Supplementary Material

Title: Deficiency of the Fanconi anemia core complex protein FAAP100 results in severe Fanconi anemia

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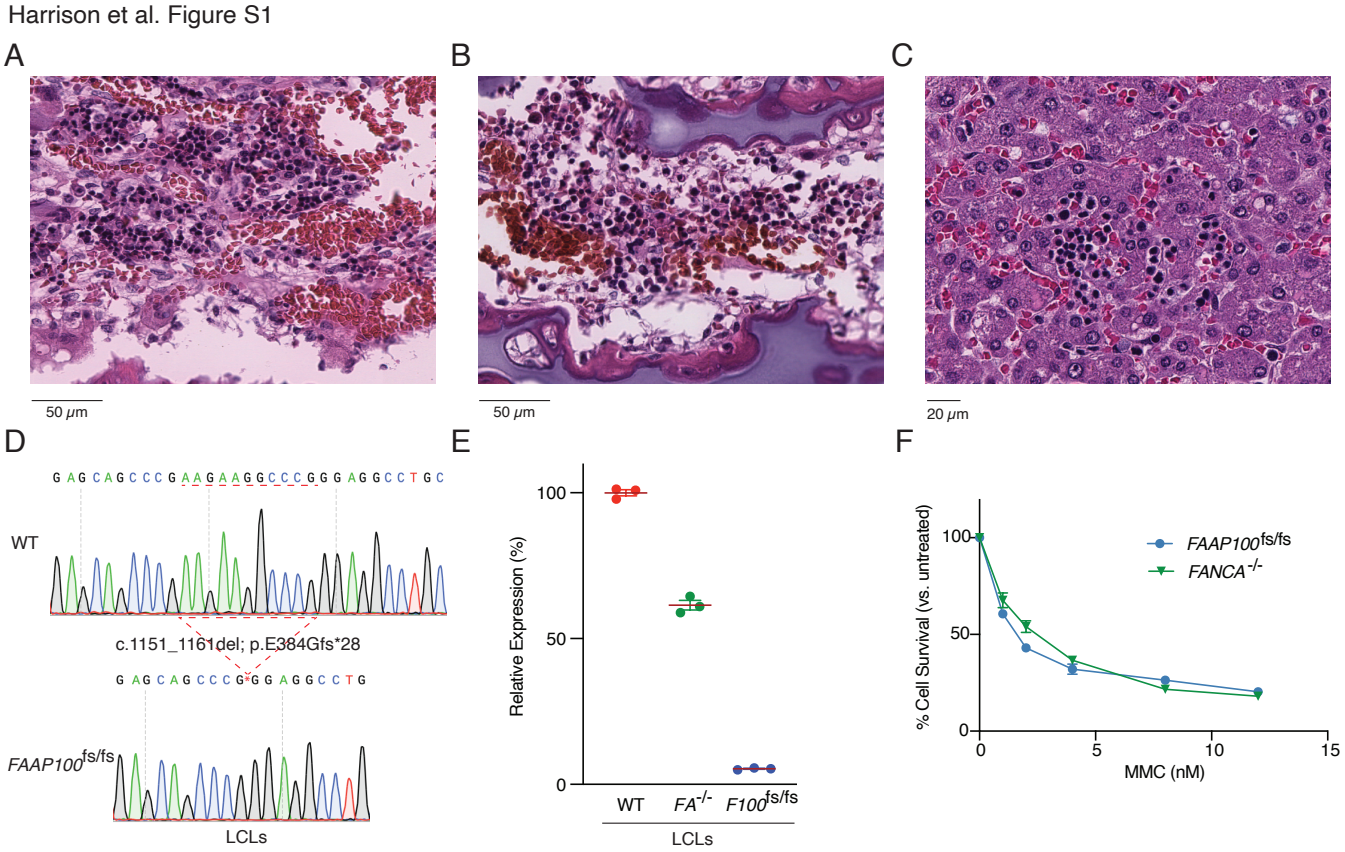

Figure S1. **(A and B)** Image of rib bone marrow exhibiting erythroid hyperplasia in subject B. (**C**) Extramedullary hematopoiesis in the liver of Subject B. **(D**) Genotyping of exon 3 of *FAAP100* in subject B's LCLs (RA3641) showing biallelic deletion of 11 nucleotides. **(E)** RT-qPCR of the *FAAP100* mRNA transcript in wild-type, *FANCA*$^{-/-}$, and subject B's LCLs showing lack of transcript expression in subject B's cell lines. **(F)** Comparison of *FANCA*$^{-/-}$ and *FAAP100*$^{fs/fs}$ fibroblast sensitivities at lower doses of MMC exhibiting similar hypersensitivity profile. Experiments were conducted at least three times in biological replicates with consistent results for **(E)**. Data are mean, error bars are SEM.

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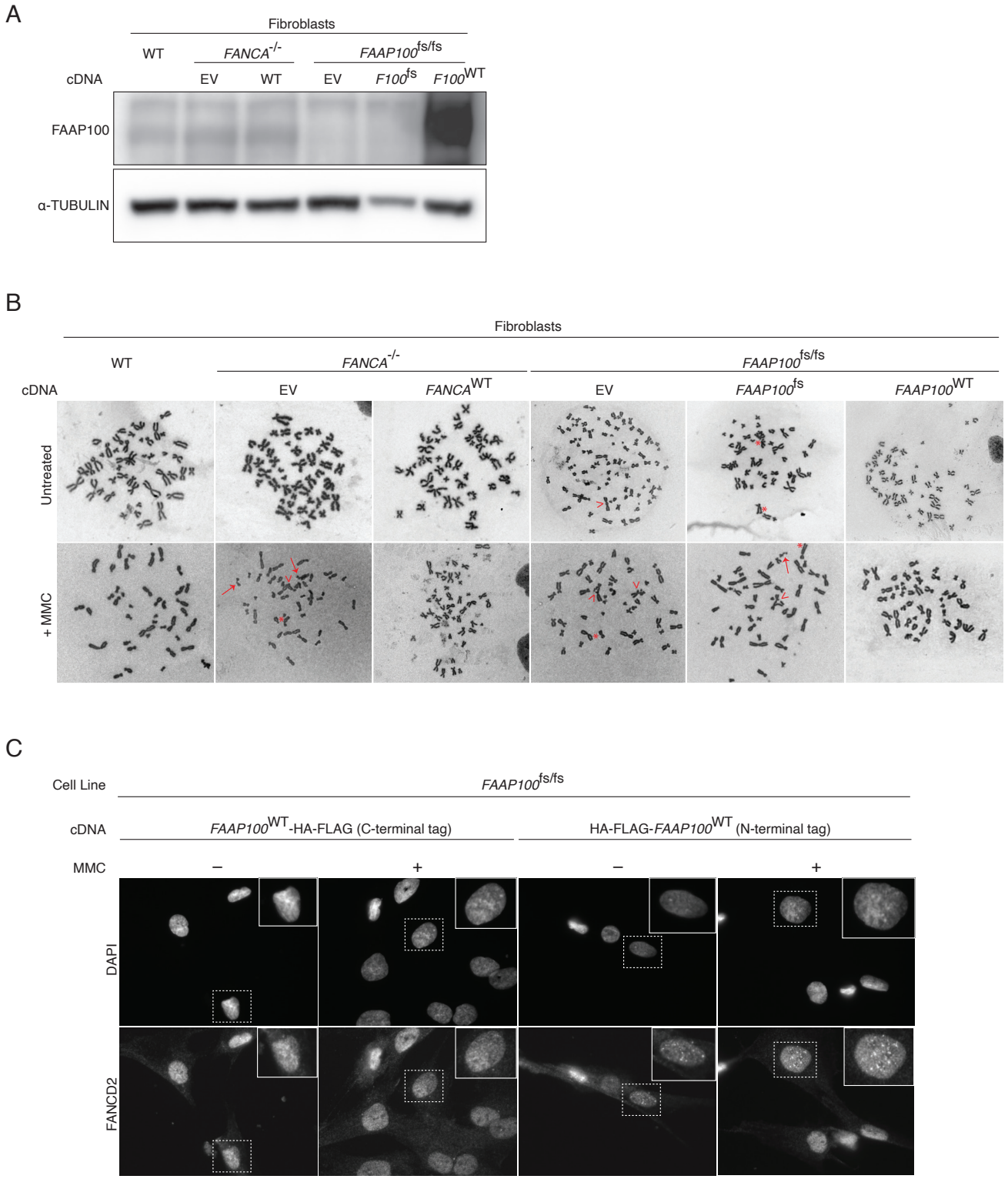

Figure S2. **(A)** Confirmation of protein expression in complemented cells using an anti-FAAP100 antibody. The tubulin blot in this figure is the same one shown in the main Figure 3A. **(B)** Representative images of metaphase spreads in complemented fibroblasts. Arrows show breaks, asterisks show gaps, and arrowheads show chromosome radials. **(C)** Representative images of FANCD2 immunofluorescence comparing cells complemented with C-terminally HA-FLAG tagged (left) vs. N-terminally HA-FLAG tagged (right) wild type *FAAP100* cDNA. Cells complemented with C-terminally tagged cDNA did not, while those complemented with N-terminally tagged cDNA did exhibit FANCD2 foci formation.

Table S1. Comparison of Clinical Findings in Subjects A-D

Feature	Subject A	Subject B	Subject C	Subject D
Sex	Male	Male	Female	Female
CNS Anomalies	+	+	Unknown	+
Ventriculomegaly	+, moderate-severe dilation of lateral ventricles with notable thinning of the parenchyma, particularly within the occipital and temporal lobes.	+	Unknown	+, dilation of lateral cerebral ventricles (right- 13 mm; left- 14 mm) at 21 weeks of gestation on ultrasonography
Hydrocephalus	+, severe	+	Unknown	Absent
Agenesis/ Absence of the Septum Pellucidum	+, absent	+, partial agenesis	Unknown	Absent
Absent pituitary stalk	+	Absent	Unknown	Absent
Septo-optic dysplasia (SOD)	Absent	+	Unknown	Absent
Polymicrogyria	Absent	+	Unknown	Absent
Olfactory nerve agenesis	Absent	+	Unknown	Absent
Cerebellar hypoplasia	Absent	+	Unknown	Absent
Fused thalami	+	Absent	Unknown	Absent
Microphthalmia	+, bilateral	Absent	Unknown	Absent
Diffusely enlarged spinal cord	+	Absent	Unknown	Absent
Thin corpus callosum	+	Absent	Unknown	Absent
HEENT Anomalies	+	+	+	+
Hypertelorism	Absent	+	Unknown	Absent
Micrognathia	Absent	+	Unknown	+
Microtia	+, bilateral. Rudimentary external ear formation with no ear canal noted	Absent	+, bilateral	Absent
Microcornea	Unknown	Absent	+, bilateral	Absent
Cleft Palate	+	Absent	Unknown	Absent
Short Palpebral Fissures	+	+	Unknown	Absent

Short neck	+	+	Unknown	Absent
Choanal stenosis/atresia	+, stenosis of right naris, atresia of left naris	Absent	Unknown	Absent
GI Anomalies	+	+	Unknown	+
Esophageal atresia	+	Absent	Unknown	Absent
Tracheoesophageal fistula	+	Absent	Unknown	Absent
Imperforate anus (anal atresia)	+	+	Unknown	Absent
Dysplastic pancreatic tail	Absent	Absent	Unknown	+
Prominent islet cell hyperplasia,	Unknown	+, highly suggestive of a diffuse form of congenital hyperinsulinemia	Unknown	Absent
Malrotation	Absent	+, appendix in midline	Unknown	Absent
Inguinal hernia	+, small, right	Absent	Unknown	Absent
Cardiovascular Anomalies	+	+	+	Absent
Dextrocardia	+	Absent	Absent	Absent
Tetralogy of Fallot	Absent	Absent	+	Absent
Patent ductus arteriosus (PDA)	+, 2.5 mm, bidirectional shunting	+	Absent	Absent
Complete atrioventricular canal defect	Absent	+, Rastelli Type B	Absent	Absent
Valve abnormalities	+, Diminutive aortic valve, and a small mitral valve, both demonstrating antegrade flow	Absent	Absent	Absent
Pulmonary anomalies	+	+	Unknown	+
Hypoplastic branch pulmonary arteries	+, mild	Absent	Unknown	Absent
Pulmonary hypoplasia	Absent	+, severe	Unknown	Absent
Incomplete lobation	Absent	+, bilateral	Unknown	+, the right lung lacked transverse fissure resulting

				in 2 lobes and measured 2.5x2.1x0.7 cm (- 2.35 SD).
Genitourinary Anomalies	+	+	+	+
Renal abnormalities/agenesis	+, solitary right kidney (3.6 cm in length) with mildly dilated right extrarenal pelvis and absent left kidney	+, rudimentary nonfunctioning left kidney, with features of hypoplasia and dysplasia, and absent right kidney	+, single ectopic kidney	+, bilateral ectopic kidneys situated in the pelvic cavity with right kidney measuring 1.2x0.9x0.5 cm (-7.5 SD), and left kidney measuring 1.1x1.8x0.4 cm (-8.3 SD)
Absent ureteric orifice	Absent	+, unilateral (absent right)	Unknown	Unknown
Hypoplastic ureter	Unknown	+, unilateral (left), extends posterior to rectum	Unknown	Absent
Hypoplastic urinary bladder	Unknown	+, contracted	Unknown	Absent
Ambiguous genitalia	+, small clitorophallic structure	Absent	Unknown	Absent
Cryptorchidism	+, bilateral	+	Unknown	Unknown
Skeletal Anomalies	+	+	+	Absent
Malpositioned scapulae	+, bilateral		Unknown	Absent
Humeral hypoplasia	+, bilateral, with the proximal segments predominantly absent, and only the distal humeral condyles visible	Absent	Unknown	Absent
Rib deformities	+, hypoplastic left first rib and a suggestion of partial fusion between the second and third left ribs	+ Right: Absent 1st and 2nd ribs. Short, free floating 3rd and 4th ribs with no sternal attachment. 5th and 6th ribs are	Unknown	Absent

		connected to a single cartilaginous bridge that extends laterally from the sternum. Free floating 7th-12th ribs. Left: 1st and 2nd ribs are fused. Free floating 4th, 5th, 7th, and 10th-12th ribs. 6th rib is branching from 7th rib. 8th and 9th ribs are fused and bridging from the sternum.		
Phocomelia	Absent	+, upper extremities	Unknown	Absent
Sacral hypoplasia	+, with evidence of partial fusion among the second to fourth sacral bodies	Absent	Unknown	Absent
Dysmorphic cervical spine	+, upper, with a reversal of the normal cervical lordosis	Absent	Unknown	Absent
Radial ray defects	+, bilateral absent radii and thumbs	+, bilateral absent radii and thumbs	+, Bilateral reduced radius size, absent thumbs, right radial club hand	Absent
Rudimentary/absent forearms	+, bilateral, rudimentary	+, absent	Unknown	Absent
Dislocated shoulder	+	Absent	Unknown	Absent
Hand anomalies	+, absent thumb and 2nd digit on the right hand, thumb-like digit fused to adjacent digit on the left	+, bilateral absent thumbs and index fingers, Soft tissue syndactyly, between the	Bilateral absent thumbs	Absent

	hand with oligodactyly, 5th finger clinodactyly bilaterally	3rd and 4th digits, Severe Clinodactyly of the 5th digits		
Foot anomalies	+, bilateral 2,3 toe cutaneous syndactyly, 4th and 5th toe clinodactyly	+, talipes equinovarus, soft tissue syndactyly between the 2nd and. 3rd toes, which overlap the great toe, bilaterally	Unknown	Absent
Other	+	+	+	Unknown
Hypotonia	+	+	Unknown	Unknown
Thoracic Cavity Deformities	Unknown	+	Unknown	Absent
Failure to Thrive	N/A	N/A	+	N/A

Table S2. Complete Blood Count (CBC) Results for Subject A. Normal ranges are indicated

Test	Value by Day of Life (DOL)				
	DOL 2*	DOL 3**	DOL 4***	DOL 5	DOL 6**
White Blood Cell Count (9.0-34.0 x10^3/µl)	5.7	3.2	3.9	2.9	3.0
Red Blood Cell Count (3.40-5.50 x10^6/µl)	2.79	3.70	4.79	4.48	3.78
Mean Corpuscular Volume (MCV) (93.1-109.8 fl)	117.0	108.1	89.1	91.3	94.4
Red Cell Distribution Width (RDW) (15.2-19.1 %)	17.3	22.8	21.2	22.7	22.4
Hemoglobin (11.1-19.9 g/dl)	11.0	13.6	15.4	14.5	12.2
Hematocrit (36.2- 58.5 %)	33.0	40.0	42.7	40.9	35.7
Mean Corpuscular Hemoglobin (MCH) (30.6-37.8 pg)	39.0	36.8	32.2	32.4	32.3
Mean Corpuscular Hemoglobin Concentration (MCHC) (31.6-35.3 g/dl)	33.8	34.0	36.1	35.5	34.2
Mean Platelet Volume (MPV) (7.1-12.0 fl)	9.0	Not measured	10.5	10.1	11.8
Platelet Count (150-590 x 10^3/µl)	74	20	Platelets clumped	82	48
Absolute Neutrophils (1.90-8.00 x10^3/µl)	2.8		0.94	0.44	0.54
Neutrophil (17.1-58.4 %)	48.7	36.4	22.0	14.0	18.0
Lymphocyte (25.0-67.3 %)	42.5	50.2	48.0	61.0	52.0
Monocyte (6.8-13.9 %)	6.8	10.2	8.0	7.0	10.0
Eosinophil (0.0-7.0 %)	1.8	1.6	12.0	9.0	12.0
Basophil (0.0- 2.7 %)	0.2	0.6	2.0	3.0	0.0

* *Subject received a pRBC transfusion after this CBC was performed*
** *Subject received a platelet transfusion after this CBC was performed*
*** *Subject received a pRBC transfusion before this CBC was performed*

Table S3. Primers used in the study

Name	Sequence
FAAP100_PCR_F	CCAAATGCCCTTGTCAAGAT
FAAP100_PCR_R	AGGCTTGTCAGTGCCTTGTT
FAAP100_Seq_F	AATTTTCTGCCTGACGAGGA
FAAP100_Seq_R	TGCACTCTCTGTGGTCATCC
FAAP100_cDNA_F_2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGCCGGCGCCGCGCCGCGGGT
FAAP100_cDNA_R_4	GGGGACCACTTTGTACAAGAAAGCTGGGTCACAGCAGGATGAGGCTGGGGT
FAAP100_Mutagenesis_F	CTGAGCAGCCCGGGAGGCCTGCCC
FAAP100_Mutagenesis_R	GGGCAGGCCTCCCGGGCTGCTCAG
FAAP100_cDNA_R_5	GGGGACCACTTTGTACAAGAAAGCTGGGCTAggcggtggcacaggagctggcctca
FAAP100_qPCR_F_9	AGCCAGGATGACCACAGAGAGT
FAAP100_qPCR_R_9	TTGTCAGTGCCTTGTTCCGCTG

Supplemental Text

Family 1:

A consanguineous Yemini couple (first cousins) presented with a history of recurrent obstetric complications and multiple fetal anomalies. Out of eight total pregnancies, six resulted in spontaneous abortions, all within the first trimester. The two other pregnancies were carried to term. Both liveborn children, designated subjects A and B, presented with severe congenital anomalies and died shortly after birth. The family history (see Main Text **Figure 1A**) is otherwise notable for a shared aunt with unexplained recurrent pregnancy loss and unspecified fetal anomalies.

Subject A was a male born at 39 weeks 0 days to a 27-year-old G5P0040 mother by spontaneous vaginal delivery. He was born with multiple congenital anomalies and subsequently died at 7 days of life. Prenatal ultrasound at 18 weeks gestation revealed multiple congenital fetal anomalies including ventriculomegaly, absent right kidney, intrauterine growth restriction (IUGR), multiple limb abnormalities, cardiac defects, and ocular, cerebellar, and vermian hypoplasia. A subsequent fetal echocardiogram at 18 weeks gestation noted hypoplasia of the proximal ascending aorta with reversed diastolic flow in the left pulmonary veins, membranous ventricular septal defect (VSD), an aberrant subclavian artery, and non-visualization of the right pulmonary artery with possible agenesis. Cell-free DNA screening and routine maternal labs were unremarkable. Amniocentesis was performed during the pregnancy, and the resulting microarray showed a normal (46, XY) chromosome number with long contiguous regions of homozygosity in multiple chromosomes.

At birth, subject A's Apgar scores were 2 at 1 minute (with 2 points for heart rate) and 7 at 5 minutes (with single point deductions for color, grimace, and tone). Birth weight and length were both below the 1st percentile, at 1.68 kg and 45 cm, respectively. Head circumference was at the 4th percentile. The subject was admitted to the NICU. Physical examination was notable for bilateral absent radii, absent thumbs, finger and toe syndactyly, microphthalmia, microtia, cleft palate, imperforate anus, microphallus with scrotal hypoplasia and other congenital anomalies (see **Table S1** for further phenotypic details). Postnatal echocardiograms revealed dextrocardia, mildly hypoplastic branch pulmonary arteries, a diminutive aortic valve and a small mitral valve. X-rays of the chest and extremities showed hypoplasia of the bilateral humeri, absent bilateral radii, hypoplastic left first rib, partial fusion between the second and third ribs and sacral hypoplasia with partial fusion of the second to fourth sacral bodies. There were no abnormalities of the femurs, tibias and fibulas. Abdominal and pelvic ultrasound showed a solitary right kidney and undescended testes bilaterally without any evidence of Mullerian structures. Brain MRI revealed severe hydrocephalus, fused thalami, absent septum pellucidum, absent pituitary stalk, diffuse white matter volume loss, extremely thinned corpus callosum and cystic dilation of the bilateral semicircular canals.

Laboratory tests were significant for hypopituitarism (low cortisol [4.9 mcg/dl], free T4 [0.72 ng/dl], and free T3 [44 ng/dl]) and pancytopenia that required several red blood cell and platelet transfusions. Leukopenia, anemia, and thrombocytopenia persisted during the hospitalization (see **Table S2** for complete blood count results).

On day of life 7, the subject began to have declines in oxygen saturation and subsequent bradycardia, eventually leading to asystole. Parents declined an autopsy.

Initial clinical genetics studies were performed based on the subject's clinical presentation. Smith-Lemli-Opitz 7-dehydrocholesterol reductase plasma level was normal. A congenital heart disease (CHD) genetic testing panel did not identify clinically relevant variants. The patient was also enrolled in the Genomic Medicine for Ill Neonates and Infants (GEMINI) research protocol, whose goal was to compare the diagnostic yield of a targeted genomic sequencing platform (Newborn Dx from Athena/Quest Diagnostics) with rapid genome sequencing (rGS).[1] This testing identified two variants that did not fully explain the subject's findings: a homozygous variant of uncertain significance (VUS) in MPDZ, c.635C>T (p.Ala212Val) and a heterozygous VUS in MAP3K1, c.1429G>A (p.Glu477Lys).

Due to phenotypic overlap with Fanconi anemia, chromosome breakage studies were performed on blood, which demonstrated breakage rates consistent with Fanconi anemia. The DEB-induced

and spontaneous breakage rates for the patient were 2.94 and 0.02 breaks per cell (b/c), respectively. The age matched control showed breakage rates of 0.06 and 0.02 b/c, respectively. A Fanconi anemia positive control cell line gave a DEB induced frequency of 7.88 b/c and a spontaneous frequency of 0.20.

After subject A's death, the couple was seen for preconception reproductive counseling. Informed by the prior chromosomal breakage findings, the clinical team ordered a genetic testing panel ('exome slice') on the subject's mother, targeting genes in the Fanconi anemia pathway. This identified a heterozygous frameshifting deletion variant of uncertain significance (VUS) at c.1151_1161del (p.E384Gfs*28) in FAAP100 (NM_025161.5). The chromosomal location is chr17:79517359-79517369del (hg19). The normal transcript sequence with the bases that are altered in brackets is: ...CCCG[del11]GGAG... Subsequent reanalysis of subject A's rGS data revealed that the subject was homozygous for this variant.

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Subject B was a male born at 40 weeks 1 day when his mother was 29 years old (G7P1051) via normal spontaneous vaginal delivery. He was born with multiple congenital anomalies and died at 17 minutes of life. Ultrasound examinations during pregnancy showed multiple fetal anomalies, including severe IUGR, hydrocephalus, multicystic dysplastic kidneys, absent urinary bladder, and imperforate anus. Genetic testing of the fetus by chorionic villus sampling revealed a normal karyotype, and microarray revealed a 70-kb homozygous deletion of unknown significance at locus 4q13.3. Due to the prior findings in this subject's brother, chromosomal breakage studies were ordered, but these failed due to poor tissue culture growth. An amniocentesis was subsequently performed, and clinical trio exome sequencing (ES) revealed that subject B was homozygous for the same FAAP100 VUS previously identified in the mother and in subject A. This ES also confirmed the father to be heterozygous for the FAAP100 variant. Given the non-diagnostic clinical evaluation, the family was enrolled in the Undiagnosed Diseases Program of the New York University Grossman School of Medicine.

At birth, subject B's Apgar scores were 1 at 1, 5, and 10 minutes. He had low tone with no spontaneous respiratory effort and a heart rate of about 40 beats per minute. Ventilation was started after birth, but the heart rate continued to decline. Resuscitation was terminated at 5 minutes, and death was pronounced 17 minutes after birth. The parents agreed to an autopsy, which revealed severe intrauterine growth restriction and multiple congenital anomalies. Birth weight and body length were both below the 1st percentile, at 1.50 kg and 28.2 cm, respectively. Head circumference was below the 3rd percentile at 29.0 cm. Musculoskeletal defects included phocomelia, absent thumbs and index fingers, absent wrist and forearm, long tapered digits, soft tissue syndactyly of the 3rd and 4th digits, clinodactyly of the 5th digit, talipes equinovarus, and thoracic cavity deformities. Craniofacial anomalies included a high forehead, recessed anterior hairline, depressed/flattened and broad nasal bridge, hypertelorism, epicanthal folds, short palpebral fissures, low set posteriorly rotated ears, and micrognathia. Genitourinary abnormalities included unilateral (right) renal and ureteric agenesis, with a rudimentary left kidney (0.8 x 0.3 cm) in the right abdominal cavity. The urinary bladder was contracted and hypoplastic. An undescended testicle on the right side showed normal parenchymal development with mild nonspecific interstitial edema. Examination of the gastrointestinal system and hepatobiliary system showed imperforate anus with marked rectal dilatation (1.6 cm), malrotation with failure of fixation of the right colon, midline appendix, and histological features suggestive of congenital hyperinsulinism, diffuse type, including mild to moderate islet cell hyperplasia, scattered islet cell nucleomegaly, and rare ductular insular complex. The autopsy also revealed severe pulmonary hypoplasia with incomplete lobation and absent/incomplete horizontal fissures in the lungs. The heart exhibited a complete atrioventricular canal defect (CAVC; Rastelli Type B). The neurological autopsy revealed an immature brain (200 grams), with polymicrogyria, olfactory nerve agenesis, partial agenesis of the septum pellucidum, cerebellar hypoplasia, and ventriculomegaly. Microscopic analysis of the ribs revealed a hypercellular marrow cavity along with trilineage hematopoiesis and hyperplasia of the erythroid lineage. Microscopic analysis of the spleen revealed persistent extramedullary hematopoiesis with increased numbers of nucleated red blood cells throughout the parenchyma. Microscopic analysis of the liver also identified persistent extramedullary hematopoiesis, including many aggregates of normal blasts and nucleated red blood cells. Additionally, mild cortical depletion was observed in the thymus gland, and mild pseudotubular changes were observed in the adrenal glands. Pathological analysis of the placenta revealed low placental weight (< 3%tile), multiple chorioangiomas (up to 2.2 cm in diameter), delayed villous maturation (moderate, diffuse), scattered small aggregates of avascular fibrotic villi adjacent to basal plate, and numerous calcified villous trophoblast inclusions. See **Table S1** for further phenotypic details.

Following subject B's demise, the parents obtained additional clinical ES, which reported variants in SURF1 and MYO1H, Reanalysis of subject B's WES data revealed a homozygous VUS in SURF1, c.684T>A (p.His228Gln). Additionally, three heterozygous variants in MYO1H were identified, including a paternally inherited likely pathogenic variant (c.2406+1G>A) and a VUS (c.3046-1G>T) with unclear inheritance due to both parents being carriers. A maternally inherited MYO1H VUS c.187G>A (p.Val63Met) was also identified, although the presence of a homozygous individual in the gnomAD database decreases the likelihood that it is pathogenic. Notably, the phenotypes associated with SURF1 and MYO1H are unrelated to the phenotype observed in Subject B.[2, 3] Therefore, despite the uncertain pathogenicity of these variants, they are likely noncontributory towards the genetic syndrome described in this case.

While *FAAP100* was suspected as the primary cause of the observed syndrome, subject B's severe presentation prompted us to further analyze subject B's WES for variants that may intensify the Fanconi Anemia phenotype. This reanalysis did not reveal any notable variants in *ALDH2* or *ADH5*, which are both recognized as modifier genes of the Fanconi anemia phenotype.[4, 5]

Prompted by variants reported in the parents' additional clinical ES performed after subject B's demise, reanalysis of subject B's WES data revealed a homozygous VUS in *SURF1*, c.684T>A (p.His228Gln). Additionally, three heterozygous variants in *MYO1H* were identified, including a paternally inherited likely pathogenic variant (c.2406+1G>A) and a VUS (c.3046-1G>T) with unclear inheritance due to both parents being carriers. A maternally inherited *MYO1H* VUS c.187G>A (p.Val63Met) was also identified, although the presence of a homozygous individual in the gnomAD database decreases the likelihood that it is pathogenic.

Family 2:

A non-consanguineous couple of South Asian ethnicity presented with a history of recurrent obstetric complications. The first and third pregnancies resulted in spontaneous abortions, occurring at six and twelve weeks of gestation, respectively. The second pregnancy culminated in the birth of a female, designated subject C, who was delivered at full term via normal vaginal delivery. Birth weight was 2350 grams, and multiple congenital anomalies were observed at birth, including bilateral microtia, reduced radius size in both forearms, absence of both thumbs, and right radial club hand. At five months old, the infant presented with a fever and at that time was diagnosed with tetralogy of Fallot, single ectopic kidney, and microcornea. Failure to thrive was noted, and at 14 months old the child had a series of febrile seizures that resulted in death due to respiratory failure. Genetic testing of the subject, including karyotype and FISH for 22q11.2 microdeletion syndrome, were non-diagnostic.

The couple's fourth pregnancy, designated subject D, was notable for prenatal ultrasound at 21 weeks gestation that revealed dilated lateral cerebral ventricles (right: 13 mm; left: 14 mm) and thoracic subcutaneous edema. The parents elected to terminate the pregnancy. Autopsy of the female fetus showed a weight of 280 grams (-0.95 standard deviations, SD), length of 25.7 cm (-0.3 SD), head circumference of 16.6 cm (-1.2 SD), and a foot length of 3.4 cm, correlating with an approximate gestational age of 20-21 weeks. The autopsy was notable for bilateral cerebral lateral ventriculomegaly, normal cerebellum, dysplastic pancreatic tail, and bilateral ectopic kidneys situated in the pelvic cavity with the right kidney measuring 1.2x0.9x0.5 cm (-7.5 SD) and the left kidney measuring 1.1x1.8x0.4 cm (-8.3 SD). The left lung was normal, while the right lung lacked a transverse fissure, resulting in two lobes, and it measured 2.5x2.1x0.7 cm (- 2.35 SD).

The parents had no known family history of genetic disorders (see Main Text **Figure 4A**), and both parents' karyotypes and hemoglobin electrophoresis results were normal. The microarray of subject D was normal, with long continuous stretches of homozygosity at 3p22.1p21.2 (11.2 Mb); 6q14.1 (4.4 Mb); 10q23.31q23.32q23.33 (4.7Mb); 17q21.31q21.32 (3.5Mb) and 7q25.3 (3.2 Mb). ES of subject D revealed a homozygous stop-gain variant, c.2590C>T (p.Gln864Ter), in exon 9 of FAAP100 (NM_025161.6).[6] The chromosomal location is chr17:79507901G>A (hg19). MutationTaster predicted the variant to be deleterious with CADD PHRED and GERP scores of 36 and 0.830, respectively. Sanger sequencing confirmed that the variant was heterozygous in both parents.

Supplemental Methods

Family 2:
Chromosomal microarray analysis performed from the fetal genomic DNA using Illumina's Infinium Global Screening Array (650k array) was normal. Whole exome sequencing was performed from the fetal genomic DNA using a SureSelect Human All Exon V8 capture kit (Agilent) followed by sequencing on a NovaSeq 6000 instrument (Illumina). Sequencing reads were aligned to the GRCh38 version of the human reference genome. Variants were annotated with ANNOVAR.[7] Variants were further analyzed with an in-house pipeline where variants were filtered based on minor allele frequency in an in-house database of 3127 whole exomes and the Genome Aggregation Database (gnomAD). Single nucleotide variants (SNV) were further filtered based on the gene's association with HPO terms and the concordance with the subject's phenotype. In silico tools' (ClinPred, MutationTaster, CADD_phred, REVEL) predictions were considered during the analysis. We did not identify any clinically relevant variants in genes associated with known Mendelian phenotypes. Sanger sequencing was performed to validate the *FAAP100* variant in the family.

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