SUPPLEMENTAL TABLES, METHODS AND FIGURES

SRP54 mutations cause syndromic neutropenia with Shwachman-Diamond-like features

Raphael Carapito[1,2,3*], Martina Konantz[4*], Catherine Paillard[1,2,5*], Zhichao Miao[6], Angélique Pichot[1,2], Magalie S. Leduc[7,8], Yaping Yang[7], Katie L. Bergstrom[9], Donald H. Mahoney[9], Deborah L. Shardy[9], Ghada Alsaleh[1,2], Lydie Naegely[1,2], Aline Kolmer[1,2], Nicodème Paul[1,2], Antoine Hanauer[1,2], Véronique Rolli [1,2,3], Joëlle S. Müller[4], Elisa Alghisi[4], Loïc Sauteur[4], Cécile Macquin[1,2], Aurore Morlon[10], Consuelo Sebastia Sancho[11], Patrizia Amati-Bonneau[12,13], Vincent Procaccio[12,13], Anne-Laure Mosca-Boidron[14], Nathalie Marle[14], Naël Osmani[1], Olivier Lefebvre[1], Jacky G. Goetz[1], Sule Unal[15], Nurten A. Akarsu[16], Mirjana Radosavljevic[1,2,3], Marie-Pierre Chenard[17], Fanny Rialland[18], Audrey Grain[18], Marie-Christine Béné[19], Marion Eveillard[19], Marie Vincent[20], Julien Guy[21], Laurence Faivre[22], Christel Thauvin-Robinet[22], Julien Thevenon[22], Kasiani Myers[23], Mark D. Fleming[24], Akiko Shimamura[25], Elodie Bottollier-Lemallaz[26], Eric Westhof[6], Claudia Lengerke[4,27*], Bertrand Isidor[20,28*], Seiamak Bahram[1,2,3*]

SUPPLEMENTAL TABLES

Supplemental Table 1 : Clinical characteristics of the patients

	AII.1	BII.1	CII.1	« Classical » Shwachman-Diamond syndrome[A]
Hematologic manifestations				
Blood				
Absolute neutrophil count, 10^9/L (normal: 1.7–9.7)[B]	0 - 0.28	0 - 0.19	0 - 2.92	90% display neutropenia (46% severe, <0.5)
Hemoglobin, g/dL (normal: 11.5–15.5)[C]	15.8	8.0	10.6	46% have anemia
Platelets, 10^9/L (normal: 150–450)[D]	265	231	342	42% have thrombocytopenia
Allo-immunization against neutrophils/granulocytes	no	no	no	no
Bone marrow[3]				
Biopsy cellularity, %	70	40	80	variable
Erythrocytic lineage, % (normal: 10-32)	11	5	32	variable
Neutrophil lineage, % (normal: 30-70)	5	21	0	variable
Lymphoid lineage, % (normal: 6-25)	48	62	40	variable
Maturation retardation at promyelocytic stage	yes	yes	yes	no
Ring sideroblast (iron staining)	no	no	no	no
Cytoplasmic vacuoles in myeloblasts/promyelocytes	yes	yes	no	no
Treatment				
G-CSF	Yes, absence of effect	Yes, absence of effect	Yes, response at 5 mcg/kg QOD	+/-
Hematopoietic cell transplantation	At 4 years of age, genoidentical unrelated allogeneic transplantation	At 1 year of age, unrelated cord blood transplantation	No	+/-
Pancreatic dysfunction				98% of patients
Diarrhea /constipation	Intermittent diarrhea	no	constipation	yes
Feeding difficulties	yes	yes	no	+/-
Steatorrhea (normal: 1-3 g/24 h)	3.4 - 4	normal	normal	+/-
Fecal elastase (normal: > 200 µg/g of stool)	< 15	< 15	> 500	Usually low
Lipase (normal: 25-110 U/L)	9	35	33	Usually low
Total amylase (normal: AII.1: 30-119 U/L; BII.1: 1-47 U/L; CII.1: 30-110 U/L)	39	4	39	Usually low
Pancreatic isoamylase (normal: AII.1: 8-34 U/L; BII.1: 2-28 U/L; CII.1: 12-52 U/L)	N/A[E]	N/A	16	Below norm if >age 3years
Pancreatic Enzyme Replacement Therapy	Creon® (12 000 UI/day) for life	no	no	+/- pancreatic enzyme supplementation
Neurological manifestations				
Autism spectrum disorder	yes	N/A (mild neuropsychological	yes	+/-

		symptoms observed but not autism before patient's death at 16 months)		
Skeletal manifestations				70% of patients
Short stature	yes	yes	initial height 13%ile, on GCSF 30%ile	66% of patients
Metaphysal dysostosis	no	no	no	53% of patients
Bone demineralization	no	yes	no (normal prior to G-CSF)	yes
Other manifestations				
Liver (elevated transaminases)	no	yes	no	61% of cases
Cardiovascular manifestations	inter-auricular and inter-ventricular septal defects	N/A	no	+/-
Rib cage abnormalities	Yes, *pectus carinatum*	no	no	yes
Teeth abnormalities	Small teeth	no	no	+/-
Other	low set asymmetric ears, thinning hair, frontal angioma, mandibular microretrognathism, high arched palate	N/A	premature adrenarche, XR bone age hand & wrist 2.5 SD advanced	variable congenital anomalies
Other features with normal results				
Clinical examination	Cerebral MRI	Cerebral MRI, abdominal and cardiac echographies	XR skeletal survey	-
Genetic testing	CGH array (180k Agilent), mitochondrial DNA sequencing, targeted sequencing of SBDS, NGS sequencing of a panel of 18 IBMFs genes	CGH array (180k Agilent), mitochondrial DNA sequencing, targeted sequencing of SBDS, NGS sequencing of a panel of 18 IBMFs genes	chromosomal microarray (CMA v7.2 105k), targeted sequencing of *SBDS*, *HAX1*, *WAS* and *COL11A1*, Fragile X repeats and sequencing, NGS sequencing of a panel of 21 IBMFs genes	-
Outcome	Alive, 6 years of age	Died, 16 months	Alive, 18 years of age	-

[A] extracted from a meta-analysis reported in Table 7-7 in Orkin et al. 2015.
[B] Observed range during evaluation of neutropenia, prior to treatment with G-CSF
[C] Mean values
[D] Measured pre-transplantation in the transplanted patients AII.1 and BII.1
[E] Information not available

Reference:

Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, and Lux S. *Nathan and Oski's Hematology and Oncology of Infancy and Childhood.* 2015.

Supplemental Table 2. Filtered variants uncovered by trio whole exome sequencing in family A, B and C

	Family	Gene name	Transcript	Exon/Intron	Chrom.	Position	cDNA	Amino acid	Zygo-sity	Inheritance	Category	References	Disease	Inheritance
Variants related to the patient's clinical phenotype	C	*DFNA5*	NM_004403	exon8	7	24745812	c.1173_1174insC	p.A392fs	Het	Father	Pathogenic	Seen in ExAc database	Deafness, autosomal dominant 5 [MIM:600994]	AD
	C	*CDH23*	NM_022124	exon26	10	73468926	c.3178C>T	p.R1060W	Het	Father	VUS	PMID 12075507, 24082139, 16679490; rs201536811; In trans with [c.8065-5C>A]	Deafness, autosomal recessive 12 [MIM:601386]; Usher syndrome, type 1D [MIM:601067]	AD/AR
	C	*CDH23*	NM_022124	intron54	10	73565920	c.8065-5C>A	N/A	Het	Mother	VUS	Seen in ExAc database; In trans with [p.R1060W]	Deafness, autosomal recessive 12 [MIM:601386]; Usher syndrome, type 1D [MIM:601067]	AD/AR
***de novo* variants In non disease-causing genes**	A	*SRP54*	NM_003136	exon8	14	35482592	c.677G>A	p.G226E	Het	De novo	N/A	Novel variant	N/A	N/A
	B	*SRP54*	NM_003136	exon4	14	35476576	c.343A>G	p.T115A	Het	De novo	N/A	Novel variant	N/A	N/A
	C	*OXA1L*[A]	NM_005015	exon10	14	23240651	c.1372C>T	p.R458X	Het	De novo	N/A	Novel variant	N/A	N/A
	C	*SRP54*	NM_003136	exon4	14	35476574	c.349_351del	p.T117del	Het	De novo	N/A	Novel variant	N/A	N/A
Compound heterozygous variants														
	A	*RELN*	NM_005045	exon34	7	103205868	c.5067C>A	p.N1689K	Het	Father	N/A	Novel variant	Lissencephaly 2 [MIM:257320]; Epilepsy [MIM:616436]	AR/AD
	A	*RELN*	NM_005045	exon30	7	103214590	c.4460G>T	p.G1487V	Het	Mother	N/A	Novel variant	Lissencephaly 2 [MIM:257320]; Epilepsy [MIM:616436]	AR/AD
In genes not related to the patient's clinical phenotype	C	*COL6A3*	NM_004369	exon38	2	238249550	c.8009C>T	p.A2670V	Het	Father	N/A	PMID 25380242; rs142851023	Bethlem myopathy 1 [MIM:158810]; Dystonia 27 [MIM:616411]; Ullrich congenital muscular dystrophy 1 [MIM:254090]	AD/AR
	C	*COL6A3*	NM_004369	exon38	2	238249370	c.8189C>A	p.A2730D	Het	Mother	N/A	rs138466455	Bethlem myopathy 1 [MIM:158810]; Dystonia 27 [MIM:616411]; Ullrich congenital muscular dystrophy 1 [MIM:254090]	AD/AR
In non disease-causing genes	B	*A2ML1*	NM_144670	exon8	12	8990121	c.814C>T	p.R272W	Het	Father	N/A	rs201215628	N/A	N/A
	B	*A2ML1*	NM_144670	exon30	12	9020598	c.3878A>G	p.N1293S	Het	Mother	N/A	rs201478459	N/A	N/A

	B	*SLC12A4*	NM_001145962	exon18	16	67980085	c.2602C>T	p.R868C	Het	Father	N/A	Seen in ExAc database	N/A	N/A
	B	*SLC12A4*	NM_001145962	exon9	16	67984928	c.1339C>T	p.R447C	Het	Mother	N/A	rs200784647	N/A	N/A
	C	*CCDC168*	NM_001146197	exon4	13	103399761	c.3286C>A	p.P1096T	Het	Mother	N/A	rs143261898; in trans with [p.Y4116N]	N/A	N/A
	C	*CCDC168*	NM_001146197	exon4	13	103390701	c.12346T>A	p.Y4116N	Het	Father	N/A	rs201767274; in trans with [p.P1096T]	N/A	N/A
	C	*CFAP46*	NM_001200049	exon12	10	134736204	c.1265C>T	p.T422M	Het	Father	N/A	rs140185143; in trans with [p.R2657C]	N/A	N/A
	C	*CFAP46*	NM_001200049	exon58	10	134622104	c.7969C>T	p.R2657C	Het	Mother	N/A	rs148457914; in trans with [p.T422M]	N/A	N/A
	C	*CNDP2*	NM_018235	exon2	18	72167187	c.-22C>T	N/A	Het	Father	N/A	Novel variant; in trans with [p.A465T]	N/A	N/A
	C	*CNDP2*	NM_018235	exon12	18	72187268	c.1393G>A	p.A465T	Het	Mother	N/A	rs144157993; in trans with [c.-22C>T]	N/A	N/A
	C	*MDM4*	NM_002393	exon11	1	204518457	c.1120A>C	p.K374Q	Het	Father	N/A	PMID 25996639; rs41299595; in trans with [c.-22C>T]	N/A	N/A
	C	*MDM4*	NM_001278516	exon6	1	204507384	c.459C>T	p.D153D	Het	Mother	N/A	Seen in ExAc database; in trans with [c.-22C>T]	N/A	N/A
Hemizigous variants														
In genes not related to the patient's clinical phenotype	C	*ANOS1*	NM_000216	exon2	X	8667780	c.214G>A	p.G72S	Hem	Mother	N/A	rs186630563	Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1) [MIM:308700]	X-linked
In non disease-causing genes	C	*MAGEB17*[B]	NM_001277307	exon2	X	16189074	c.569T>A	p.L190X	Hem	Mother	N/A	Novel variant	N/A	N/A
	C	*YY2*	NM_206923	exon1	X	21875099	c.497C>T	p.T166M	Hem	Mother	N/A	Novel variant	N/A	N/A
	C	*DCAF8L2*	NM_001136533	exon1	X	27765065	c.53G>A	p.S18N	Hem	Mother	N/A	rs183301101	N/A	N/A
In non disease-causing genes	C	*RGAG4*	NM_001024455	exon1	X	71350598	c.793T>G	p.L265V	Hem	Mother	N/A	Seen in ExAc database	N/A	N/A
	C	*ZXDA*	NM_007156	exon1	X	57936736	c.119C>T	p.P40L	Hem	Mother	N/A	Seen in ExAc database	N/A	N/A

[A] 93 loss of function (mostly p.A52fs) are known in the internal exome database of Baylor College of Medicine without any overlapping clinical phenotype (no neutropenia), including this case and including one homozygous.

[B] 2 hemizygous loss of function (and 3 female het) are known in the internal exome database of Baylor College of Medicine without any overlapping clinical phenotype, including this case. 7 hemizygous LOF individuals reported in ExAC.

Supplemental Table 3: Pathogenic CNVs encompassing *SRP54* as reported in public databases

Database	Variant ID	Variant	Type	Size	Clinical Assertion	Phenotype(s)	References
Decipher[A]	286741	14:29639741-35604711	copy number loss	5.96 Mb	Definitely pathogenic	Agenesis of corpus callosum, Hypertonia, Intellectual disability, Postnatal microcephaly, Short stature	-
Decipher	289774	14:35007710-44901392	copy number loss	9.89 Mb	Definitely pathogenic	Anal stenosis, Congenital hypothyroidism, Dry skin, Frequent falls, Generalized muscle weakness, Global developmental delay, Intellectual disability, Retinal dystrophy	-
Decipher	300434	14:33974276-37707521	copy number loss	3.73 Mb	Definitely pathogenic	Global developmental delay	-
Decipher	337740	14:33086546-44485372	copy number loss	11.40 Mb	Definitely pathogenic	Macroglossia, Neonatal hypotonia	-
dbVar[B]	nsv2778054	14:19794561-107234280	copy number gain	87.44 Mb	Pathogenic	Developmental delay AND/OR other significant developmental or morphological phenotypes	(1)
dbVar	nsv2775779	14:34049147-49348823	copy number loss	15.3 Mb	Pathogenic	Global developmental delay	(1)
dbVar	nsv2772240	14:23164384-54733411	copy number gain	31.57 Mb	Pathogenic	Delayed gross motor development; Intellectual disability	(1)
dbVar	nsv996197	14:20511672-49111245	copy number gain	28.6 Mb	Pathogenic	Abnormal facial shape; Failure to thrive; Flexion contracture; Global developmental delay; Hypothyroidism; Seizures; Short neck	(1)
dbVar	nsv996041	14:34349618-42828688	copy number loss	8.48 Mb	Pathogenic	Delayed gross motor development; Delayed speech and language development; Morphological abnormality of the central nervous system; Muscular hypotonia	(1)
dbVar	nsv995182	14:20511673-107285437	copy number gain	86.77 Mb	Pathogenic	Abnormal facial shape; Abnormality of cranial sutures; Ectrodactyly; Hearing impairment; Jaundice; Retrognathia; Sandal gap; Short stature; Sparse hair; Thrombocytopenia; Ventricular septal defect	(1)
dbVar	nsv931929	14:20490852-44562875	copy number gain	24.07 Mb	Pathogenic	Cleft palate; Cleft upper lip; Delayed gross motor development; Intellectual disability; Intrauterine growth retardation; Talipes equinovarus; Transposition of the great arteries	(1)
dbVar	nsv917265	14:31261477-45154334	copy number loss	13.89 Mb	Pathogenic	Abnormal facial shape; Abnormality of mouth size; Cupped ear; Epicanthus; Gastroesophageal reflux; Global developmental delay; Hearing impairment; Infantile axial hypotonia; Inguinal hernia; Limb dystonia; Prominent forehead	(1)
dbVar	nsv532465	14:20619108-40215358	copy number gain	19.6 Mb	Pathogenic	Developmental delay AND/OR other significant developmental or morphological phenotypes	(2)
dbVar	nsv532462	14:20468770-39453619	copy number gain	18.98 Mb	Pathogenic	Craniofacial Abnormalities; Hypertonia; Intellectual disability	(2)
dbVar	nsv531003	14:31139520-45459798	copy number gain	14.32 Mb	Pathogenic	Coarse facial features	(2)
dbVar	nsv530375	14:20619308-	copy	86.64	Pathogenic	Global developmental delay	(1)

		107263478	number gain	Mb			
dbVar	nsv530014	14:34920140-37349356	copy number loss	2.43 Mb	Pathogenic	Developmental delay AND/OR other significant developmental or morphological phenotypes	(2)
dbVar	nsv530012	14:24018169-42452605	copy number loss	18.43 Mb	Pathogenic	Developmental delay AND/OR other significant developmental or morphological phenotypes	(2)
dbVar	nsv530007	14:20665104-45754005	copy number loss	25.09 Mb	Pathogenic	Global developmental delay	(2)
dbVar	nsv498512	14:30851760-38181546	copy number loss	7.33 Mb	Pathogenic	Developmental delay AND/OR other significant developmental or morphological phenotypes	(1)

[A] DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources , DECIPHER; https://decipher.sanger.ac.uk/application

[B] Database of Structural Variation, dbVAR; http://www.ncbi.nlm.nih.gov/dbvar

References

1. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *American journal of human genetics.* 2010;86(5):749-64.
2. Kaminsky EB, Kaul V, Paschall J, Church DM, Bunke B, Kunig D, et al. An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2011;13(9):777-84.

Supplemental Table 4. Possibly pathogenic variants of *SRP54* reported in ExAc

Chrom.	Position	Variant	Frequency	Allele count	Number of hom.	GERP++_NR	LRT	Mutation Assessor	Mutation Taster	PhyloP	Polyphen2_HDIV	rsID	Effect
14	35465929	A>G	0.000008242	1	0	6.04	0	2.995	1	1.178	0.983	rs777914961	missense
14	35465938	G>T	0.000008242	1	0	6.04	0	3.465	1	1.03	1	rs771241246	missense
14	35465939	A>T	0.000008242	1	0	6.04	0	1.675	1	1.178	1	rs774740121	missense
14	35465962	C>T	0.00002473	3	0	6.17	0	3.135	1	0.92	0.996	rs775147862	missense
14	35468768	T>C	0.000008316	1	0	5.64	1.00E-06	3.585	1	0.964	1	rs766140384	missense
14	35477846	A>T	0.0001027	12	0	5.03	1.00E-06	1.16	0.999999	1.135	1	rs191230389	missense
14	35477881	G>T	0.000008406	1	0	5.03	0	2.865	1	0.993	1	rs761089126	missense
14	35477980	G>C	0.000008288	1	0	5.03	0	1.05	1	0.993	1	rs775741587	missense
14	35478009	A>T	0.000008314	1	0	5.03	0	1.5	1	1.135	[0.999, 0.992]	rs764460322	missense
14	35480822	A>G	0.000008255	1	0	5.81	0	3.92	1	1.14	1	rs766018104	missense
14	35480840	A>T	0.000008264	1	0	5.81	0	5.2	1	1.14	1	rs369651041	missense
14	35482589	T>C	0.00001648	2	0	5.5	0	1	1	1.061	[0.999, 0.998]	rs757036271	missense
14	35482693	C>G	0.000008242	1	0	5.65	0	2.315	0.999999	0.935	[0.986, 0.95]	rs750331626	missense
14	35483018	A>G	0.000008255	1	0	5.76	0	3.475	1	1.199	[0.928, 0.873]	rs778122134	missense
14	35483028	C>T	0.00001649	2	0	5.76	0	5.2	1	0.935	0.999	rs749487051	missense
14	35483058	T>C	0.00000824	1	0	5.76	0	2.29	1	1.061	[0.938, 0.891]	rs776316751	missense
14	35487898	G>A	0.000008274	1	0	5.77	0	2.98	1	0.998	1	rs755358034	missense
14	35492205	G>T	0.00000824	1	0	5.54	0	3.09	1	1.048	[0.967, 0.942]	rs770936481	missense
14	35492208	G>A	0.00000824	1	0	5.54	0	2.78	1	1.048	0.751	rs374841884	missense

Supplemental Table 5. Primers used for PCR amplifications and Sanger sequencing of *SRP54*

Exons	Identity	Sequence (5' -> 3')
Exon 1	SRP54-ex1-F	ACAGGTTGAGGCTATGGAAGGTG
	SRP54-ex1-R	AGCCATCTTCCCAATGCTAGTGAC
Exon 2	SRP54-ex2-F	ACCATGCCGGGCCTATAATTACTT
	SRP54-ex2-R	CACTTCCAGTTAGCACCTCTTTCCA
Exon 3	SRP54-ex3-F2	GTCTGTAGTAATTACATGCATGTTAATTGGAAGAC
	SRP54-ex3-R2	CCACTGAGTATAGAATCACAGCATAATCTTC
Exon 4	SRP54-ex4-F4	GGGTTTTGAATTTATATGTATGTTAACCTGATT
	SRP54-ex4-R4	ACAAAACTTGATTTATACAAACCTATCTTCCAT
Exons 5,6	SRP54-exs5-6-F	GAATGCTACTGCATGACAATTAGAATATAAAACTTTGTAGG
	SRP54-exs5-6-R	GCTGACAGACAAGAAGAGGGATATAAGTG
Exon 7	SRP54-ex7-F	TTGAAATTGGGGTCATTTTGGCTTT
	SRP54-ex7-R	ATTACTGGGGATCTGCCTCACA
Exon 8	SRP54-ex8-F	GCCACTGCACCTGGCTCTAAATA
	SRP54-ex8-R	TCCCAAGACAGAGGACAACAGTATC
Exon 9	SRP54-ex9-F	TAGCTTTGGAGGCGGATTCACT
	SRP54-ex9-R	ACCACAGAGGGCAGATAACCATT
Exon 10	SRP54-ex10-F	CCTGGCCATGCCACTTAACTTT
	SRP54-ex10-R	CTGTAACTGCTCAGGTACCTGAGA
Exons 11,12	SRP54-ex11-12-F2	GATAGGACCTGTCTTTGTCTATGTATTCTCCC
	SRP54-ex11-12-F2	GCCTGAGGAAAATAATCTGCCATCTTAC
Exon 13	SRP54-ex13-F	TTGCACATAACTGCTTTGATGGTGA
	SRP54-ex13-R	TTTTCCAATACTGGCAGCACTCTTT
Exon 14*	SRP54-ex14-F	CCTCCTACGCTGACTCAAAATCTTT
	SRP54-ex14-R	AAACTGCATTAGCAACTGCCAAC
Exon 15	SRP54-ex15-F	TCGCAATAACTCCGTAAGATAGGCT
	SRP54-ex15-R	GAAACGCTGAGGTCTCAGCAAA

* Sequencing primers were the same as amplification primers, except for exon 14 where the sequencing primers were the following : forward (SRP54-ex14A-F): TGACTCAAAATCTTTTTTTTTTTCCCCTCAG and reverse (SRP54-ex14A-R): GCAACTGCCAACAACTTGGTATTTAC.

NB.: The following primers were used to confirm the variant in patient CII.1: GCCTGGGTGACAAGAGTGTG and CAAACCTATCTTCCATATGACTTCTTTTT.

SUPPLEMENTAL METHODS

MITOCHONDRIAL DNA ANALYSIS

The whole mitochondrial genome was amplified in two overlapping fragments of 8009 bp (amplicon A) and 8994 bp (amplicon B). The primer pairs used for both PCRs - tested on DNA extracted from cells devoid of mtDNA-(Rho Zero cells) to avoid pseudogene amplification - were located on the cytochrome B and cytochrome c oxidase 1 genes: 5'-TACGTTGTAGCCCACTTCCACT-3' and 5'-GCCCGATGTGTAGGAAGAG-3' for amplicon A; 5'-AACTTCGGCTCACTCCTTGG-3' and 5-'AGTAACGTCGGGGCATTCCG-3' for amplicon B. Long-range PCR was performed with KapaLongRange DNA polymerase according to the manufacturer's recommendations (Kapa Biosystems, Boston, MA). The concentrations of PCR products were quantified by the Qubit® 2.0 Fluorometer using the Qubit® dsDNA HS Assay Kit (Thermo Fischer technologies, Waltham, MA). For each sample, amplicons A and B were pooled in an equimolar manner. Libraries were compiled from 50-200 ng of pooled PCR products with the enzymatic fragmentation method of the Library Builder Automate using the dedicated Ion Xpress™ Plus Fragment Library Kit (Thermo Fischer technologies). The enriched mtDNA samples were sequenced using an Ion Proton high-throughput sequencing platform according to the manufacturer's protocols (Thermo Fischer technologies). Sequencing reads were mapped to an mtDNA reference sequence (NC_012920) before being analysed with Mitomaster software (http://www.mitomap.org/MITOMAP) and a dedicated in-house pipeline integrating various modules for coverage analysis, variant calling, annotation and prioritization.

KNOCK-DOWN OF *SRP54* BY SHRNA IN CELL LINES.

HL60 is a human cell line derived from a patient with acute promyelocytic leukemia (ATCC® CCL-240™). Cells were grown in suspension in RPMI 1640 medium (ThermoFisher Scientific, Waltham, MA) supplemented with GlutaMAX™, 1% penicillin/streptomycin, 1 mM Sodium Pyruvate, and 10% heat-inactivated fetal bovine serum. HeLa (ATCC® CCL-2™) is a human

cell line derived from cervix adenocarcinoma. HeLa cells were grown in DMEM medium (ThermoFisher Scientific, Waltham, MA) supplemented with GlutaMAX™, 1% penicillin/streptomycin, and 10% heat-inactivated fetal bovine serum. All cultures were maintained at 37°C in a 5% CO_2-humidified atmosphere. HeLa and HL60 cells were transfected using Lipofectamine™ 3000 Reagent (ThermoFisher Scientific, Waltham, MA). Twenty four hours prior to transfection, the cells were seeded in a 6-well plate at a cell density of 0.25 x 10^6 cells per well. First 3.75 µl of Lipofectamine™ 3000 were added to 125 µl of Opti-MEM™ medium, followed by addition of a mix containing 5 µg shRNA in 125 µl of Opti-MEM™ medium. The mixture was incubated at room temperature for 10 min and then added to the cells. In each case, the entire solution was added to the cells and mixed by gently swirling the plate. The plate was incubated at 37°C for 72 h in a 5% CO_2 incubator. The sequence of shRNA directed against *SRP54* was GAGGAAAGGUUGGAAGACCUGUUUA (Thermo Fisher Scientific). The control used was the Stealth RNAi Negative Control Duplex medium GC (45-55%) (Reference 10143902, ThermoFisher Scientific, Waltham, MA). Cells from each well were collected, and after centrifugation, washed once in D-PBS. The final pellet was suspended in 60 µl of lysis buffer (150mM NaCl, 1% NP40, 50mM Tris pH 8) including protease inhibitors (cOmplete, Roche Diagnostics GmbH) and left on ice for 20 min. The total cellular extract was centrifuged 30 min at 13000 rpm to remove cell debris. A Bradford assay was performed for quantifying proteins (BIO-RAD laboratories, Hercules, CA).

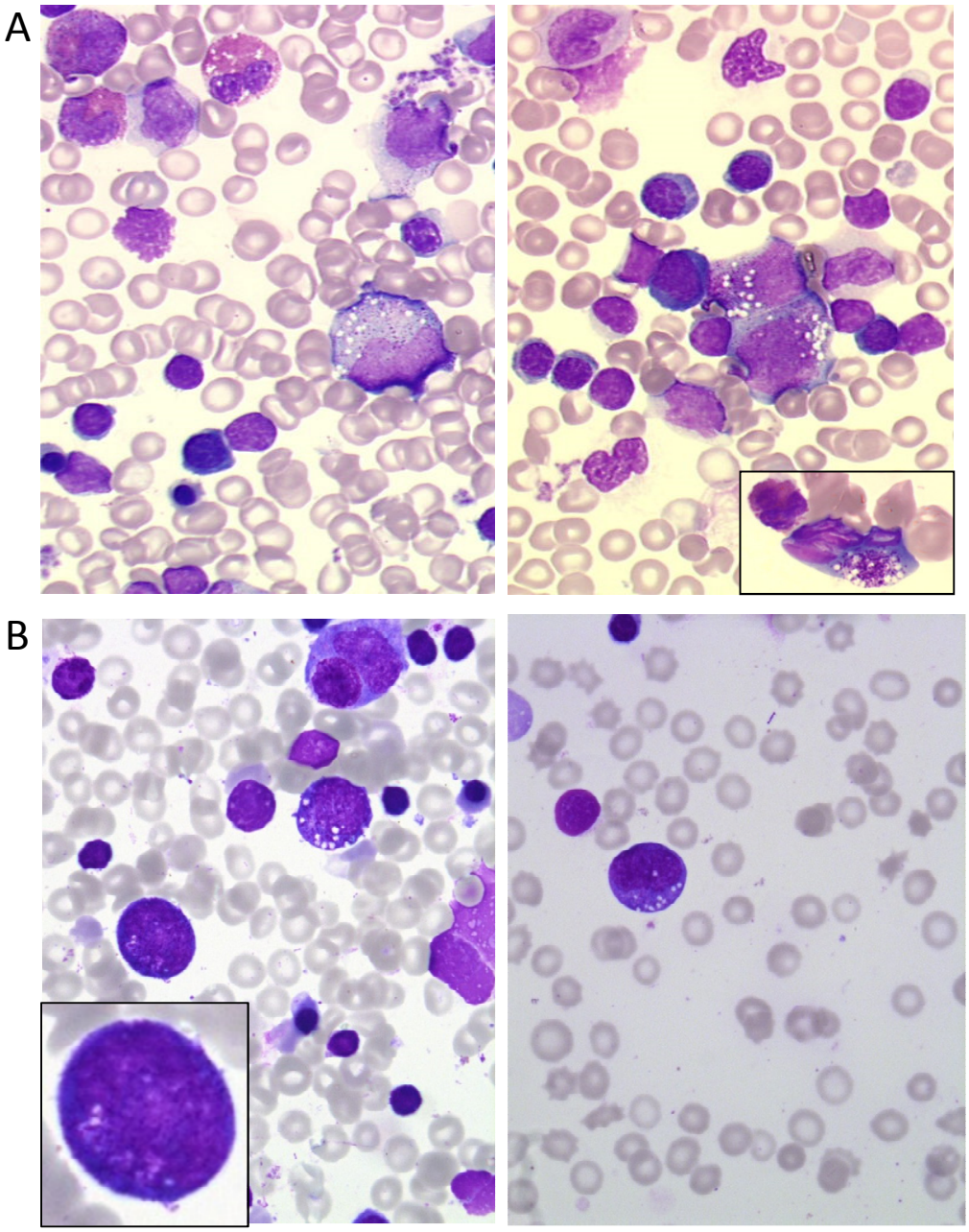
For Western blotting, 20 µg of cellular total extracts were loaded on 8% polyacrylamide gels (for mTOR and phospho-mTor), 12% acrylamide gels (for SRP54 and GAPDH) and 10% acrylamide gels (for DNAJC21 and SBDS). After migration at 150V, proteins were transferred onto PVDF membrane via semi-dry transfer (Trans-Blot, BIO-RAD laboratories, Hercules, CA). Membranes were blocked for 1h in 5% skimmed milk, TBS 0.1% Tween®20 (for mTOR and phospho-mTor) and 5% skimmed milk, PBS 0.05% Tween®20 (for SRP54, GAPDH, DNAJC21 and SBDS). Anti-mTOR (7C10, Cell Signaling, Danvers, MA) and anti-phospho mTOR Ser2448 (D9C2, Cell Signaling, Danvers, MA) antibodies were incubated overnight at

4°C in 5%BSA-TBS 0.1% Tween®20 at 1/1000 dilution. Anti-DNAJC21 (Proteintech, Rosemont, IL), anti-SBDS (ab 128946; Abcam, Cambridge, UK), anti-SRP54 (SC393855; Santa Cruz Biotechnology, Dallas, TX) were incubated overnight at 4°C in 5% skimmed milk PBS 0.05% Tween®20 at the following dilution : 1/1000 for DNAJC21, 1/10 000 for SBDS and 1/1000 for SRP54. Membranes were then incubated with secondary antibodies coupled to Horseradish peroxidase (HRP) (BIO-RAD laboratories, Hercules, CA). Antibodies were revealed with an enhanced chemioluminescence detection system using ChemiDoc XRS (BIO-RAD laboratories, Hercules, CA). Loading control was performed with an anti-GAPDH antibody (MAB374, Merck-Millipore, Molsheim, France).

SUPPLEMENTAL FIGURES LEGENDS

Embedded within figures

Supplemental Figure 1



Supplemental Figure 1. Bone marrow smears of patients AII.1 and BII.1 at diagnosis.

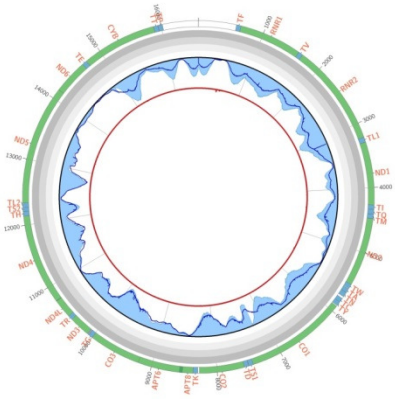
(A) Bone marrow smear of patient AII.1 was hypocellular with regards to age and disclosed a hypoplasic neutrophil lineage (5%) with retarded granulocytic maturation. By contrast, hypereosinophilia was observed at 8.5% as well as 13% monocytes. There were 39.5% lymphocytes and a subset of 10% hematogones. Myeloblasts and promyelocytes displayed numerous cytoplasmic vacuoles suggesting the onset of apoptosis, confirmed by a few images of apoptotic cells (right panel, lower right box). (B) Bone marrow smear of patient BII.1 showed a typical maturation arrest at the promyelocyte stage with presence of vacuolated promyelocytes. Other cell lineages were normal.

Supplemental Figure 2

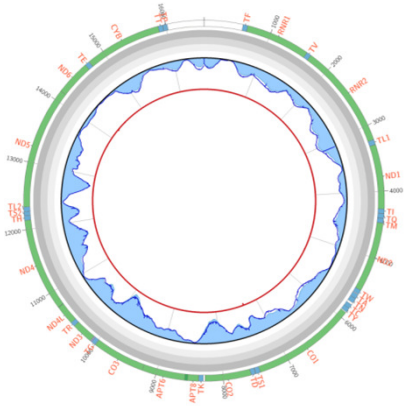
A

	Gene Locus	Sequence variation	Mutation type	Phylogenetic conservation (%)	Status	Patient haplogroup
Patient AII.1	D-Loop	m.513 G>GCACA	non-coding	-	Poly	K1d1
	D-Loop	m.567 A>ACCC	non-coding	-	Poly	
	MTCO3	m.9287 G>A	synonymous	-	Poly	
	MTCO3	m.9591 G>A	non-syn: p.Val129Ile	97	Poly	
	MTND4	m.10907 T>C	non-syn:p.Phe50Leu	13	Poly	
	MTND5	m.12501 G>A	synonymous	-	Poly	
	D-Loop	m.16184 C>T	non-coding	-	Poly	
	D-Loop	m.16223 CT>C	non-coding	-	Poly	
	D-Loop	m.16361 GT>G	non-coding	-	Poly	
Patient BII.1						
	MTCOX1	m.6719 T>C	synonymous	-	Poly	H1b
	MTND5	m.12618 G>A	synonymous	-	Poly	
	MTND5	m.13419A>G	synonymous	-	Poly	
	D-Loop	m.16265 A>G	non-coding	-	Poly	
	D-Loop	m.16390 G>A	non-coding	-	Poly	

B



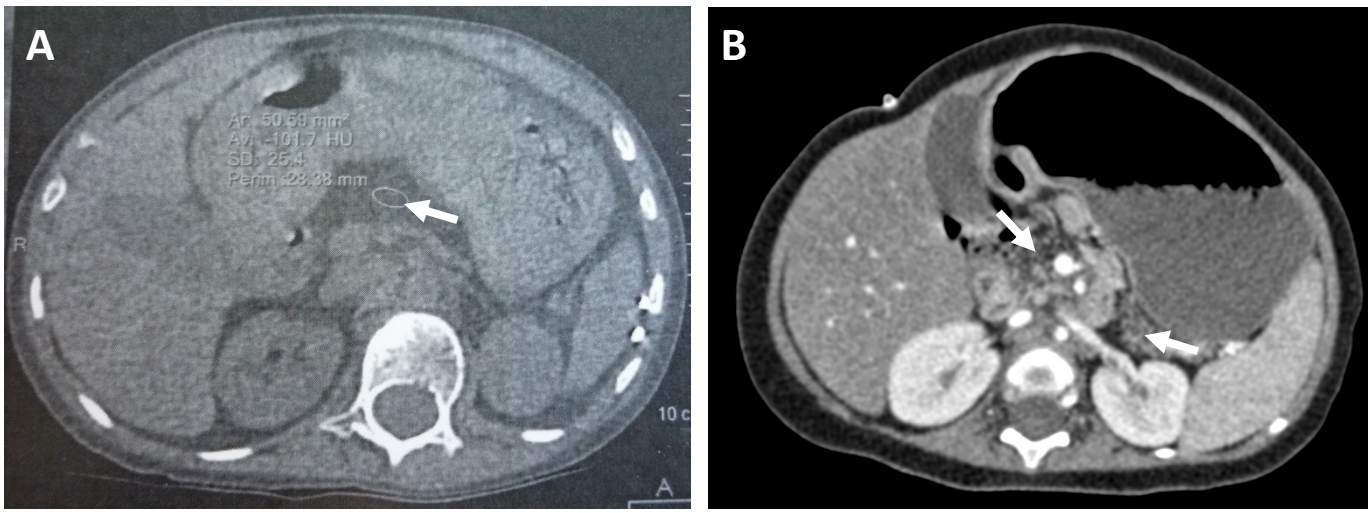
Patient AII.1



Patient BII.1

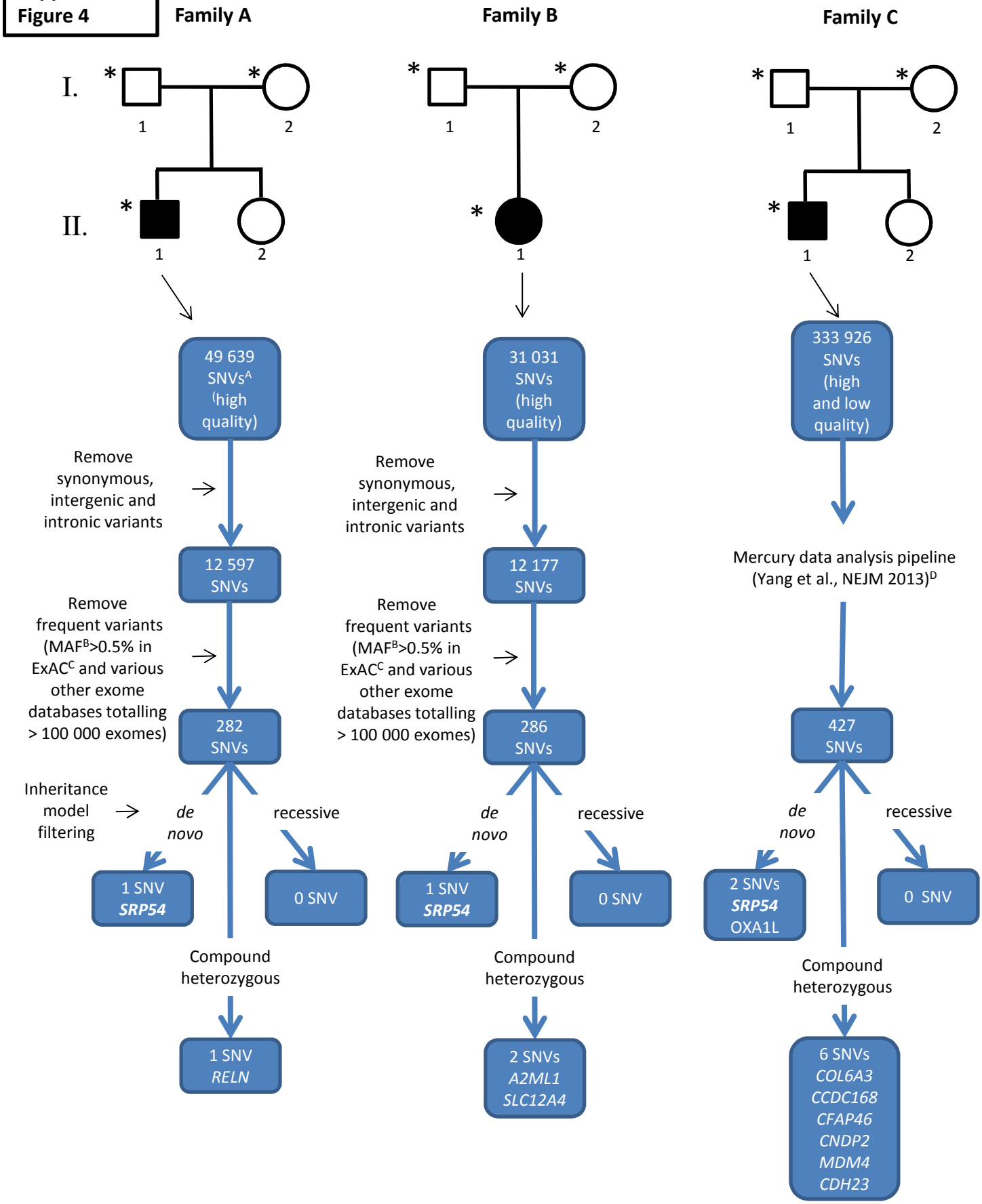
Supplemental Figure 2. Mitochondrial genome analysis.
(A) Mitochondrial DNA variants identified by Next-Generation Sequencing (NGS). The analysis did not reveal any pathogenic mutations or rearrangements of the entire mitochondrial DNA. **(B) Circos graphical representation of the circular whole mitochondrial genome.** The analysis of the mtDNA shows the absence of mtDNA rearrangements. In addition, long range PCR products loaded on an agarose gel did not reveal any mtDNA rearrangements (not shown).

Supplemental Figure 3



Supplemental Figure 3. Fatty infiltration of the pancreas in patients AII.1 and BII.1.
Panels A and B represent CT scans showing a fatty infiltration of the pancreas (white arrow) in case of patients AII.1 and BII.1, respectively.

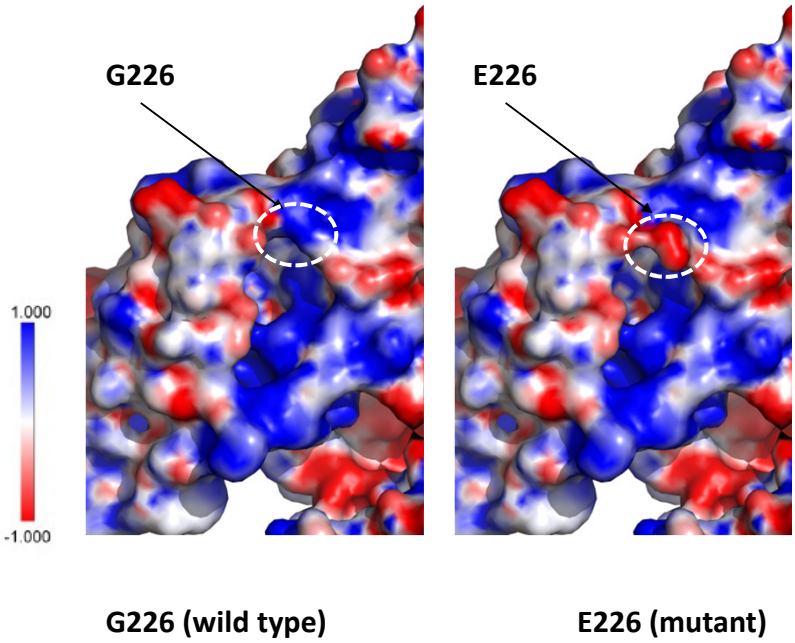
Supplemental Figure 4



Supplemental Figure 4. Variant filtering strategy in trio whole exome sequencing in families A, B and C.

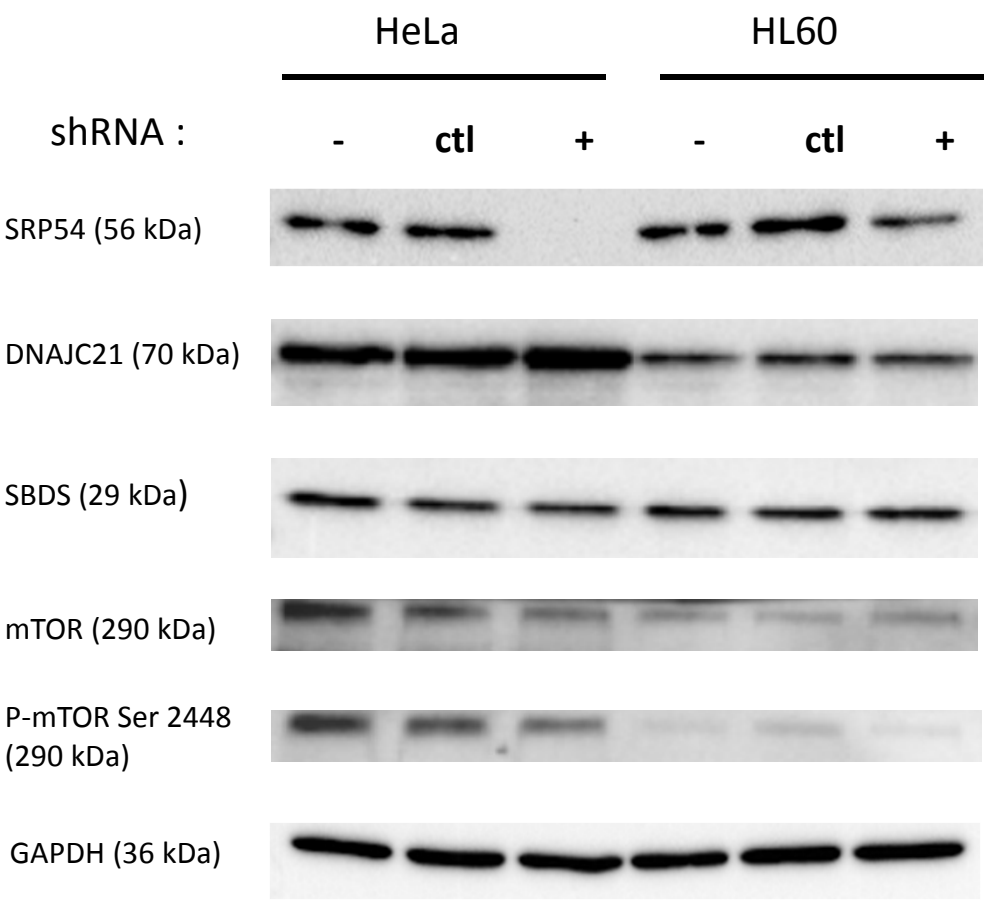
In pedigrees, black icons denote affected individuals. Asterisks indicate subjects who underwent exome sequencing. [A] SNV: single nucleotide variant. [B] Minor Allele Frequency. [C] The Exome Aggregation Consortium, ExAC; http://exac.broadinstitute.org. [D] Reference: Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. The New England Journal of Medicine. 2013;369(16):1502-11.

Supplemental Figure 5



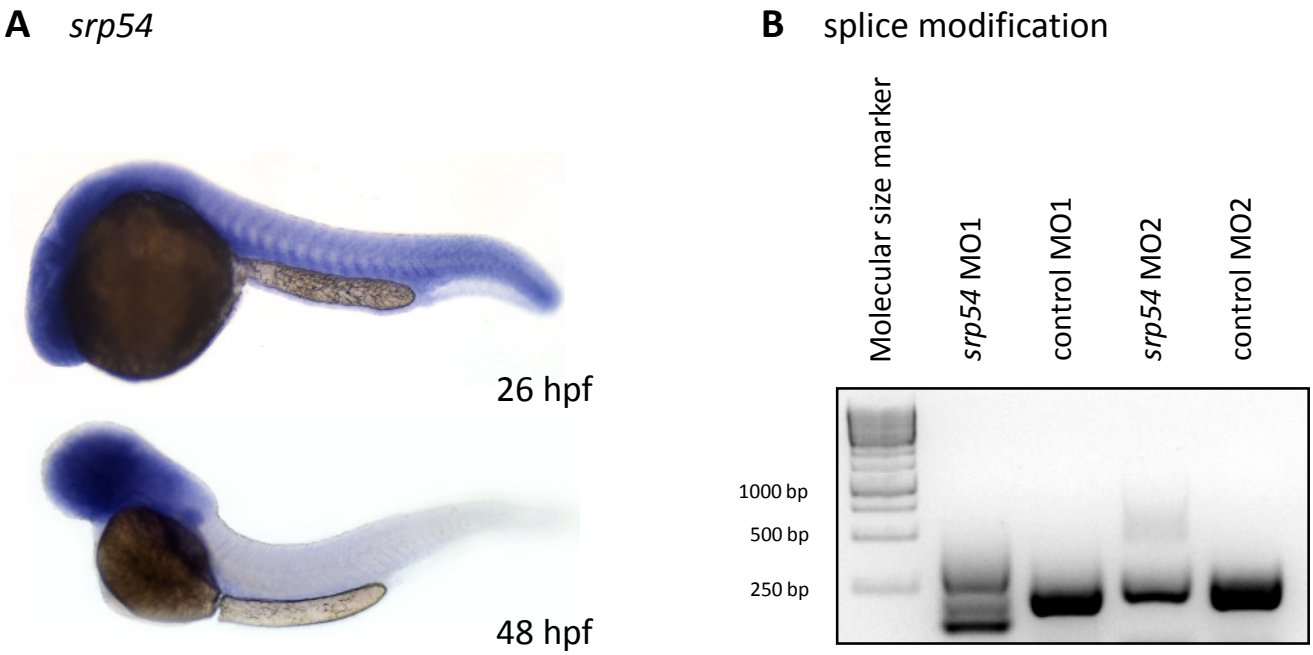
Supplemental Figure 5. Structural impacts of the SRP54 mutation p.G226E.
Electrostatic potential surface of SRP54 (based on PDB 2j37 chain W), centered on residue 226. The negative (red) and the positive charges (blue) are shown.

Supplemental Figure 6



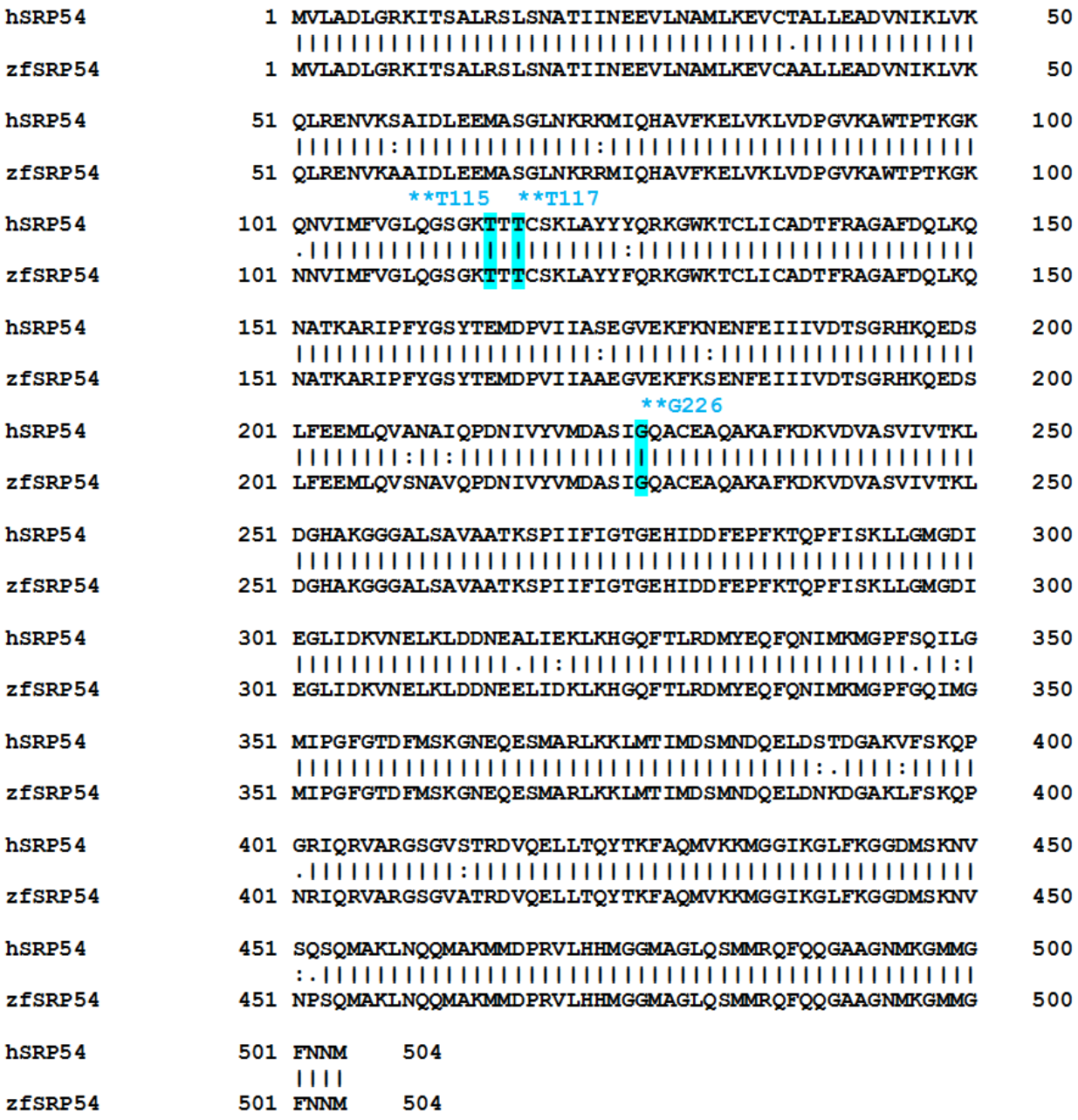
Supplemental Figure 6. Knockdown of *SRP54* by shRNA in two cell lines.
-: non-transfected control; ctl: negative shRNA control, +: shRNA targeting SRP54. In HL60, the silencing of *SRP54* was estimated at 72% as compared to the control shRNA using the Image Lab software (BioRad laboratories, Hercules, CA). SRP54 and GAPDH analyses were performed on the same gel/membrane. DNAJC21, SBDS, mTOR and P-mTOR analyses were done on different gels/membranes, but using identical quantities of material originating from the same original protein extract.

Supplemental Figure 7



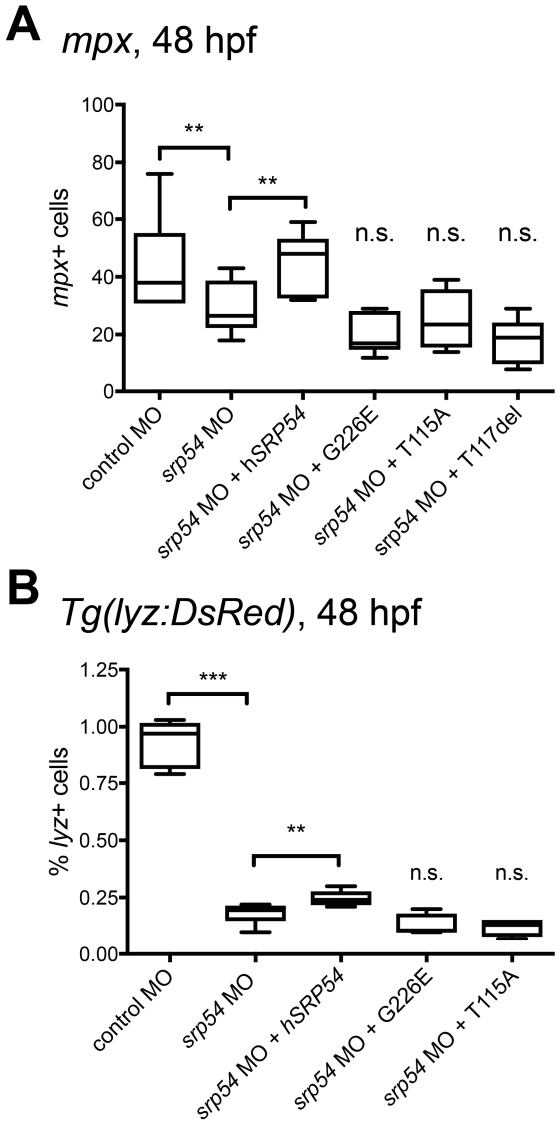
Supplemental Figure 7. Confirmation of *srp54* expression and splice modification after *srp54* MO injection.
(A) WISH of *srp54* showing ubiquitous expression at 26 hpf (upper panel) and decrease over time (48 hpf, lower panel). (B) RT-PCR of *srp54* in embryos injected with *srp54* MO1 or MO2 indicate splice modification.

Supplemental Figure 8



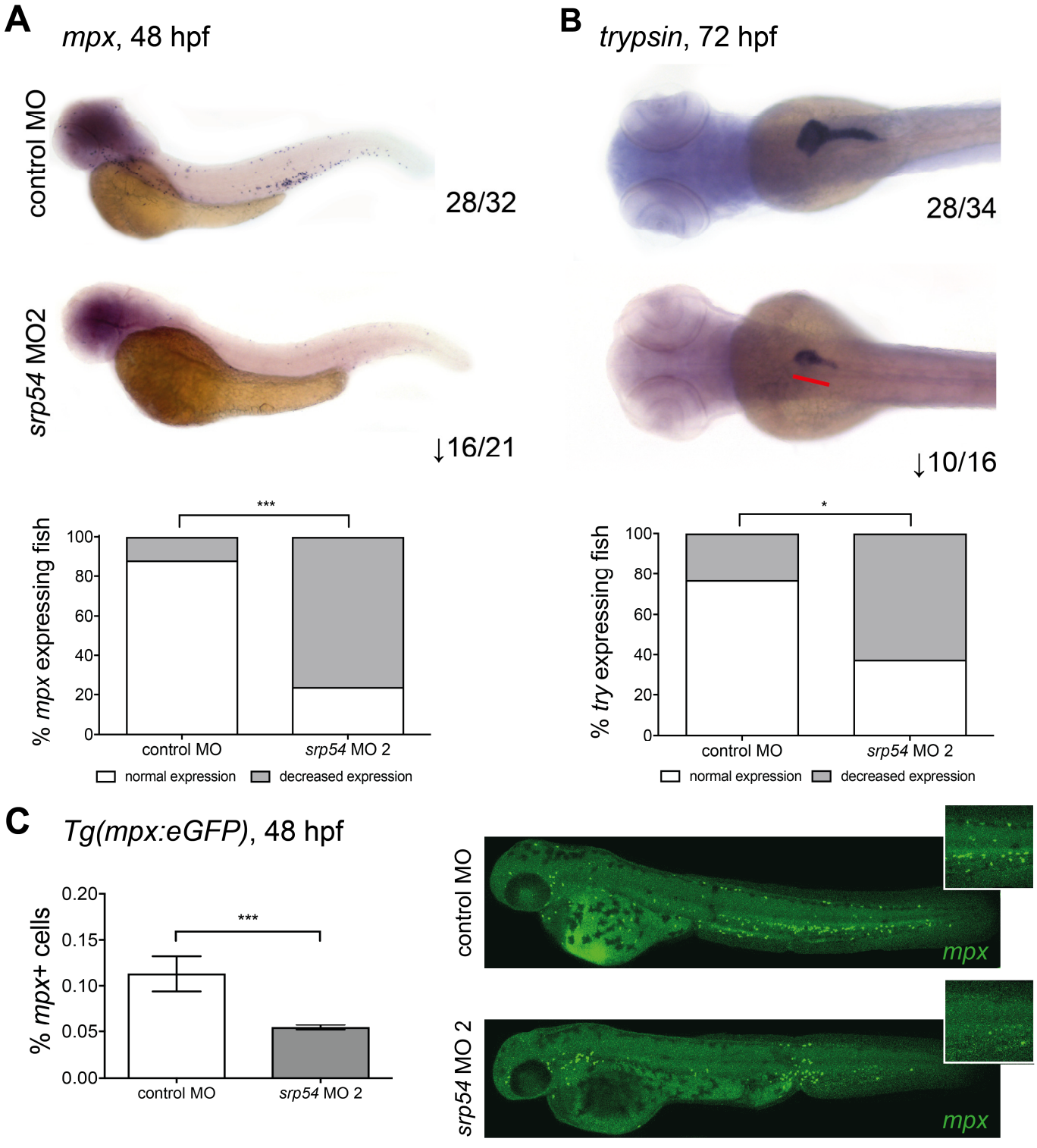
Supplemental Figure 8. Alignment of human and zebrafish SRP54 proteins.
The localization of the three mutations presented in this work are highlighted in blue.

Supplemental Figure 9



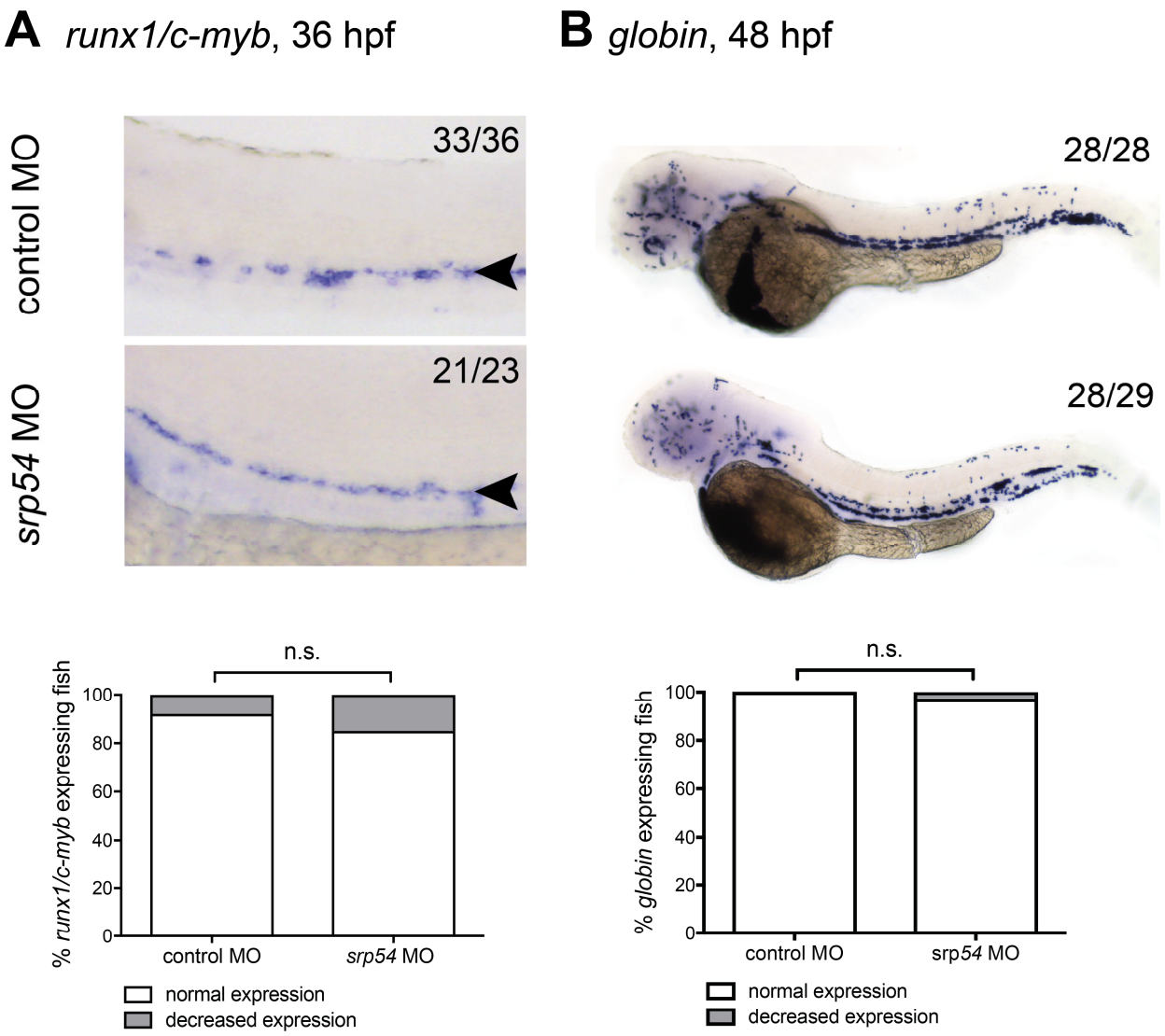
Supplemental Figure 9. Co-injection of mutated versions of *hSRP54* mRNA fails to rescue neutropenia in morphants.
(A) Semi-automated quantification of *mpx+* cells after WISH from control, *srp54* MO injected, MO + h*SRP54* mRNA, MO + G226E mRNA, MO + T115A mRNA and MO + T117del co-injected transgenic embryos. For each group, five or more embryos were analyzed. In **(B)**, percentages of positive fluorescence from dissociated *lyz+* cells are shown. A 1-way ANOVA with multiple comparisons was used to test for statistical significance and error bars are shown as ± standard deviation ($p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *). Four biological replicates with at least 5 embryos per experiment were performed.

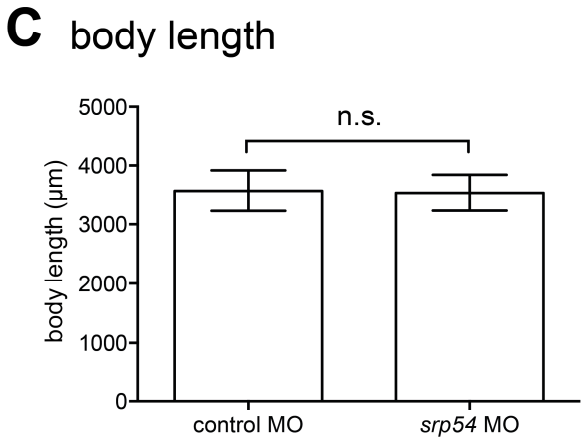
Supplemental Figure 10



Supplemental Figure 10. Effects of *srp54* gene knockdown in zebrafish embryos by using an alternative morpholino oligonucleotide (MO2).
(A) WISH of *mpx* at 48 hpf in control injected (upper) and *srp54* morpholino 2 (MO2) injected (lower) zebrafish embryos. **(B)** WISH of *trypsin* at 72 hpf in control injected (upper) and MO2 injected (lower) zebrafish embryos. A Fisher's exact test was performed to test for statistical significance. **(C)** Flow cytometry quantification of dissociated Tg(*mpx:eGFP*) embryos at 48 hpf confirming results from (A). Percentages of positive fluorescence in both dissociated control and *srp54* MO injected transgenic embryos are shown. On the right, representative pictures of control and *spr54* MO injected Tg(*mpx:eGFP*) embryos are depicted.
In A & C, lateral views are shown, with anterior to the left, dorsal up. In B, dorsal views are shown with anterior to the left. Numbers indicate the amount of embryos presenting with the phenotype shown in the picture/total number of embryos analyzed in each experiment. The arrow (↓) indicates the diminution of expression in knockdown versus wild type embryos. A minimum of three biological replicates was performed for each marker with at least n=5 embryos per experiment for each condition. A Mann-Whitney U-test was used to test for statistical significance and error bars are shown as ± standard deviation. ($p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *).

Supplemental Figure 11





Supplemental Figure 11. *srp54* knockdown does not influence c-myb and globin gene expression or early body size growth.
Representative pictures of WISH of *runx1/c-myb* at 36 hpf (A) or *globin* at 48 hpf (B) are shown. Note that hematopoietic stem cells (*runx1/*c-myb+ cells) or erythrocytes (globin+ cells) are not altered upon *srp54* MO knockdown. Graphs display quantitation of results, indicating normal and decreased gene expression. Lateral views are shown, with anterior to the left, dorsal up. Numbers indicate the amount of embryos with the respective phenotype/total number of embryos analyzed in each group from three independent biological replicate experiments. (C) Body length assessed at 5 dpf is not affected in *srp54* morphants. Shown are mean values from at least n=10 embryos, from 3 or more independent biological replicate experiments. A Mann-Whitney U-test was used to test for statistical significance and error bars are shown as ± standard deviation (n.s. = non-significant).