The American Journal of Human Genetics, Volume *110*

Supplemental information

Null and missense mutations of *ERI1* cause a recessive phenotypic dichotomy in humans

Long Guo, Smrithi Salian, Jing-yi Xue, Nicola Rath, Justine Rousseau, Hyunyun Kim, Sophie Ehresmann, Shahida Moosa, Norio Nakagawa, Hiroshi Kuroda, Jill Clayton-Smith, Juan Wang, Zheng Wang, Siddharth Banka, Adam Jackson, Yan-min Zhang, Zhen-jie Wei, Irina Hüning, Theresa Brunet, Hirofumi Ohashi, Molly F. Thomas, Caleb Bupp, Noriko Miyake, Naomichi Matsumoto, Roberto Mendoza-Londono, Gregory Costain, Gabriele Hahn, Nataliya Di Donato, Gökhan Yigit, Takahiro Yamada, Gen Nishimura, K Mark Ansel, Bernd Wollnik, Martin Hrabě de Angelis, André Mégarbané, Jill A. Rosenfeld, Vigo Heissmeyer, Shiro Ikegawa, and Philippe M. Campeau

Supplementary Note: Case Reports

Individual 1A

She was born at term and she had bilateral severe vesicoureteral reflux causing antenatally diagnosed hydronephrosis, which required bilateral reimplantation. She has asthma, partial conductive hearing loss, hypernasal speech, radial head dislocation, scoliosis and vertebral anomalies. She has chronic intermittent right hip pain, with decreased range of motion. She has joint stiffness especially in the morning, treated with ibuprofen. She has recurrent gingival infections. She has a past medical history of bronchitis, mastitis, and a Bartholin cyst. She has a normal intelligence. She enjoys cycling, talking walks and painting. She works as a babysitter and in a shop.

Examination: Height 112 cm (-8 SD). Weight 24 kg (-5 SD). Head circumference 50.6 cm (-3.5 SD). Hands 12 cm (-6 SD), feet 13 cm (-8 SD). Face is long and narrow. She has proptosis with slightly upslanting palpebral fissures and full eyebrows. Zygomatic hypoplasia. Prominent alveolar processes of the maxilla and mandibule. Facial features are somewhat coarse. The speech is hypernasal. The ears are low-set and small. Dolicocephaly. Limited elbow extension (150 degrees). Limited hip abduction. Joint hypermobility wrists and fingers. On the right, she has only one phalanx on the thumb; she has clinodactyly with ulnar deviation of the 2nd and 3rd fingers at the level of the PIP, she has a short 5th finger with clinodactyly (radial deviation). On the left hand, she has clinodactyly with ulnar deviation of the 3rd finger at the level of the PIP and she has a short 5th finger with clinodactyly (radial deviation). She has pes planus with prominent heels. She has large 2nd toes, and syndactyly of the 2nd and 3rd toes on the right, and 4th and 5th toes on the left.

Radiology: Decreased tubulation of long bones with decreased medullary space. Hypoplastic femoral heads.Bilateral radioulnar subluxation. Dextroconvex lumbar scoliosis. Increased height of the vertebrae, with decreased anterior-posterior diameter. Narrow metacarpal bones. Bone mineral density at the lower limit of normal, no fractures.

There were no hematological anomalies.

Individual 1B

Antenatally diagnosed hydronephrosis. Term delivery His birth weight was 4 pounds and his length was 14 ¾ inches. He is known for bilateral severe vesicoureteral reflux causing severe hydronephrosis, impaired renal function and hypertension. Bilateral reimplantation of ureteras at age 3. He also has velopharyngeal insufficiency, a submucous cleft palate, mild conductive hearing

loss, asthma, 2 febrile seizures, congenital right hip dislocation which was repaired at 3 years of age. History of transient neutropenia, but otherwise no hematological anomalies. Bilaterally dislocatable patellae, operated at age 20. Some neck pain and ankle rigidity. Normal development and intelligence.

Physical examination. Height 128 cm (-7 SD), Weight 26 kg (-5 SD) Head circumference 53 cm (10th centile), Hands 15 cm (-3 SD), feet 17.5 cm (-5 SD). There is bitemporal narrowing. Proptosis with slightly upslanting palpebral fissures and full eyebrows. The chin is small but there are prominent alveolar processes of the maxilla and mandibule. The facial features are somewhat coarse. Zygomatic hypoplasia. The speech is hypernasal and the palate is high. The ears are low-set with prominauris. The chest is wide with mild pectus excavatum. The muscle mass is decreased. The elbows can't be extended beyond 150 degrees but there is wrist hypermobility. Beighton score of 5/9, with points for hypermobility at the level of the hands and back. There is extendable camptodactyly of the 2nd-5th fingers. Tapering fingers. Pes planus with prominent heels. There were no abnormalities on the neurological exam except for bilateraly brisk deep tendon reflexes. There is normal neck range of motion. Feet: left 4-5 cutaneous syndactyly.

Radiology: slight scoliosis with vertebral bodies of increased height, decreased anterio-posterior diameter, and irregular vertebral plates. The pelvis is dysplastic with right coxa vara and left coxa valga. The diaphyses are thin and the medullary space is narrow for multiple bones. There is hypoplasia of the olecranon process, the distal radial and ulnar epiphyses, the proximal radius and the carpal bones. There was bilateral dislocation of radio-ulnar joints and patellae. Narrow metacarpal bones with narrow medullary space.

Individual 2

The Individual was a male neonate who was admitted to the neonatal ICU after birth due to respiratory distress. He was noted to have short limbs on prenatal ultrasound at 24 weeks. An amniocentesis was performed and testing for mutations in *FGFR3*, *COL1A1* and *COL1A2* did not identify rare variants.

At birth he was noted to be short, with a birth length of 40cm (-5.17SD). Additionally, he had several dysmorphic features: facial dysmorphic features included a prominent forehead, flat nasal bridge, micrognathia, large ears. He also had bilateral complete cutaneous syndactyly of fingers 3-5 and toes 4-5, 5th finger clinodactyly and a narrow thorax. Further testing included a chromosomal microarray and FLNB gene sequencing, which were both normal. No hematological abnormalities were noted. Due to pulmonary hypertension and right heart failure, he passed away at age 5 months. On autopsy, giant chondrocytes were seen in the epiphyseal plates of the long bones. No other systemic or organ malformations were noted.

Individual 3

The boy was born to non-consanguineous Japanese parents at 39 weeks and 6 days of gestation. His birth length was 41.7 cm (-4.3 SD) and weight 2,000 g (-3.3 SD). He presented with tachypnea leading to a conventional respiratory support (nasal directional positive airway pressure). He showed oligosyndactyly of the right hand, proximally set left thumb, post-axial syndactyly of the feet, dislocation of the right elbow, and ulnar deviation of the left wrist. He was bottle-fed. His weight was 3,340 g at 3.5 months of age (< -3 SD). Respiratory support was weaned to nasal continuous positive airway pressure, and continued until hospital discharge at 4 months. Soon after then, he developed upper airway infection and subsequent respiratory failure, and came to an emergency room. Ultrasonography revealed pulmonary hypertension with severe tricuspid regurgitation. Despite ventilator support, he suddenly died the next day after hospital admission.

His X-rays showed platyspondyly and metaphyseal dysplasias of the long tubular bones. The left hand showed type A1 brachydactyly, while the right hand showed oligosyndactyly. Both feet showed post-axial syndactyly. The test of blood cells showed macrocytic anemia and congenital neutropenia.

Individual 4

The male child was noted to have severe intrauterine growth retardation. He was born prematurely and had postnatal growth restriction with short stature. His past medical history included mild conductive hearing loss of left ear, delayed dentition with abnormal enamel and eruption, kidney hydronephrosis with grade 5 vesicoureteral reflux, small atrial septal defect, 2 small ventricular septal defects and a prominent aortic root. He had generalized hypotonia, delayed motor milestones, delayed speech. His failure to thrive was severe and he passed away at 26 months.

Examination showed dysmorphic facial features (trigonocephaly, frontal bossing, small jaw, sparse hair, small and low-set ear, posteriorly rotated, prominent pre-maxilla and a recessed chin retrognathia). He had puffy hands and feet, nonpitting edema, and undescended testes. Limb anomalies included dysplastic digits of both feet (rudimentary 2nd and 3rd toes and absent 4th and 5th toes on the left, right foot with 4th and 5th toe syndactyly)

Skeletal abnormalities including pectus carinatum deformity, skeletal dysplasia (osteopenia, wormian bones, severely delayed bone age).

There were no hematological anomalies.

Individual 5

She is female born at term with a weight of 2.760 kg. There is no known parental consanguinity. Her past medical history includes bilateral inguinal hernias, a ventricular septal defect (perimembranous), and pulmonary vein stenosis. She has global developmental delay and autism. She was reported as F5 in a previous paper[1], since she is homozygous for a *KYNU* variant (NM_003937.3:c.616G>A, p.Glu206Lys) which affects a non-conserved amino acid but may perhaps still impact her clinical picture.

Examination at 8y and 9 mo: Weight 22 kg (8th centile) height 130.8 cm (46th centile), head 48.9 cm (just <2SD) . Brachycephaly with a flat occiput. Bitemporal narrowing, small nose ears cupped and prominent. The right hand showed a transverse terminal limb reduction with a rudimentary 5th digit. Her left hand was less affected with hypoplastic and deep-set nails. Her right foot only had digits 1 and 5 which however displayed anonychia. The left foot showed an oligodactyly with digits 2 to 4 being absent. Digits 1 and 2 had syndactyly and anonychia.

There were no skeletal anomalies (normal spine, metaphyses and epiphyses) apart from the extremities.

She had a normal complete blood count at 11 months.

Individual 6

He is a male born at 39 weeks (weight 2810 g (-1,6 SD), length 49 cm (-1,3 SD), head circumference 35 cm (-0,2 SD)). His parents are first cousins and he has a healthy younger brother. He has strabismus and mild intellectual disability, but is otherwise functioning well.

Examination at 13y 3m showed a height of 155 cm (25th centile) and height of 62 kg (85th centile) and a head circumference of 56 cm (75th centile). He has high arched eyebrows, a low-set forehead hairline and low-set ears. His hands showed a clinodactyly 5th finger bilaterally, brachydactyly, slightly dysplastic nails. Feet examination showed short wide feet, short, low set 4th left toe, very slight partial syndactyly 2-3.

Radiographies showed a normal skeleton apart from extremities. Hands: Brachymesophalangy 5, pseudoepiphyses, hypoplastic distal phalanges. Feet Short and thin left metatarsal 4, short left metatarsal 5

Individual 7

The proband is the first child of consanguineous parents (first cousins) born at term by emergency Caesarean section due to fetal distress. Pregnancy itself was unremarkable. Birth weight was 2.6kg (0.4th centile). There were no concerns regarding the probands early development. The parents became concerned when she appeared not to be coping well at school. She is reported to have poor memory with limited concentration. She is sociable but mum has concerns that she can sometimes be inappropriately social towards strangers. She attends mainstream school with a statement of educational needs. At the age of 5 years 11 months (71 months), she underwent a Griffiths Mental Development assessment. Her developmental age at this time was 54.5 months for eye-hand coordination, 35 months for performance and 48 months for practical reasoning.

The proband had a urine infection when 5 months of age and she was subsequently found to have vesicoureteric reflux and hydronephrosis with scarring of her left kidney. She was commenced on prophylactic antibiotics.

On examination at 7 y, Weight 27.6 kg (50th centile) Height 130 cm (90th centile). She had prominent epicanthic folds and columella with upturned earlobes. She had shortening of the 5th metacarpals bilaterally and absence of the 5th toe on the right foot with overriding of the 3rd and 4th toes. The 4th toe on the left appeared absent and there was duplication of the 5th toe with syndactyly.

There were no radiological anomalies apart from those at the extremities. Abnormal appearance of the epiphyses of both distal radius and probably also distal ulna with overlapping proximal carpal bones. At age 8 years, bone age of 11 years on radiographs of hands and feet. Hands: Short right 1st metacarpal. Bilaterally short 5th metacarpals and mild shortening of 4th. Proximal phalanx right thumb is short. The right 5th finger distal phalanx is small with an abnormal epiphysis which is thickened.

Feet: Right: Missing the 5th ray. The fourth digit proximal phalanx and epiphysis are abnormal in shape. Overriding of the 3rd, 4th and 5th toes. Proximal 3rd metatarsal is fused to the 4th metatarsal. 4th metatarsal is widened. Left: There are five rays in the left foot. Proximal fusion of 4th and 5th metatarsals. Bilaterally: The 1st metatarsals are broadened and the shaft of the metatarsals and multiple epiphyses and phalanges have abnormal shapes in both feet.

Urine organic acids, urine amino acids, serum urea and electrolytes, full blood count, ferritin and thyroid function were all within normal limits.

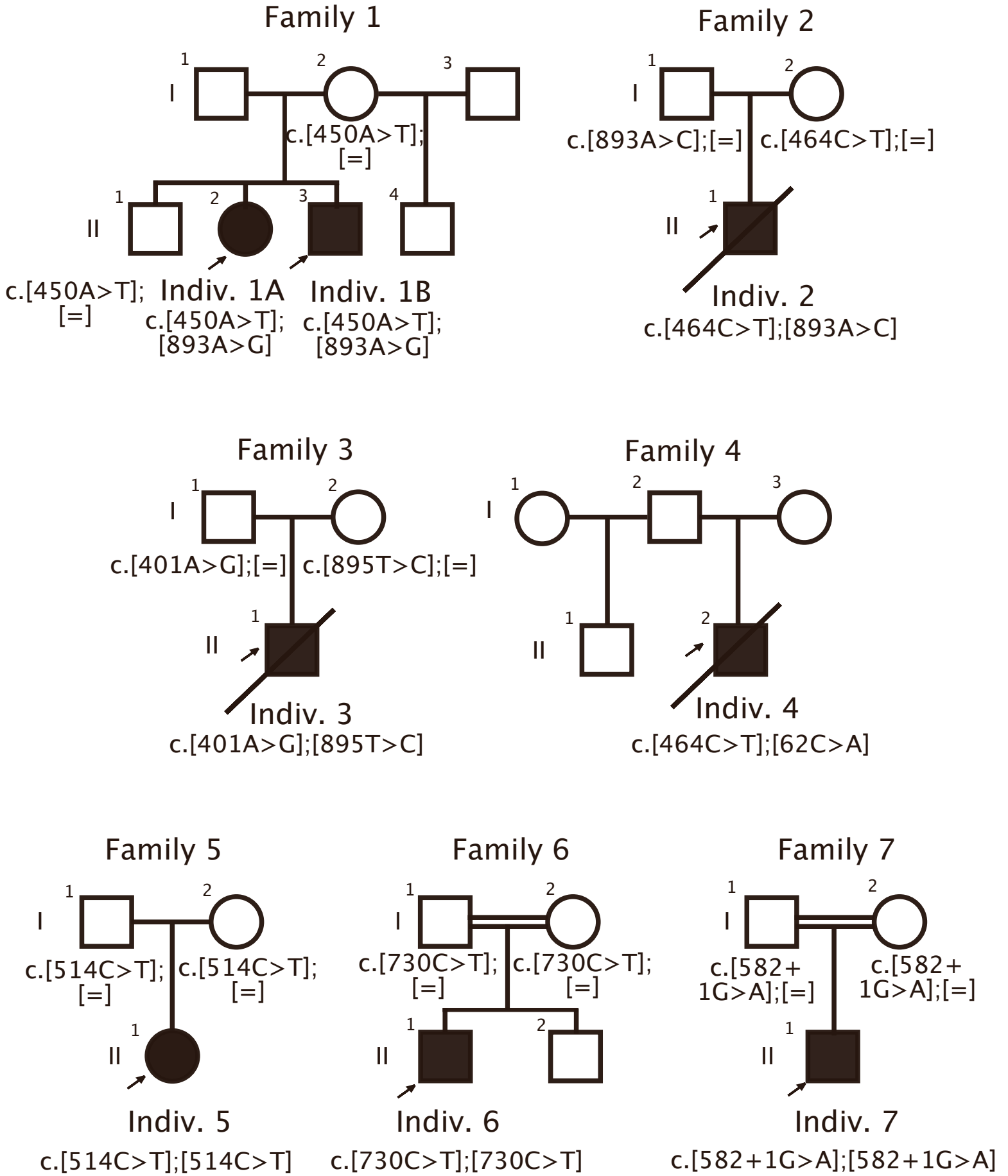

Figure S1. Pedigrees of the families described. Variants are named according to NM_153332.4.

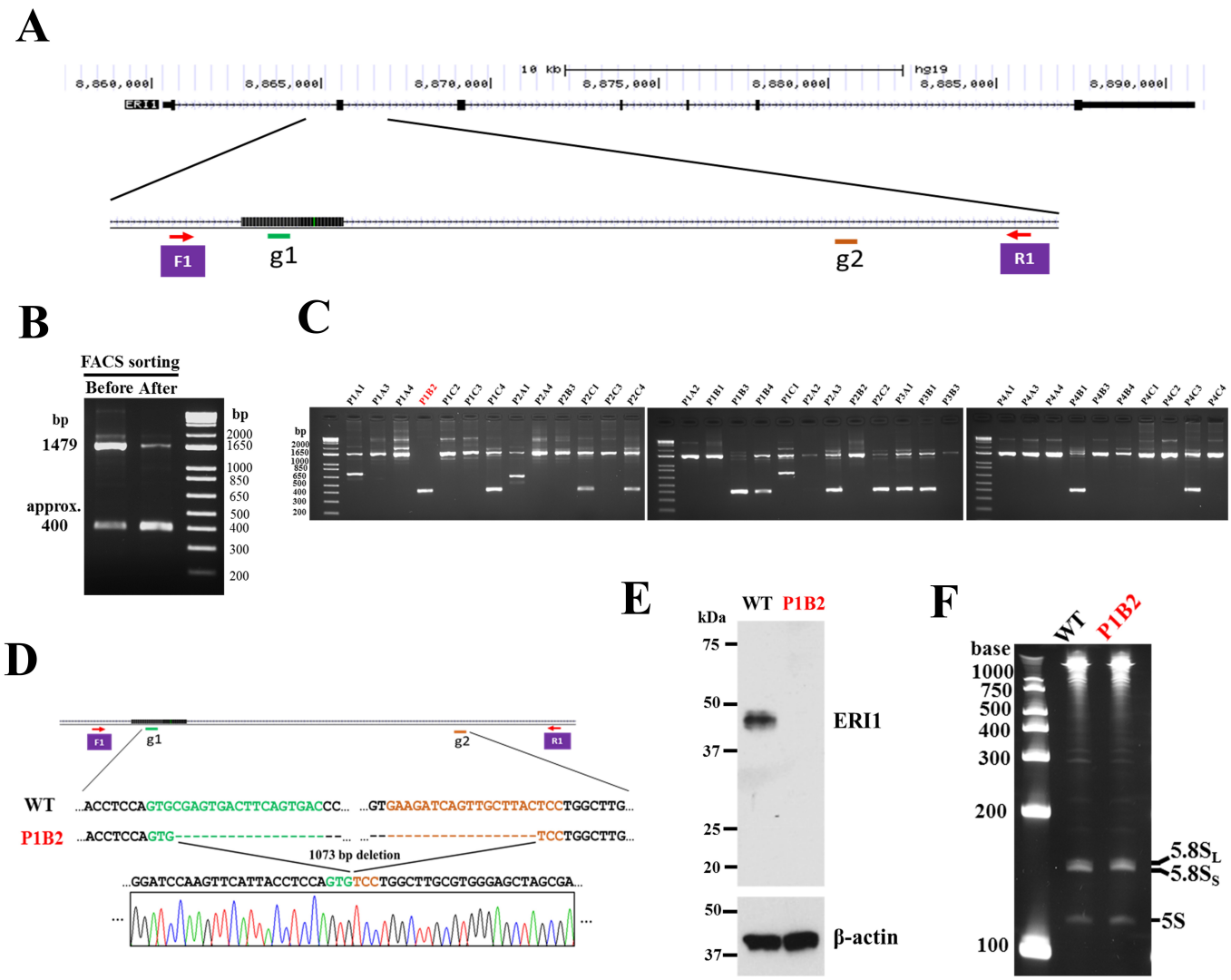

Figure S2. Generation of the *ERI1* knock-out Hela cell line by CRISPR/Cas9-mediated gene editing. A, Two guide RNAs (g1, 2) were designed to target genomic DNAs within exon 2 and intron 2 of *ERI1*, respectively. Mixture of g1 and g2 were introduced into Hela cells with Cas9. **B,** The proportion of the cells with a genomic deletion generated by g1 and g2 was increased after FACS sorting. **C**, Monoclonal screening for *ERI1* knock-out lines. The clone P1B2 without the PCR band corresponding to the wild type (marked in red) was selected. **D,** Sanger sequencing confirming a 1,073-bp genomic deletion in the P1B2 line. **E**, Western blot validation of the knock-out effect. **F,** Ethidium bromide-stained RNA. $5.8S_L$, long form of 5.8S rRNA; $5.8S_S$, short form of 5.8S rRNA.

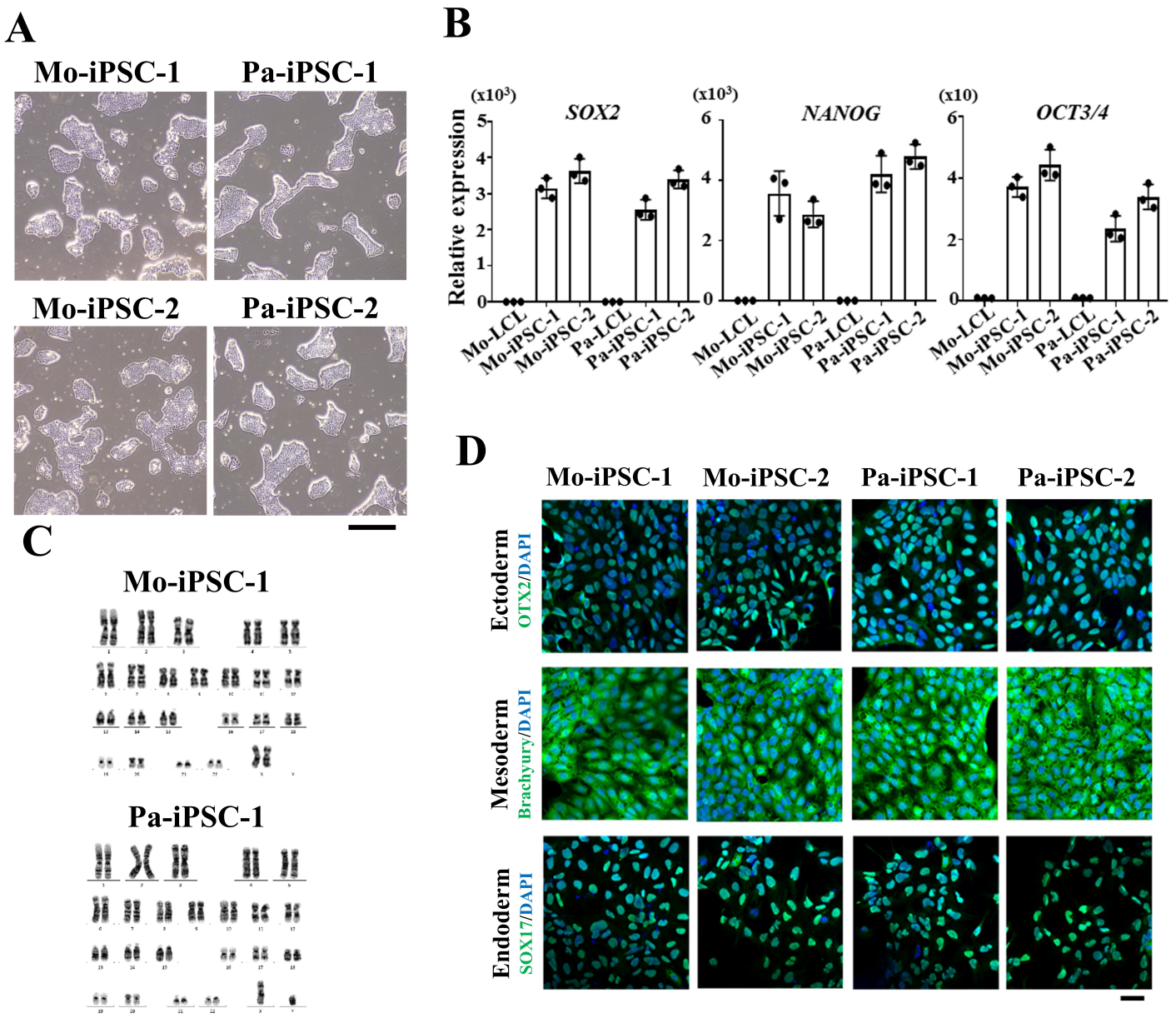

Figure S3. Characterization of induced pluripotent stem cell lines (iPSCs). A, Representative bright-field views of iPSCs of the healthy mother (Mo-iPSC-1 and 2) and affected individual 3 (Pa-iPSC-1 and 2) generated from the lymphoblastoid cell lines (Mo- and Pa-LCLs). Scale bars, 500 µm. **B,** RT-qPCR analysis showing the stem cell markers (*SOX2*, *NANOG* and *OCT3/4*) expressed in the iPSCs. n = 3 biologically independent samples. The data indicate mean ± SD. **C,** Normal karyotypes in Mo- and Pa-iPSCs by chromosomal G-banding analysis. **D,** Evaluation of differentiation capacity into three germ layers of the iPSC lines by immunostaining for SOX17 (endoderm), Brachyury (mesoderm) and OTX2 (ectoderm). Scale bars; 50 µm.

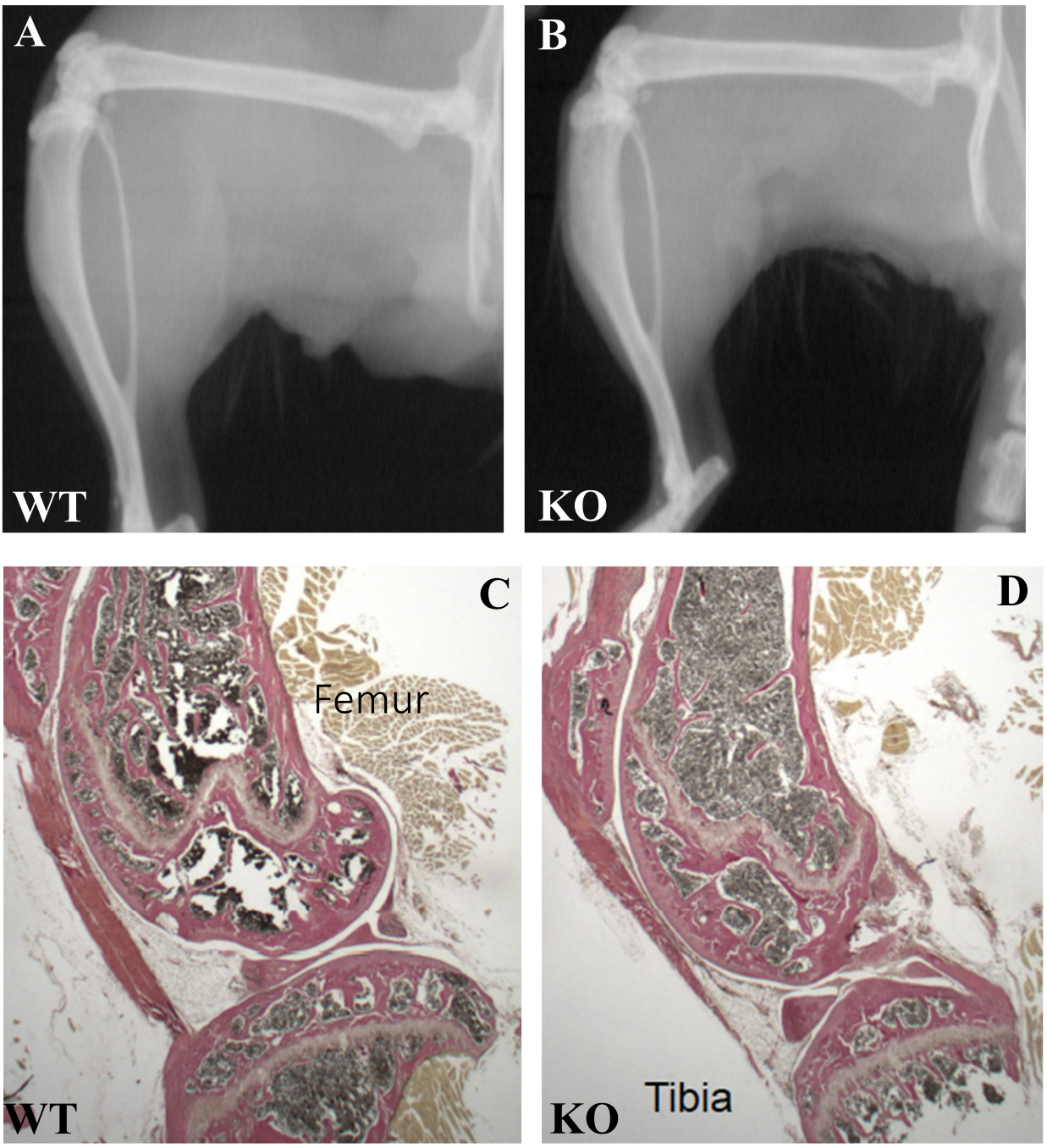

Figure S4: Evaluation for the skeletal phenotype of hind limbs. A-B, X-ray images of the wild type (WT) and *Eri1* knockout (KO) mice at 14 weeks. Compared with WT mice, KO mice do not show significant morphological changes in the epimetaphyseal portions of the femurs and tibias. **C-D**, Van Gieson's staining for the epimetaphyseal portions of femur and tibia. KO mice do not show significant changes in the morphology and ossification of the epimetaphyses.

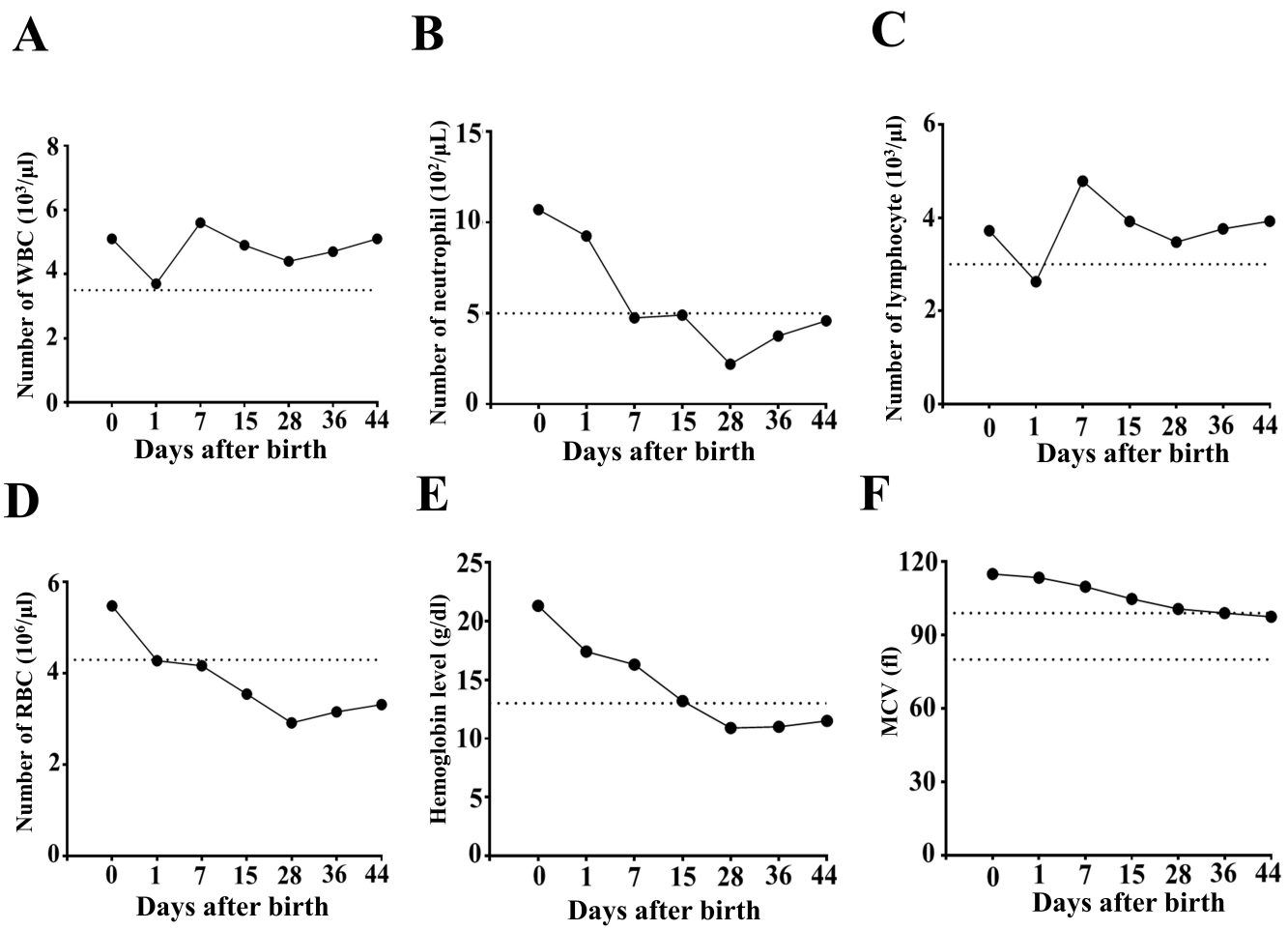

Figure S5. Blood tests of Individual 3. A, Number of white blood cells (WBCs). Normal range: 3.5-8.5 [$\times 10^3$/μl]. The dotted line shows the lower limit of the normal value. **B,** Number of neutrophils. Normal range: [27-144] $\times 10^2$/μl from birth to 2 weeks; 10-50 [$\times 10^2$/μl] since 2 weeks. The dotted line shows the diagnosis standard for severe congenital neutropenia. **C,** Number of lymphocytes. Normal range: 3.0-9.5 [$\times 10^3$/μl]. The dotted line shows the lower limit of the normal value. **D,** Number of red blood cells (RBCs). Normal range: 4.3-5.6 [$\times 10^6$/μl]. The dotted line shows the lower limit of the normal value. **E,** Hemoglobin level. Normal range: 13-17 [g/dl]. The dotted line shows the lower limit of normal value. **F,** Mean corpuscular volume (MCV). Normal range: 80-99 [fl]. The dotted lines show the lower and upper limits of the normal value.

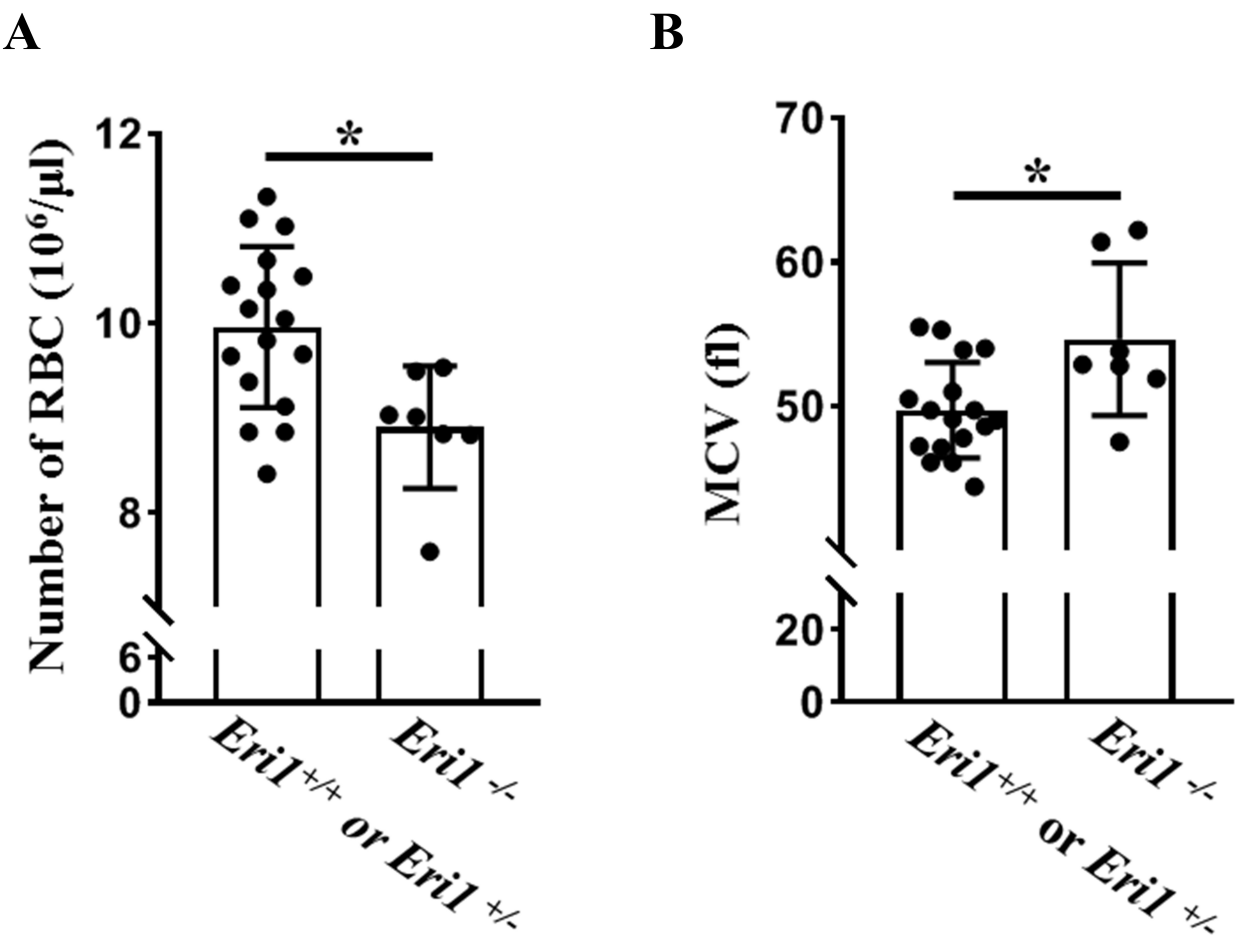

Figure S6. Blood tests of mice at 8 weeks. A, Number of red blood cells (RBCs). *Eri1*$^{+/+}$ or *Eri1*$^{+/-}$ mice, n=17; *Eri1*$^{-/-}$ mice, n=7. *$P < 0.05$. **B,** Mean corpuscular volume (MCV). *Eri1*$^{+/+}$ or *Eri1*$^{+/-}$ mice, n=17; *Eri1*$^{-/-}$ mice, n=7. *$P < 0.05$. The above quantitative data indicate mean ± SD and statistical significance was assessed using two-sided t-test.

Supplementary table 1. Primers used in this study

Primer name	Sequence (5'-3')
Expression plasmid construction	
Sal1 ERI1 CDS f	TCGGTCGACCATGGAGGATCCACAGAGTAAAGAGC
Not1 ERI1 CDS r	TACGCGGCCGCGTTACTTTCTAAAATGTGGCATTTGTGGTG
ERI1 D134G f	CCACTTGTGAAGAAGGAAACCCA
ERI1 D134G r	CTTCAAAGcCAATAATACAAATGTAGTCATAATAACTGT
ERI1 E150 f	TACATGAcATAATTGAATTTCCGGTTGTTTTACTG
ERI1 E155 f	TACATGAAATAATTGAATTTCtGGTTGTTTTACTG
ERI1 E150 P155 r	CAAACTCAGGTGGGTTTCCTTCT
ERI1 S298 f	GATGgCTCTAAGAATATCGCCCGAATAG
ERI1 S298 r	AAGACCACAGTGAGGCCGC
ERI1 S299P f	GACcCTAAGAATATCGCCCGAATAGCAG
ERI1 S299P r	ATCAAGACCACAGTGAGGCCG
Sanger sequencing	
ERI1 EX1 f	TCAACGGAGAAAGGCGAG
ERI1 EX1 r	TGAACACAGAATTAAGTTTAAAGCAA
ERI1 EX3 f	ATGGTTTGAAGTACCAGTATATGTTGGAG
ERI1 EX3 r	GATGTCAGAGGCCCTATCAGAGAAC
ERI1 EX4 f	GCTGTGATGAATTCGTCGTG
ERI1 EX4 r	CCAAGCAAGGTATTAAATTGTTCAC
ERI1 EX6 f	TTTGTAGGAAGCAAGGTTAATTTTC
ERI1 EX6 r	TTTGAACATAAGGAATTTGGAATC
ERI1 EX7 f	CTGGTCTCATACTCCTGACCTCAAG
ERI1 EX7 r	GCTGGTGACAAAGAATTCAGTATGCC
Genome editing for Hela cells	
ERI1 e2 g1 f	CACCgGTCACTGAAGTCACTCGCAC
ERI1 e2 g1 r	AAACGTGCGAGTGACTTCAGTGACc
ERI1 i2 g2 f	CACCgGAAGATCAGTTGCTTACTCC
ERI1 i2 g2 r	AAACGGAGTAAGCAACTGATCTTCc
PCR for ERI1 g1g2del f1	CATGTAGCATGAAGAGGCTTCAGAAG
PCR for ERI1 g1g2del r1	TTCTCATGCTGTACCTGGCCAG
RT-qPCR	
COL2A1_RT_F	GGTGGCTTCCATTTCAGCTA

COL2A1_RT_R	TACCGGTATGTTTCGTGCAG
OCT3/4_RT_F1	GACAGGGGGAGGGGAGGAGCTAGG
OCT3/4_RT_R2	CTTCCCTCCAACCAGTTGCCCCAAAC
SOX2_RT_F	GGGAAATGGGAGGGGTGCAAAAGAGG
SOX2_RT_R	TTGCGTGAGTGTGGATGGGATTGGTG
NANOG_RT_F	CAGCCCCGATTCTTCCACCAGTCCC
NANOG_RT_R	CGGAAGATTCCCAGTCGGGTTCACC
ACTB_RT_F	AGAAAATCTGGCACCACACC
ACTB_RT_R	AGAGGCGTACAGGGATAGCA
HPRT_ F	GCCTATAGACTATCAGTTCCCTTTGG
HPRT_ R	TGCTGTGGTTTAAGAGAATTTTTTCA
HIST1H2AB_F	ACTCGGTCTTCTCGTGCAG
HIST1H2AB_R	GCTCCTCGTCATTGCGGAT
HIST2H2BE_F	CCGCAAAGAGAGCTACTCCA
HIST2H2BE_R	GTGGAGCGCTTGTTGTAGTG
HIST2H3C_F	CTACCAGAAGTCCACGGAGC
HIST2H3C_R	TGGATGGCGCACAGGTTC
HIST2H4A_F	GGCGGAAAAGGCTTAGGCAA
HIST2H4A_R	CCAGAGATCCGCTTAACGCC
HIST1H1A_F	TGGGCATTAAGAGCCTGGTAA
HIST1H1A_R	ACCCGTTGCCTTAGTTTTTGTA
TBP_F	TCTGGAATTGTACCGCAGCTT
TBP_R	GCTCCTGTGCACACCATTTT

Acknowledgements

We thank the affected individuals and their families for their participation. We would like to thank Lucie Dupuis and Vanda McNiven for help with patient recruitment and patient care. This study was supported by research grants from Japan Agency For Medical Research and Development (22ek0109486h0003 for S.I., Z.W., L.G.; JP21ek0109486, JP21ek0109549, and JP21ek0109493 for N.Ma.), JSPS KAKENHI (JP19H03621 for N.Mi.), Japanese Society for Bone and Mineral Research (Rising Stars Grant; for L.G.), the Suzuken Memorial Foundation (for L.G.), Xi'an Jiaotong University (Young Talent Support Plan; for L.G.), and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC; EXC 2067/1-390729940) and the DZHK (German Centre for Cardiovascular Research; partner site Göttingen) grant 81Z0300112 (for B. W.). One of the authors of this publication is a member of the European Reference Network for Rare Malformation Syndromes, Intellectual and Other Neurodevelopmental Disorders ERN-ITHACA [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516]. We thank Mrs. Tomoko Kusadokoro for the technical support. The Toronto authors thank the SickKids Centre for Genetic Medicine. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]. This study makes use of DECIPHER (http://www.deciphergenomics.org), which is funded by Wellcome [grant number WT223718/Z/21/Z]. See Nature PMID: 25533962 or www.ddduk.org/access.html for full acknowledgement. AJ and SB acknowledge the support of Solve-RD. The Solve-RD project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 779257.

References

1. Szot, J.O., Slavotinek, A., Chong, K., Brandau, O., Nezarati, M., Cueto-González, A.M., Patel, M.S., Devine, W.P., Rego, S., Acyinena, A.P., et al. (2021). New cases that expand the genotypic and phenotypic spectrum of Congenital NAD Deficiency Disorder. Hum Mutat *42*, 862-876. 10.1002/humu.24211.