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Supplemental Data

Mutations in *ACTL6B* Cause Neurodevelopmental Deficits and Epilepsy and Lead to Loss of Dendrites in Human Neurons

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Supplemental Case Reports

Recessive mutations

Individual R1

Stable epilepsy, on a 4/1 ketogenic diet, lamictal, rivotril, frisium and Keppra. Very sensitive to heat and infections, with increased seizures at those time, but did not require changing the treatment. Stable global developmental delay, goes to school, has good waking periods, but there is no significant gain. Fine from the digestive point of view, still gavage-fed, which is well tolerated (she has had in the past periods with little tolerance to gavage-feeding and nausea, but this has not happened for about a year. Last aspiration pneumonia in December 2017). Hospitalized for a first treatment of bisphosphonate, this due to a worsening of her osteoporosis. The treatment was well tolerated, it caused a little fever, but without seizures. However, she received only two-thirds of the half-dose that she was to receive, but it is enough to reassure the team that she will be able to receive the next treatments.

Individual R2

The individual is a 5-year-old boy who was originally seen for genetic evaluation at 4 months of age due to history of hypotonia, developmental delay and failure to thrive as well as a family history of a sibling who died at age 5 years with history of intractable seizures, global developmental delay and hypotonia. There was no concerns or complications during pregnancy or delivery. Baby was born full term with normal vaginal delivery. His birth weight was 3.76 kg, length 48 cm and OFC was 35.5 cm. The individual also had history of GERD (gastroesophageal reflux disorder) with recurrent emesis as well as dysphagia. He underwent gastric tube placement with Nissen fundoplication at 14 months of age and his GI symptoms greatly improved. His first onset of seizures was at 3 years of age with complex partial epilepsy.

His brain MRI at 3 years of age was unremarkable but no recorded seizures on the EEG. He also had significant cortical visual impairment. His symptoms including hypotonia, global developmental delay, intellectual impairment and seizures continued to deteriorate and he died at 5 years of age. He underwent at 2 years of age whole exome sequencing which identified compound heterozygous mutations in the *ACTL6B* gene, c.695delC (p.P232fs) and a c.1275C>A (p.C425X). Both of these mutations were identified in his deceased sibling with a similar phenotype. Each parent was carrier for one of the mutations. The individual's brother had history of global developmental delay and hypotonia which were first noted around 4 months of age. He also has cortical visual impairment with intractable mixed seizures with onset at 2 years of age. His brain MRI showed cerebral atrophy and delayed white matter maturation followed by white matter loss including corpus callosum. He had history of GERD and underwent Nissen Fundoplication with gastric tube placement

Individual R5

Age 1 year and 11 months. She has a pharmacoresistent epilepsy. EEG shows a multifocal epileptiform activity and non-normal background activity. The girl has a severe global developmental delay, has some eye contact, no verbal communication and has no voluntary motor function. Sha has a severe dystonic motor pattern. She uses a jejunostomy for feeding.

Individuals R8a and R8b

Full sisters of Mexican descent. Their parents are second cousins. Both individuals have severe global developmental delay. They are nonambulatory and nonverbal. Both have been diagnosed with spastic quadriplegic cerebral palsy GMFCS level V. Both have a history of

aspiration pneumonias and chronic lung disease. Both have seizure disorders with the elder sister having seizures by 5 months of age and the younger sister within the first month of life. Both have had MRIs of the brain which show thinned corpus callosum and white matter volume loss.

Individual R9

This girl is the second child of non-consanguineous parents. She was born at term (40+3 weeks) with a birth weight of 3270 gram. At the age of 5 weeks her length was 53 cm (-0,5 SD), weight 4,2 kg (+0,1 SD) and her head circumference 35,5 cm (-1,1 SD). There is no relevant family history. She has a healthy older brother who was born with preaxial polydactyly of his left foot. The brother was tested negative for both ACTL6B mutations. Both parents are healthy carriers. She had severe epilepsy with antenatal onset; the mother reported “hick-ups with strange child movements” during pregnancy. EEG shows multifocal EEG abnormalities with intensive epileptic abnormalities of the left hemisphere. She had myoclonic seizures as well as tonic seizures. MRI cerebrum showed a thin corpus callosum, high signal intensity of dorsal globus pallidum/putamen an showed some asymmetric gyral patern. At the age of 14 months she was examined: she had a significant developmental delay, head lag and she was not able to sit yet. Besides the seizures which are difficult to control with medication she had severe axial hypotonia, hypertonia of the extremities (spastic tetraparese) and hyperreflexia. Also she needs feeding by gastric tube because of severe feeding problems and vomiting. She had extreme discomfort, which might be partially caused by chronic sever obstipation en possible a minor congenital anorectal malformation. The last examination was by a paediatrician at the age of 2 years and 4 months. She had an infection of the gastric tube which she was operated on and now gets a daily enema. This resulted in less discomfort. However, there is still some discomfort

with vomiting during the day. She has less seizures; but despite high doses different anti-epileptic medication she still has a lot of (mostly mild) seizures every day and also at night. She doesn't develop much (she makes contact and smiles, some babbling, but still head lag and no sitting).

Dominant mutations

Individual D3

Patient was enrolled in the Pediatric Genomics study conducted by HudsonAlpha Institute for Biotechnology in Huntsville, AL, USA in collaboration with Children's Rehabilitation Service and the Alabama Department of Rehabilitation Services. The *de novo* missense mutation NM_016188.4:c.1027G>A p.Gly343Arg in *ACTL6B* was identified using whole genome sequencing of the affected child and both of his unaffected parents was performed and the result was confirmed by Sanger sequencing. This young boy was enrolled at 6 years and 6.5 months of age and at that time, he presented with global developmental delay, severe ID, hypotonia, absent speech, tracheomalacia, and ambulation difficulties. EEG was normal and no seizure activity was detected. Brain MRI, CGH, Microarray, and hearing screening were not remarkable. Gene panel testing for genes associated with Myotonic Dystrophy, Fragile X Syndrome, and Prader-Wili Syndrome were all normal. He has mild facial dysmorphism (hypertelorism, spaced teeth, low set ears, everted upper lip, and wide mouth). He also wears glasses (specific condition is unknown), has poor muscle tone, sensory processing abnormalities, and extreme joint laxity. At 7 years of age, the child was reported to be able to crawl, pull up, and feed himself using a spoon.

Individual D9

9-year-old female, who has been followed since age 2 with a negative evaluation for Angelman syndrome that included SNP CMA, PWS/AS methylation testing and UBE3A sequence analysis. MECP2 sequencing was also negative. She has an unsteady gait with improving tone, strength, and fine motor skills. She is non-verbal and uses a picture device for communication (combination PECS/I-Pad system). She has some hand stereotypies and poorly coordinated eye movements with severe intellectual disability. Her receptive skills are better than her expressive skills, and she has a treatment diagnosis of autism. She is unable to cooperate with formal psychometric testing. She is partially toilet-trained, with very sensitive skin and a high pain threshold, and she had early onset of puberty with early pubertal dentition. She had a single febrile seizure that did not recur. She has not had an MRI. She has a normal sister and a less severely-affected female cousin born to her dad's brother with seizures and intellectual disability. Exam revealed height at the 75^{th}-90^{th} centile, weight 75^{th}-90^{th} centile, and head circumference 50^{th}-75^{th} centile. She had a 5 x 3 cm mole on her right flank. She had ocular hypertelorism (OCD 9.8 cm,+4 SD above the mean; IPD 6.0 cm, +2 SD; and ICD 3.5 cm, +2 SD). There is a disruption of the medial eyebrows with nearly absent medial portion. She has a somewhat squared off nasal tip. She has a high, narrow anterior palate with mixed dentition and a short philtrum. A bifid uvula was noted previously. Breast buds were apparent, Tanner II-III. Long appearing palms with 5th fingers remarkably shortened bilaterally. There are prominent digital pads on the thumb and the 4^{th} fingers bilaterally. She has malalignment of her toes, with her 3rd toe underneath her 4^{th} toe and broad halluces. Her toes are also broad with somewhat small nails, and she has broad distal phalanges on her fingers with small nails. She was non-verbal with relatively normal tone and severe intellectual disability.

Individual D10

Female patient with developmental delay, intellectual disability. global hypotonia, walking at 6y3m, ataxic gait with hyperlordosis, no language but says “mama”, no seizures. kindness but very anxious; stereotypies strabismus, astigmatism. One brother with same problems, better evolution/better management and no problems at birth, same mutation. Father : intellectual disability, abnormal behavior (psychiatric).

Supplementary Figures and Legends

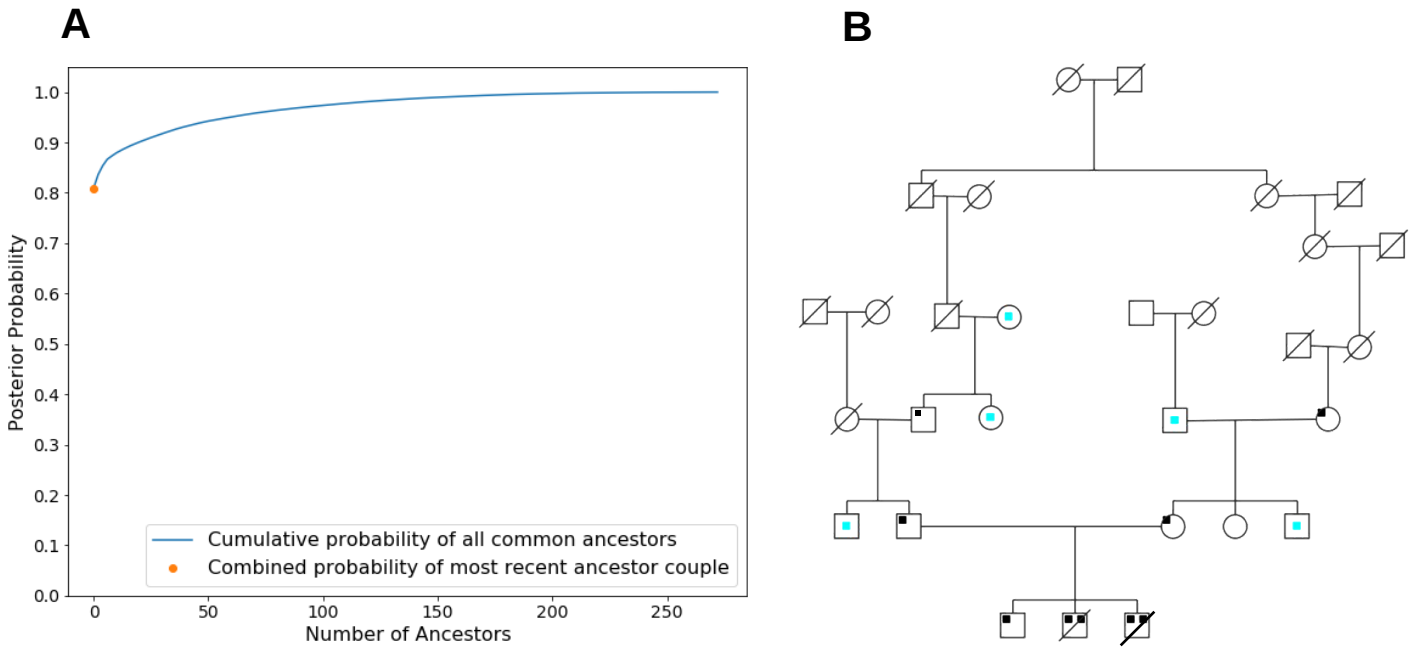


Figure S1. A) The most recent common ancestor couple of the parents of family R3 are the most likely origin of the affected *ACTL6B* allele, with a posterior probability of 0.809. Details on how this probability was calculated can be found in the supplemental methods. Church marriage records from Quebec revealed that the father and mother of R3a and R3b had a common ancestor 4 and 5 generations back, respectively. Common ancestor also independently confirmed by family. B) Pedigree of family R3. Church marriage records from Quebec revealed that the father and mother of R3a and R3b had a common ancestor 4 and 5 generations back, respectively. Common ancestor independently confirmed by family. Black squares denote presence of mutation; blue squares signify that the individual was assessed for the presence of the mutation, but that it was absent.

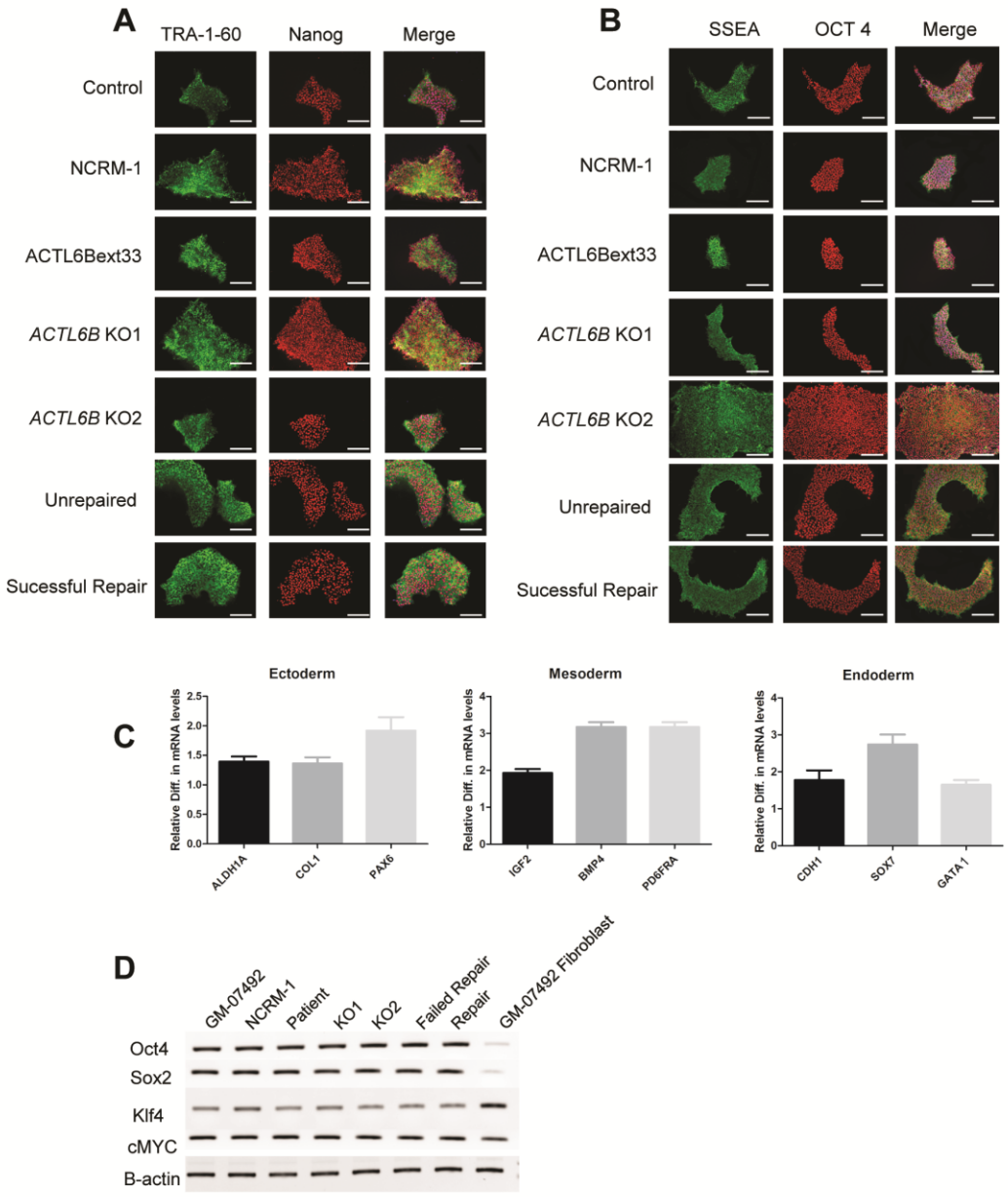


Figure S2. Characterization of pluripotency markers in induced plurpotent stem cell lines. A-B) Immunocytochemistry of four pluripotency markers (TRA-1-60, Nanog, SSEA and OCT4) in all iPSC lines used in this study. Scale bars represent 200µm. C) qPCR assessment of key genes in all cell lines utilized in this study following differentiation for eight days into a ectoderm, mesoderm and endoderm lineage. Scale is relative to expression of genes in a sample composed of equal parts of all three germ layers. D) Assessment of the expression of pluripotency genes in cDNA obtained from iPSCs. Primers bind only to endogenously expressed genes.

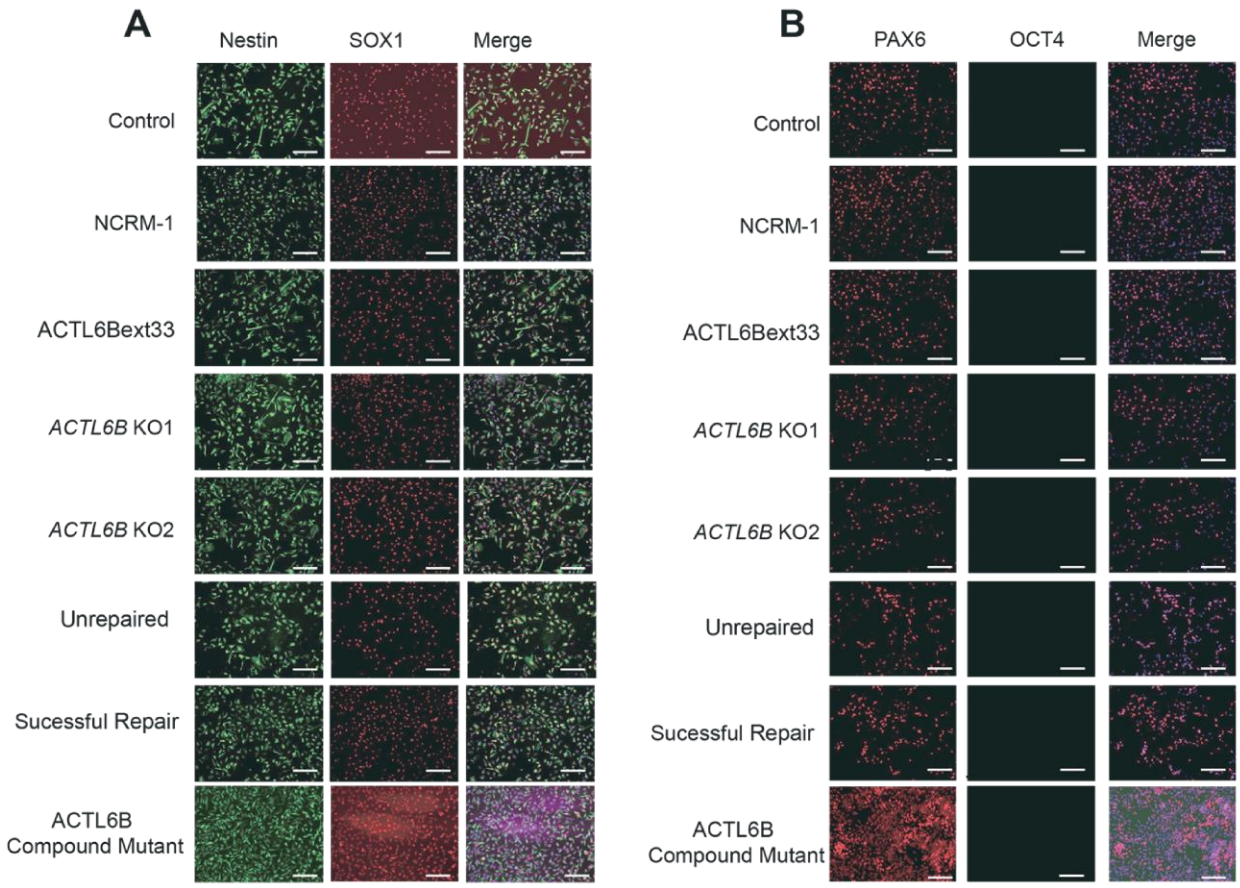


Figure S3. Characterization of neural lines generated for this study. A-B) ICC staining of all NPC lines generated in this study, showing positive staining of forebrain NPCs (Nestin, SOX1, PAX6) and negative staining pluripotency markers (OCT4). Scale bars represent 200µm.

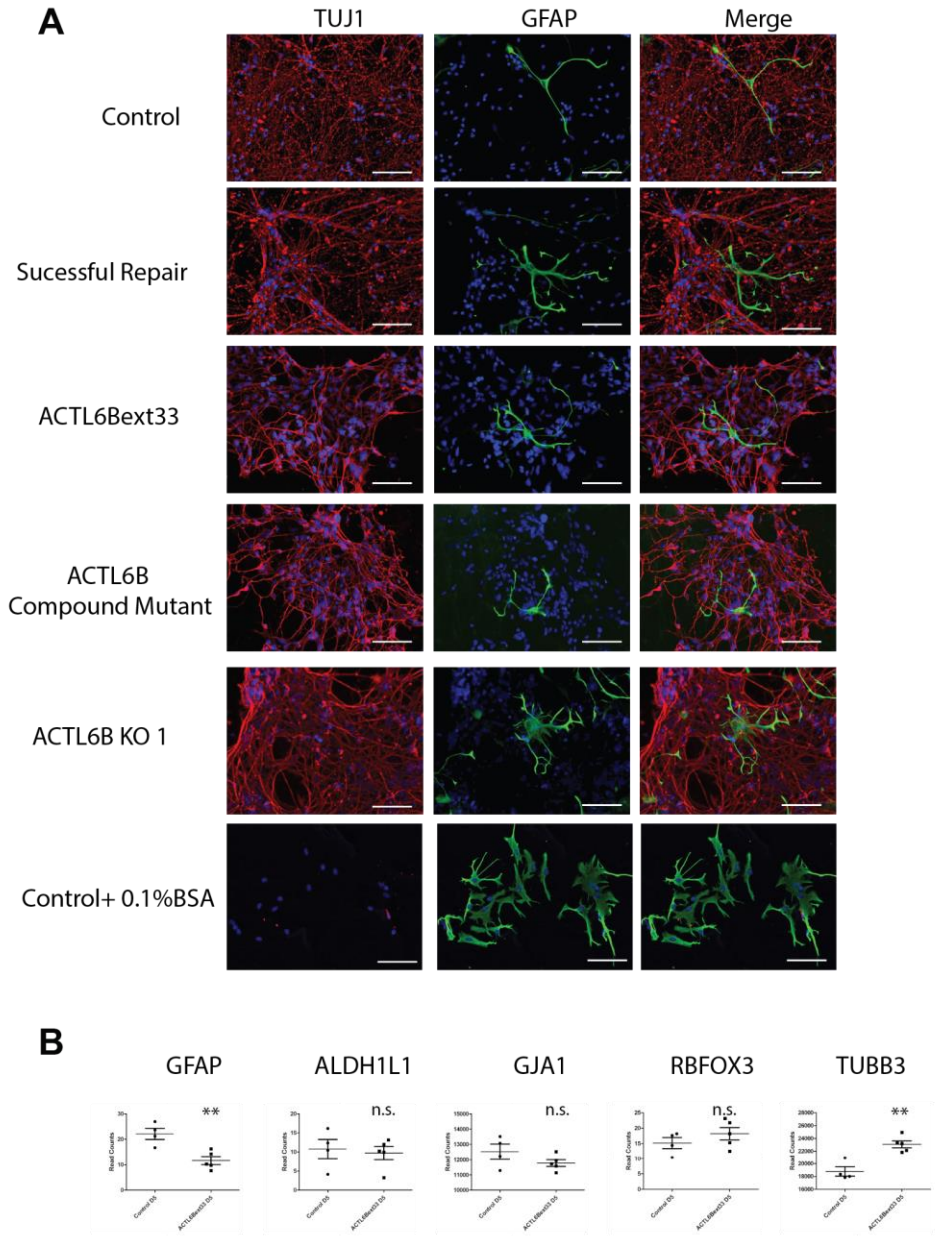


Figure S4. No difference in GFAP-positive cells in mutant, wildtype, or engineered cell lines

A) Staining of control, ACTL6Bext33, ACTL6Bcompoundmutant, and gene-edited lines for TUJ1 and GFAP, demonstrating a small percentage of cells are positive for GFAP staining. B) Read counts from D5 RNA-SEQ data from control and ACTL6Bext33 neurons for select astrocytic and neuronal genes (N=5). $P<0.05$, $^{**}P<0.01$.

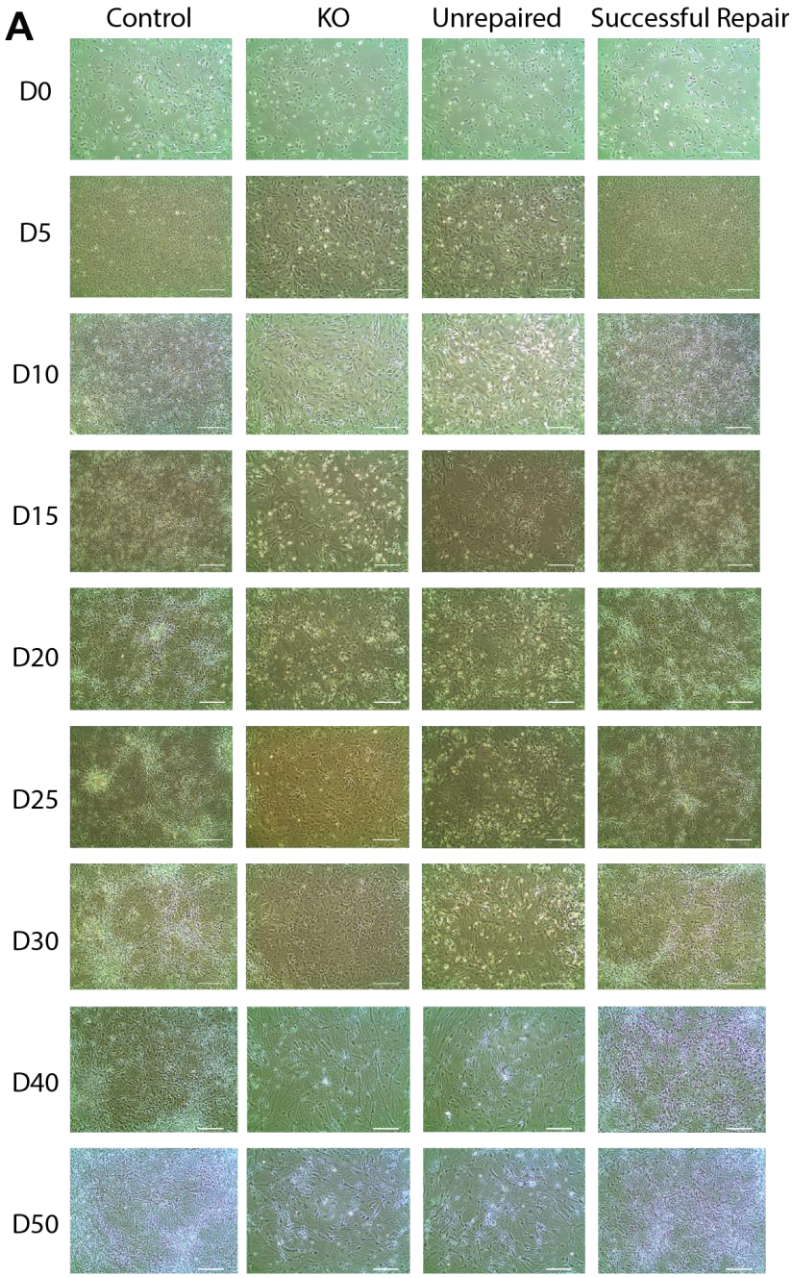


Figure S5. Morphology of CRISPR modified neuronal lines during differentiation.

Bright field images of Control, KO, Unrepaired, and Successful Repair cell lines during fifty days of neuronal differentiation. Scale bars represent 100µm. Note that both KO and Unrepaired lines consistently show larger nuclei.

Supplementary Tables

Table S1. Additional clinical details for patients with bi-allelic mutations in *ACTL6B*.

Individual	R1	R2	R3	R4	R5	R6	R7	R8a	R8b	R9	R10
Family history		Family history is significant for a brother who passed away at 5 years old who had a history of developmental delay,hypotonia, intractable seizures, hypotonia, gastroesophageal reflux disease (GERD), and cortical visual impairment	Full brother also affected with epileptic encephalopathy, died at 2 years old. Healthy older brother who carries heterozygous mutation and has no pathological phenotype. 23 family members sequenced (incl great parents of R3), and no homozygotes found.	Healthy younger brother	Single child of healthy unrelated parents	Parents healthy. Healthy brother	Healthy parents	Full sister also affected. No other siblings. Parents are second cousins. No other affected relatives.	Full sister also affected. No other siblings. Parents are second cousins. No other affected relatives.	Healthy older brother has preaxial polydactyly left foot, both parents are healthy No relevant family history, except for nephew of mother (son of brother of her father) who died at age of 7 months because of (genetic) liver disease.	No affected relative
Birth growth parameters	3020g, 51cm, OFC 34 cm		2972g, 49cm, OFC 33.5 cm	2314g, 47.3cm, OFC 32.0cm	prenatal microcephaly birthweight 3280 grams, length 51 cm, OFC 32 cm	7pds 5 oz, L 20 and ¼ in	NM_016188:exon4: c.C289T:p.R97X (homozygous)	2.62kg at 36 weeks EGA	3.486kg at 38 weeks EGA	Birthweight: 3270 gr (40+3 wk) At age 5 weeks: 53 cm (-0,5 SD), 4,2 kg (+0,1 SD) and OFC 35,5 cm (-1,1 SD)	NA
Age at last visit	3 y.o. F	3 1/2y.o. M	11 m.o. (death at 4 yo)	8 yo F	5 months	12 m.o.	4 y.o.	6yo F	5yo F	14 months	4.5y F
Height	73 cm (-5.5 SD)	failure to thrive	75 cm (50th)	117.8 cm	60,5 cm (10th %ile)	70 cm (1%ile)	Nl	18mo 72cm (-2.5 SD)	4mo 59.5cm (22nd %ile)	79 cm (+0,4 SD)	NA
Weight	10th %ile	Short stature	8.58 kg (10th)	17.9 kg	5,8 kg (10th %ile)	7.2 kg (1%ile)	Nl	18mo 9.985kg (17th %ile)	4mo 5.63kg (25th %ile)	9,4 kg (-0,8 SD)	11.2 (-3.8 SD)
ID, DD	Significant developmental delay	Delayed motor milestones, delayed speech, delayed visual maturation.	Developmental delay	Developmental delay	Severe global developmental delay	Severe GDD/ID. Nonverbal, non ambulatory	Yes, severe GDD	severe global developmental delay, nonverbal, nonambulatory	severe global developmental delay, nonverbal, nonambulatory	Significant developmental delay, not able to sit yet	Yes
Neuro findings	Axial hypotonia, spasticity 4 limbs, dystonia neck and L arm, choreoathetotic mouth movements (resolved)	Axial hypotonia, lower extremities hypertonia/spasticity,	Axial hypotonia, spasticity 4 limbs	Axial hypotonia	Severe stimulus induced dystonia. Generalized hypertonia hyperreflexia and spasticity..	Hypertonia, spasticity, cerebral palsy, cortical visual impairment	Hypotonia, lower limbs spasticity, developmental delay	spastic quadriplegia and dystonia with truncal hypotonia, GMFCS level V	spastic quadriplegia and dystonia with truncal hypotonia, GMFCS level V	Axial severe hypotonia, severe epilepsy (antenatal onset), hypertonia extremities (spastic tetraparese), hyperreflexia	Axial hypotonia, Limb spasticity
Feeding difficulties	Yes	Feeding difficulties Dysfunctional swallow), GERD	Difficulty swallowing	Yes, difficulties in swallowing	increasing feeding difficulties Gastrostomy from the age of five months	G-tube fed, GERD	Yes, difficulties in swallowing	A: Completely G-tube dependent, fundoplication, GERD	B: Completely GJ tube dependent, GERD	Severe (recently gastric tube)	
Epilepsy	Started at 3 months. Myoclonias 2-6 per day. Multifocal EEG anomalies	Complex partial epilepsy with onset at age 3 years. Seen recently at 5 years of age by neurology and he seems to have complex partial epilepsy with possible tonic epilepsy that is intractable and getting daily.(deceased	Multifocal EEG abnormalities	No EEG abnormalities	Started at 2 months of age. EEG shows multifocal epileptiform activity and generalized slowing of background patterns.	Started at 3 w.o. with infantile spasms. Epileptic encephalopathy	Multifocal EEG abnormalities, seizures since infancy	Seizures since infancy, usually daily seizure episodes, generalized slowing of background rhythms on EEG	Seizures since infancy, usually daily seizure episodes, generalized slowing of background rhythms on EEG	Started antenatal (mother felt "hick-ups with strange childmovements" during pregnancy) Multifocal EEG abnormalities, intensive epileptic abnormalities left hemisphere myoclonic seizures AND tonic seizures	Infantile spasms, then Tonic and myoclonic seizures

		brother with complex partial, possible atonic/tonic seizures)									
MRI	Prominent subarachnoid spaces and small corpus callosum	MRI of brain at 4 years of age was normal.Deceased brother: white matter loss. Interval enlargement of sulci and ventricles suggesting atrophy or Possibly pseudoatrophy)	Mild T2 hyperintensity in frontal periventricular white matter	Mild T2 hyperintensity in frontal periventricular white matter	The first MRI of the head performed at 1 week of age was normal. The second MRI of the head performed at 2 months of age showed symmetric signal changes in the brainstem and in the dorsal medulla oblongata, possibly also around the dentate nucleus	3 w.o.: asymmetric ventricles, cortical dysplasia right parietal lobe. 9 m: cerebral atrophy, hypoplasia of corpus callosum	NA	MRI at 5mo: Significantly decreased white matter throughout, extremely thin corpus callosum. Normal MR spectroscopy	MRI at 10mo: Periventricular leukomalacia with white matter volume loss and overall brain volume loss, delayed myelination and thinning of corpus callosum. Normal MR spectroscopy	Thin corpus callosum High signal intensity doral globus pallidus/putamen Some asymmetry gyral patern	At 3.5yo: Cerebral and cerebellar atrophy, thin corpus callosum
Rx	Mogadon, valproate, Topamax, prevacid and bicarbonate	Oxcarbazepine. .Deceased brother had vagal nerve stimulator implantation	Tegretol, rivotril, Neurontin, prevacid, melatonin	No medication	Valproate Levetiracetam Nitrazepam Esomeprazol Midazolam (as emergency medication) Botulinum toxin injections in the hip-adductors	Sabril, onfi, felbamate, augmentin, baclofen. Did not tolerate leviteracepam, topiramate, prednisolone	NA	Baclofen, QVAR, Klonopin, Lamictal, Keppra, lansoprazole	Baclofen, Vimpat, Keppra, lorazepam, ompeprazole	Different medication: At this moment: fycompa perampanel, clobazam, levetiracetam, chloralhydraat, baclofen, Nexium, forlax	NA
Dysmorphisms	High arched palate, thick gums, strabism,	Scaphocephaly with bi-termporal narrowing, otherwise non-dysmorphic. Deceased brother not dysmorphic	Narrow palate, Strabismus,	Rt outer strabismus	She does not look dysmorphic	Nondysmorphic. Microcephaly, micrognathia, high-arched palate	Not available	Microcephalic but not overly dysmorphic	Microcephalic but not overly dysmorphic	Asymmetric skull/face (right side fuller) , microcephaly, but no evident dysmorphic features	NA
Other		Chronic aspiration needed salivary gland botox	Increased inhibin A during pregnancy. Heart US normal. Abdominal US normal. Orange hue to skin and tongue		spectroscopy did not document a lactate peak	Aspiration pneumonia. Metab wo nl, died at age 2.		Both: chronic lung disease with aspiration pneumonias,asthma, and frequent viral infections	chronic lung disease with aspiration pneumonias,asthma, and frequent viral infections	- Chronic severe constipation possibly due to minor anorectal malformation and vomiting	NA
Site for sequencing	Genome Quebec	Illumina	Genome Quebec	Yokohama City University Graduate School of Medicine	Haukeland University Hospital, University of Bergen	GeneDx	University College London	GeneDx	UCLA Clinical Genomics Center	Radboud University Medical Center in Nijmegen	Genome Quebec
WES or WGS	WGS	WGS	WES	WES	WES	WES	WES	WES	WES	WES	WGS
Library capture	Illumina TruSeq	Illumina TruSeq	Agilent SureSelect Human All Exon v4	Agilent SureSelect Human All Exon v4	Roche-NimbleGen Sequence Capture EZ Exome v2 kit	Agilent SureSelect Human All Exon v4	Illumina's Nextera Rapid Capture	Agilent SureSelect Human All Exon v4	Agilent SureSelect Human All Exon 50 Mb kit	Agilent SureSelect Human All Exon v4	Illumina TruSeq
Sequencing	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq
Alignment	BWA-mem v.0.7.10 and Picard	Illumina WGS Informatics Pipeline version 2.01-02 using the iSAAC package	BWA	NovoAlign, Picard	BWA v.0.5.8c and Picard	BWA-Mem v.0.7.8, GATK v.1.6	Novoalign	BWA-Mem v.0.7.8, GATK v.1.6	Novoalign, SAMtools, GATK	BWA, GATK	BWA-mem v.0.7.10 and Picard
Variant calling, filtering and annotation	GATK, Annovar	Same as above	GATK, in house pipeline	GATK, Annovar	GATK, Annovar	SAMtools v.0.1.18	Annovar	SAMtools v.0.1.18	GATK	in house pipeline	GATK, Annovar
ACMG classification of variants	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance
Reference for exome method	[1]	[2]	[1]	[3]	[4]	[5]	[6]	[5]	[7]	[8]	[1]

Table S2. Additional clinical details for patients with *de novo* mutations in *ACTL6B*.

Individual	D1	D2	D3	D4	D5	D6	D7	D8	D9
De novo mutation	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.1027 G>A, p.Gly343Arg	NM_016188.4:c.230 A>G, p.Asp77Gly
Birth growth parameters	Weight 50TH CENTILE Length 50th centile OFC 2nd centile	NA	3355g, 53 cm	Weight 2.75kg 7th centile	normal parameters	Weight : 2976g Length: 53 cm	3.5 Kg, 51 cm length, OFC 35	39+5w, 2680 g	NA
Age at last visit	5y6m M	29y F	6y6m M	5y9m F	4y6m F	3 F	21y F	2y 6m F	8y F
Height in cm	110 (5th %ile)	163 (50th %ile)	114 (-2.0 SD)	NA	109 (63th %ile)	91.4 cm (17th %ile)	166 cm (67th %ile)	Length 89cm (-0.7SD);	(75th-90th %ile)
Weight in kg	18 (5th %ile)	63 (60th %ile)	18.2 (-2.1 SD)	16.5 (-2.2 SD)	19 (69th %ile	13.1 kg (18th %ile)	44.7 kg (3rd %ile)	Weight 11.1 kg (-1.8 SD);	(75th-90th %ile)
ID, DD	severe ID, absent speech and ambulation	Global DD (sitting position without help: 24 months, first steps without help: 42 months), severe ID, absent speech at time of the inclusion	Global DD, severe ID, absent speech and ambulation difficulties	Severe ID 10-20 words, occasionally 2 words together. Receptive skills better than expressive skills.	severe mental retardation,	Sits independently and roll over but can't crawl, cruise or walk. She is nonverbal but very alert and interactive.	Severe ID, absent speech . Limited ambulation, uses wheelchair, rolls over to move around, can walk with extensive support from walker aid.	IQ test showed developmental age of 9 months at chronological age 29 months (~IQ 30).	Severe ID, with absent speech and communicated mostly using gestures and sounds. stronger receptive skills than her expressive language skills
Neuro	NA	Severe hypotonia, wide based gait (cerebellar syndrome), feeding difficulties	Hypotonia	Autistic features Happy disposition, wide based gait, Obsessions, easily excitable, short attention span	hypotonia , stereotypies	Hypotonia	Rett/AS-like handwringing;	Able to stand but not walk. Can stands on her toes.	autism
Epilepsy	No seizures, EEG nl	No seizures and EEG in the normal range	EEG-nl ; no seizures	No	No	No seizures	infantile spasms first episode, 3 months; subsided at 18 months. unresponsive to vigabitrin nocturnal GTCS with focal onset at starting age 18;lasted 3 years.	No	NA
MRI	Normal	None	Normal on several examinations	Normal	Normal	reportedly showed some thinning of the corpus callosum (done at the outside institution)	Generalised atrophy 2y	mild periventricular gliosis, possibly consistent with perinatal damage.	NA
Rx	NA	None	None	NA	None.	none	zonegran, keppra, midazolam, chloralhydrate, Lamotrigin, Diazepam if needed.	No	NA
Dysmorphisms	Prominent forehead, hypertelorism, wide mouth, brachycephaly	Prominent forehead, hypertelorism, broad and bulbous nasal tip, low set ears, short philtrum, thin and everted upper lip, wide mouth, widely spaced teeth, long palms with short 5th fingers,	Hypertelorism, spaced teeth, low set ears, everted upper lip, and wide mouth	Broad forehead Deep set eyes Narrow palpebral fissures Upturned nasal tip Flushed cheeks Carp shaped mouth Thickened helices of the ears Narrow feet Sandal gap	No dysmorphisms, no short phalanges or nails, no prominent or wide forhead	Normocephalic, mild pseudostrabismus and telecanthi. Ears were normal.	Forehead slightly high broad, wide nostrils, upturned tip of nose wide mouth, short philtrum, thick everted lips lower lip>upper lip, widely spaced teeth especially between upper incisives	No dysmorphic features.	Ocular hypertelorism, with absence of the medial eyebrows, a broad nasal tip, a short philtrum and high narrow anterior palate. Long palms with 5th fingers, prominent persistent fetal pads

				Short broad nails on the feet			short broad and spatulated distal phalanx of thumb wiht short broad nails		on thumbs and 4th fingers bilaterally, malaligned toes with the 3rd toe under 4th toe bilaterally and broad halluces.
Other	Feeding difficulties chokes on lumpy food, GERD, oesophagitis,	Feeding difficulties and gastroesophageal reflux disease, delayed sleep phase disorder, atopic dermatitis	Tracheomalacia, poor muscle tone, wears glasses condition unknown, sensory processing abnormalities, extreme joint laxity	Angelman methylation normal	Diastema, sleep difficulties, frequent sharp screaming, hyperactive behavior, head circumference 48 cm (2^{nd} %ile)	On thickened liquids due to dysphagia. Mild OSA. Torticollis. Negative microarray, metabolic studies, Prader Willi and myotonic dystrophy. Muscle biopsy showed slightly low muscle CoQ10 and low I+III complex but insufficient for a Walker criterion.	right hip contracture adduction --> adductor release operation middle ear infections/glue ears in the past. Very friendly / happy personality. Frequent RTI's in first year and frequent UTI's up till age 4. She has also a de novo c.535C>T p.(Arg179Trp) change in the MED8 gene	Pneumonia at age 13 months. No food aversion, but takes little interest in food and eating.	early pubertal development at age 8 years Tanner 2-3, breast buds
Site for sequencing	DDD	Institut Pasteur	HudsonAlpha Genomic Services Laboratory	DDD	Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany	GeneDx	GeneDx	Radboud University Medical Center in Nijmegen	GeneDx
WES or WGS	WES	WES	WES	WES	WES	WES	WES	WES	WES
Exome library capture	Agilent SureSelect 55MB Exome Plus	Agilent SureSelect Human All Exon v4	Nimblegen SeqCap EZ Exome v3	Agilent SureSelect 55MB Exome Plus	Agilent SureSelect Human All Exon Kit	Agilent SureSelect Human All Exon v4	Agilent SureSelect Human All Exon v4	Agilent SureSelect Human All Exon v4	Agilent SureSelect Human All Exon v4
Sequencing	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq
Alignment	BWA	BWA	BWA	BWA	SOLiD LifeScope software	BWA-Mem v.0.7.8, GATK v.1.6	BWA-Mem v.0.7.8, GATK v.1.6	BWA, GATK	BWA-Mem v.0.7.8, GATK v.1.6
Variant calling, filtering and annotation	GATK, SAMtools, Dindel, CoNVex, DeNovoGear, VEP version 2.6	In-house pipeline	GATK, CarpeNovo	GATK, SAMtools, Dindel, CoNVex, DeNovoGear, VEP version 2.6	LifeScope and GATK	SAMtools v.0.1.18	SAMtools v.0.1.18	in house pipeline	SAMtools v.0.1.18
ACMG classification of variants	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance	Uncertain Significance
Reference for exome method	[9]	[10]	[11]	[9]	[12]	[5]	[5]	[8]	[5]

Table S3. Information about cell lines obtained and generated in this study.

Information regarding the condition, sex, and sorce of cell lines used in this experiment.

NDD= Neurodevelopmental Disorder.

ID	Condition	Sex	Obtained from	Lines obtained as:	RRID
GM-07492	Healthy	M	Coriell	Fibroblasts	CVCL_7467
NCRM1	Healthy	M	Coriell	iPSCs	CVCL_1E7
ACTL6Bext33	NDD	M	Biopsy	Fibroblasts	N/A
ACTL6B KO1	N/A	M	GM-07492	Fibroblasts	N/A
ACTL6B KO2	N/A	M	GM-07492	Fibroblasts	N/A
Unrepaired	N/A	M	Patient	Fibroblasts	N/A
Successful Repair	N/A	M	Patient	Fibroblasts	N/A

Table S4. Genes that are significantly different in both ChIPseq and RNAseq, within subjects, comparing control cells to patient *427Aspext*33.

	ChIPSeq		RNAseq	
Gene	**fold change in D5**	**FDR p**	**log2 fold change in D5**	**padj**
Control (D0 vs D5)				
A4GALT	-1.78	0.008	-2.27	3.44E-17
CNNM2	-1.68	0.01	0.77	4.40E-13
NID1	-1.52	0.023	-1.63	1.12E-09
CERK	-1.84	0.004	0.45	2.72E-09
BTBD11	-1.36	0.024	1.17	5.32E-08
SCUBE3	-1.44	0.023	0.7	6.23E-08
PRTG	-1.44	0.024	1.09	1.50E-07
PHLDA2	-1.31	0.048	-1.57	1.19E-05
ST8SIA3	-1.55	0.023	1.05	8.09E-05
NAA20	-1.35	0.044	-0.47	0.0004

VAV2	-1.79	0.008	0.37	0.0007
PITPNC1	-1.39	0.027	-0.51	0.01
FGF8	-1.43	0.031	1.79	0.013
NELFA	-1.38	0.031	0.25	0.017
CHPF	-1.49	0.023	-0.62	0.021
ZYX	-1.38	0.024	-0.47	0.025
FSCN1	-1.38	0.044	-0.22	0.031
CCDC85A	-1.33	0.038	1.2	0.044
Patient (D0 vs D5)				
SOX8	1.56	0.044	-1.45	2.49E-76
KCNJ12	1.55	0.026	2.36	3.98E-21
MAP2K3	2.13	0.004	0.5	2.02E-07
MAP2K3	2.17	0.0003	0.5	2.02E-07
TPPP	2.69	0.0002	1.22	2.34E-07
ESPNP	1.89	0.024	-3.2	0.011

Table S5. Antibodies used in Immunocytochemistry, Western Blots, and Chromatin Immunoprecipitation. Concentration used, Supplier and Catalog number is provided for each antibody. The BAF53B antibody used in this experiment was a gift from the lab of Dr. Crabtree. ICC= Immunocytochemistry, WB= Western Blot, ChIP= Chromatin Immunoprecipitation

Antibody	Use	Concentration Used	Supplier	Catalog Number
Tuj1	ICC	1/2000	Abcam	ab14545
Nestin	ICC	1/2000	Stem cell Technologies	60091
SOX1	ICC	1/1000	Stem cell Technologies	60095
OCT4	ICC	1/100	Stem cell Technologies	60093
PAX6	ICC	1/500	Stem cell Technologies	60094
TRA-1-60	ICC	1/100	Abcam	ab109884
Nanog	ICC	1/100	Abcam	ab109884
SSEA	ICC	1/100	Abcam	ab109884
MAP2	ICC	1/100	Abcam	ab109884
ALEXA 488	ICC	1/2000	Invitrogen	A-11008
ALEXA 555	ICC	1/2000	Invitrogen	A-21422
BAF53B	WB	1/1000	Crabtree Lab	N/A

BAF53A	WB	1/2000	Abcam	ab3882
SMARCA4	WB	1/5000	Santa-Cruz	sc-10768
β-Actin	WB	1/10000	Abcam	ab49900
Goat Anti-Rabbit IgG H&L (HRP)	WB	1/5000	Abcam	ab6721
Rabbit Anti-Mouse IgG H&L (HRP)	WB	1/5000	Abcam	ab6728
SMARCA4	ChIP	1/200	Santa-Cruz	sc-17796
SMARCA4	CHiP	1/100	Santa-Cruz	sc-10768

Table S6. Primers used in qPCR analysis

Forward and Reverse primers used in this study. All Primers were used at a final concentration of 10µM.

Gene	Forward	Reverse
ACTL6B	CCTTACATGATCGCAGCCAA	CAGCCACCTGTTCATCGTAG
ACTL6A	CAGTCACTTCGCCAGTTAGC	CACCAGCATAACCAGCTCTC
SMARCA4	CTAACCCACCCAACCTCACC	TAGTACTCGGGCAGCTCCTT
SEMA4D	AGGCCCTGAAGAAGTATCAA	TCCACATTTCCCAGTTCTCC
GAPDH	TTCTCAAGCTCATTTCCTGG	TGTGAGGAGGGGAGATTCAG
ALDH1A	CTGCTGGCGACAATGGAGT	CGCAATGTTTTGATGCAGCCT
COL1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
PAX6	AACGATAACATACCAAGCGTGT	GGTCTGCCCGTTCAACATC
IGF2	AGACGTACTGTGCTACCCC	TGCTTCCAGGTGTCATATTGG
BMP4	GCACTGGTCTTGAGTATCCTG	TGCTGAGGTTAAAGAGGAAACG
PDGFRA	AACCGTGTATAAGTCAGGGGA	ATTTCTTCCAGCATTGTGAT
CDH1	CGAGAGCTACACGTTCACGG	GGGTGTCGAGGGAAAAATAGG

SOX7	TCGACGCCCTGGATCAACT	CTGGGAGACCGGAACATGC
GATA1	TGCGGCCTCTATCACAAGATG	CTGCCCGTTTACTGACAATCA
TPPP	TGCCGGACCATCTTTG	TGCTCTTGTCTTTGAATCGCTTC
FSCN1	CACAGGCAAATACTGGACGTT	CCACCTTGTTATAGTCGCAGAAC
PRTG	AAAAGCTGATCTTGCCTTATCAA	GAGGGTGGGATGAAATCTTG

Supplementary Methods

Method S1: Ancestry mapping for family R3

In order to determine the probability of the allele having originated in the known common ancestor of the parents, we linked all individuals with known genotypes to the BALSAC population database, which contains the genealogical relationships between 2.9 million current and past individuals within the province of Quebec, Canada, and compared the probability that the known common ancestor contributed the mutation to the probability that the mutation was introduced by any founder of the population and inherited by both parents without being present in the known common ancestor.

We used an importance sampling Monte Carlo method for efficiently simulating the transmission histories of rare alleles through a known genealogy, and obtained probability estimates for the mutation having originated in any common ancestor of the known carriers within the BALSAC genealogy.

Starting from known carriers in the genealogy, we simulated the inheritance of each affected allele through the maternal or paternal sides and backwards through the genealogy until all alleles had coalesced, or founders of the genealogy were reached (i.e., until we encountered individuals with no known parents in the genealogy). When two alleles are inherited through a single individual, they have probability 0.5 of coalescing in the individual, else the individual is simulated to be a homozygote. Because the allele considered here produces a severe phenotype, the true inheritance history was assumed to not contain ancestral homozygotes.

Representing the set of observed carriers within the genealogy as *S*, a possible ancestral origin of the allele as *a*, and the inheritance history as Γ, we defined the indicator function

$\mathbb{1}_a(\Gamma) = \begin{cases} 1, & \text{if } \Gamma \text{ coalesces to } a \text{ and contains no ancestral homozygotes} \\ 0, & \text{otherwise} \end{cases}$

Our simulations then gave us the probability of the observed carriers given that the true ancestral origin *A* is ancestor *a* as:

$$\mathrm{P}(S|A=a) \;=\; \mathrm{P}(\Gamma \text{ coalesces to } a) \;=\; \mathrm{E}[\mathbb{1}_a(\Gamma)] \;\simeq \frac{1}{M}\sum_{i=1}^{M} \mathbb{1}_a\,(\Gamma_{\mathrm{i}})$$

where the last step is a Monte Carlo integration and Γ_i is the tree created in simulation i. We used a new importance sampling scheme to vastly accelerate convergence of the Monte-Carlo integral in Equation – details of the importance sampling scheme will be described elsewhere[13].

Assuming a flat prior for all ancestors, Bayes theorem provides the normalized posterior probability that *a* is the founding carrier:

$$\mathrm{P}(A=a|S) = \frac{\mathrm{P}(S|A=a)\mathrm{P}(A=a)}{\sum_{a\prime\in A}\mathrm{P}(S|A=a')\mathrm{P}(A=a)} = \frac{\mathrm{P}(S|A=a)}{\sum_{a\prime\in \mathrm{A}}\mathrm{P}(S|A=a')}$$

Method S2: *ACTL6B* CRISPR KO Experiments

Type of CRISPR System used: Double Nickase

Section of *ACTL6B* Gene targeted: hg19_dna range=chr12:13722703-13722942

AGGTGACAGGCCCCGGGGTGCCCAGAGCTCGGATGGATGGCGCGGGTTTGGAACGCAGGGCTGCGGGAGCCGGG

GGCCCGAGGCTCGCTCACCTCCGCCGTAGACGCCCCCGCTCATAGTGCCCGCTGCGCTGCTAGCGGCCCGTGGGC

GGTGGCGGGATCAGCACCGAGGCGGCCGGACAGCTCCCGGGATCCCTGGCGGGGCGGGACTCTCAGCGGCCAAT

TGGGAGGCCGGATCCCCCGC

FWD CRISPR gRNA sequence:

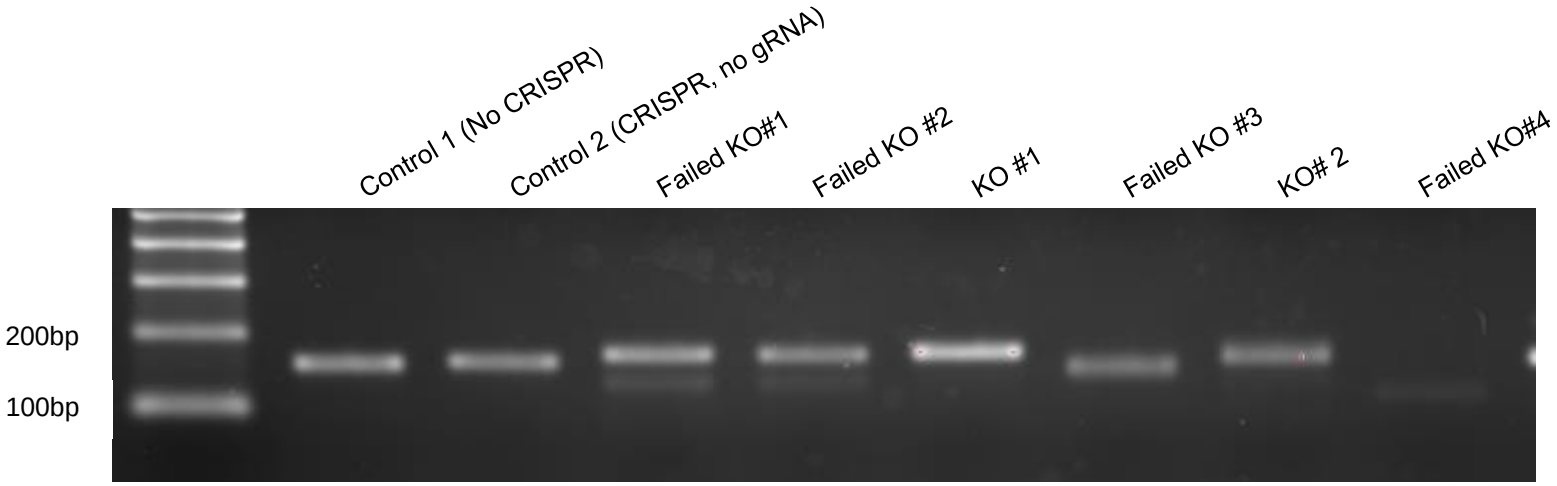
CCGTAGACGCCCCCGCTCAT

REV CRISPR gRNA sequence:

ACGGGCCGCTAGCAGCGCAG

Sanger Forward Primer sequence: CAGAGCTCGGATGGATGG

Sanger Reverse Primer sequence: AATTGGCCGCTGAGAGT



Raw Fasta sequences from Sanger sequencing (reverse strand)

Control

ttttcccgctgcgcgcgttgacgggggatccggcctccaattggccgctgagagtcccgccccgccagggatcccgggagctgtccggccgcctcggtgctgatcccgccaccgcccacg

ggccgctagcagcgcagcgggcactatgagcgggggcgtctacggcggaggtgagacaatgaccnaccgg

ACTL6B KO1

cgctgcgcgcgttgacgggggatccggcctccaattggccgctgagagtcccgccccgccagggatcccgggagctgtccggccgcctcggtgctgatcccgccaccgcccacgggcc

gctagcagcgcagcgggcactatg**ctgggcactatg**agcgggggcgtctacggcggaggtgagacaatgaccgaccgga

ACTL6B KO2

ccgctgcgcgcgttgacgggggatccggcctccaattggccgctgagagtcccgccccgccagggatcccgggagctgtccggccgcctcggtgctgatcccgccaccgcccacgggc

cgctagcagcgcagcgggcactatgagcgggggcgtcta**gctgg**cagcggaggtgagacaatgaccgaccgg

Alignment (sense strand)

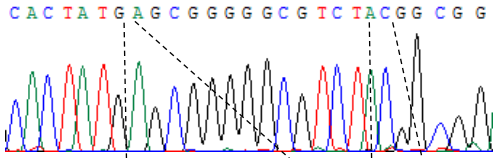
Control cgggcactatgagcgggggcgtctacggcggaggtgagacaatgaccgaccgg

ACTL6B KO1 cgggcactatg**ctgggcactatg**agcgggggcgtctacggcggaggtgagacaatgaccgaccgga

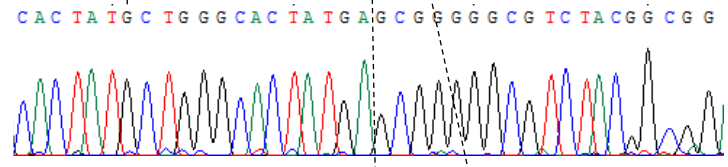
ACTL6B KO2 cgggcactatgagcgggggcgtcta**gctgg**cagcggaggtgagacaatgaccgaccgg

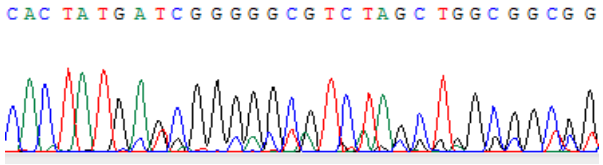
Chromatograms (sense strand)

Control



ACTL6B KO1
[c3_4ins(11)]





ACTL6B KO2
[c17_18ins(5)]

Method S3: *ACTL6B* Repair CRISPR Experiment

Type of CRISPR System used: Wild Type

Section of patient ACTL6B gene targeted: >hg19_dna range=chr7:100240745-100240994

GTGTAGAAGTGAGACTCCCTCCCCTCACCTCCCTCCAGACCCCTGTGCCCACTCCCCACCCCACGGAGGCCAAACT

TTTTTTTCTTAAAACATTTTTACTTCTTTCAACCCAGAAACATCACCATTAATGAGGACAAGAGGGAAAGGAGGAGGGG

GGCAATGTGGCATGGGGGTTAAGGGACTTCCATCTGAGCTTGGGAGCAGGTGTGTGGGGAGGAGTGCCATCGGGG

CACTTTCGCTCCACGCACTGCTTCCCGCCCTCCTCATATTCCTGCTTGGAGATCCACATCTGCTGGAAAGTGCCCTG

GGTGGAGGGGGTAATATCAGGGTCAGGGTGGCCTTGGAGGGAGGTGTGGACAGGCAGGTGCAGCCCCTGGGAATC

CTCCAACAGGCCCCAACCACCCCTTGGGTTCACATCCCTCTGAAGCCCCTCCCCACCTCATCCTCCC

Control Sequence: GTGTGGGGAGGAGTGCCATCAGGGGCACTTT (Control)

Patient Sequence: GTGTGGGGAGGAGTGCCATC-GGGGCACTTT (patient)

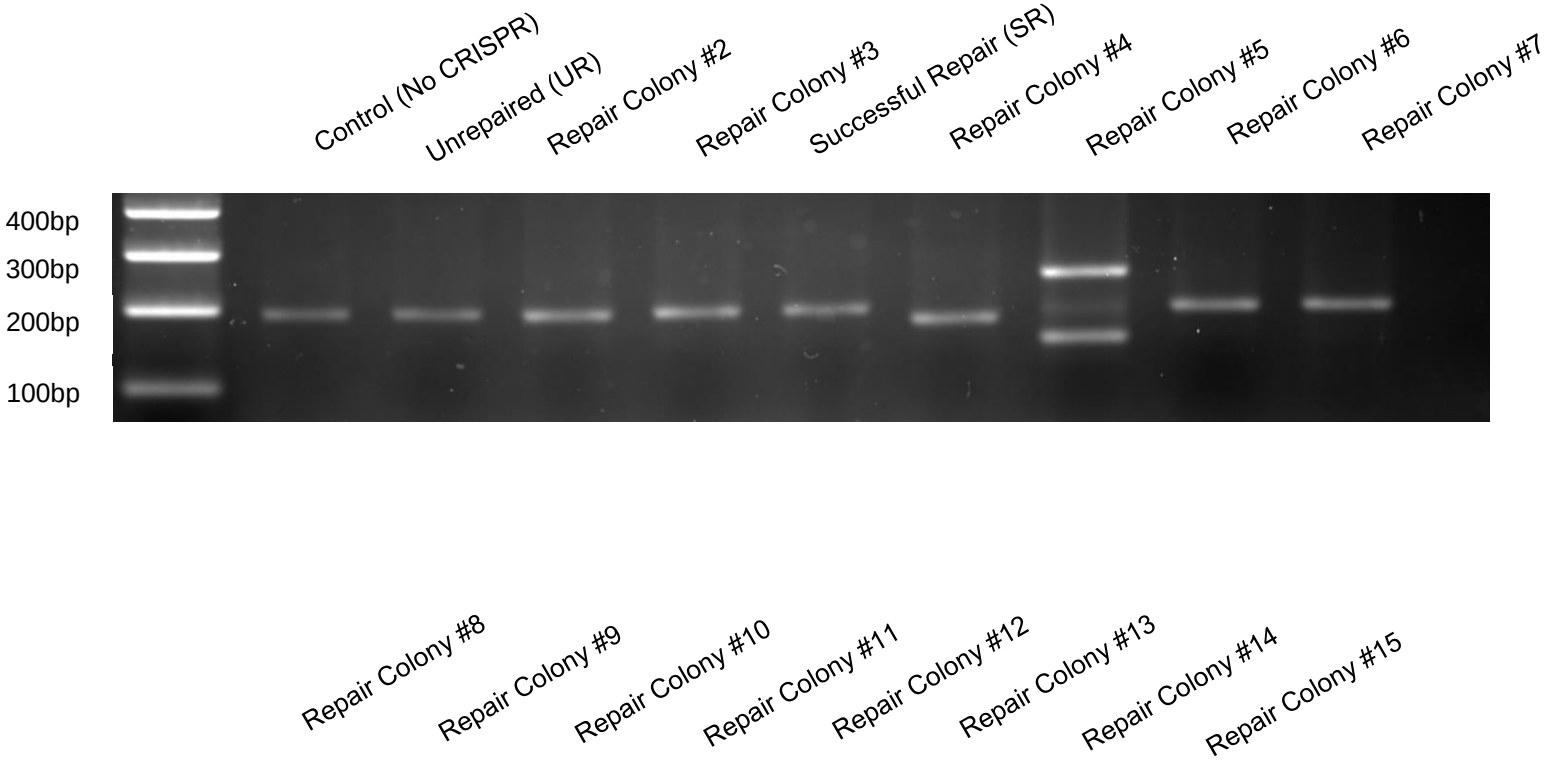
gRNA Sequence: TGTGGGGAGGAGTGCCATC-G

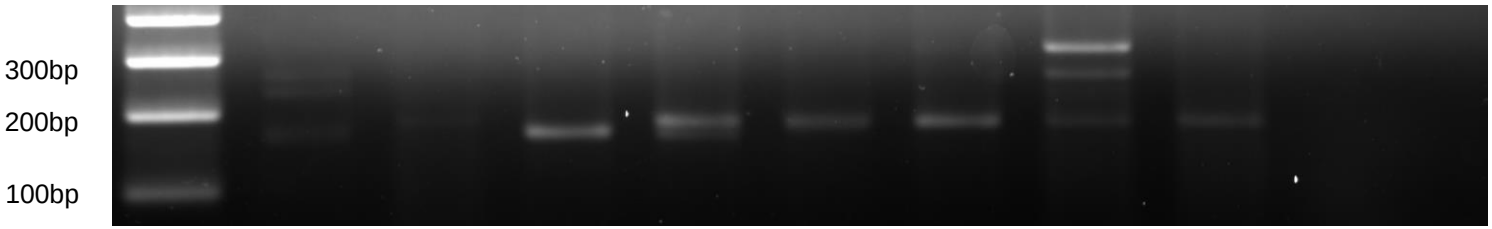
Repair Sequence:GGGTTAAGGGACTTCCATCTGAGCTTGGGAGCAGGTGTGTGG

GGAGGAGTGCCATCAGGGGCACTTTCGCTCCACGCACTGCTTCCCGCCCTCCTCATATTCCTGCTTGG

Sanger Forward Primer sequence: CTTTCAACCCAGAAACATCACCATTAAT

Sanger Reverse Primer sequence: AGCAGATGTGGATCTCCAAGCAG





Raw Fasta sequences from Sanger sequencing (Sense Strand)

Unrepaired (UR)

gggggcatgtggcatgggggttaagggacttccatctgagcttgggagcaggtgtgtggggaggagtgccatcgggg cactttcgctccacgcactgcttcccgccctcctc atattcctgcttggagatccacatctgc

Successful Repair (SR)

ggacttccatctgagcttgggagcaggtgtgtggggaggagtgccatcagggg cactttcgctccacgcactgcttcccgccctcctcatattcctgcttggagatccacatctg

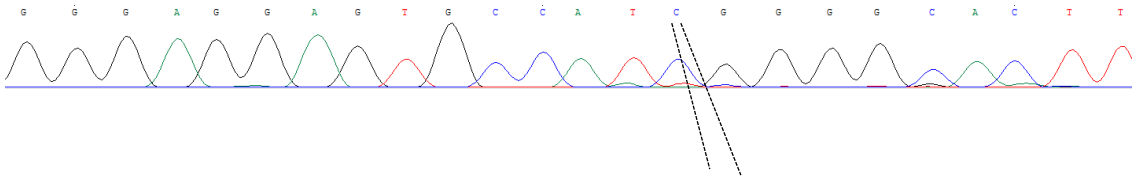
Alignment (Sense Strand)

Unrepaired (UR) gaggagtgccatc-ggggcactttcgctccacgcactgcttcccgccctcc

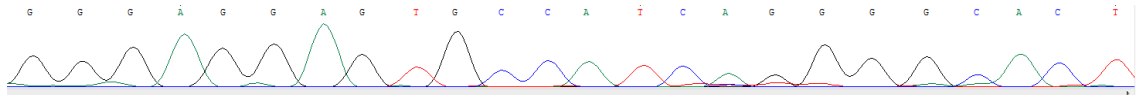
Successful Repair (SR) gaggagtgccatcaggggcactttcgctccacgcactgcttcccgccctcc

Chromatograms (Sense Strand)

Unrepaired (UR)



Successful Repair (SR)



Method S4: Confirmation of homozygous CRISPR KO and Repair

Note: all PCR reactions conducted with 100ng of gDNA, all bar graphs show N=3 data points, with error bars representing standard error around the mean.

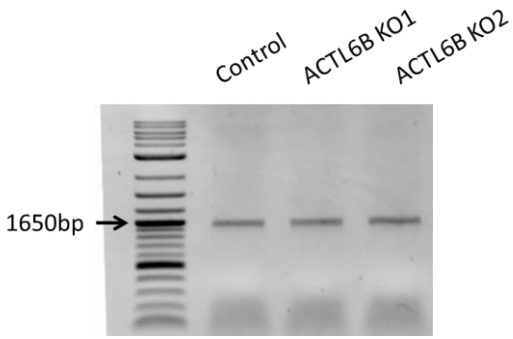
In order to confirm that all of our gene editing events were homozygous, and not the result of off-target gene editing events that have resulted in a large deletion one allele which prevents PCR amplificanion, we first designed primers that covered ~800bp both upstream and downstream of both the ACTL6B KO and ACTL6B repair gene editing site. We then ran the PCR products on an agarose gel and screened for any bands that would indicate large deletions or insertions.

Section of genome targeted centered on ACTL6B KO site : >hg19_dna range= chr7:100,253,189-100,254,802

Forward Primer: CACTGTGGTGGGGAAGTCAG

Reverse Primer: AGATGGAGACACACCCCCTT

Size of Product: 1614

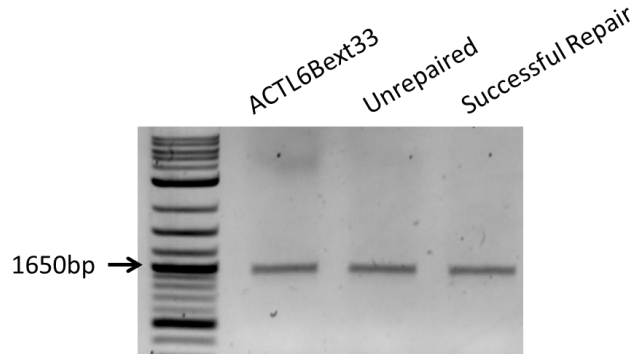


Section of genome targeted centered on ACTL6B Repair site: >hg19_dna range= chr7:100,239,869-100,241,468

Forward Primer: GTTTGGTCACCGAAGGACTTG

Reverse Primer: ATGCTCATTGCAGGAACCCTC

Size of Product: 1600



We did not observe any bands in any lines that would suggest that there were any large insertions or deletions. However, this did not eliminate the possibility that a large deletion had deleted the entire ACTL6B gene on one allele. To rule out this possibility, we designed primers to the center of the ACTL6B gene and loaded equal amount of DNA from all cell lines. We examined the resulting products for a large reduction of product, as would happen if only one allele of the gene was accessible for PCR amplification. In

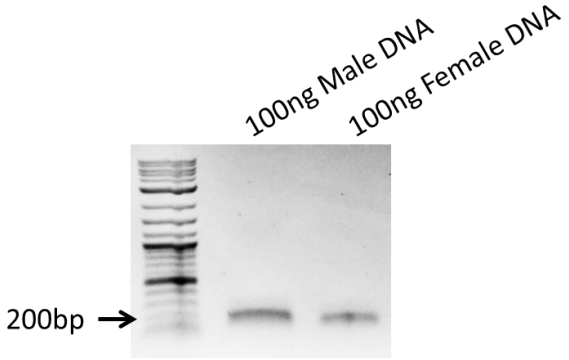
order to have a positive control for the experiment, we ran a separate qPCR reaction with primers designed to the X chromosome with equal amounts of male and female DNA.

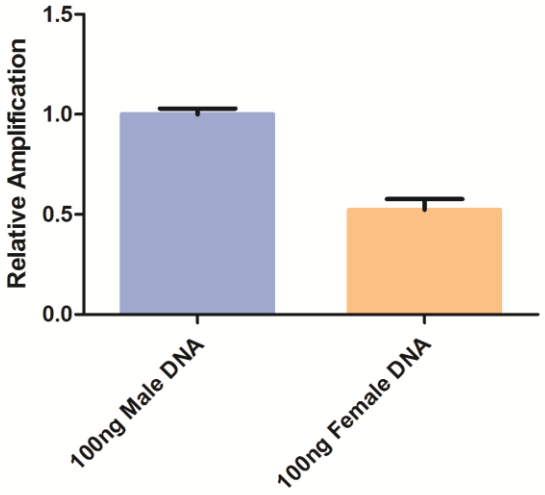
Test of system conducted using primers targeting sequences on X-chromosome

Section of genome targeted: >hg19_dna range=chrX:133612614-133613151

Forward Primer: ATTGCCTGGGGATTCCAAATAC

Reverse Primer: AATATCACAGGGCAGAAAAGGTCA



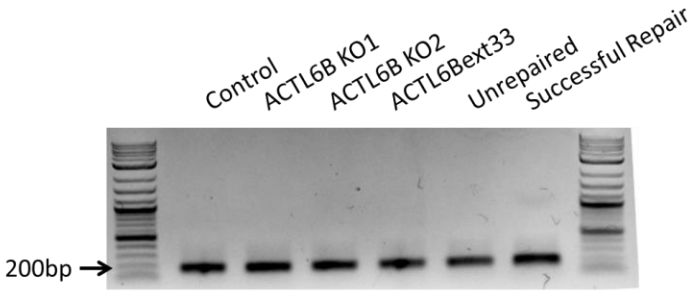


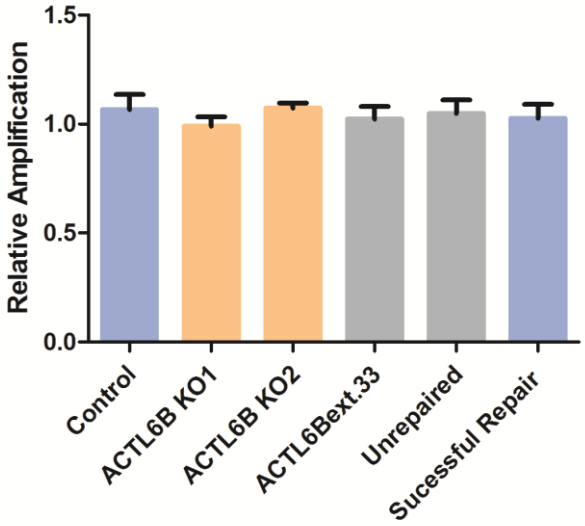
Section of genome targeted on ACTL6B gene : >hg19_dna range= chr7:100,247,392-100,247,578

Forward Primer: TTCCAGCCCCTACTCTCTCC

Reverse Primer: GTCAGGCCTGGTTCTCTGAA

Size of Product: 187bp





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