
cg00901687	17	48585270	MYCBPAP	TSS1500	0.51	0.40	0.54
cg07442736	17	65040607	CACNG1	TSS200	0.90	0.34	0.92
cg01147067	17	78233766	RNF213;RNF213	TSS1500;TSS1500	0.58	0.27	0.84
cg02398342	17	80708632	TBCD;FN3K	TSS1500;3'UTR	0.53	0.36	0.41
cg10004653	18	713071	ENOSF1;ENOSF1;ENOSF1	TSS1500;TSS1500;TSS1500	0.54	0.28	0.33
cg07100532	18	713085	ENOSF1;ENOSF1;ENOSF1	TSS1500;TSS1500;TSS1500	0.66	0.37	0.42
cg25317025	18	47019823	RPL17;RPL17;RPL17;RPL17;RPL17; RPL17-C18orf32;RPL17;RPL17;RPL17	TSS1500;TSS1500;TSS1500;TSS1500; TSS1500;TSS1500;TSS1500;TSS1500; TSS1500	0.41	0.17	0.46
cg04547181	19	6721855	C3	TSS1500	0.47	0.70	0.43
cg14279361	19	6721955	C3	TSS1500	0.23	0.39	0.23
cg12768975	19	6721965	C3	TSS1500	0.24	0.42	0.21
cg16474696	19	13875014	MRI1;MRI1	TSS1500;TSS1500	0.24	0.44	0.22
cg25755428	19	13875111	MRI1;MRI1	TSS1500;TSS1500	0.34	0.67	0.34
cg19882830	19	21264948	ZNF714;ZNF714;ZNF714;ZNF714	TSS200;TSS200;TSS200;TSS200	0.21	0.50	0.12
cg18805164	19	36265700	SNX26	TSS1500	0.52	0.08	0.55
cg23489630	19	44645078	ZNF234;ZNF234	TSS1500;TSS1500	0.71	0.94	0.76
cg22459517	19	55587193	EPS8L1	TSS200	0.59	0.68	0.51
cg07461715	19	57989332	ZNF772;ZNF772	TSS1500;TSS1500	0.62	0.95	0.51
cg25325723	20	6104886	FERMT1	TSS1500	0.74	0.96	0.98
cg14752227	20	34000481	UQCC;UQCC	TSS1500;TSS1500	0.80	0.74	0.73
cg04305670	20	43937273	MATN4;MATN4;MATN4;RBPJL; RBPJL;RBPJL	TSS1500;TSS1500;TSS200;Body; Body;Body	0.59	0.78	0.74
cg18287711	20	62288918	RTEL1;RTEL1;RTEL1;RTEL1;RTEL1- TNFRSF6B	TSS1500;TSS1500;TSS1500;TSS1500; TSS1500	0.70	0.24	0.31
cg12690462	21	43822540	UBASH3A;UBASH3A;UBASH3A	TSS1500;TSS1500;TSS1500	0.67	0.79	0.42
cg10296238	21	47605174	C21orf56;C21orf56	TSS1500;TSS1500	0.30	0.05	0.19
cg11466708	22	23974816	C22orf43	TSS1500	0.70	0.86	0.56
cg24989447	22	31730238	PIK3IP1-AS1;PATZ1;PATZ1;PATZ1	TSS1500;Body;Body;Body	0.53	0.68	0.79
cg01124132	22	32599511	RFPL2;RFPL2;RFPL2	TSS200;5'UTR;TSS1500	0.48	0.23	0.16
cg27308932	22	32600139	RFPL2;RFPL2	5'UTR;TSS1500	0.49	0.46	0.21
cg05019187	22	32601185	RFPL2	TSS1500	0.56	0.34	0.34
cg08161306	22	47169227	TBC1D22A;TBC1D22A;TBC1D22A; TBC1D22A;TBC1D22A	TSS1500;5'UTR;Body;Body;Body	0.53	0.41	0.46

Table S5. Gene set enrichment analyses of KEEG pathway and GO cellular component terms associated with the 1,000 most differentially methylated probes among affected subjects heterozygous for the *HIST1H1E* frameshift mutations and controls.

GO term	Description GO term (Cellular Component)	FDR	Gene Symbol
GO:0098590	Plasma membrane region	5.69e-11	ABCG2;ADORA2A;ANXA2;ARHGEF18;BCR;C2CD5;CDH13;CPEB4;DISC1;DLGAP1;EHD2;EPS8L1; ERC1;EXOC1; FERMT1;ITGB2;KANK1;KCNC1;KCNC4;KCNJ10;KIFAP3;MLC1;MYO1D;NDRG4;NRCAM;NRP1;P2 RX1;PARD3B; PDE6B;PKD1L1;PLB1;PRR12;PTH1R;RGS14;SCN10A;SCRIB;SLC1A1;SLCO1B1;STK39;SYNE1;SY NJ2;TACSTD2;TANC1;ZFYVE19
GO:0045202	Synapse	5.69e-11	ABR;ADORA2A;APBB2;BCL11A;BCR;CDH13;CPEB4;CPT1C;CYFIP1;DISC1;DLGAP1;DOCK10; ERC1;FGFR2;HDAC4;ITSN1;KCNC1;KCNC4;KCNJ10;MAGI2;MDGA1;NCK2;NRCAM;NRP1; P2RX1;PDE4B;PHACTR1;PLCB1;PRR12;PTPRN2;RAB6B;RAP1A;RGS14;RPS6KB1;SCN10A;SCRI B;SDK1; SLC2A8;SYN3;SYNE1;SYNJ2;TANC1;UCN3
GO:0097458	Neuron part	7.07e-11	ADORA2A;APBB2;ARID1B;ASRGL1;BCL11A;BCR;BRSK2;CCSAP;CDH13;CNTN4;CPEB4;CPT1C; CROCC;CYFIP1;DISC1;DLGAP1;DOCK10;ERC1;FBXO31;ITSN1;KCNC1;KCNC4;KCNJ10; KCNN3;KIFAP3;LMTK2;MAG;MAGI2;MBP;MTPN;MYO1D;MYO3B;NCK2;NFASC;NRCAM;NRP1;P 2RX1; PDE4B;PDE6B;PRPH2;PRR12;PTPRN2;RAB6B;RAP1A;RGS14;RPS6KB1;RPTOR;SCN10A;SCRIB; SLC2A8; SYN3;SYNJ2;TANC1;TSHZ3;UCN3
GO:0042613	MHC class II protein complex	0.001	HLA-DPB1;HLA-DQB1;HLA-DQB2;HLA-DRB1;HLA-DRB5
GO:0033267	Axon part	0.001	ADORA2A;APBB2;BRSK2;CPEB4;CYFIP1;ITSN1;KCNC1;KCNC4;LMTK2;MAG;MBP; MYO1D;NFASC;NRCAM;NRP1;PTPRN2;SYNJ2;TANC1;TSHZ3;UCN3
GO:0044463	Cell projection part	0.001	ADORA2A;AGBL2;APBB2;BRSK2;C2CD5;CATSPER4;CCSAP;CPEB4;CPT1C;CROCC; CYFIP1;DISC1;DNAH9;DOCK10;DUSP22;EPS8L1;FERMT1;ITSN1;KANK1;KCNC1; KCNC4;KIFAP3;LMTK2;MAG;MAGI2;MBP;MYO1D;MYO3B;NDRG4;NEDD1;NFASC;NRCAM;NRP1 ; PDE4B;PDE6B;PKD1L1;PLB1;PRPH2;PTH1R;PTPRN2;RGS14;RPTOR;SYNJ2;TANC1;TSHZ3;UCN 3;WDR66
GO:0120038	Plasma membrane bounded cell projection part	0.001	ADORA2A;AGBL2;APBB2;BRSK2;C2CD5;CATSPER4;CCSAP;CPEB4;CPT1C;CROCC;CYFIP1;DISC1 ; DNAH9;DOCK10;DUSP22;EPS8L1;FERMT1;ITSN1;KANK1;KCNC1;KCNC4;KIFAP3;LMTK2;MAG; MAGI2; MBP;MYO1D;MYO3B;NDRG4;NEDD1;NFASC;NRCAM;NRP1;PDE4B;PDE6B;PKD1L1;PLB1;PRPH 2;PTH1R;PTPRN2;

			RGS14;RPTOR;SYNJ2;TANC1;TSHZ3;UCN3;WDR66
GO:0044304	Main axon	0.003	ADORA2A;KCNC1;MAG;MBP;MYO1D;NFASC;NRCAM;UCN3
GO:0042611	MHC protein complex	0.003	HLA-DPB1;HLA-DQB1;HLA-DQB2;HLA-DRB1;HLA-DRB5
GO:0030424	Axon	0.004	ADORA2A;APBB2;BRSK2;CCSAP;CNTN4;CPEB4;CPT1C;CYFIP1;ITSN1;KCNC1;KCNC4;LMTK2; MAG;MBP;MTPN;MYO1D;NFASC;NRCAM;NRP1;PTPRN2;SCN10A;SYNJ2;TANC1;TSHZ3;UCN3
PATHWAY: KEGG number	**Description**	**FDR**	**Gene Symbol**
hsa04514	Cell adhesion molecules (CAMs)	0.001	CDH4;HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;ITGB2;MAG;NFASC;NRCAM;NRXN3;SELL
hsa04940	Type I diabetes mellitus	0.002	HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;PTPRN2
hsa05330	Allograft rejection	0.008	HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5
hsa05332	Graft-versus-host disease	0.008	HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5
hsa05416	Viral myocarditis	0.008	HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;ITGB2
hsa05310	Asthma	0.016	HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;PRG2
hsa05320	Autoimmune thyroid disease	0.024	HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5
hsa05150	Staphylococcus aureus infection	0.026	C3;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;ITGB2
hsa04145	Phagosome	0.026	C3;COLEC11;COLEC12;HLA-A;HLA-C;HLA-DPB1;HLA-DQB1;HLA-DRB1;HLA-DRB5;ITGB2
hsa04650	Natural killer cell mediated cytotoxicity	0.031	HLA-A;HLA-C;IFNAR2;ITGB2;KLRC4-KLRK1;MICA;PPP3R1;SH2D1B;SH3BP2

GO, Gene Ontology; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes.