Supplemental data for

Human genetic defects in SRP19 and SRPRA cause severe congenital neutropenia with distinctive proteome changes

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Supplemental methods

Human subjects

We present clinical, genetic and biological data from 6 pedigrees with 11 patients carrying variants in SRP-complex subunits. A novel heterozygous *de novo* mutation in SRPRA was found in one pedigree with one patient (family A: II.3). A novel homozygous mutation in SRP19 was identified in two related pedigrees with 5 patients affected (family B: IV.2; IV.3; IV.5 and V.1; V.2). SRP54 variants were found in three pedigrees with 5 patients. Patients were referred to pediatric centers in Germany (LMU Munich, Dr. von Hauner Children's Hospital), Iran (Immunology, Asthma and Allergy Research Institute and Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Teheran), and Turkey (Department of Pediatrics, Division of Pediatric Hematology & Oncology, Erciyes University, Written informed consent was given by the parents/legal guardians in accordance with the Declaration of Helsinki and European regulatory principles. Children gave their informed assent. The study was approved by the LMU Munich ethics committee as well as ethics boards of local clinical institutions. Healthy individuals included unaffected family members.

Reprogramming of HMGU1 and iPS cell culture

On day 1 of reprogramming, BJ fibroblast cultures (ATCC, cat# CRL-2522) of 80% confluency were detached using 0.25% Trypsin-EDTA (Life Technologies, cat# 25200056), counted and seeded on the NuFF3-RQ cells at two different densities: $2x10^4$ cells/well and $4x10^4$ cells/well. On day 2, medium was changed to Pluriton Reprogramming Medium (Stemgent, cat# 00-0070) supplemented with 500ng/ml carrier-free B18R Recombinant Protein (Stemgent, cat# 03-0017). Day 3-18, the modified mRNAs (mmRNAs) cocktail containing OCT4, SOX2, KLF4, LIN28, and

C-MYC mRNAs at a 3:1:1:1:1 stoichiometric ratio was transfected as described previously[1]. The first morphological changes were noted as early as day 5 after the first transfection, and the first induced pluripotent stem cell (iPSC) colonies appeared by day 14. On day 16, medium was changed to STEMPRO hESC SFM (Thermo Fisher Scientific, cat# A1000701) for the colonies to mature and subsequently they were passaged as a bulk population on γ-irradiated mouse embryonic fibroblasts (MEFs) and grown in STEMPRO for 10 additional passages before adapting the iPSCs to a feeder-free culture system using Matrigel (Corning BioLogica, cat# FALC354230) and mTeSR1 media (StemCell Technologies cat# 85850). iPSCs were maintained on Geltrex (Thermo Fisher scientific, cat# A1413302) with mTeSR1 medium.

Maintenance and differentiation of human iPS cells

iPS cells were maintained on a tissue culture dish coated with growth factor-reduced Matrigel (Corning, cat# 356231) in mTeSR1 serum-free medium (Stemcel, cat# 5850). For differentiation of iPS cells into neutrophil-like cells, undifferentiated iPS cell colonies were prepared at the density of 6 colonies per well of a 6-well tissue culture plate. When individual colonies grew up to approximately 500 mm in diameter, mTeSR1 maintenance medium was supplemented with BMP4 (80 ng/ml, Peprotech cat# 120-05), CHIR99021 (4μM, millipore cat# 361571) and VEGF (80 ng/ml, Peprotech cat# 100-20). This day was defined as day 0 of neutrophil differentiation. On day 2, medium was replaced by essential 6 medium (Thermo Fisher scientific, cat# A1516401) supplemented with VEGF (80 ng/ml), basic FGF (25 ng/ml, Peprotech cat# 100-18B), SCF (50 ng/ml, Peprotech cat# 300-07) and SB431542 (2μM, selleckchem cat# S1067). On day 4, StemPro-34 SFM medium (Thermo Fisher scientific, cat# 10639011) was supplemented with SCF (50 ng/ml),

IL-3 (50 ng/ml, Peprotech cat# 200-03), Flt-3 (50 ng/ml, Peprotech cat# 300-19), TPO (5 ng/ml, Peprotech cat# 300-18) and VEGF (50 ng/ml). On day 8, medium was replaced by StemPro-34 SFM medium supplemented with SCF (50 ng/ml), IL-3 (50 ng/ml), Flt-3 (50 ng/ml) and TPO (5 ng/ml). Thereafter, half medium was changed every 3-4. On day17, the cytokines were changed to G-CSF (50 ng/ml, Peprotech cat# 300-23), SCF (50 ng/ml) and IL-3 (50 ng/ml). A detailed protocol is described in Supplemental Figure 4A.

Cell Lines

HeLa cells were grown at 37°C in Dulbecco's modified eagle medium (DMEM) with 10% tetracycline-free fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin in a humidified incubator at 37°C and 5% CO_2. EBV-LCL cells were grown in RPMI1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 10mM Hepes in a humid atmosphere at 37°C and 5% CO_2.

Exome sequencing analysis

Whole Exome Sequencing (WES) was performed at the Dr. von Hauner Children's Hospital NGS facility. Genomic DNA was isolated from whole blood (Qiagen) for generation of whole exome libraries using the SureSelect XT Human All Exon V6+UTR kit (Agilent Technologies). Barcoded libraries were sequenced with a NextSeq 500 platform (Illumina) to an average coverage depth of 90x. Bioinformatics analysis used Burrows-Wheeler Aligner (BWA 0.7.15), Genome Analysis ToolKit (GATK 3.6) [2] and Variant Effect Predictor (VEP89) [3]. The frequency filtering used allele frequencies from the public gnomAD database and a comprehensive in house database. Novel sequence variants were confirmed by Sanger sequencing for the patient and informative family members.

Whole Genome Sequencing analysis

For family A, whole genome sequencing (WGS) of the patient, four siblings and the parents was performed at the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama. WGS data was analyzed using the workflows provided by the toolbox KNIME4NGS[4]. Paired sequence reads of 151 base pairs length were mapped to the human reference genome GRCh37 with BWA (0.7.12) [5]. The Genome Analysis Tool Kit (GATK 3.5) was used to identify variants according to the provided best practice guidelines[2]. Functional annotation was performed with Variant Effect Predictor (VEP) using Ensembl release 83[3]. Variants were filtered and analyzed using Gemini (0.20.0) [6]. Rare variants were identified based on allele frequencies of the gnomAD data set. The functional effect of variants was predicted using the predicted impact of VEP and CADD scores[7]. The remaining variants were compiled and filtered for *de novo* variants assuming a dominant effect as well as rare homozygous and compound heterozygous variants following a pattern of autosomal recessive inheritance.

About 6,000,000 variants were found in the WGS data of the seven family members. Variant filtering and prioritization revealed only two variants with CADD scores higher than 25. Both variants are *de novo* variants in the genes METTL26 (NM_001040160.1:c.26G>A, NM_001040160.1(C16orf13_i001):p.(Arg9Gln)) and SRPRA (NM_003139.3:c.1390C>G, NM_003139.3(SRPRA_i001):p.(Gln464Glu)). The effect of the amino acid exchange in the SRPRA-SRP54 complex shown in Figure 1E and 1F was modelled based on structure 5L3Q[8] in the Protein Data Bank using PyMOL (2.3.2).

Neutrophil granulocyte proteome sample preparation

Venous blood anticoagulated with EDTA (Ethylenediaminetetraacetic acid) was drawn and processed within a 4hour time window. Neutrophils were isolated with the

MACSexpress human neutrophil isolation kit (Miltenyi, cat# 130-104-434) according to the vendor's protocol. For total erythrocyte depletion we used the MACSxpress Erythrocyte Depletion Kit (Miltenyi, cat# 130-098-196) according to the vendor's protocol. After isolation, neutrophils were washed twice in PBS (Gibco, cat# 14190250), and divided into aliquots of $1x10^6$ cells. Purity, assessed on May-Grunwald Giemsa stained cytospins, reached > 96% for all samples. After pelleting, the supernatant was removed and 5 µl of 25X protease inhibitor was added (Roche, cat# 04693159001). Cells were then frozen and stored in liquid nitrogen. Purified neutrophil samples were processed with the Filter-Aided Sample Preparation (FASP) method as follows: ~$1x10^6$ cells were lysed using 50 µl of SDS lysis buffer (0.5% SDS, 0.1 M DTT in 0.1 M Tris-HCl pH 7.6) and sonicated for 30 sec using a Branson Ultrasonics 250A analog sonifier (10% duty cycle, energy level 1). Samples were then heated for 5 min at 95°C. Subsequently, 150 µl of UA buffer (8 M urea in 0.1 M Tris-HCl pH 8.5) were added to the samples to a total volume of 200 µl, loaded onto 0.5 ml Microcon 30 kDa-cutoff Ultracel membrane filters (Merck, cat# MRCF0R030) and centrifuged in an Eppendorf 5418 centrifuge for 20 min at 14,000 rcf. Next, 200 µl of UA buffer was added and the centrifugation step was repeated. Subsequently, 50 µl of IAA buffer (0.05 M iodoacetamide in UA buffer) were added to the filters and incubated in darkness for 20 min at room temperature. Next, two washing steps using 150 µl and 200 µl of UA buffer were performed, each time spinning down the samples for 20 min at 14.000 rcf. As a final washing step, the samples were washed twice with 200 µl of ABC buffer (50 mM ammonium bicarbonate in The filters with washed samples were then transported to new collector tubes and MS-grade trypsin (Thermo Fisher, cat# 90057) in digestion buffer (1 M urea in 0.1 M Tris-HCl 8.5) was added in 1:100 ratio. The filters were wrapped in parafilm and placed in a wet

chamber for overnight digestion at 37°C. ddH_2O) and spun down as described above. Finally, the samples were spun down for 15 min at 14.000 rcf, 50 µl of ABC buffer were added to the filters and the centrifugation step repeated. The samples were then acidified to pH ~2.5 using 20% trifluoroacetic acid (TFA). The peptide yield was estimated using Thermo Fisher NanoDrop 2000c.

The samples were cleaned-up and desalted using a C18-StageTip approach as described in[9] and stored at -20°C.

Data-independent acquisition of neutrophil proteomes

The data-independent acquisitions were performed on Thermo Fisher QExactive HF mass spectrometer coupled to a Dionex UltiMate 3000 HPLC system using a 50 cm C-18 Thermo Fisher EasySpray column, heated to 50°C. Each patient sample was measured once. All samples contained spiked-in iRT peptides (Biognosys, cat# Ki-3002-2) for retention-time alignment. The mass spectrometry settings were as follows: one MS1 scan was performed between 350 - 1300 m/z at resolution of 120.000, AGC target of 5e6 and a maximum IT of 100 ms, followed by ten 12.5 m/z MS2 windows, ten 37.5 m/z MS2 windows and a final single 450 m/z MS2 window (21 MS2 windows total). In case of all the MS2 windows the resolution was set to 30.000, AGC target to 1e6, maximum IT to “auto” and the collision energy to 30. Both MS1 and MS2 scans were recorded in profile mode. The DIA method resulted in a median of 7 data points per peak. The data of our mass spectrometry proteomics have been deposited to the ProtreomeXchange consortium via the partner repositories PRIDE[10] and iPeX[11,12]. All healthy donor data are accessible via PRIDE under the accession number PXD010701, while all patient data are accessible via iPeX under the accession number IPX0004836001.

Protein identification and quantification

Biognosys Spectronaut 14 was used for DIA search and protein quantification. The DIA raw files were converted into HTRMS format using Biognosys HTRMS converter.

Protein identification and quantification was performed using the "direct DIA" approach (non library based) with the default settings. Protein FDR was set to 5%, data filtering was set to "Qvalue percentile 0.5", cross-run normalization was set to "Qvalue complete". Protein inference was set to automatic.

As a final filtering step, known contaminants according to MaxQuant[13] were removed from the list of quantified proteins.

Proteome data interpretation and bioinformatic tools

All data analysis was performed using R-studio (Version 1.2.5019) running R (version 3.6.1). Limma R package[14] was used to perform differential protein expression analysis using empirical Bayes moderation. The log2-transformed expression data were normalized using VSN normalization (A). Batch effect analysis was done using PCAtools (B) identified batch effects were corrected using LIMMA for graphical data representation and clustering analysis but not in differential analysis. Only proteins identified by two or more peptides were used for differential abundance testing. Hits with Benjamini-Hochberg P-adjusted value <0.05 were considered statistically significant. All plots were generated using ggplot2 from the tidyverse package (C). GO analysis was performed using clusterProfiler (D). PCA analysis and plotting was performed using before mentioned PCAtools (A), kmeans clustering was performed using the kmeans algorithm included in base R stats, for AGNES clustering we used the package cluster (E). Due to sensitivity of k-means and AGNES to large differences in group sizes, we excluded healthy donors from cluster

analysis. The number of clusters to analyze for was optimized using "Within sums of square" (WSS) and "silhouette" scoring and resulted in two clusters as the optimal number.

Volcano plots were created using ggplot from the tidyverse package (C). Protein annotations were derived by querying the uniprot database (https://www.uniprot.org/) using uniprot identification numbers of the UNIPROT system. We used entry associated uniprot-keywords to annotate protein localisations and protein domains (https://www.uniprot.org/keywords/). In addition we used neutrophil specific protein localization studies as mentioned in the main text (granules and plasma membrane proteins ref[15], granule proteins ref[16]). To derive Figure 3J and K, we first used the proteome ruler[17] and used a dot plot to show the combined information of the total molecular number (dot size) and the resulting total mass (x-axis) in each cellular compartment (y-axis).

For hydrophobicity testing, we recapitulated the approach of a previous study[18]. First, AA sequence of each protein in our neutrophil proteome was fetched from Uniprot. Next, AA hydrophobicity scores were fetched from the Kyte Doolittle database (https://web.expasy.org/protscale/pscale/Hphob.Doolittle.html). [19]

Subsequently hydrophobicity was calculated for each protein in the first 60 AA from the N-terminus (or less if shorter) in sequence windows of 9 AA. Each protein was assigned a score value composed of the maximal hydrophobicity multiplied by the length of the strongest window. Hydrophobicity scores were compared from differentially expressed protein of individual genotypes.

References of bioinformatic analysis:

A) Huber W, von Heydebreck A, Sueltmann H, Poustka A, Vingron M (2002). "Variance Stabilization Applied to Microarray Data Calibration and to the

Quantification of Differential Expression." *Bioinformatics*, 18 Suppl. 1, S96-S104 (www.bioconductor.org/packages/vsn)

B) Kevin Blighe (2019). PCAtools: PCAtools: Everything Principal Components Analysis. R package version 1.2.0. https://github.com/kevinblighe/PCAtools

C) Wickham et al., (2019). Welcome to the tidyverse. Journal of Open Source Software, 4(43), 1686 (https://doi.org/10.21105/joss.01686)

D) Guangchuang Yu, Li-Gen Wang, Yanyan Han and Qing-Yu He. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS: A Journal of Integrative Biology 2012, 16(5):284-287

E) Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K (2021). *cluster: Cluster Analysis Basics and Extensions*. R package version 2.1.2 (https://CRAN.R-project.org/package=cluster)

Expression of pluripotency associated genes

Protein expression levels of Oct4 and SSEA4 were assessed in each iPS cell clone derived from control, $SRPRA^{+/MUT}$ and $SRP19^{MUT/MUT}$ before differentiation using Millipore's FlowCellect™ Human iPS Cell Characterization Kit (Millipore, cat# FCSC100107) according to the manufacturer's instructions. Validation of marker Oct 4 and SSEA4 was performed by flow cytometry using the BD LSRFortessa and the software FlowJo.

Cytospin and light-microscopy

2×10^4 differentiated iPS cells were centrifuged at 500 rpm at RT for 5 min utilizing a Tharmac Cellspin I Cytocentrifuge (THARMAC) on to air-dried slides. The prepared cytospin slides were stained with May-Grünwald Giemsa (Merck, cat# 1014241000) for 2 minutes, following Giemsa`s azur eosin methylene blue solution (Merck, cat# 1092040500, diluted 1:20 in sörengen buffer) for 17 minutes. Distribution of the

maturation stages and morphology of cells were analyzed with a Zeiss Axioplan 2 imaging microscope (Carl Zeiss). 200 cells of each genotype were analyzed.

FACS analysis and sorting

Peripheral blood and bone marrow samples were washed with PBS and stained with the following antibodies for 20min at RT: PE anti-CD45 (Pharmingen), V500 anti-CD45 (HI30, BD), PE-Cy7 anti-CD33 (P67.6, Biolegend), APC anti-CD16 (HIT3a, Biolegend), PE anti-CD10 (HI10a, BD). Red blood cells were lysed using 1× BD FACS Lysing Solution (BD Biosciences) according to the manufacturer's instructions. For experiments with iPSC-derived cells, floating cells were harvested at day 29, washed with FACS-buffer (2% FCS in PBS), incubated with human Trustain FcX (Biolegend, cat# 422302) followed by staining in Brillant stain buffer (BD Horizon, cat# 563794) with Fixable Viability Stain 780 (BD biosciences, cat# 565388), PE anti-CD49d (BD, Biolegend cat#555503), APC anti-CD33 (Biolegend, cloneWM53, cat# 303408), BB515 anti-human CD11b (BD, cat# 564518), PE-Cy7 anti-human CD16 (Pharmingen, cat#557744), APC-R700 anti-human CD45 (BD, cat#566041). Live/CD49d^{low}/CD33^{low}/CD11b^{+}/HLA-DR^{-}/CD14 cell population was sorted using BD FACSAria and prepared for immunoblotting. The samples were acquired using a LSRFortessa (BD Bioscience) cytometer. Data were analyzed using FlowJo Software (TreeStar) v9 and v10.

Immunoblotting

All cells were lysed in Laemmlibuffer and boiled. The following primary antibodies were used: mouse monoclonal anti-SRP19 (Santa Cruz, cat# sc-393775), mouse monoclonal anti-SRP54 (BD, cat# 610940), rabbit polyclonal anti-SRPRA (Biorbyt, cat# orb 412406), mouse monoclonal anti-GFP (B2, Santa Cruz, cat# sc9996), mouse monoclonal anti-actin (C4, Santa Cruz, cat# sc-47778 HRP), rabbit polyclonal anti-

CRISP3 (Proteintech, cat#14847-1-AP), rabbit polyclonal anti-XBP-1 (Cell Signaling, cat#12782S), mouse monoclonal anti-HAX1 (BD Biosciences, cat# 610824), Apoptosis Western Blot Cocktail (pro/p17-caspase-3, cleaved PARP1, muscle actin) from Abcam [20] ab136812.

The following secondary antibodies were used: HRP goat anti-mouse IgG (BD, cat# 554002), anti-rabbit IgG, HRP-linked antibody (Cell signaling, cat# 7074S). Bound antibodies were detected with the Molecular Imager Chemic Doc XRS+ (Biorad). Immunoblotting of BiP (mouse monoclonal anti-BiP, BD Biosciences, cat# 610978) and β-actin (mouse monoclonal anti-actin (C4) HRP, Santa Cruz, cat# sc-47778 HRP) was performed essentially as described[21].

Immunofluorescence

For immunofluorescence, cells were fixed with 4% paraformaldehyde, washed with PBS and permeabilized for 5 min in 0.2% TritonX-100. For immunostaining, cells were blocked with blocking solution (BS: 2% BSA in PBS) at RT for 1 hr or o/n at 4°C. Primary antibodies were diluted in BS and used as follows: mouse monoclonal anti-GM130 (BD cat# 610823), rabbit polyclonal anti-Calnexin (Enzo life sciences, cat# ADI-SPA-860-F), mouse monoclonal anti-eIF6 (Santa Cruz, cat# sc390441), rabbit polyclonal anti-CRISP3 (Proteintech, cat#14847-1-AP). After three washing steps with BS, fluorescent secondary antibodies were used as followed: goat anti-rabbit 594 (Invitrogen, cat# A11037) and goat anti-mouse 594 (Invitrogen, cat# A11032). After incubation at RT for 1 hr, cells were washed with BS and coverslips were mounted with the Dako mounting medium. Confocal microscopy was performed with a Zeiss LSM-800 upright microscope using a 63x 1.4NA Oil DIC Plan-Apochromat immersion lens.

Migration assays

Human polymorphonuclear neutrophils (PMNs) were isolated from healthy volunteers as previously described[22] and resuspended in adhesion medium (HBSS (Biochrom) supplemented with 20 mM Hepes (Sigma-Aldrich), 0.25% BSA (Sigma-Aldrich), 0.1% glucose (Sigma-Aldrich), 1.2 mM $Ca^{2+,}$ and 1 mM Mg^{2+} (Sigma-Aldrich)). PMNs or differentiated, mature iPSC-derived neutrophils (2.5 x 10^5 per sample) in adhesion medium were allowed to adhere for 5 min in chambers of µ-slides $VI^{0.1}$ (ibidi) coated with ICAM-1 (10 µg/ml) and IL-8 (1 µg/ml). Shear stress of 1 dyne/cm^2 was applied for 10 min and time-lapse videos were recorded with an Axiovert 200M microscope equipped with a Plan-Apochromat 20x/0.75 NA objective, AxioCam HR digital camera, and a temperature-controlled environmental chamber (Zeiss, Germany).

Afterwards cells were washed once with PBS and fixed with 4% PFA in PBS. For F-actin staining, PMNs and mature iPSC-derived neutrophils were labeled with silicon rhodamin (SiR)-actin (100 nM, Spirochrome) and nuclei were stained with the nuclear dye Hoechst 33342 (5 µM, Thermo Fisher Scientifc) and visualized using an upright spinning disk confocal microscope (Examiner, Zeiss, Germany) equipped with a confocal scanner unit CSU-X1 (Yokogawa Electric Corporation, Japan), a CCD camera (Evolve, Photometrics, USA) and a 20x/0.75 NA water immersion objective (Plan Apochromat, Zeiss, Germany).

Apoptosis assay

Floating iPS cells were collected on day 26 after differentiation and stained with AnnexinV-FITC (Biolegend, cat# 640906), APC anti-CD33 (Biolegend, cat# 303408) and PE anti-CD49d (Biolegend, cat# 304304). Apoptotic cells in $CD33^{high}CD49d^{high}$ or $CD33^{low}CD49d^{low}$ population were evaluated by flow cytometry using the BD

LSRFortessa (BD Biosciences). The percentage of Annexin V-positive cells was analyzed by FlowJo software (Tree Star Software).

Reactive oxygen species (ROS) assay

Catalase (1000 U /ml, Sigma Aldrich, cat# C9322-1G) and Dihydrorhodamine (DHR123 10 μM, Sigma Aldrich, cat# D1054) was added to 100,000 differentiated iPS cells suspended in Hank's Balanced Salt Solution. After 5 min incubation in a 37°C shaking water bath, cells were divided into 2 new tubes, with or without PMA (6 μM, Sigma Aldrich, cat# P8139) and incubated for additional 15 min before analysis by flow cytometry.

Phagocytosis assay

iPS cells on day 43 after differentiation were evaluated in opsonophagocytic assay as previously described.[23] For the assay, *Enterococcus faecalis* was used as bacterial target, baby rabbit serum as complement source and rabbit serum raised against lipoteichoic acid as a source of opsonic antibodies[24]. Briefly, *E. faecalis* strain 12030 was grown in tryptic soy broth (Roth, cat# X938.1) to an OD600 of 0.40 and resuspended to a final concentration of $5.6x10^6$ cells/ml in RPMI 1640 medium supplemented with 15% FBS (RPMI-FBS). Lyophilized baby rabbit complement (Cerdalane, cat# CL3441) was reconstituted with RPMI-FBS medium to a final concentration of 6.7% and absorbed with *E. faecalis* 12030 for 60 min at 4°C on a rotor rack. After incubation, complement was centrifuged at 1,580 g for 10 min at 10°C and sterile-filtered. Equal volumes of rabbit serum raised against lipoteichoic acid (25 μg IgG/ml in RPMI-FBS), absorbed baby rabbit complement (6.7%), bacterial suspension ($5.6x10^6$ CFU/ml) and RPMI-FBS medium (negative sample) or suspension of iPSC-derived/purified neutrophil granulocytes in RPMI-FBS ($2x10^7$ cells/ml) were mixed and incubated on a rotor rack at 37°C. After 90 min incubation,

25 µl of samples were plated on Tryptic soy agar (Carl Roth, cat# X937.1) in quadruplicate. On the following day, the bacterial colony forming units (CFUs) were enumerated and percentage of killing was calculated by comparing the surviving bacterial CFUs from negative sample using following formula: % Killing =100 - [100 x mean CFU of assay sample / mean CFU of negative sample].

Molecular Cloning

The coding regions of all genes used in this study were amplified from human cDNA ORF clones obtained from Genscript. All primers have been synthetized by Eurofins Genomics. CRISP3 (Accession No. NM_006061.4; Clone ID OHu12339), GUSB (Accession No. NM_000181.4; Clone ID OHu27733), PTX3 (Accession No. NM_002852.4; Clone ID OHu14877), Prolactin (Accession No. NM_000948.6; OHu26664), Nup53 (Accession No. NM_138285.5; Clone ID OHu00973), SRP19-isoform1 (Accession No. NM_003135.3 Clone ID OHu26556) and SRP19-isoform3 (Accession No. NM_001204194.1; Clone ID OHu26495) were cloned into pcDNA5/FRT using the following primer pairs:

CRISP3 was amplified with the following primers: 5’ -attaGGCCGGCCgatgaaacaaatac-3’ (forward primer) and 5’-attaGGCGCGCCgataaatgctgtttg-3’ (reverse primer). GUSB was amplified with the following primer: 5’-attaGGCCGGCCgatggcccgggggtcg-3’ (forward primer) and 5’-attaGGCGCGCCgagtaaacaggctg-3’ (reverse primer). PTX3 was amplified with the following primers: 5’- TTTAAACttaagggccggatgcatctccttgc-3’ (forward primer) and 5’-CTTGCTCACCATggcgcgtgaaacatactgagctc-3’ (reverse primer). Prolactin was amplified with the following primers: 5’- attaGGCCGGCCgatgaacatcaaagg-3’ (forward primer) and 5’-attaGGCGCGCCgttagcagttgttgttg-3’ (reverse primer). Nup53 was amplified with the following primers: 5’-attaGGCCGGCCgatggcagcctttgc-3’

(forward primer) and 5'-attaGGCGCGCCgctaccagccaaacatg-3' (reverse primer). SRP19-isoform1 was amplified with the following primers: 5'-attaGGCCGGCCgatggcttgcgc-3' (forward primer) and 5'-attaGGCGCGCCgttacttctttttc-3' (reverse primer). SRP19-isoform3 was amplified with the following primers: 5'-attaGGCCGGCCgatggcttgcgc-3' (forward primer) and 5'-attaGGCGCGCCgttacttctttttc-3' (reverse primer).

All plasmids were sequence-confirmed.

sgRNA design and cloning for targeted gene correction

sgRNA-Cas9 with fusion GFP plasmid was purchased from Addgene (PX458, cat# 48138). sgRNAs targeting SRPRA (TACATTTCGTGCTGGGGCCGT), SRP19 (GTATTTCTTGAGGTATGACG) and HAX1 (AGTACGAGATTTCAATAGCA) genes were designed with the the online CRISPR Design tool developed by Feng Zhang's Lab (http://crispr.mit.edu/). sgRNA with a lower number of predicted potential off target sites in coding region were selected. sgRNA-Cas9 vector and single-stranded oligodeoxynucleotide (ssODN, bought from Integrated DNA Technologies,IDT) were co-transfected into healthy control-derived iPS cells using Amaxa Nucleofector® II Device (Lonza, program B-016) using Human stem cell nucleofector kit 2 (Lonza, cat# VPH-5022). pCas-Guide vector with a scrambled sequence was used as control. Two days after transfection, GFP positive cells were sorted by BD FACS Aria (BD Biosciences) and 3000 iPS cells were seeded on a 10cm dish pre-coated with mouse embryonic fibroblasts. 10 days after seeding, each colony derived from a single cell was picked up for genotyping and further expansion. Genotyping primers are as follows:

SRPRA; (forward primer): GGCGTTAATGGAGTGGGGAAAT;

SRPRA; (reverse primer): AGCAGAGCATGGCAAGTAACTG;

SRP19; (forward primer): TGGGCACACAGTTACGCATTAGTG;

SRP19; (reverse primer): CTCTCCATGAGCAGTAGTCCTGTC

sgRNA targeting the HAX1 (AGTACGAGATTTCAATAGCA) gene were also designed with the online CRISPR Design tool developed by Feng Zhang's Lab. sgRNA design and cloning for targeted gene knock-out for HAX1 was carried out with same procedure as described above.

RT-PCR for myeloid specific gene expression in iPSC-derived myeloid cell

Total RNA were extracted from differentiated iPSC using RNEasy Plus Mini Kit (250) (Qiagen, cat# 74136) and converted to cDNA using high capacity cDNA reverse transcription kit (Thermo Fisher scientific, cat# 4368814) according to manufacture's instruction.

The following primers were used: F (forward primer), R (reverse primer):

ACTB; F GCCAACACAGTGCTGTCT; R AGGAGCAATGATCTTGATCTT;

AZU1; F TCAGCAGCATGAGCGAGAAT; R GCAGAGGCAGTGGCAGTATC;

MPO; F ATCAGGTGAGCTGTGGAGGT; R TCAGAGGGCCCTGTCTATGG;

ELANE; F CGTGGCGAATGTAAACGTCC; R TTTTCGAAGATGCGCTGCAC;

RUNX1; F CCCTAGGGGATGTTCCAGAT; R TGAAGCTTTTCCCTCTTCCA;

CSF3R; F CTCAGGCCTTTCTCTCCTGC; R GAGAGGTTGTGGGGTATGGC

RT-PCR for SRP19 transcripts (neutrophil from patients and family)

Total RNA was extracted from neutrophils using RNeasy micro kit (Qiagen) and reverse transcribed using the High Capacity cDNA Reverse Transcripition kit (Applied Biosystems) as well as random hexamers combination with oligo^{21}18 primers (3:1). RT-PCR was performed using oneTaq polymerase (NEB) and the primer sets, 5'-CTCTTGTGAAGATGGCTTGCG-3' (forward primer) and 5'-TTACAGACTCCCCTGTCCCC-3' (reverse primer) for SRP19 transcripts, and 5'-

GCCAACACAGTGCTGTCT-3' (forward primer) and 5'-AGGAGCAATGATCTTGATCTT-3' (reverse primer) for beta-actin. For the amplification of SRP19 transcripts in neutrophils, semi-nested PCR was performed. Prior to the amplification using SRP19 transcript forward and reverse primers, cDNA was amplified using 5'-GGACGCGGAACCATTTCTTG-3' (forward 1st primer) and SRP19 transcript reverse primer. For RT-PCR from EBV-LCL, total RNA was extracted using the RNeasy plus mini kit (Qiagen).

SRP19 exon2-4 Minigene splicing assay

SRP19 exon 2, and 500 bp 5' sequence of intron 2 was PCR amplified by 5'-GTTTATTTGTATCTATCCTGCTTATTTA-3' (forward primer 1) and 5'-agatgtactcatcatgggaga-3' (reverse primer 1) and 5' overlap sequence to pcDNA3.1 (+) vector and 3' overlap sequence with 500 bp 3' sequence of intron 2 were added by PCR using 5'-cttggtaccgagctcggatcc-3' (forward primer 1') and 5'-ttttccatccagatgtactcatcatg-3' (reverse primer 1'). 500 bp 3' sequence of intron 2, exon 3, intron 3 and exon 4 was amplified by 5'- ctctggatggaaaattggattat-3' (forward primer 2) and GTGATGGGAACTGTACAAGG-3' (reverse primer 2) and then 5' overlap sequence to 500 bp 5' sequence of intron 2 and 3' overlap sequence to pcDNA3.1(+) vector were added by PCR using 5'-gagtacatctggatggaaaatt-3' (forward primer 2') and 5'- acgggccctctagactcgagGTGATGGGAACTGTACA-3' (reverse primer 2'). Two amplicons were inserted into pcDNA3.1 (+) vector using NEBuilder HiFi DNA assembly (NEB). A single nucleotide mutation in the intron 3 (+5) was introduced by site direct mutagenesis using 5'-TCTTGAGgtatgacgtggttcttcactattttcca-3' (forward primer) and 5'-atagtgaagaaccacgtcatacCTCAAGAAATACG-3' (reverse primer). Wild-type (WT) and mutated (mut) SRP19_2-4 minigene plasmids were transfected into HeLa cells or

into 293T cells, using Lipofectamine reagent or calcium-phosphate, respectively. 36 hours after transfection, total RNAs were extracted and cDNA was sythesized by oligo ^{21}18 primer using high capacity reverse transcription kit. WT and mut minigene transcripts from HeLa and 293T cells were detected by PCR with 5'-TAATACGACTCACTATAGG -3' (forward primer) and 5'-CTTACTTATGGGGATTCGCC-3' (reverse primer) using OneTaq polymerase (NEB). Transfection efficiency of minigene plasmids were confirmed by PCR with 5'-TAATACGACTCACTATAGG-3' (forward primer) and 5'-CCCGGACTCTGCCTCTGTATT-3' (reverse primer).

Generation of Reporter Cell Lines

HeLa reporter cell lines carrying tetracycline-inducible genes were generated using a HeLa Flp-In T-Rex cell line according to this published study[25].

Treatment of Reporter Cell Lines

Expression of reporters was induced by addition of 1 μg/ml tetracycline (Sigma) for 24h.

Tunicamycin Treatment

Tunicamycin (Sigma) was added at a final concentration of 2 μg/ml for 4 hours.

siRNAs and siRNA transfections

The nontargeting (NT) siRNA oligonucleotide (5-UGGUUUACAUGUCGACUAAdTdT-3) was not complementary to any known human and mouse mRNAs. HeLa cells were seeded in six-well plates at 2×10^5 cells per well, grown for 6 h and then transfected with siRNA oligonucleotides at a final siRNA concentration of 20 nM for SRPRA (5-GAGCUUGAGUCGUGAAGACdTdT-3) and SRP19 (5-CGUAAGUCAGUAAUGUUGUdTdT-3) and 10 nM for SRP54 (5-

GAAAUGAACAGGAGUCAAUTTdTd-3) using INTERFERin (Polyplus Transfection, cat# VWR, 409-10DE) for 48h according to the manufacturer's instructions. After 48h, tetracycline was added for 24h. Cells were washed once with PBS and lysed in Laemmli sample buffer.

Transient Transfection

HeLa cells were seeded at a density of 10^5 cells per well of a 6 well plate. The next day, plasmids encoding for GFP-SRP19-isoform1 and GFP-SRP19-isoform3 were transiently expressed in HeLa cells using Extremegene (Roche), fixed with 4% PFA after 24h and processed for immunostaining as decribed below.

Quantification of iPS cell data

Figure legends describe the applied statistical tests and statistical significance, with n; number of samples, SEM; standard error of the mean. Statistical significance was performed with GraphPad PRISM 7.0.

Image processing and quantification

Images were taken at identical exposure conditions within each experiment.

For co-localization analysis, a polygon selection covering the region of GM130 and CRISP3 localization was drawn and pearson values were calculated using the Zen software (Zen 2.3). The mean values of 3 independent experiments were then plotted using Prism (GraphPad). Statistical significance was performed with GraphPad PRISM 7.0. P values were calculated by using the unpaired, two-tailed Student's t-test. n=3 independent experiments.

Quantification of migration assays

Quantification of the number of adherent iPSC-derived neutrophils and human PMNs interacting with the surface was performed offline using ImageJ software (NIH). Cells were defined as adherent when an arrest at the same spot was longer than 15 sec.

Relative adhesion was quantified by dividing the accumulated number of adherent cells by the accumulated number of all interacting cells (100%). Migration tracks were analyzed in ImageJ with the implemented manual tacking plugin (Fabrice Cordeliès, Institute Curie) and migration velocity and Euclidean distance were calculated using the ibidi Chemotaxis software (Germany).

Data availability

Patient data cannot be shared publicly because of European and national data protection regulations that require that genomic data must not be made public to protect the privacy of patients. Data are available from the Dr. von Hauner Children's Hospital, Medical Center of the LMU Munich for researchers who meet the criteria for access to confidential patient data. Please direct your requests to the Dr. von Hauner Children's Hospital (sekretariat.kinderklinik@med.uni-muenchen.de) or the Institutional Review Board of the LMU (ethikkommission@med.uni-muenchen.de).

Zebrafish

Fish were held in FishCore (Monash University) using standard practices. Transgenic strains, carried on a predominantly Tübingen background, were: *Tg(mpx:EGFP)*^{i114}[25] and a compound triple transgenic line for neutrophil-specific gene editing designated "*mpx*-Cas9" carrying three transgenes, *Tg(mpx:KalTA4;cryaa:EGFP)*^{gl28}, *Tg(UAS:Cas9;cmcl2-RFP)*^{gl37}, and *Tg(UAS-E1b;Eco:NfsB-mCherry)*^{c264} as previously detailed[266]. Embryos were incubated in egg water (0.06 g/L salt (Red Sea, Sydney, Australia)) or E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, pH 7.0). Following collection, embryos were incubated at 28°C (Thermoline Scientific). To block pigmentation, 0.003% (w/v) 1-phenyl-2-thiourea (Sigma-Aldrich) was added from 12 hpf. Zebrafish experiments followed National Health and Medical Research Council (NHMRC) guidelines under protocols

approved by the Monash University Animal Ethics Committee MARP3 (Project IDs: MAS-2010-18 and 17270).

***srpra* CRISPR/Cas9 gene editing**

Human and zebrafish *srpa* and *srp19* gene sequences (ENSG00000182934, ENSDARG00000061124; ENSG00000153037, ENSDARG00000035699) were extracted from Ensembl databases. Sequence alignments were performed in the Multiple Sequence Comparison by Log- Expectation (MUSCLE) web tool[27]. The human missense *srpra* allele c.1390C>G, p.Gln464Glu corresponded to zebrafish c.1426C>G, p.Gln476Glu. The human *SRP19* putative disease allele c.189+5G>A corresponded to zebrafish c.186+5G>A. For genome editing of *srpra*, the Alt-R CRISPR-Cas9 System RNAs (Integrated DNA Technologies, IDT) were used. The approach anticipated gene disruption from gene editing followed by non-homology end joining (NHEJ) repair, but also intentionally provided opportunity for targeted engineering of a zebrafish equivalent of the candidate missense disease allele by homology directed repair (HDR). The gene editing strategy is summarised in supplemental Figure 8A; for oligonucleotide sequences, see supplemental Table 4. Of 2 *srpra*-crRNAs (20 nt *srpra* targeting sequence plus 16 nt of common overlap sequence to anneal to tracrRNA) suggested by the IDT Online Design Tool, the one predicted to induce Cas9-mediated double strand break 8 nts upstream of the nucleotide of *srpra* corresponding to the human missense mutation was chosen (Fig S1A). For homology-directed repair, the antisense asymmetric donor oligomer was designed based on the approach of Richardson et al[28], optimised in zebrafish by Prykhozhij et al[29]. Briefly, a sense oligonucleotide (sense to gRNA) symmetric in length from the cutting site of the CRISPR/Cas9 complex consisted of 120 nucleotides. An antisense oligonucleotide (antisense to gRNA) was asymmetric from

the cutting site and consisted of 36 nucleotides in 5'-direction and 84 nucleotides in 3' direction from cutting site on the sense strand.

To generate the annealed crRNA/tracrRNA complex, 2 µl *srpra*-crRNA (1 µg/µl) and 4 µl tracrRNA (1 µg/µl) were mixed, heated in a thermocycler at 95 °C for 5 min and then slowly cooled to room temperature[30]. 2 µl of the annealed gRNA were transferred to another tube, to which the symmetric sense and asymmetric antisense DNA oligonucleotides (0.5 µl each), 0.5 µl Cas9 enzyme (10 mg/ml) and 7.5 µl Duplex buffer (30 mM HEPES, pH 7.5; 100 mM Potassium Acetate) were added. This mix was allowed to sit to form complexes for 15 min at room temperature. This injection mix was loaded into pulled glass microinjection needles and single-cell embryos aligned in an agarose bed were injected using a PLI-100 Pico-Injector (Harvard Apparatus).

***srp19* morpholino oligonucleotide gene knockdown**

A splice-blocking morpholino oligonucleotide was designed to target the exon 3 splice donor site of zebrafish *srp19* (supplemental Table 4, supplemental Figure 9A). The stock concentration was 1000 µM, from which 500 µM and 200 µM dilutions were prepared. A control morpholino was only injected at its stock concentration of 1000 µM. 5 µl of each dilution of the *srp19* MO and control MO were mixed with 0.5 µl rhodamine dextran (Sigma; 5% w/v in 0.2M KCl) before injection, loaded into microinjection needles and injected into 1-cell embryos.

Microscopy and neutrophil scoring

An Olympus MVX10 microscope fitted with an Olympus DP72 camera and CellSens software version 1.11 was used for fluorescence and bright field imaging. EGFP- or mCherry-expressing neutrophil numbers were quantified at various time points in the tail region caudal to the yolk extension, which includes the leukocyte-rich caudal

haematopoietic tissue. Trunk neutrophil numbers were counted manually as previously described[26-28]. The experiment of Figure 6J was scored blinded to treatment group.

Zebrafish embryo DNA preparation

DNA was extracted from zebrafish embryos by the HotSHOT method[32]. Single tricaine-euthanased embryos were selected and put into labelled PCR tubes separately. 100 µl of 50 mM NaOH was added to each tube and the sample heated to 95 °C for 20 min. The solution was then stabilised by adding 10 µl of Tris-HCL pH 7.5 to each tube. The samples were either stored at -20 °C or used directly.

Sample preparation for RT-PCR

Approximately 50 embryos were pooled for each sample. Embryos were collected in a 1.5-ml microfuge tube, excess water removed, 1 ml of TRIzol reagent (Thermo Fisher Scientific) added, embryos homogenized by repeated aspiration through a 21-gauge needle for 5 min at room temperature and 0.2 ml of chloroform added to each sample with vigorous shaking for 15 s to mix. Samples were incubated at room temperature for 2 min and centrifuged at 12,000 *g* for 15 min at 4 °C. The top aqueous layer was transferred and 0.5 ml isopropanol was added to precipitate the RNA. The sample was allowed to sit at room temperature for 10 min and was then centrifuged at 12,000 *g* for 10 min at 4 °C. The supernatant was removed with a pipette, the pellet washed with 1 ml 75 % Ethanol and mixed by gentle inversion of the tube. Samples were centrifuged at 7,500 *g* for 5 min at 4 °C. The ethanol was carefully removed with a pipette and the sample allowed to dry for 10 min. Pellets were resuspended by adding 30 µl of RNAse-free water and incubation for 10 min at 55 °C. Sample quality and concentration was determined using a NanoDrop spectrophotometer.

To remove residual DNA, 3 µl 10x TURBO DNase Buffer and 2 µl TURBO DNase was added to the extracted RNA and the solution gently mixed and incubated at 37 °C for 30 min. To inactivate the DNAse, 6 µl DNase Inactivation Reagent was added and the samples were incubated for 5 min at room temperature, with occasionally shaking. The microfuge tubes were centrifuged at 10,000 *g* for 2 min and the upper layer containing the RNA was transferred into a fresh microfuge tube.

For the cDNA synthesis, 3 µl DEPC-treated water, 1 µl dNTP mix and 1 µl 50 ng/µl random hexamer primers for each sample were combined with 8 µl of DNase-treated RNA. This RNA-primer mix was heated at 65 °C for 5 min. A master mix consisting of 4 µl 5x SSIV Buffer, 1 µl 100 mM DTT, 1 µl Ribonuclease Inhibitor and 1 µl SuperScript IV Reverse Transcriptase was prepared for each sample. After cooling of the RNA-primer mix on ice, 7 µl of the master mix was added to each sample and mixed. The samples were at first incubated at 23 °C for 10 min, after that at 55 °C for 10 min and finally at 80 °C for 10 min before storing them at -20 °C.

FACS purification of leukocytes for DNA preparation

Tricaine-euthanased zebrafish embryos were divided to only collect their yolk and tail. These were pooled and transferred into a 1.5 ml tube with 1 ml of FACS medium (PBS with 0.25% heat-inactivated fetal bovine serum) and stored on ice. Samples were homogenized by repeated aspiration with an 18.5-gauge needle. The sample was added to 49 ml of FACS medium in a 50 ml Falcon tube, centrifuged at 1500 *g* for 8 min and the supernatant removed. The pellet was resuspended in 500 µl of FACS medium, filtered through blue cap filters (Falcon, BD352235) into polypropylene tubes. For viability testing, 0.5 µl DAPI (1 mg/ml) was added. Samples were sent for FACS sorting in the Monash University Flowcore facility. The viable, collected cell population was defined as the DAPI-negative, GFP/mCherry-positive cells.

PCR – genotyping and RT-PCR

Each PCR reaction consisted of 11.5 µl Milli-Q Water, 5 µl Phusion High Fidelity buffer, 2 µl 10 mM DNTP mix, 0.5 µl of each primer (10 uM; primer sequences in supplemental Table 4), 0.5 µl Phusion enzyme, and 5 µl of DNA sample. A Bio-Rad T100 thermal cycler used. For amplification of the *srpra* DNA: 90 seconds at 95 °C as initial denaturation, followed by 34 cycles of 30 s at 95 °C for denaturation, 45 s at 65 °C for annealing, 30 s at 72 °C for extension, and final extension at 72 °C for 5 min. For amplification of the *srp19* cDNA, the following program was used: 90 seconds at 95 °C as initial denaturation followed by up to 40 cycles of 30 s at 95 °C for denaturation, 45 s at 61 °C for annealing, 45 s at 72 °C for extension, and final extension at 72 °C for 5 min. The PCR product was mixed with 5 µl of Orange-G loading dye (Sigma-Aldrich) and pipetted into a gel consisting of 200 ml TAE-Buffer with 1.5 % agarose (w/v) and 0.33 % ethidium bromide (w/v), using a 1 Kb Plus DNA Ladder (Thermo Fisher Scientific). The gel was run on 90 V for ≈45 min and examined under a UV transilluminator.

Sanger sequencing

Single bands were excised from gels and DNA purified using the AccuPrep Gel (Bioneer) purification kit according to the manufacturer's instructions. Products were submitted for Sanger sequencing at the Monash University Micromon facility. Sequencing traces were analyzed using SnapGene Viewer (Version 4.2.6).

NGS sequencing

NGS sequencing was performed as previously described[266].

Statistics

For quantitative analyses, group sizes of >10 animals/experiment were intended but determined by the number of embryos laid on the day, with 2-3 replicates/experiment.

Anticipated effect sizes were not pre-specified. The contributions of individual experiments to pooled data are colour coded within figures. Embryos were randomly assigned to experimental groups. Scoring was not blinded except where stated. Descriptive and analytical statistics were prepared using Prism 8 Version 8.3.1(332) (GraphPad Software, CA, USA). Data are mean ± standard deviation, with P-values generated from tests as indicated. P-values <0.05 were considered significant. No data points were excluded as outliers.

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Supplemental Tables

Supplemental Table 1. Genetic characteristics of all SCN patients analyzed in the study.

Patient-ID	gene	ensemble transcript ID	genotype cDNA	genomic position	CADD v1.6	gnomAD AF	gnomAD count	zygosity	effect on protein
A.II.3	*SRPRA*	ENST00000332118	c.1390C>G	11-126134989-G-C	26.8	0		Het - de novo	p.Gln464Glu
B.IV.2	*SRP19*	ENST00000505459	c.189+5G>A	5-112200230-G-A	14.3	0.000003978	1 het in European population	Hom	aberrant splicing
B.IV.3	*SRP19*	ENST00000505459	c.189+5G>A	5-112200230-G-A	14.3	0.000003978		Hom	aberrant splicing
B.IV.5	*SRP19*	ENST00000505459	c.189+5G>A	5-112200230-G-A	14.3	0.000003978		Hom	aberrant splicing
B.V.1	*SRP19*	ENST00000505459	c.189+5G>A	5-112200230-G-A	14.3	0.000003978		Hom	aberrant splicing
B.V.2	*SRP19*	ENST00000505459	c.189+5G>A	5-112200230-G-A	14.3	0.000003978		Hom	aberrant splicing
SRP54.1	*SRP54*	ENST00000556994	c.349_351delACA	14-35476574-AAAC-A	22.5	0		Het	p.Thr117del
SRP54.2	*SRP54*	ENST00000556994	c.349_351delACA	14-35476574-AAAC-A	22.5	0		Het	p.Thr117del
SRP54.3	*SRP54*	ENST00000556994	c.349_351delACA	14-35476574-AAAC-A	22.5	0		Het	p.Thr117del
SRP54.3	*SRP54*	ENST00000556994	c.349_351delACA	14-35476574-AAAC-A	22.5	0		Het	p.Thr117del
SRP54.4	*SRP54*	ENST00000556994	c.349_351delACA	14-35476574-AAAC-A	22.5	0		Het	p.Thr117del
HAX1.1	*HAX1*	ENST00000328703	c.130_131insA	1-154245888-T-TA	31	0			p.Trp44Ter
HAX1.2	*HAX1*	ENST00000328703	c.130_131insA	1-154245888-T-TA	31	0			p.Trp44Ter
HAX1.3	*HAX1*	ENST00000328703	c.556_840del	1:154247477_154248177del	-	-			p.Asp186_Arg279delinsProCys
HAX1.4	*HAX1*	ENST00000328703	c.376delC	1-154246308-TC-T	33	0			p.Arg126GlyfsTer88
HAX1.5	*HAX1*	ENST00000328703	c.130_131insA	1-154245888-T-TA	31	0			p.Trp44Ter
HAX1.6	*HAX1*	ENST00000328703	c.130_131insA	1-154245888-T-TA	31	0			p.Trp44Ter
ELANE.1	*ELANE*	ENST00000263621	c.545delG	19-855741-CG-C	22.3	0			p.Arg182LeufsTer9
ELANE.2	*ELANE*	ENST00000263621	c.308G>T	19-853345-G-T	20.9	0			p.Arg103Leu
ELANE.3	*ELANE*	ENST00000263621	c.597+5G>A	19-855799-G-A	22.4	0			intronic variant

ELANE.4	*ELANE*	ENST00000263621	c.508C>T	19-855705-C-T	40	0.000007191	1 het in European, 1 het in African/African-American population		p.Gln170Ter

Supplemental Table 1. Genetic characteristics of all SCN patients analyzed in the study.

Supplemental Table 2. Clinical characteristics of all SCN patients analyzed in the study.

Patient-ID	Clinical phenotype	ANC	Bone marrow Histology	Extrahematopoietic features	Stool elastase in µg/g (normal > 200)	Weight percentile	Height percentile	Therapy - outcome	Quantification of Giemsa stains
A.II.3	SCN	100	myeloid maturation arrest	exocrine pancreatic insufficiency, growth deficiency	86 (severely reduced)	<1	<1	G-CSF:10µg/kg/d/ - alive and healthy	Purity > 99%, S: 60% / B: 30% / I: 10%
B.IV.2	SCN	340	myeloid maturation arrest	growth deficiency	>500	1	<1	G-CSF: 5µg/kg/w - alive and healthy	NA
B.IV.3	SCN	400	myeloid maturation arrest	growth deficiency	>500	Normal BMI	Short statue (150cm)	G-CSF:10µg/kg/w - alive and healthy	Purity > 99%, S: 30% / B: 40% / I: 30%
B.IV.5	SCN	450	not available	growth deficiency	>500	Normal BMI	Short statue (150cm)	G-CSF:10µg/kg/w- alive and healthy	NA
B.V.1	SCN	430	not available	growth deficiency	>500	<1	<1	G-CSF:10µg/kg/w - alive and healthy	Purity > 95%, S: 50% / B: 30% / I: 20%
B.V.2	SCN	310	myeloid maturation arrest	growth deficiency	>500	<1	<1	G-CSF:10µg/kg/w - alive and healthy	NA
SRP54.1	SCN	not available	not available	exocrine pancreatic insufficiency	reduced (values missing)	17	24	G-CSF:14µg/kg/d - alive and healthy	Purity >99%, S: 90% / B: 10%
SRP54.2	SCN	430	myeloid maturation arrest	exocrine pancreatic insufficiency	49 (severely reduced)	30	50	G-CSF:13µg/kg/d - alive and healthy	Purity >99%, S: 80% / B: 20%
SRP54.3	SCN	250	myeloid maturation arrest	exocrine pancreatic insufficiency	reduced (values missing)	Normal BMI	Normal statue (178cm)	G-CSF:34µg/kg/d - died	NA
SRP54.4	SCN	500	myeloid maturation arrest	none	207 (normal low)	83	32	G-CSF: 6µg/kg/w - alive and healthy	NA
SRP54.5	SCN	320	myeloid maturation arrest	none	208 (normal low)	63	20	G-CSF:10µg/kg/w - alive and healthy	Purity >99%, S:30% / B: 50% / I:20
HAX1.1	SCN	not available	not available	none	not available	not available	not available	transplanted - alive and well (before G-CSF 10µg/kg/d)	Purity > 99%, S: 30% / B: 50% / I: 20%
HAX1.2	SCN	not available	not available	none	not available	not available	not available	transplanted - alive and well (before G-CSF 10µg/kg/d)	NA

HAX1.3	SCN	not available	not available	none	not available	not available	not available	G-CSF:1.5µg/kg/d - alive and healthy	NA
HAX1.4	SCN	not available	not available	Epilepsy and severe mental retardation	not available	not available	not available	unknown	NA
HAX1.5	SCN	not available	not available	none	not available	not available	not available	G-CSF: 3.5µg/kg/d - alive and healthy	Purity > 95%, S: 50% / B: 20% / I: 30%
HAX1.6	SCN	not available	not available	none	not available	not available	not available	unknown	NA
ELANE.1	SCN	not available	myeloid maturation arrest	none	not available	not available	not available	G-CSF: 4µg/kg/d - alive and healthy	NA
ELANE.2	SCN	not available	myeloid maturation arrest	none	not available	not available	not available	G-CSF: 20µg/kg/2d - alive and healthy	Purity > 99%, S: 40% / B: 50% / I: 10%
ELANE.3	SCN	not available	myeloid maturation arrest	none	not available	not available	not available	G-CSF: 20µg/kg/2d - alive and healthy	NA
ELANE.4	SCN	not available	myeloid maturation arrest	none	not available	not available	not available	G-CSF: 20µg/kg/2d - alive and healthy	NA

Supplemental Table 2. Clinical characteristics of all SCN patients analyzed in the study.

supplemental Table 2 summarizes clinical features associated with the genetic variants of all SCN patients analyzed in this study. SCN: Severe Congenital Neutropenia; ANC: Absolute Neutrophil Count (before G-CSF therapy); G-CSF: Granulocyte-colony stimulating factor. allo-HSCT: allogeneic hematopoietic stem cell transplantation. Post-isolation microscopy evaluation of Giemsa stains: purity = overall neutrophil population. S = segmented nuclear stage as percentage, B = banded nuclear stage as percentage, I = immature stages as percentage.

Supplemental Table 3. Autozygosity values

Sample	Autozygosity	Causal gene
scn190ch	0.29	
scn190fa	0.43	
scn190mo	0.12	
scn190pa	0.38	SRP54
scn209fa	1.23	
scn209mo	1.39	
scn209pa	1.97	SRP54
scn213fa	5.19	
scn213mo	7.46	
scn213pa	9.92	SRP54
scn276pa	11.68	HAX1
scn281pa_ba	6.16	SRP19
scn281pa_fa	7.53	SRP19
scn282fa	0.48	
scn282mo	0.35	
scn282pa_md	3.71	SRP19
scn282pa_mg	7.18	SRP19
scn284fa	0.29	
scn284mo	0.29	
scn284pa	2.13	SRP54
scn299pa	7.06	HAX1
scn305br1	7.5	
scn305pa	10.83	HAX1
scn305si1	6.54	
scn306br2	2.42	
scn306br3	3.14	
scn306fa	4.74	
scn306mo	3.1	
scn306pa	3.18	SRPRA
scn309fa	1.13	
scn309mo	0.73	
scn309pa	0.55	HAX1
scn311pa	1.18	HAX1
scn331pa	0.09	SRP54
scn339pa	4.88	HAX1
scn357pa	2.79	SRP54
scn379pa	2.6	SRP54
scn58pa	4.65	HAX1
u139pa	8.45	HAX1

Supplemental Table 3. Autozygosity values.

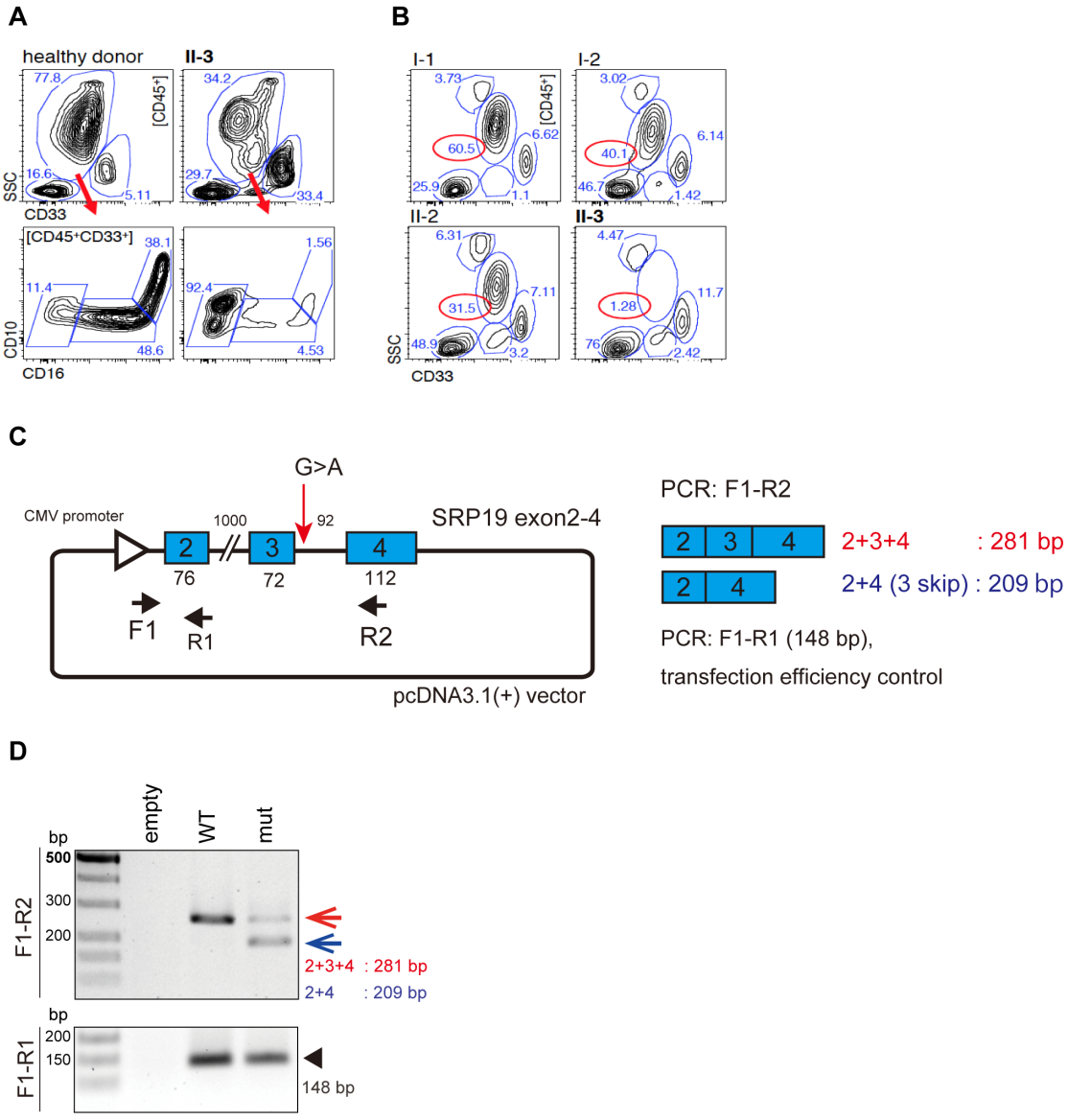
Supplemental Table 4. Oligonucleotides used for zebrafish studies.

Name	Purpose	Sequence (5' → 3')	Amplicon size (bp)
srpa-crRNA	crRNA specific sequence to target selected PAM site	TGACACGTTCCGTGCTGGAG	n/a
srpa-HDR-sense	sense symmetric DNA oligo template for HDR	ATTGGCTGATTGAGAACGGCTTCAC-TGTGCTGATCGCGGCCTGTGACAC-GTTCCGTGCTGGAGCGGTGGAG-CAGCTGAGGACTCAC-CAGCGCCGGCTGAACTCTCTACATCCAC-CTGAGA	n/a
srpa-HDR-antisense	antisense asymmetric DNA oligo template for HDR	GGACGGCGGGCTGGCCTCCGTGTTTCTCAGGTG-GATGTAGAGAGTTCAGCCGGCGCTGGTGAG-TCCTCAGCTCCTCCAACGCTCCAGCAC-GGAACGTGTCACAGGCCGCGATCAGCACAG	n/a
srp19-MO	Intron 3 donor site splice-blocking MO	TTATAATATCCAGACCTCCACGTAG	n/a
Control-MO	Random sequence GeneTools control MO	CCTCTTACCTCAGTTACAATTTATA	n/a
srpra-F	RT-PCR	GCAAGGGTTAAAAGGGTTCG	262
srpra-R	RT-PCR	TTTTACCAAAGGCAATGGCC	
srp19-F	RT-PCR	GCAGAAGGCAGAAGAATACC	268
srp19-R	RT-PCR	GCGTTCGGCTCTTCAGTTTC	
srp19-intron3-R	RT-PCR	GGGGCTCATAATATTTTTGC	590
β-actin-F	RT-PCR	GCTGACAGGATGCAGAAGGA	285
β-actin-R	RT-PCR	TAGAAGCATTTGCGGTGGAC	

Supplemental Table 4. Oligonucleotides used for zebrafish studies.
crRNA, CRISPR RNA; HDR, homology-directed repair; MO, morpholino oligonucleotide; PAM, protospacer adjacent motif; gRNA, guide RNA; n/a, not applicable.

Supplemental Figures

supplemental Figure 1



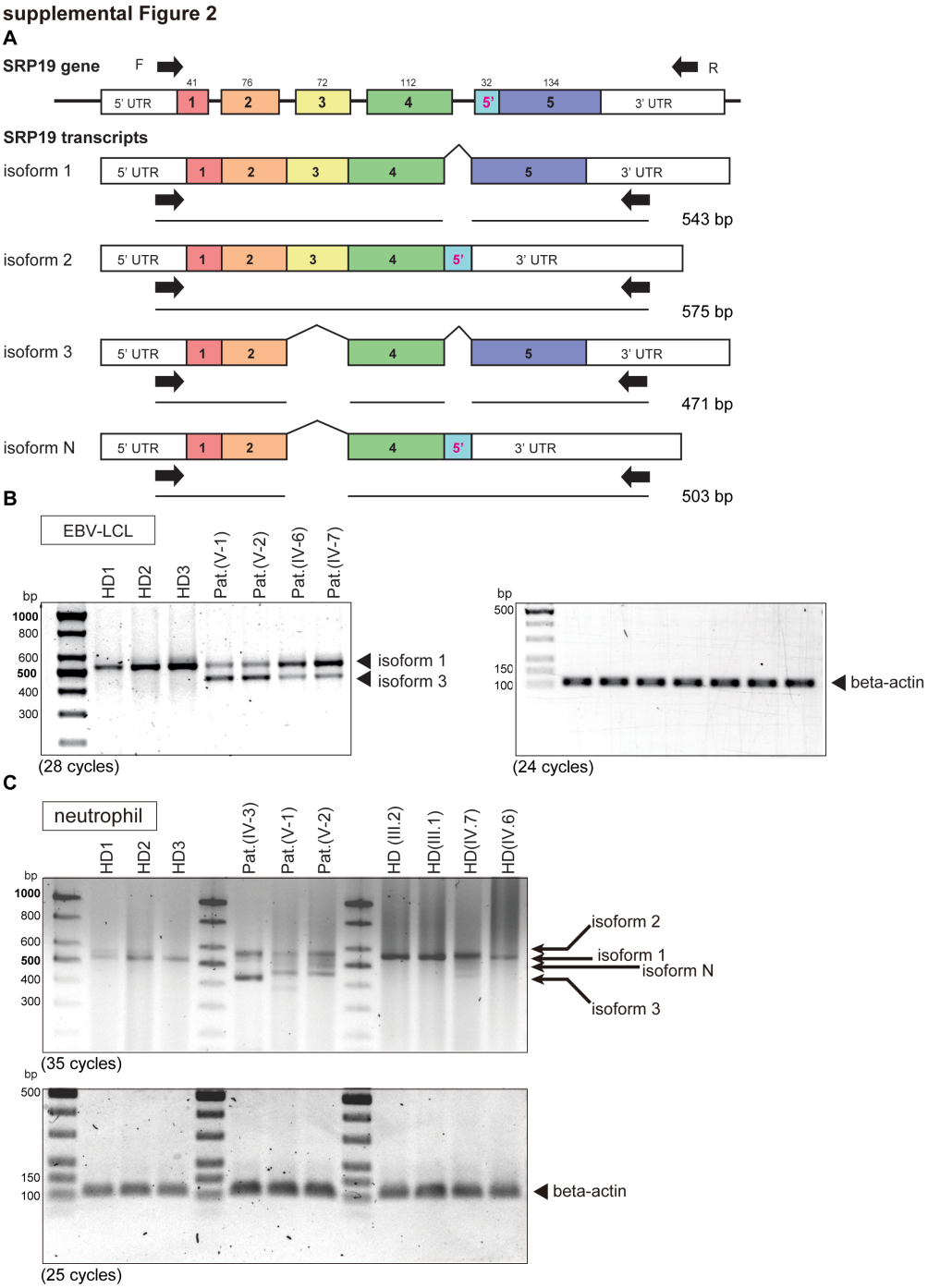
Supplemental Figure 1. Hematological phenotype of patient II-3 (family A) and validation of genetic SRP19 mutation (family B).

(A) Contour plots of mononuclear bone marrow (BM) cells of HD and the index case (A.II.3) generated by antibody staining and FACS analysis. Mature neutrophils were defined as $CD10^{lo}$ $CD16^{hi}$. Numbers adjacent to the gates indicate percentages.

(B) Contour plots of peripheral blood mononuclear cells of A.I-1 (mother), A.I-2 (father), A.II-2 (brother) and the index patient (A.II.3). Numbers adjacent to the gates indicate percentages.

(C) Scheme of SRP19 minigene.

(D) RT-PCR results documenting wildtype (WT) and mutated (mut) SRP19_2-4 minigene transcripts in HEK293T cells. F1-R1 and F1-R2 are the primer pairs used to amplify the indicated SRP19 exons. The primer pair F1-R2 amplifies SRP19 exon 2-4 as indicated with a red arrow (281 bp). The SRP19 variant results in a PCR product of only 209bp (blue arrow). The primer pair F1-R1 amplifies parts of the SRP19 Exon 2 and is used as a transfection efficiency control. Experiments were performed in triplicate.



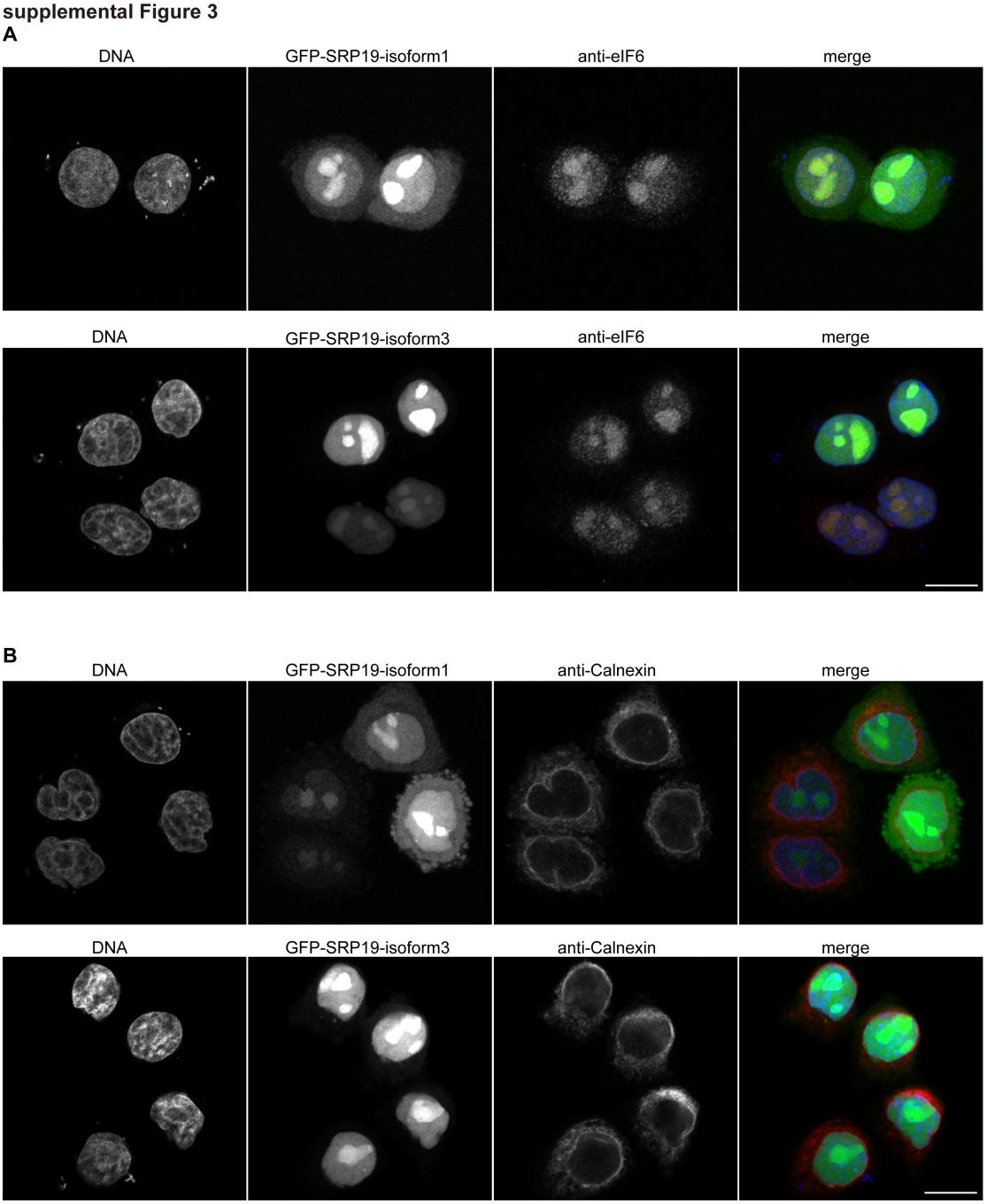
Supplemental Figure 2. Expression analysis of SRP19 gene variant.

(A) Schemes of the SRP19 gene and SRP19 transcripts.

(B) RT-PCR of SRP19 isoforms and β-actin in EBV-LCL from healthy donors (wildtype) and SRP-variant patients with homozygous splice site variant. Experiments were performed twice.

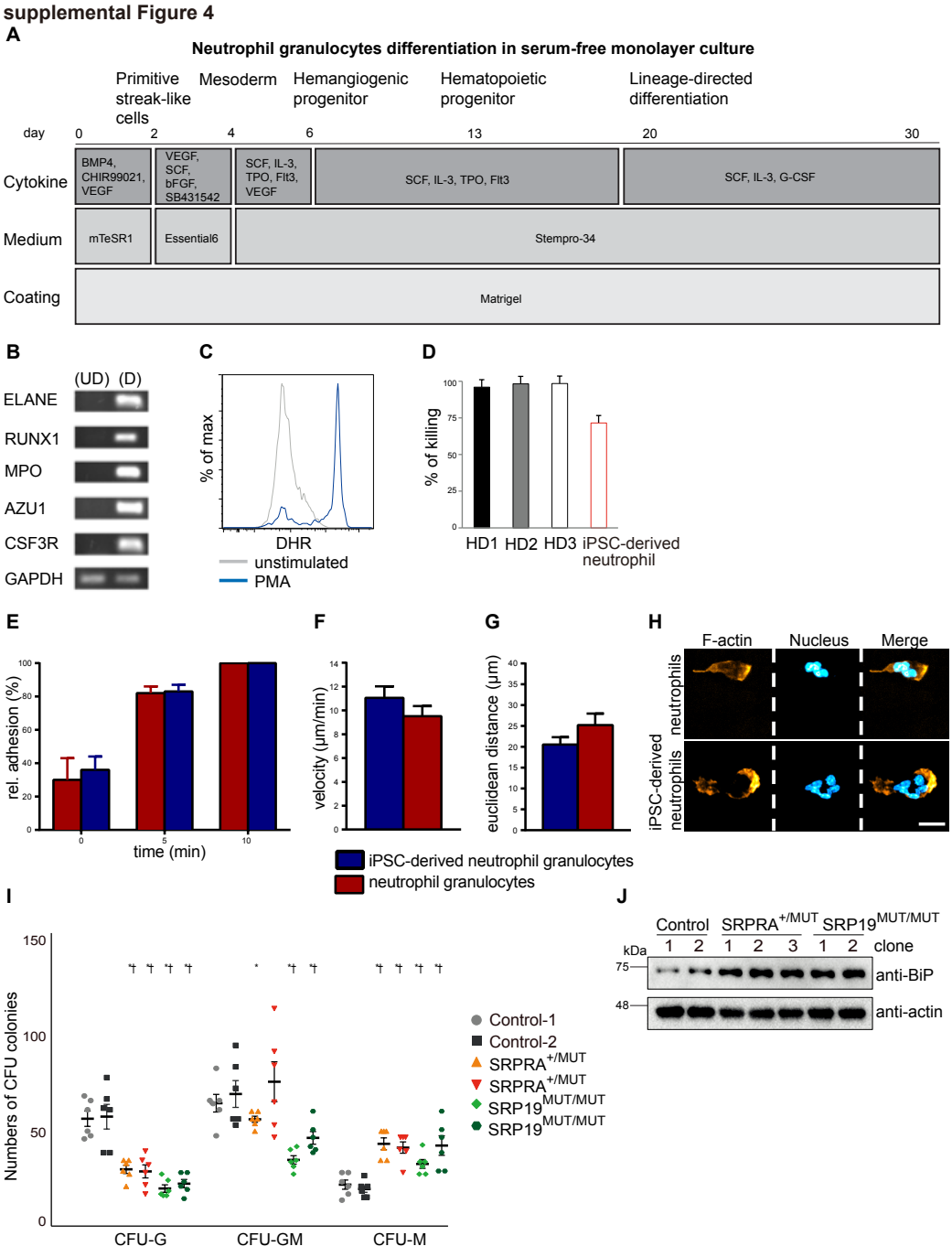
(C) RT-PCR of SRP19 isoforms and β-actin in neutrophils from healthy donors (wildtype) and SRP patients with the homozygous splice site variant. Experiments were performed in triplicate.

supplemental Figure 3



(A) Confocal microscopy images of immunostaining of eIF6 in HeLa cells transiently expressing GFP-SRP19-isoform1 and GFP-SRP19-isoform3. DNA was visualized with DAPI. Scale bar, 10 μm. Experiments were performed in triplicate.

(B) Confocal microscopy images of immunostaining of Calnexin in HeLa cells transiently expressing GFP-SRP19-isoform1 and GFP-SRP19-isoform3. DNA was visualized with DAPI. Scale bar, 10 μm. Experiments were performed in triplicate.



Supplemental Figure 4. Scheme of *in vitro* differentiation of neutrophil granulocytes and characterization of iPSC-derived neutrophil granulocytes.

(A) Scheme illustrating differentiation protocol of neutrophil-like granulocytes from iPS cells.

(B) RT-PCR analysis of lineage-specific marker genes: neutrophil elastase (Elane), runt-related transcription factor 1 (RUNX1), myeloperoxidase (MPO), azurocidin (AZU), colony stimulating factor receptor 3 (CSFR3) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in undifferentiated (UD) and differentiated iPSCs (D).

(C) FACS histogram showing superoxide production in iPS-derived wildtype myeloid cells with or without PMA stimulation. Activity was assessed using Dihydrorhodamine.

(D) Opsonophagocytic killing activity in wildtype iPSC-derived neutrophil granulocytes on day 43 after differentiation.

Motility studies in iPSC-derived wildtype neutrophil granulocytes compared to neutrophil granulocytes isolated from HD.

iPSC-derived neutrophils were sorted by FACS and seeded into ICAM1 and CXCL8 coated chambers followed by time-lapse microscopy. Parameters indicate in:

(E) Relative adhesion, (F) Velocity and in (G) Euclidean distance.

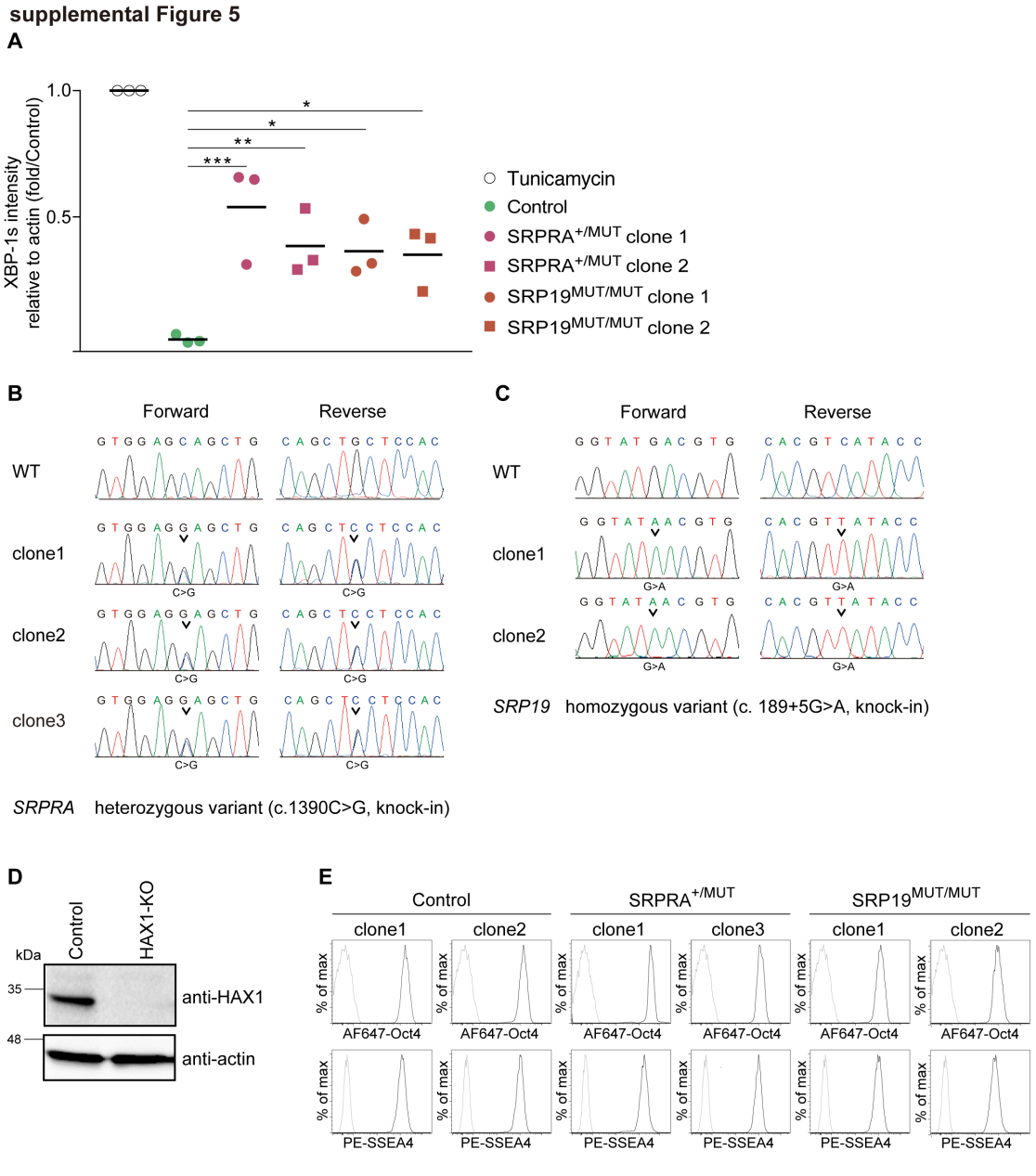
n=6 experiments. Errorbars indicate SEM.

(H) Confocal microscopy analysis of neutrophils and iPSC-derived wildtype neutrophils. Cells were fixed and stained for F-actin and DNA followed by confocal microscopy analysis. Scale bar, 10 μm.

(I) Quantification of Colony forming unit formation by iPSC-derived hematopoietic progenitor cells on day 17 after differentiation. Data are presented as mean ± SEM (n=*3 dishes* per each clone, 2 *independent experiments*. Two-*tailed Mann–Whitney U test*, *P<0.05 vs control1, †P<0.05 vs control2). CFU-G; colony-forming unit-

granulocyte CFU-GM; colony-forming unit-granulocyte/macrophage CFU-M; colony-forming unit-macrophage.

(J) Immunoblot analysis of BiP expression in sorted (CD49low/CD33low) iPSC-derived neutrophil granulocytes.



Supplemental Figure 5. Characterization of iPS cells and edited derivative cell clones.

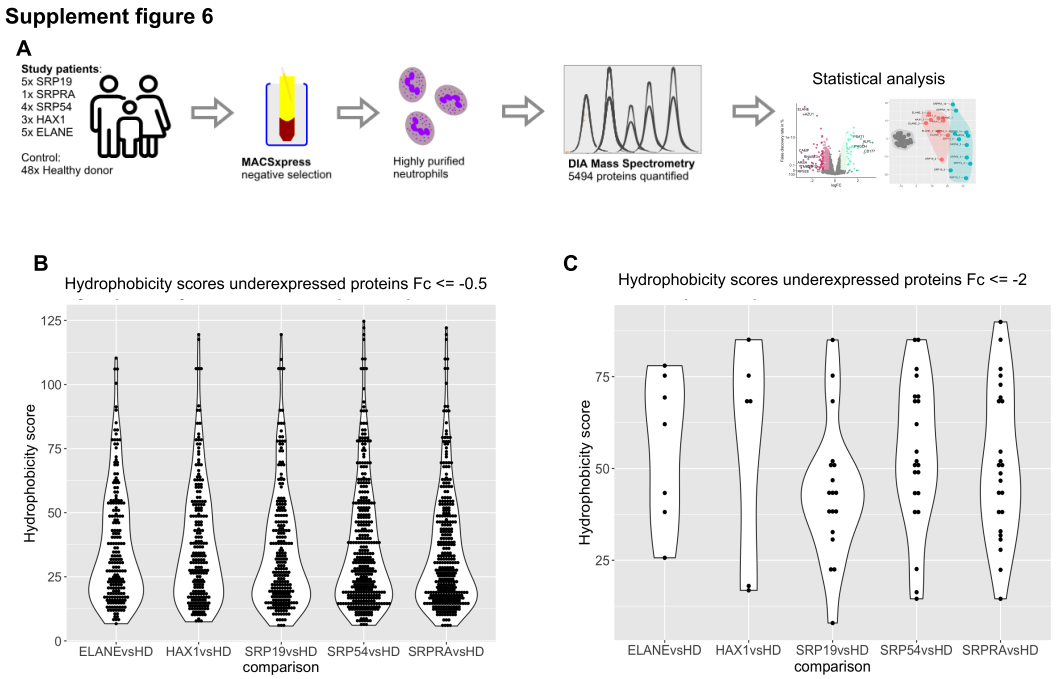
(A) Quantification of endogenous levels of XBP-1s of Tunicamycin-treated (2µg/ml for 4 hours), control and SRP-mutated lysates from cells harvested at day 14 at neutrophil differentiation. Statistical analysis using One-way ANOVA. $SRPRA^{+/MUT}$ clone 1: p= 0.0007; $SRPRA^{+/MUT}$ clone 2: p=0.0098; $SRP19^{MUT/MUT}$ clone 1 p=0.0144; $SRP19^{MUT/MUT}$ clone 2 p= 0.0184. Experiment was performed in triplicate.

(B) Chromatogram of CRISPR-Cas9 edited iPS cells modeling the patients' heterozygous variant SRPRA (c.1390C>G).

(C) Chromatogram of CRISPR-Cas9 edited iPS cells modeling the patients' homozygous variant SRP19 (c.189+5G>A).

(D) Immunoblot analysis of control and HAX1-deficient iPS cells using the indicated antibodies.

(E) Oct4 and SSEA4 expression analysis in each iPS clone before differentiation. (All clones showed high expression of Oct4 and SSEA4 before differentiation.)

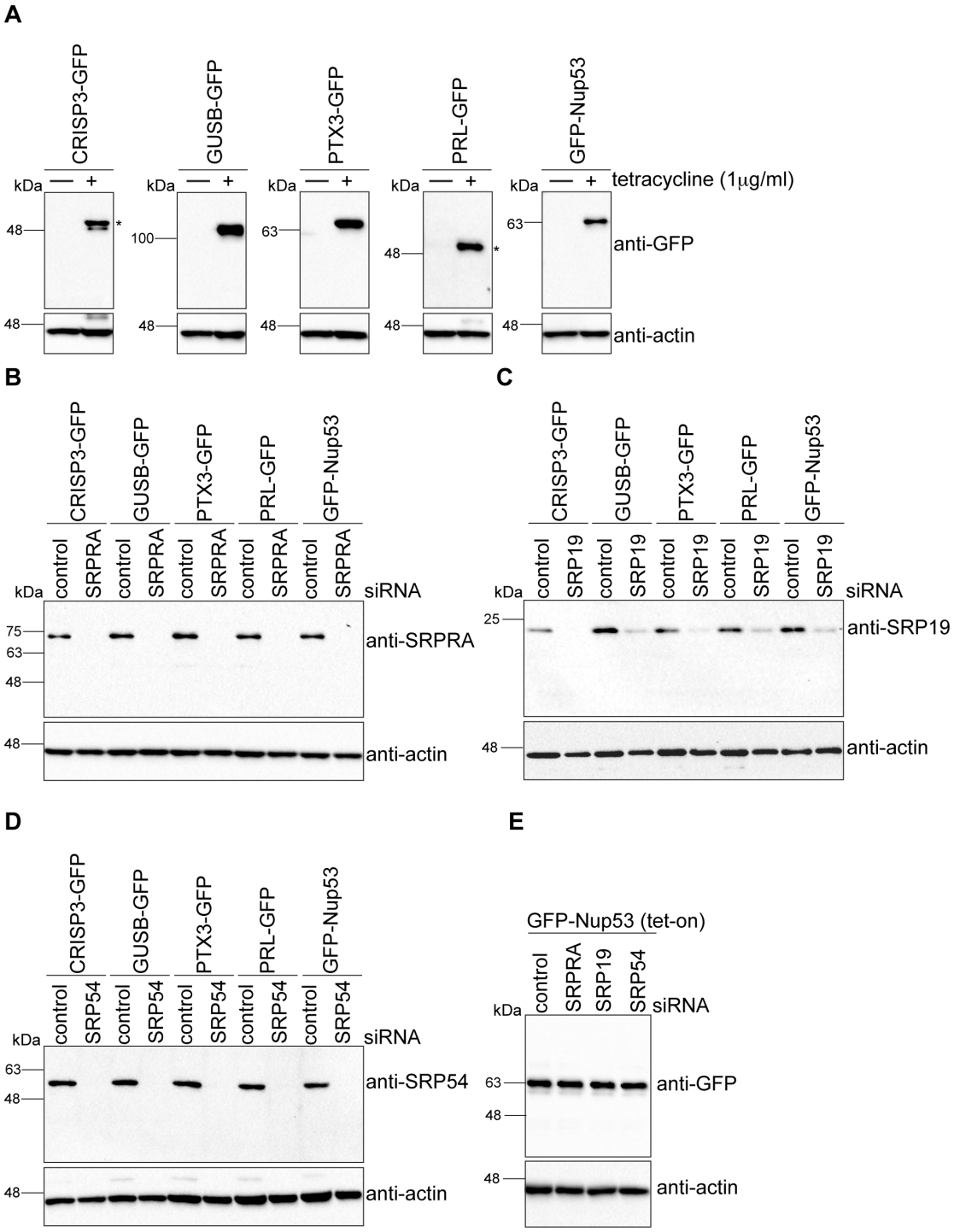


Supplemental Figure 6. Proteome analysis of patient derived primary neutrophil granulocytes.

(A) Scheme of the proteome sample preparation and analysis workflow.

(B-C) Hydrophobicity scores as described in Figure 3L but with lesser (G = abs(Fc) > 0.5) and higher (H = abs(Fc) > 2) threshold levels.

supplemental Figure 7



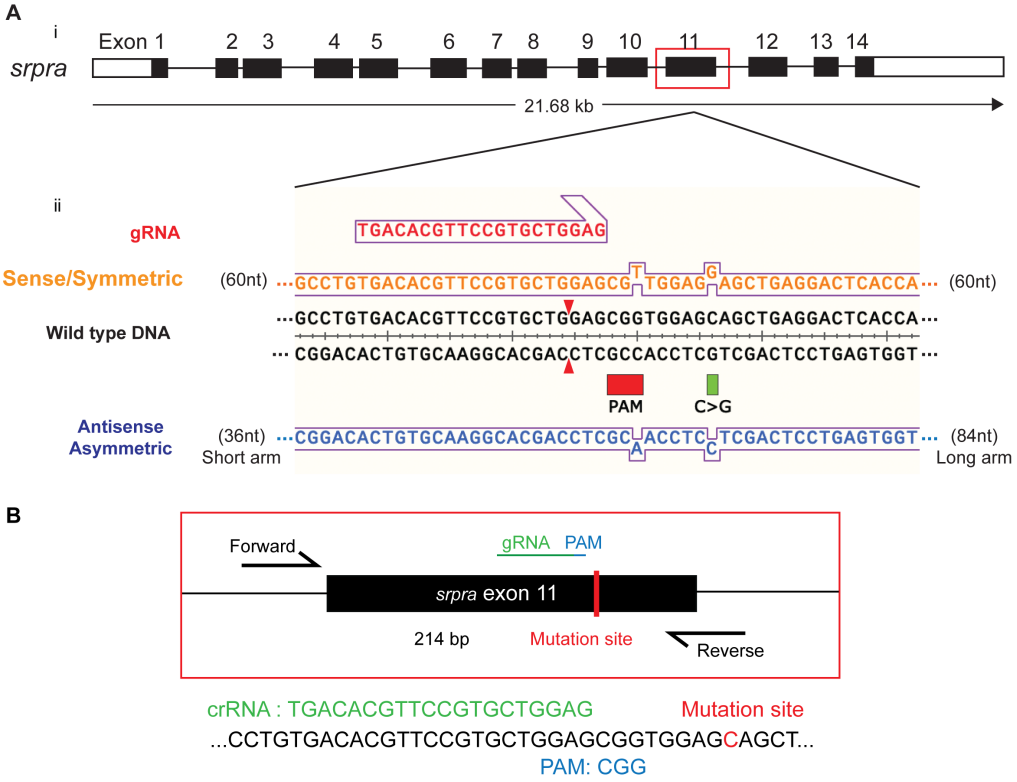
supplemental Figure 7. Characterization of tet-on reporter cell lines.

(A) HeLa cells were treated with or without tetracycline for 24h and cell lysates were analyzed by immunoblotting with the indicated antibodies.

(B)-(D) Cells stably expressing tetracycline-induced GFP-tagged reporters were treated with siRNAs targeting control, SRP19 (B), SRPRA (C) and SRP54 (D). Cell lysates were analyzed by immunoblotting with the indicated antibodies.

(E) Cells stably expressing tetracycline-induced GFP-Nup53 were treated with siRNAs targeting control, SRPRA, SRP19 and SRP54. Cell lysates were analyzed by immunoblotting with the indicated antibodies. All experiments were performed in triplicate.

supplemental Figure 8



C i

```
WT:...CACGTTCCGTGCTGGAGCGGTGGAG...
Var1:...CACGTT--------GAGCGGTGGAG... -8bp (13%)
Var2:...CACGTTCCG------AGCGGTGGAG... -6bp (5%)

WT:...AGCGGTGG..40bp..CACCTGAGA...
Var3:...AGCGGTGG..40bp..CACCTTAGA... G>T (7%)
```

ii

```
WT: .. AACDTFRAGAVEQLRTHQRRLNSLHPPEKHGGQPAVQLFEKGYGKDAAGIAMEAI..
Var1: .. AACDTLSGGAAEDSPAPAELSTST*
Var2: .. AACDTFRA--VEQLRTHQRRLNSLHPPEKHGGQPAVQLFEKGYGKDAAGIAMEAI..
Var3: .. AACDTFRAGAVEQLRTHQRRLNSLHPP*
```

iii

Variant	gDNA coordinate	cDNA	Protein	VAF
Var 1	18:44832662_44832669	c.1407_14a14del	p.(Phe469Leufs*488)	13% (38804/291662)
Var 2	18:44832661_44832666del	c.1410_1415del	p.(Ala471_Gly472del)	5% (15728/291865)
Var 3	18: 44832605 C > A	c.1471G>T	p.(Glu491Ter)	7% (13471/193076)

Supplemental Figure 8. Gene editing of zebrafish *srpra*.

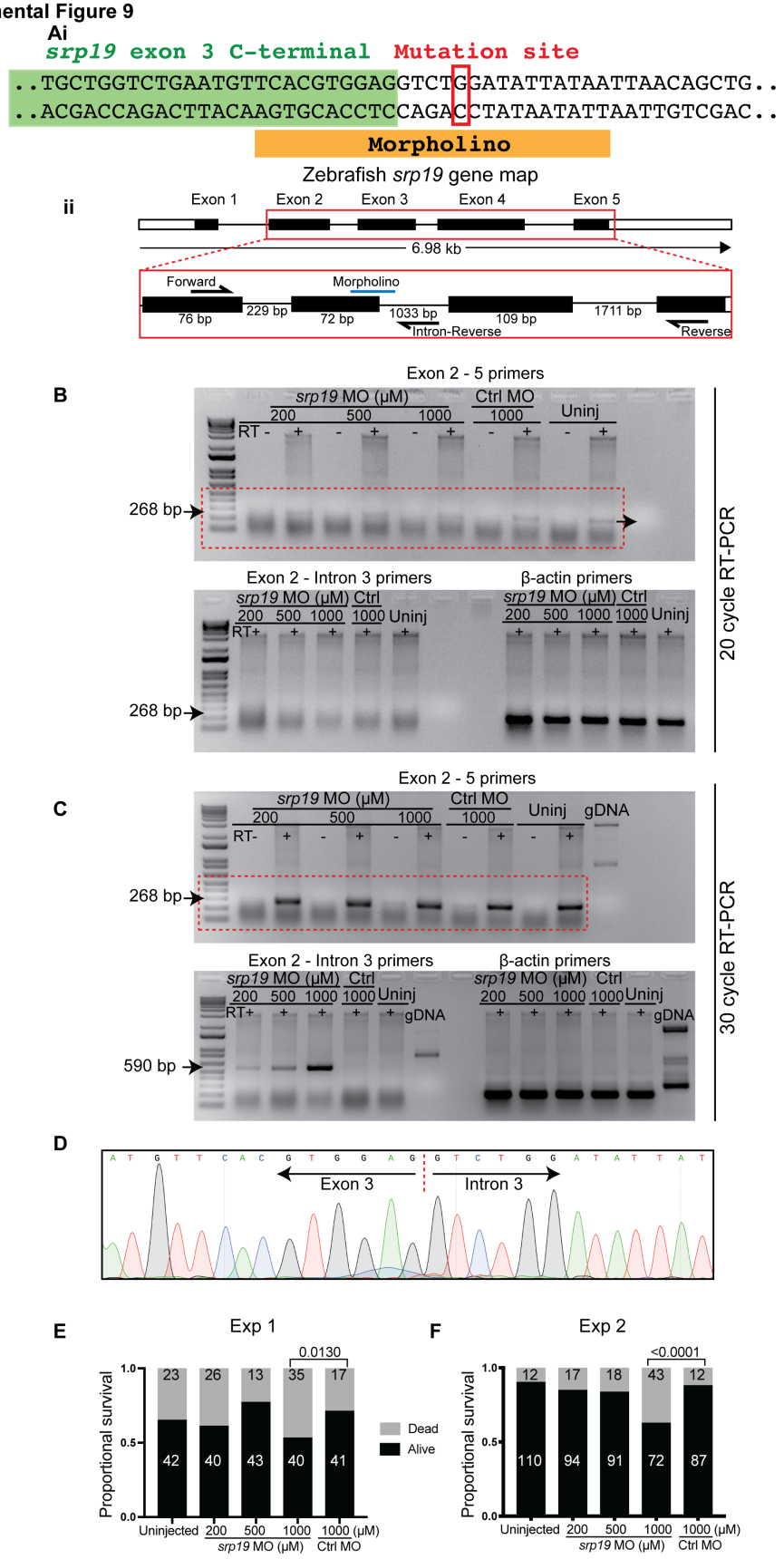
(A) Schematic showing design and components of the *spra* CRISPR/Cas9 gene editing approach, targeted by a guide RNA (gRNA) to a protospacer adjacent motif (PAM) site in exon 11. Sense and antisense DNA oligonucleotides were designed to encourage homology-directed repair, which, were it to occur, would ablate the PAM

site (by creating a G>T substitution) and generate the C>G missense mutation corresponding to the patient's putative disease missense allele.

(B) Schematic showing the *srpra* PCR-based genotyping approach.

(C) NGS data showing 3 commonest variants in DNA prepared from neutrophils of *mpx-cas9* neutrophil-lineage crispants. (i) Nucleotide sequences. (ii) Predicted protein sequences. (iii) Tabulation with variant allele frequencies (VAF).

supplemental Figure 9



Supplemental Figure 9. Morpholino-induced mis-splicing of zebrafish *srp19*.

(A) (i) Schematic showing design of *srp19* exon 3 donor site splice-blocking morpholino, corresponding to the antisense sequence above the orange bar. (ii) Schematic showing primer location for RT-PCR to demonstrate normally spliced and aberrantly-spliced intron-retaining transcripts.

(B) RT-PCR (20 cycles) showing no detectable 268 nt normally-spliced *srp19* transcript in *srp19*-MO injected embryos despite its presence in control groups, consistent with reduced abundance compared to normal of the normally-spliced *srp19* transcript. The intron-retaining transcript was not detected after 20 cycles.

(C) RT-PCR (30 cycles) showing detectable 268 nt normally-spliced *srp19* transcript in all *srp19*-MO injected embryos and control groups, along with increasing amounts of an aberrantly-spliced 590 nt transcript corresponding to intron 3 retention, consistent with a MO dose-dependent increase in aberrant *srp19* splicing.

(D) Excerpt of Sanger sequencing chromatogram of the aberrant 590 nt *srp19* product in (C), confirming that it represents intron 3 retention.

(E-F) Embryo survival rates at 2 dpf for the two independent *srp19*-MO experiments of Figure 6J and 6K respectively, showing that in both experiments, *srp19*-MO injection was only associated with significant reduced survival at the highest (1000 μM) dose. Importantly, there was no survival disadvantage in Experiment 1 (Figure 6J) at the 500 μM dose, where reduced neutrophil counts were observed from blinded scoring.