An atypical form of 60S ribosomal subunit in Diamond-Blackfan anemia linked to *RPL17* variants

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Supplemental Case Reports

Family 1 Case report

1-IV-2 (Index case). Morphological fetal ultrasound of a 31 y.o nulliparous patient of Northern European ancestry (Swiss; 1-III-5) at 21 weeks gestational age revealed a bilateral hand malformation (four metacarpals and four fingers) and retrognathism; fetal growth was in the normal range for gestational age. The index case was delivered at 41 weeks 4 days; weighed (W) 2940 g (<10th centile); with a length of 50 cm (10th centile); with a head circumference (HC) of 33.5 cm (<10th centile). Upon physical examination at birth, we noted numerous craniofacial features that were reminiscent of Treacher-Collins syndrome including: low-set ears; severe microretrognathism; glossoptosis; high arched palate; and posterior median cleft palate. He also displayed hypoplasia of the ascending branches of the mandible and bilateral pretragian fistula. Consistent with fetal ultrasonography, he displayed agenesis of the left thumb; right floating thumb; hypoplastic fifth fingers; and bilateral clinodactyly of the fifth toes. We also noted an anorectal malformation with perineal fistula. During the neonatal period, he needed non-invasive ventilation by bilevel positive airway pressure (BPAP) due to Pierre Robin sequence. The evoked auditory potentials were abnormal, showing right sensorineural deafness (50 dB). The index case since underwent multiple corrective surgeries. First, the anorectal malformation with perineal fistula was treated surgically at 14 months. When he was 16 months old, he had surgery for gastroesophageal reflux; gastrostomy and resection of the pretragian fistula; the gastrostomy was closed when he was 5 years 8 months of age. Furthermore, pollicization of the right index finger was performed at 3 y.o.; and pollicization of the left index was completed at 5 y.o.. At 4 y.o., he had a first bilateral mandibular osteotomy. At 13 y.o. he underwent a second bilateral sagittal mandibular osteotomy and placement of a mandibular distractor with the ultimate aim to perform a laryngoplasty. During this time, his growth parameters followed the 10th percentile for weight and height until age 12 y.o. when his height increased to the 25th-50th percentiles. Complete blood counts (CBC) identified moderate anemia soon after birth but he did not require any treatment or

transfusion. Hemoglobin (HGB), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) are as follows: 16 months, HGB: 10.7 g/dL (N: 10.5-13.5 g/dL), with macrocytosis, MCV: 95 fl (N: 70-86 fl); 20 months, HGB: 11.1 g/dL (N: 10.5-13.5 g/dL); MCV: 95 fl (N: 70-86 fl), MCH: 31 pg (N: 23-31 pg); 2 years 3 months, HGB: 11.2 g/dL (N: 10.5-13.5 g/dL), MCV: 94 fl (N: 70-86 fl), MCH: 31.6 pg (N: 23-31 pg). From age 6 to 13 y.o. blood counts were in the following ranges: HGB 11.7-14.1 g/dL, without macrocytosis (MCV 87-94 fl, MCH 31.6-32.8 pg), thrombocytes 187-238 G/L and no leuko/neutropenia except for one isolated para-viral episode age 8 y.o. where absolute neutrophil counts dropped to 0.94 G/L and since recovered to baseline. His adenosine desaminase (ADA) levels were in the normal range: 930 U/l_{EC} (N: 160-1030 U/l_{EC}). although new normal values in the reference laboratory have since changed to 100-400 U/l_{EC}. His HbF was 4.2-5.2 % (age 6-11 yo).

1-III-5 (Mother of the index case). Individual 1-III-5 is the second child of an unrelated couple who was born at term. She was small for gestational age with a birth weight of 2850 g (<10^{th} centile) and length of 46 cm (<10^{th} centile). At birth, she presented with microretrognathism and bilateral pes adductus. A partial cleft palate was diagnosed and treated surgically at 2 years of age and 6 years of age. Anemia was discovered when she was 3 months-old; HGB levels were 8.5 g/L (Normal: 10.5-13.5 g/dL). At age 4, her psychomotor development was in the normal range, and she had, H: 100 cm (25^{th} centile); W: 16 kg (50^{th} centile); HC: 52.5 cm (97^{th} centile); her anemia stabilized (HGB: 9 g/dL) and did not necessitate any treatment, however she presented with leukopenia (3.8 G/L; N: 5.0-21.0 G/L). Fanconi anemia was ruled out as no significant excess of chromosome breakage was observed after Mitomycin C testing. Upon physical examination, she displays a small chin, left thumb hypoplasia and limited pronosupination. Hematological follow-up as an adult revealed moderate, but constant anemia: HGB: 9.5-12.1 g/dL (N: 11.7-15.7 g/dL); Erythrocytes: 2.87-3.89 T/L (N: 3.8-5.2 T/L); MCV: 92-99 fl (Normal: 81-99 fl). No leukopenia was detected; leukocytes: 3.9-6.7 billion cells/L (N: 4-10 G/L).

Her ADA levels are in the normal range: 191 U/L (N: 160-1030 U/L). Her height at the last physical examination is 161 cm (-0.4 SD). She underwent genetic testing of all known causal Diamond-Blackfan genes; Sanger sequencing of *RPL5*, *RPL11*, *RPS19*, *RPS15*, *RPS10*, *RPS17*, *RPS24*, *RPS26* and *RPL35A* were negative.

1-IV-3 (Sibling of the index case). During the second pregnancy of 1-III-5, prenatal genetic testing was conducted for the *RPL17* c.217-3C>G variant. He is not a mutation carrier and is reported to be healthy.

1-III-6 (**Maternal uncle)**. Individual 1-III-6 was born at term with bilateral absent thumbs; these were treated surgically by pollicization of the index fingers. He was followed during infancy for a moderate anemia which did not necessitate any treatment. At age 35, his CBC values were within normal limits, except for a low erythrocyte count (4.22 T/L); HGB: 13.5 g/dL; Leukocytes: 4.9 G/L.

1-II-4 (Maternal grandfather). Upon physical examination, individual 1-II-4 displays a soft palate malformation, nasal voice, and hypoplasia of 5th finger. He does not present with anemia: HGB: 13.9 g/dL; Erythrocytes: 4.58 T/L; MCV: 89 fl , but he does present with leukopenia; leukocytes: 2.5 G/L.

1-II-2 (Maternal great-uncle). This individual presents with retrognathism and a nasal voice. He has been monitored clinically for leukopenia for decades (consistently ~3.0 G/L; Normal: 4-10 G/L), with a major episode when he was 30 years old. His other CBC values were within normal limits at the most recent examination, age 71 yo: HGB: 13.9 g/dL; MCV: 91 fl, total leukocytes 2.7 G/L (mild leukopenia).

1-II-1 (**Maternal great-uncle)** He is reported to be healthy and does not carry the *RPL17* c.217-3C>G mutation. His most recent CBC values were within normal limits, HGB: 15.4 g/dL; leukocytes: 6.9 G/L; erythrocytes: 4.73 T/L.

1-I-1 (Maternal great-grandmother). Her medical history is unremarkable except hypertension. Upon physical examination, we noticed a small chin and bilateral 5th digit clinodactyly. At her last clinic visit at 83 years of age, she displayed leukopenia: 2.5 G/L and moderate thrombocytopenia (140,000-180,000/μl) but no anemia was observed for the past several decades that she was followed by our clinic (erythrocytes: 4.62 T/L ; HGB: 13.8 g/dL). She developed an acute myeloid leukemia and died soon after the diagnosis. She had two sisters and one brother; and three out of four nieces and nephews (children of her deceased brother) had no particular medical history and were tested negative for the *RPL17* c.217-3C>G mutation.

1-III-3. She was born at term following an uneventful pregnancy; birth weight: 3050 g (<25th centile); length: 47 cm (<25th centile). Severe anemia (HGB: 3.5 g/dL; Normal: 10.5-13.5 g/dL) was discovered when she was 13 months old, and was treated initially by packed red blood cell transfusions and later on by corticosteroids (prednisone: 12 mg/2d). She also presented thrombocytopenia. A diagnostic bone marrow aspirate/biopsy showed severe hypocellularity with marked erythroid lineage hypoplasia and dyserythropoiesis (incl. megaloblastic forms) and mild dysmyelopoiesis. The pattern was deemed to be compatible with DBA. After introduction of prednisone therapy, repeat bone marrow aspirates/biopsies showed progressive correction of the previously observed features without complete correction of the hypocellularity. Prednisone treatment was diminished progressively (5 mg/2 days at age 3.5 years old). When she was 4 years 6 months, steroid treatment was stopped; her HGB and platelet levels remained stable, but leukoneutropenia was observed. Upon physical examination, she displays a small chin, and fifth finger clinodactyly. At age 32, a hematological evaluation confirmed the diagnostic of a minor

functional platelet disorder. There was no anemia, HGB: 14.3 g/L; Erythrocytes: 4.55 T/L; MCV: 94 fl. However, mild leukopenia was observed; leucocytes: 3.0 G/L without neutropenia. Follow-up leukocyte levels corrected (3.8-4.3 G/L). Her ADA level is in the normal range: 219 U/l$_{EC}$ (N: 100-400).

1-IV-1. The son of 1-III-3 was born at 38 weeks 2 days gestational age, with a birth weight 2680 g (5th-10th centile), and a length of 49 cm (<50th centile). No malformations were detected upon ultrasonography during pregnancy. Prenatal diagnosis showed that the fetus carried the *RPL17* c.217-3C>G mutation. At birth ankylosis of the metacarpo-phalangeal and interphalangeal joints of the left thumb were noted. Growth (age 1-7 y.o) was on the 75th-90th percentiles for weight and 75th percentile for height. From age 4 to 7 y.o. blood counts were in the following ranges: HGB 11.3-12.9 g/dL, with mild macrocytosis (MCV 92-95 fl, MCH 32.5-33.4 pg), thrombocytes 98-221 G/L and no significant leuko/neutropenia. His ADA level is in the normal range: 232 U/l$_{EC}$ (N: 100-400). His HbF was high in early infancy (7% at age 4 y.o.) and then decreased to 2.3-2.9 % (age 4-7 y.o.).

1-IV-4 (cousin of the index case). This female is the healthy child of 1-III-6. The parents declined genetic testing of the *RPL17* c.217-3C>G mutation.

Family 2 Case report

The index case of Family 2 (2-II-1) is a male delivered at 39 weeks gestation to his primigravid mother by vaginal delivery. Although no pregnancy complications were reported, ultrasound exams were notable for poor fetal growth from early on and he weighed 4 pounds 10 ounces at birth. His neonatal hospital stay was six days due to poor weight gain and hypoglycemia. He was followed by his pediatrician for poor growth. He presented at 8 months of age with acute vomiting and a CBC revealed pancytopenia with macrocytic anemia (HGB of 4.8 g/dL; Normal: 10.5-13.5

g/dL), neutropenia with an absolute neutrophil count (ANC) of 30 G/L (Normal: 1-8 G/L) and mild thrombocytopenia with platelet count of 117 G/L (Normal: 150-450 G/L). A bone marrow aspirate was performed, which showed no evidence of malignancy; testing for EBV, CMV, HIV, and parvovirus was negative. Further workup for congenital bone marrow failure syndromes was recommended but was never sent. He was less than the third percentile for both height (65 cm) and weight (6.5 kg) and was noted to have an incarcerated inguinal hernia which was surgically repaired. At follow up in hematology/oncology clinic at 9 months of age, his counts showed recovery (HGB 9.3 g/dL, WBC 7.85 G/L, platelet 224 G/L) and it was thought that he had recovered from a viral suppression of his marrow. However, he was readmitted to hospital at 10 months of age for fever, pancytopenia and growth failure. Genetics consultation at the time noted mild dysmorphia including a tall forehead, epicanthal folds, a high nasal root with narrow nasal base, full lips with a mildly triangular facial shape. Negative etiologic testing included an infectious disease work-up, cystic fibrosis, vitamin B12/ folate deficiency, karyotype, microarray-CGH, DEB clastogen assay for Fanconi anemia, sequencing for Noonan syndrome (*BRAF, HRAS, KRAS, MAP2K1, MAP2K2, PTPN11, RAF1*, and *SOS1)*, *TIN2, TERC, SBDS,* and *DKC1*. Upon evaluation for Dyskeratosis Congenita, his telomere length on 3 cell lines was short (<1%); repeat detailed testing demonstrated telomere abnormalities in the following cell types: lymphocytes- low; granulocytes- very low; Naive T cells- low; Memory T cells- low; B cells -very low; NK cells- low. These results were reviewed by experts at the National Institutes of Health (NIH) and thought not to be low enough to be diagnostic of Dyskeratosis Congenita. His anemia was initially transfusion dependent, but the last transfusion needed was at 15 months of age. His neutropenia also improved without requiring treatment with granulocyte stimulating factor. His most recent CBC was conducted at 6 years of age: HGB 11.4 g/dL (Normal: 11.5-15.5 g/dL), platelets 143 (Normal: 150-450 G/L) and ANC of 3.3 G/L (Normal: 1.5-9 G/L). His developmental milestones were met on time. He was last seen at the age of 8 and was diagnosed with autism and ADHD; he was following his own growth curve at the 3-5% for both height and weight. His mother and

father are 5’5” and 5’8” tall, respectively; both are healthy, with no history of anemia or developmental problems. A paternal aunt is noted to have a hole in her heart, wide neck and large forehead, resembling the patient’s father in both stature and appearance. A paternal uncle is 5 feet 6 inches tall and with a heart murmur. The paternal grandparents are 5’2” and 5’3” tall and healthy.

2-I-1 (Father of the index case). The father, heterozygous for the *RPL17* c.452delC variant, is reportedly healthy. He is 5 feet 8 inches tall.

Supplemental Tables

Supplemental Table 1. Whole exome sequencing coverage statistics

	1-III-3	1-III-6	1-IV-2	2-I-1	2-I-2	2-II-1
Affected status	Affected	Affected	Affected	Healthy	Healthy	Affected
Total raw reads	75477130	75487432	70931422	177442862	188120990	172289661
Reads mapped to genome	68458983	68516261	65160239	136813684	145138617	132682615
% of target bases covered >10x	92.98	92.8	92.66	94.90	94.86	94.71
% of target bases covered >20x	85.53	85.29	85.25	93.04	93.17	92.80
Mean target coverage	97.32	98.39	91.99	142.46	151.47	137.61

Supplemental Table 2. Variant filtering strategy for Family 1.

Individual	**1-III-3**	**1-III-6**	**1-IV-2**
Variant filtering step	**Number of variants**		
Impact protein sequence and splice junctions	50968	50544	50624
Allele frequency <1% in ExAC and in-house database	1965	2011	1958
Quality score pass (>30)	280	282	267
Heterozygous	274	275	264
Common across 3 individuals	10		
Absent from exome databases (ExAC, in-house database)	2 *DIS3L2*: NM_152383, c.1945G>A; p.G649R [OMIM: 267000, AR] *RPL17*: NM_000985, c.217-3C>G; p.A73_K105del		

Supplemental Table 3. Variant filtering strategy for Family 2.

Individual	**2-II-1**
Variant filtering step	**Number of variants**
OMIM genes nominated by SSAGA and MAF<1% in non-Finnish Europeans (ExAC)	95
MAF<1% in all populations (Finnish Europeans; South Asians; Africans; ExAC)	81
ACMG category 1-3	ACMG 3: 57
	ACMG 2: 3 *ARL13B*: NM_182896.2, c.131-1G>C [OMIM: 612291, AR] *FYCO1*: NM_024513.3, c.3327_3328del; p.C1110Pfs*7 [OMIM: 610019, AR] *NEB*: NM_001164507.1, c.13147C>T; p.Q4383* [OMIM: 256030, AR]
	ACMG 1: 0
OMIM genes that when mutated result in a phenotype overlapping that of the proband	0

Supplementary Table 4. Representative erythroid cell counts from lateral images of *gata1:dsRed* zebrafish larvae at 2 dpf.

Injection	Measurement				p-value (unpaired Student's t-test, two-sided)		
	n	Mean	S.E.M.		vs Control	vs. gRNA alone	vs. MO
In vivo complementation: Figure 3B							
Controls	20	133	2.9		-	-	-
100 pg *rpl17* gRNA + 200 pg Cas9	22	74	5.2		3.90E-12	4.56E-09	-
100 pg *rpl17* gRNA	24	120	3.7		0.0082	-	-
4 ng *rpl17* sb MO	38	69	1.2		3.69E-31	-	-
4 ng *rpl17* sb MO + 100 pg WT *RPL17* mRNA	40	125	2.5		0.0513	-	3.17E-31
rpl17 morpholino dose curve: Supplemental Figure 3D							
Controls	52	133	1.3		-	-	-
1 ng *rpl17* sb MO	49	130	1.4		0.1164	-	-
2 ng *rpl17* sb MO	37	143	1.4		3.62E-06	-	-
4 ng *rpl17* sb MO	38	69	1.2		4.00E-53	-	-

Supplemental Table 5. Representative ceratohyal angle measurements from ventral images of *-1.4col1a1:egfp* zebrafish larvae at 4 dpf (degrees).

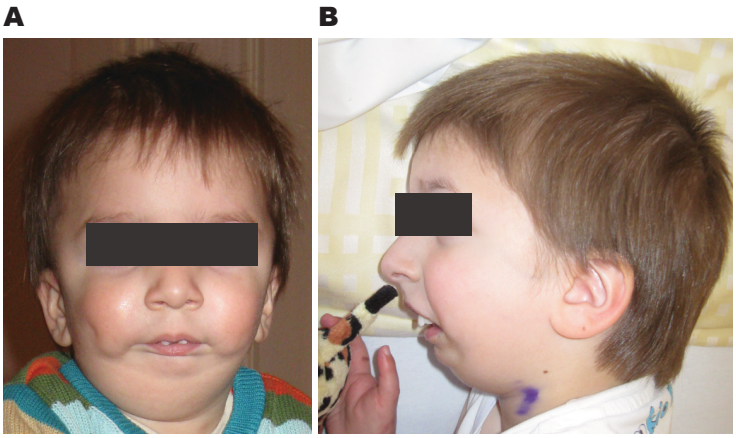
Injection	Measurement				p-value (unpaired Student's t-test, two-sided)			
	n	Mean	S.E.M.		vs Control	vs. gRNA alone	vs. MO	vs. WT rescue or mRNA alone
In vivo complementation: Figure 3D								
Controls	28	81	0.7		-	-	-	-
100 pg *rpl17* gRNA + 200 pg Cas9	16	140	6.1		9.57E-16	6.05E-14	-	-
100 pg *rpl17* gRNA	27	86	1.0		0.0008	-	-	-
4 ng *rpl17* sb MO	26	127	3.0		1.55E-20	-	-	-
4 ng *rpl17* sb MO + 100 pg WT *RPL17* mRNA	29	105	2.2		5.36E-14	-	2.33E-07	-
4 ng *rpl17* sb MO + 100 pg p.A73_K105del *RPL17* mRNA	24	125	3.8		2.56E-16	-	7.20E-01	1.67E-05
4 ng *rpl17* sb MO + 100 pg p.(T151Rfs*25) *RPL17* mRNA	25	117	3.7		3.10E-13	-	0.0435	0.0064
4 ng *rpl17* sb MO + 100 pg p.Q56L *RPL17* mRNA	32	104	3.0		9.23E-09	-	2.10E-06	0.7789
rpl17 morpholino dose curve: Supplemental Figure 3E								
Controls	60	82	0.8		-	-	-	-
1 ng *rpl17* sb MO	41	83	0.9		0.132	-	-	-
2 ng *rpl17* sb MO	51	79	1.5		0.0745	-	-	-
4 ng *rpl17* sb MO	55	134	2.9		5.61E-35	-	-	-
RPL17 mRNA alone (100pg): Supplemental Figure 4								
Controls	30	79	1.9		-	-	-	-
100 pg WT *RPL17* mRNA	22	76	0.9		0.2187	-	-	-
100 pg p.A73_K105del *RPL17* mRNA	36	76	0.7		0.1595	-	-	0.8509
100 pg p.(T151Rfs*25) *RPL17* mRNA	23	75	0.8		0.1259	-	-	0.5891
100 pg p.Q56L *RPL17* mRNA	23	75	0.9		0.0658	-	-	0.2551

Supplemental Table 6 – Ribosome Footprint Density – Gene Level Data
Deposited on Zenodo. https://doi.org/10.5281/zenodo.12571975

Supplemental Table 7 – Ribosome Footprint Density – Gene Ontology Analysis
Data deposited on Zenodo. https://doi.org/10.5281/zenodo.12571975

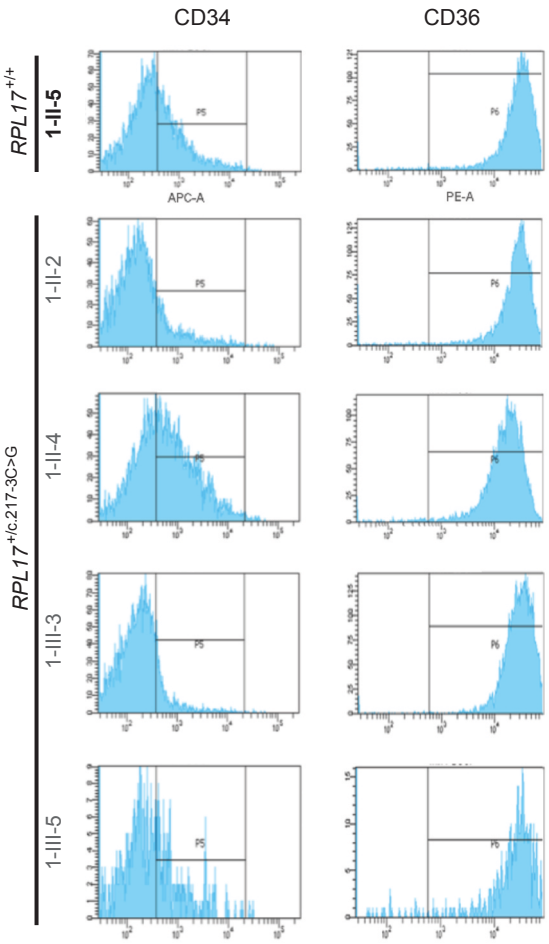
Supplemental Table 8. Oligonucleotide sequences

Oligo name	Species	Sequence (5'-3')	Purpose
RPL17_ex2_F	Human	AGAGACGGCACTCACTACCT	Targeted sequencing of RPL17 coding regions
RPL17_ex2_R	Human	GCCAAGCCCCAAGTAGGAAA	Targeted sequencing of RPL17 coding regions
RPL17_ex3_F	Human	TTTCCTACTTGGGGCTTGGC	Targeted sequencing of RPL17 coding regions
RPL17_ex3_R	Human	TCTTTGGGGGCAAGGCAATT	Targeted sequencing of RPL17 coding regions
RPL17_ex4_F	Human	AGTGTGCAGATCAAGATGGGG	Targeted sequencing of RPL17 coding regions
RPL17_ex4_R	Human	ACCATCAATCCAAGGTGTCCTG	Targeted sequencing of RPL17 coding regions
RPL17_ex5_F	Human	TTCATTGAGGCAGGACTCTG	Targeted sequencing of RPL17 coding regions
RPL17_ex5_R	Human	CAGGTGAAAGGGTTGCTCAG	Targeted sequencing of RPL17 coding regions
RPL17_ex6_F	Human	TCTCCACCCGTCTAAGGATCTT	Targeted sequencing of RPL17 coding regions
RPL17_ex6_R	Human	AGGTGAAAGGGTTGCTCAGAAT	Targeted sequencing of RPL17 coding regions
RPL17_ex7_F	Human	ACCTCAGGCCCGTATGATTATC	Targeted sequencing of RPL17 coding regions
RPL17_ex7_R	Human	TGGTGCAGGGTAAACAATCTTG	Targeted sequencing of RPL17 coding regions
RPL17-RTEx2F	Human	CACTCGTGAAACTGCTCAGGCCA	RT-PCR to monitor mRNA splicing in Family 1
RPL17-RTEx6R	Human	ATGGCACGGGAGTAAATTCAGCAT	RT-PCR to monitor mRNA splicing in Family 1
rpl17 sb MO	Zebrafish	AGAGTTAAATCTTACCTTGAAGTGA	morpholino for transient suppression of rpl17
rpl17-DR-rt-F	Zebrafish	TGAAAATGGTCCGCTACTCTCT	RT-PCR to test MO efficiency
rpl17-DR-rt-R	Zebrafish	AGCTCATGTAGGGGTTGATGC	RT-PCR to test MO efficiency
rpl17_G1_F	Zebrafish	TAATACGACTCACTATAGGGCAAGGGGAGCTCATGTAG	template for rpl17 gRNA synthesis
rpl17_G1_R	Zebrafish	TTCTAGCTCTAAACCTACATGAGCTCCCCTTGCC	template for rpl17 gRNA synthesis
rpl17_G1_Flanking_F	Zebrafish	CCTGGCTTGGCTGTATTTAATC	PCR amplify rpl17 gRNA target
rpl17_G1_Flanking_R	Zebrafish	CATTCACCAACAGCAGATCCTA	PCR amplify rpl17 gRNA target
siRpl17-1	Human	GUGUACCAUUCCGACGUUA	siRNA to knock down RPL17 in HeLa cells
siRpl17-2	Human	CGCAGAGAGUAAUGCUGAA	siRNA to knock down RPL17 in HeLa cells
siRpl17-3	Human	CUCUGGUCAUUGAGCAUAU	siRNA to knock down RPL17 in HeLa cells
5'-ITS1	Human	CCTCGCCCTCCGGGCTCCGTTAATGATC	Northern blot probe
ITS2-1	Human	CTGCGAGGGAACCCCCAGCCGCGCA	Northern blot probe
ITS2-2	Human	GCGCGACGGCGGACGACACCGCGGCGTC	Northern blot probe
ITS1-5.8S	Human	CTAAGAGTCGTACGAGGTCG	Northern blot probe
5'-5.8S	Human	CACCGCTAAGAGTCGTACGA	Northern blot probe
5.8S	Human	GTTCTTCATCGACGCACGAGC	Northern blot probe
18S	Human	TTTACTTCCTCTAGATAGTCAAGTTCGACC	Northern blot probe
28S	Human	CCCGTTCCCTTGGCTGTGGTTTCGCTAGATA	Northern blot probe
7SK-1	Human	CATGGAGCGGTGAGGGAGGA	Northern blot probe
7SK-2	Human	GTGTCTGGAGTCTTGGAAGC	Northern blot probe



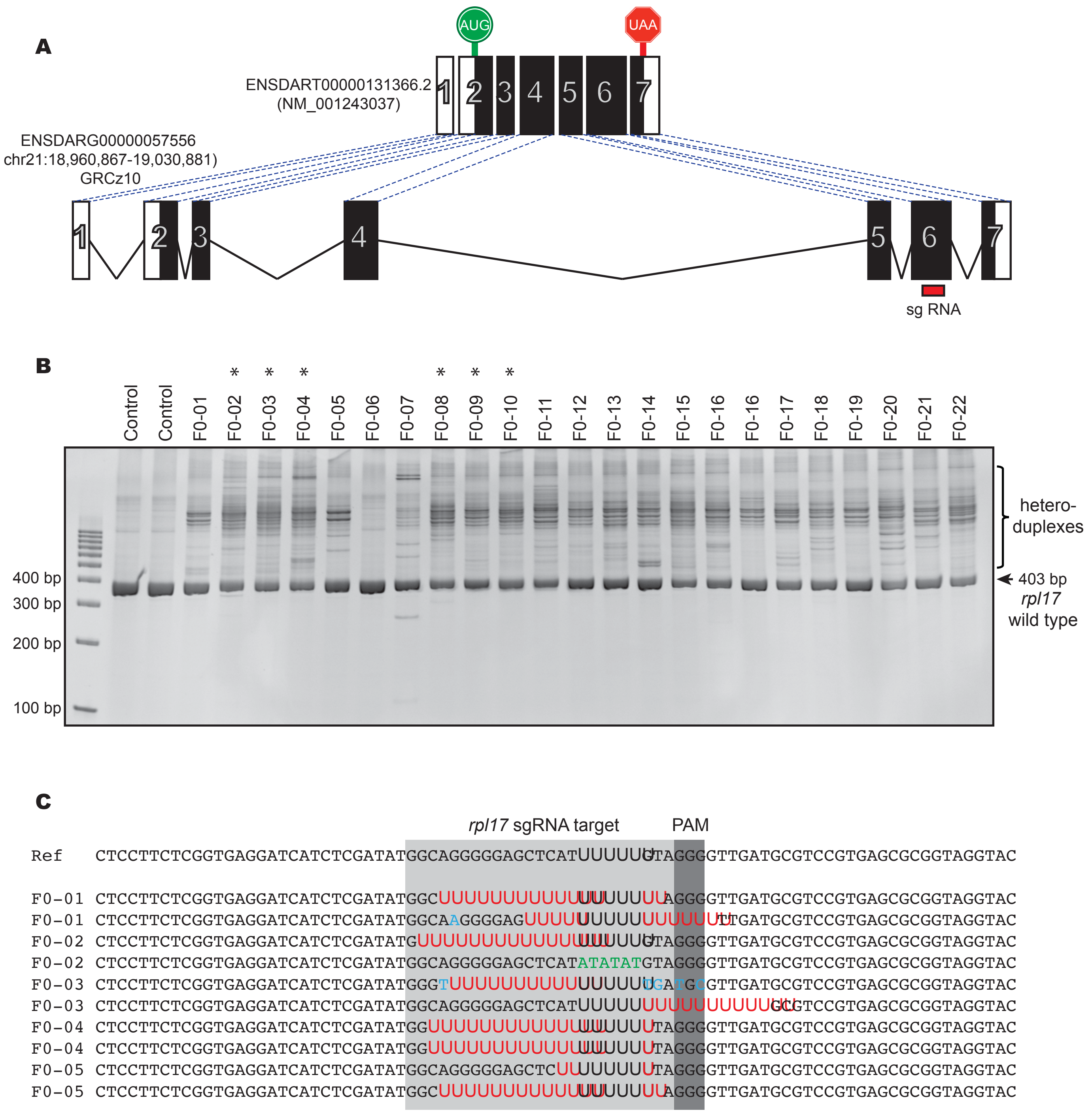
Supplemental Figure 1. Facial features of the Family 1 index case (1-IV-2).

(**A**) Front, and (**B**) Side profile images taken at 3 years of age. Note the microtia, micrognathia, and malar hypoplasia.



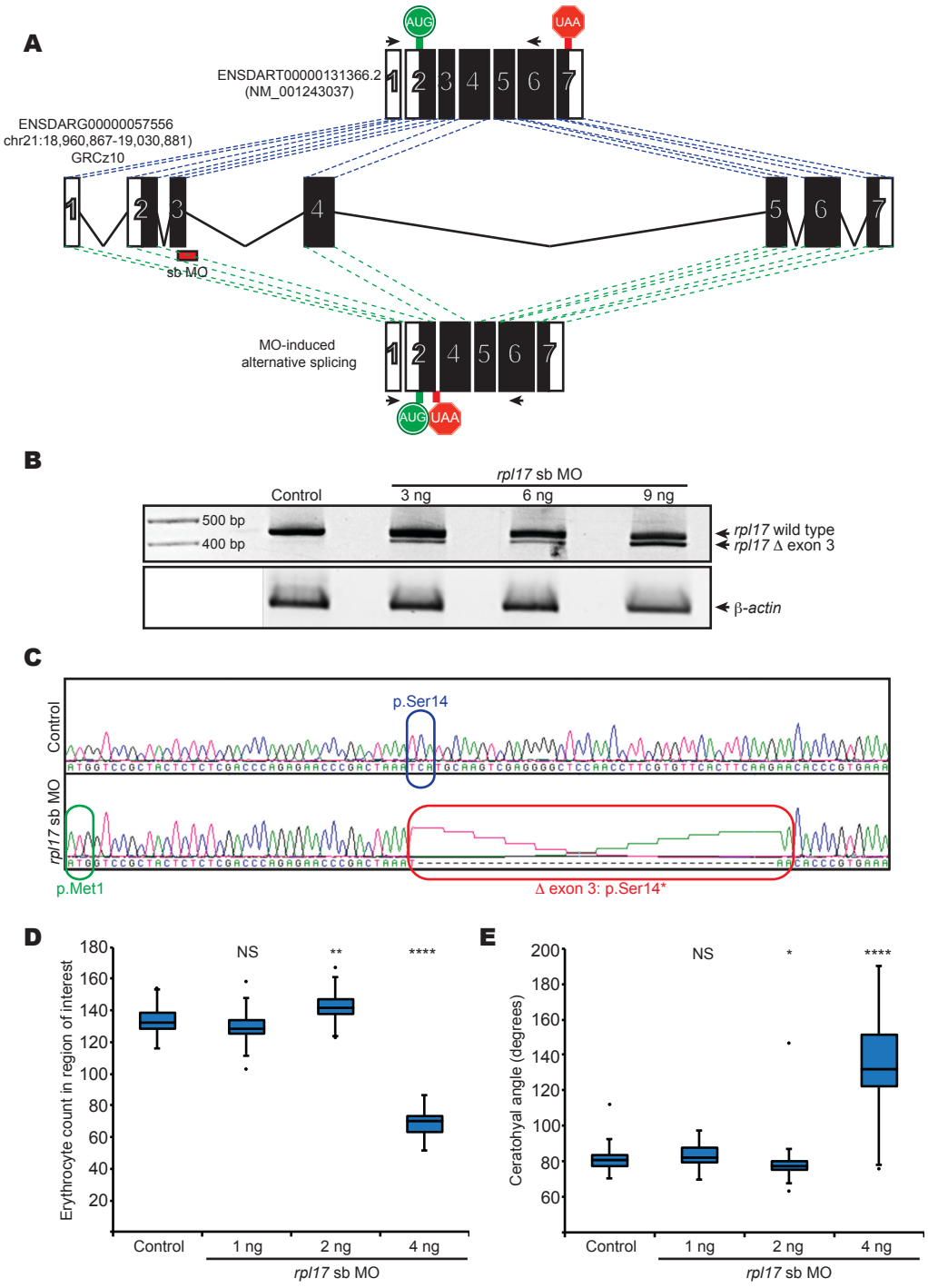
Supplemental Figure 2. Characterization of erythroid maturation defects in cells from family 1 cultured *in vitro*: CD34 and CD36 labelling at D7

FACS analysis at D7 shows no consistent change in the percentage of BFU-e ($CD34^+$/ $CD36^-$) or CFU-e ($CD34^-$/ $CD36^+$) progenitor cells with *RPL17* variants when compared to cells from the unaffected individual. These distribution graphs correspond to the same data as in Figure 2B for D7, but the two markers are shown independently.



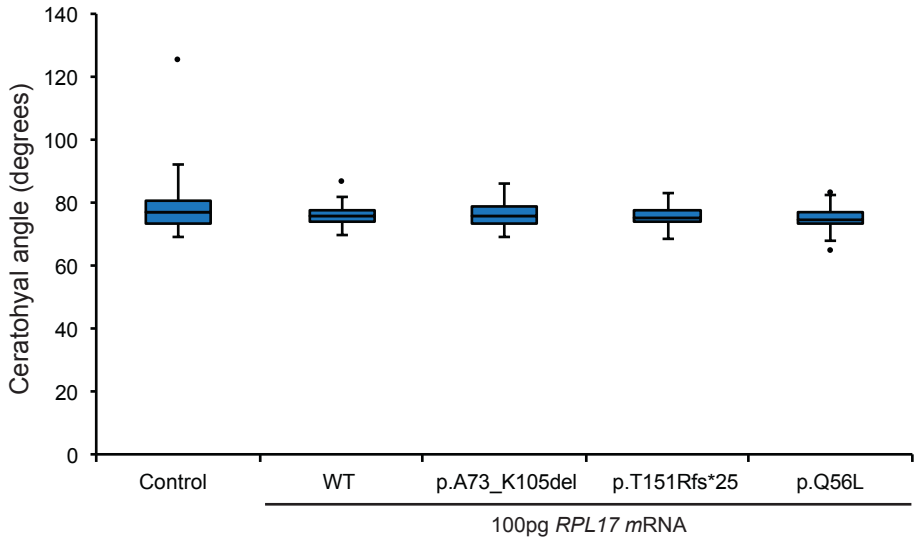
Supplemental Figure 3. CRISPR/Cas9 genome-editing of *rpl17* in zebrafish

(**A**) Schematic of the zebrafish *rpl17* locus on *D. rerio* chromosome 21 (bottom) and the mRNA transcript (top). Black boxes, coding exons; white boxes, untranslated regions; solid black lines, introns; green "AUG", start codon; red "UAA", stop codon; red box, single guide (sg)RNA target site. (**B**) Heteroduplex analysis of two uninjected controls and 22 individual *rpl17* F0 CRISPR/Cas9 mutants. Embryos were injected with 100 pg gRNA and 200 pg Cas9 protein into the cell at the single-cell stage; harvested for genomic DNA extraction at 2 days post-fertilization; the region flanking the sgRNA target was PCR-amplified; denatured; reannealed slowly and migrated by polyacrylamide gel electrophoresis (PAGE). Asterisks indicate embryos used to estimate mosaicism. (**C**) Alignment of representative sequences obtained from cloned PCR fragments flanking the sgRNA target (n=6 F0 embryos and 1 control embryo; 12 colonies sequenced per embryo). Light gray shading, sgRNA target; dark gray shading, protospacer adjacent motif (PAM); red dashes, deletions; green text, insertions; blue text, single nucleotide changes. 68% of F0 sequences contained mutations (averaged across 6 embryos).



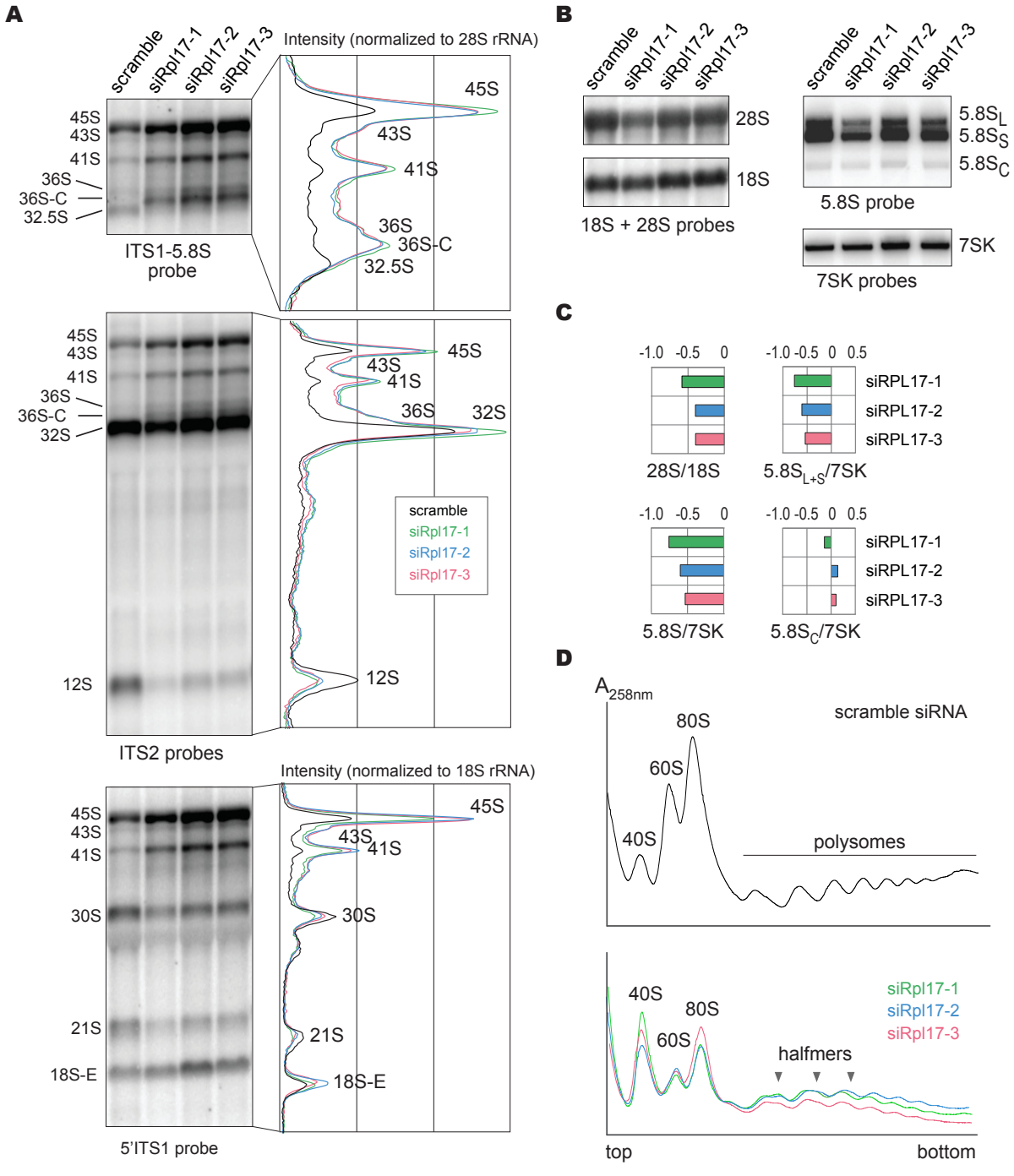
Supplemental Figure 4. Transient suppression of *rpl17* in zebrafish using morpholino (MO) antisense oligonucleotides

(**A**) Schematic of the zebrafish *rpl17* locus on *D. rerio* chromosome 21 (center); wild type (WT) mRNA transcript (top); and transcript resulting from MO-induced blocking of the exon 3 splice donor. Black boxes, coding exons; white boxes, untranslated regions; solid black lines, introns; green "AUG", start codon; red "UAA", stop codon; red box, MO target site; black arrows, primers used for RT-PCR validation of the MO. (**B**) Agarose gel image showing dose-dependent induction of aberrant *rpl17* mRNA splicing. *beta-actin* was used to control for mRNA integrity. (**C**) Chromatograms showing the WT mRNA and MO-targeted *rpl17* transcript; the MO results in exclusion of exon 3, resulting in the introduction of a p.S14* premature termination codon. (**D**) Injection of *rpl17* sb-MO into *gata1 dsRed* transgenic embryos at progressively increasing doses (1 ng, 2 ng, and 4 ng) followed by quantitative scoring of erythroid precursors on lateral fluorescent images at 3 days post fertilization (dpf) results in a significant reduction of fluorescent cells at 4 ng. (**E**) Injection of *rpl17* sb-MO into *-1.4col1a1:egfp* transgenic embryos at progressively increasing doses (1 ng, 2 ng, 4 ng) followed by ventral imaging of transgenic signal at 4 dpf results in altered cartilage patterning at the 4 ng dose, as indicated by the width of the ceratohyal angle. NS, not significant; * p<0.05; **p<0.01; **** p<0.0001; Kruskal-Wallis with Dunn's multiple comparisons test.



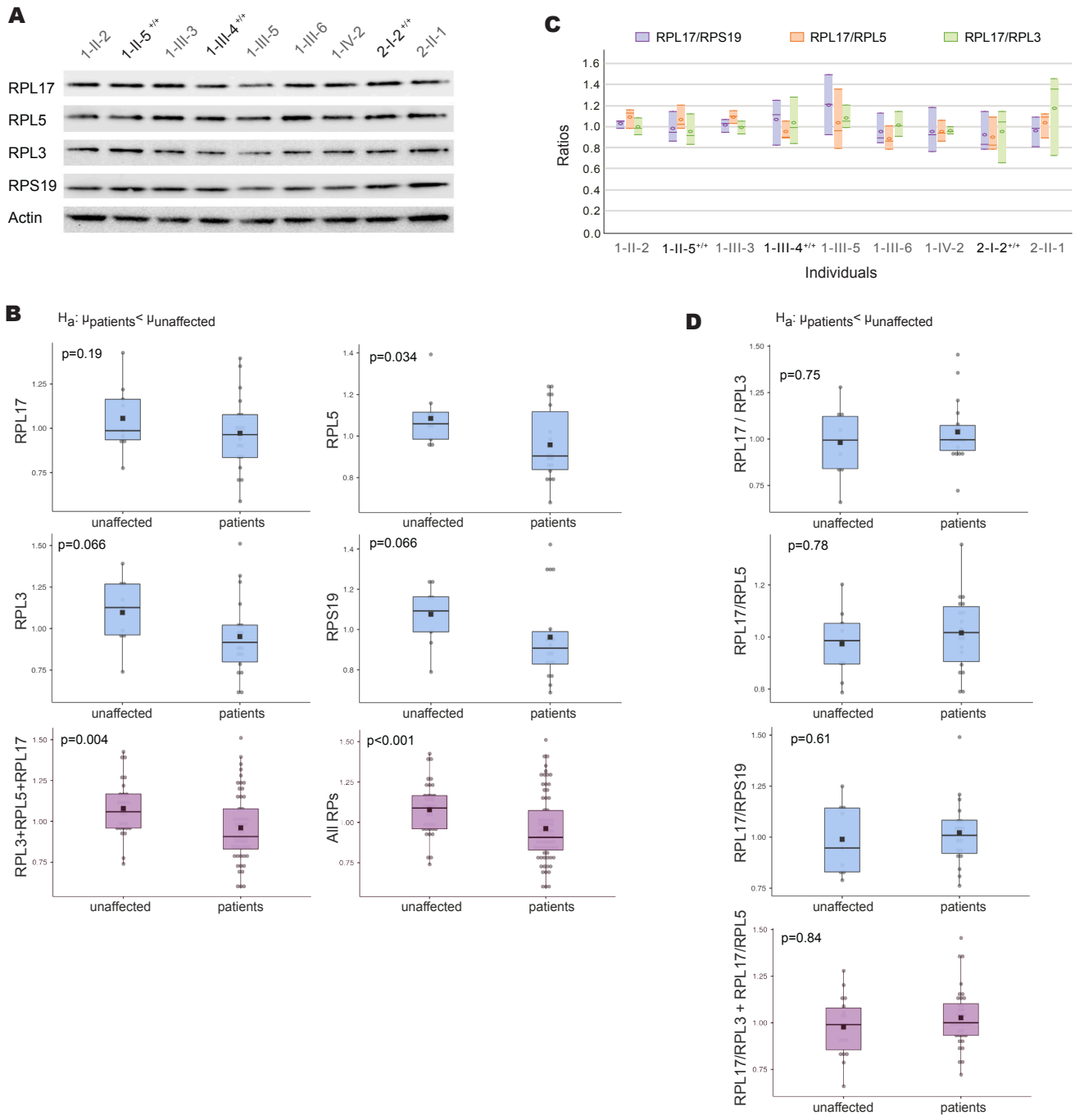
Supplemental Figure 5. Zebrafish larvae injected with human *RPL17* mRNA show no detectable phenotype

Quantification of the ceratohyal angle in -*1.4col1a1:egfp* transgenic zebrafish embryos injected with 100 pg wild type (WT) or mutant *RPL17* mRNA and imaged live at 4 days post-fertilization (See Figure 3C); n=22-30 larvae/batch, repeated at least twice. mRNA encodes predicted proteins p.A73_K105del and p.(T151Rfs*25) corresponding to Family 1 and Family 2, respectively. mRNA coding for p.Q56L is present in public databases (rs753489644; gnomAD browser). The ends of the whiskers are set at 1.5 times the interquartile range (IQR) above the third quartile and below the first quartile, respectively. Black dots, minimum and maximum outliers; no significant differences were detected in pairwise comparisons between batches (Kruskal-Wallis with Dunn's multiple comparisons test).



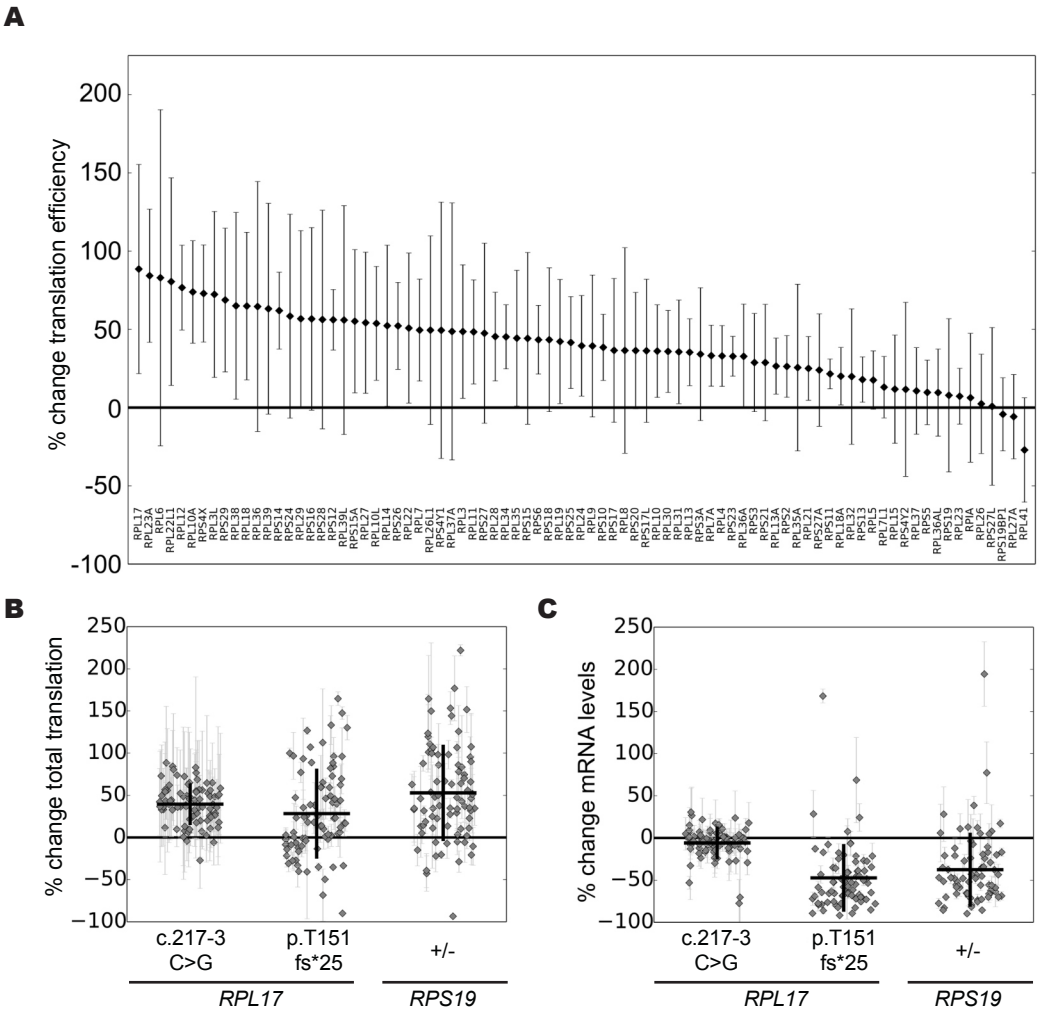
Supplemental Figure 6. Depletion of RPL17 in HeLa cells impairs 5.8S rRNA production

(**A**) Total RNAs from HeLa cells treated with the 3 different siRNAs targeting *RPL17* mRNA were analyzed by northern blot and hybridized with the indicated probes. Intensity profiles corresponding to precursors in control (black) or siRNA-treated samples (gray) were normalized relative to 28S or 18S rRNAs. (**B**) The membrane displayed in (**A**) was hybridized with a mixture of probes specific to 18S and 28S rRNAs. After separation on a 6% polyacrylamide gel, 5.8S rRNA species were identified with 5.8S or 5'-5.8S probes, and quantified relative to 7SK snRNA. (**C**) Pre-rRNAs were quantified by using MultiGauge software. Log2 ratios were normalized to the value obtained for cells treated with a scramble siRNA. The ratios were obtained for 28S relative to 18S rRNAs, and to 5.8S rRNA species relative to 7SK snRNA in the corresponding sample. Both 28S and 5.8S rRNA levels were decreased upon lack of RPL17, besides the level of 5.8SC rRNAs remained identical in control and RPL17-depleted HeLa cells. (**D**) Cytoplasmic fractions prepared from control cells treated for 48 h with a scramble siRNA (top) or with the corresponding siRNA (bottom) were analyzed on 10-50% sucrose gradients.



Supplemental Figure 7. Evaluation of RPL17 stoichiometry relative to other ribosomal proteins in cells from patients and unaffected individuals

(**A**) RPL17, RPL5, RPL3, RPS19 and actin were detected by western blot and their levels were quantified in whole cell extracts prepared from LCLs. The figure shows one of three independent experiments made on different cell extracts. (**B**) Ratios of the four ribosomal proteins relative to actin in patients and unaffected individuals measured in three independent experiments. In each experiment, for a given ratio, values were normalized by dividing by the mean. Graphs in blue aggregate the data from the three experiments for each ribosomal protein. The graphs in purple aggregate the data either for the three large ribosomal subunit proteins (RPLs), or for the four ribosomal proteins. A unilateral Mann-Whitney U-test was performed to assess the hypothesis that ribosomal protein levels were lower in patients when compared to unaffected individuals. The results suggest a slight but significant decrease in ribosomal protein levels in patients. (**C**) Ratios of RPL17 levels relative to RPS19, RPL5 and RPL3 in patients and unaffected individuals. In each experiment, for a given ratio, values were normalized by dividing by the mean. Horizontal bars correspond to the ratios measured in three independent experiments, and ellipses indicate the average value. (**D**) Ratios of RPL17 relative to RPL3, RPL5 and RPS19 in patients and unaffected individuals. The data are the same as in C, but were grouped in patients and unaffected individuals. The graph in purple aggregates the RPL17/RPL3 and RPL17/RPL5 ratios to increase statistical power. A unilateral Mann-Whitney U-test was performed to assess the hypothesis that the level of RPL17 relative to the other ribosomal proteins was lower in patients when compared to unaffected individuals. The results show no significant difference between patients and healthy individuals. Statistical analyses and graphs were made with Jamovi: The jamovi project (2023). *jamovi*. Computer software. (Version 2.3). Retrieved from https://www.jamovi.org.



Supplemental Figure 8. Enhanced ribosomal protein translation in DBA cells

(**A**) Percentage change in translation efficiency for all ribosomal proteins across *RPL17* c.217-3C>G cells relative to controls. Errors bars represent ±SD; n=6 for DBA and n=7 for WT. (**B**) Dot plot indicated percentage change in total translation for each ribosomal protein in each of the three DBA genotypes analyzed. (**C**) As in panel B, except analyzing mRNA levels only.