CRISPR/Cas9-mediated *ELANE* knockout enables neutrophilic maturation of primary hematopoietic stem and progenitor cells and induced pluripotent stem cells of severe congenital neutropenia patients

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Supplemental Information

Supplemental Methods

Generation of mutant p.P139L and p.C151Y *ELANE* HL60 cell lines

Zhang lab's CRISPR Design tool (http://crispr.mit.edu/) was used to identify a guide RNA to target *ELANE* with minimal off-target effects. This guide was cloned into the pSpCas9(BB)-2A-GFP vector (Addgene, #48138). The Guide-it sgRNA Screening Kit (Clontech, #631440) was used to test the efficacy of different single guide RNAs (sgRNAs) *in vitro* prior to using them in the HL60 cells. A 100nt single-stranded DNA oligonucleotide (ssODN) was designed as an HDR repair template, containing the desired mutation with ~50 nt of homology on either side of the predicted cut site. HL60 cells were co-transfected with the ssODN repair template and the Cas9 vector containing the sgRNA using electroporation. 24 hours after transfection, the cells were single-cell sorted using GFP selection into two 96 well plates containing 50% conditioned media. The cells were expanded for three weeks. After the first week, the media was refreshed every 2 - 3 days. After three weeks the presence of mutations was confirmed by Sanger sequencing.

ELANE knock-down using small inhibitory RNA (siRNA) in HL60 cells

Silencer-Select anti-ELANE (s4601) and scrambled (4390843) siRNAs were purchased from Thermo Fisher Scientific. Mutant and wild-type HL60 cells were electroporated with siRNAs using the Neon Transfection system (Thermo Fisher Scientific, #MPK5000) following the recommended guidelines of the manufacturer. After the transfection, cells were maintained in the complete medium. The expression of NE was

assessed at day 4 by western blot analysis using anti-NE monoclonal antibody (Abcam, #121260) with actin-specific monoclonal antibody as a loading control (Santa Cruz, #47778).

Myeloid differentiation of HL60 cells

After siRNA transfection, cells were maintained in the complete medium for 24 hours. Then, myeloid differentiation was stimulated by addition of 2µM all-trans retinoic acid (ATRA) to cell culture. Cells were harvested at day 5, labeled with CD11b-PE granulocytic differentiation surface marker (BD Biosciences, #561001) and evaluated by flow cytometry.

Reprogramming of peripheral blood mononuclear cells (PB MNCs)

1.5 x 10^6 PB MNCs were cultured in Stemline II medium (Sigma) supplemented with 10 % FCS, 1 % penicillin/streptomycin (Biochrom, #A2213), 1 % glutamine (Biochrom, #K0283) and cytokines: 20 ng/µl IL-3 (PeproTech, #200-03), 20 ng/µl Il-6 (PeproTech, #200-06), 20 ng/µl TPO (R&D Systems, #288-TP-200), 50 ng/µl SCF (R&D Systems, #255-SC-200) and 50 ng/µl FLT-3L (BioLegend, #550604) for 7 days. On day 7, cells were transferred to Retronectin (Clontech)-coated 12-well plates and lentiviral supernatants with Oct3/4, Klf4 and Sox2 cDNAs (pRRL.PPT.SF.hOct34.hKlf4.hSox2.i2dTomato.pre.FRT kindly provided by A. Schambach) with a multiplicity of infection (MOI) of 2. After 4 days, cells were transferred to SNL-feeder cells and cultured in the mixture of a half of iPSCs- maintenance medium (DMEM F12 (Sigma, #D6421-6x) supplemented with 20 % knock out serum replacement (Invitrogen, #10828028), 1 % non-essential amino acids solution (Invitrogen, #11140-050), 100uM 2-Mercaptoethanol and 2mM L-Glutamine) and a half of $CD34^+$ cells expansion medium supplemented with 2 mM valproic acid and 50 µg/ml vitamin C. Medium was gradually changed to iPSCs medium only. First iPSCs colonies appeared approximately three weeks after initiation of reprogramming.

Quantitative Real Time PCR

RNA was isolated using RNeasy Micro Kit (Qiagen) and cDNA was prepared from 1 µg of total RNA with Omniscript RT Kit (Qiagen). qPCR was performed using SYBR Green

qPCR master mix (Roche) and Light Cycler 480 (Roche). Target genes were normalized to GAPDH and/or ACTB genes. Primer sequences are presented in **Supplemental Table 2**.

Characterization of reprogrammed iPSC clones

For iPSCs characterization, the stem cell surface markers TRA1-60 (BD, #560380) and SSEA4-FITC (BD, #560126) were analyzed. Anti-mouse IgGk beads (BD Biosciences) were used for compensation. Alkaline phosphatase assay was performed for iPSCs colonies on SNL-feeders at day 10 of culture were washed with PBS, fixed in 4 % PFA /10 % Sucrose in water and stained with NBT/BCIP staining dye (Sigma. # 11681451001) for 20 min at room-temperature. RNA was isolated from iPSC clones using RNeasy Micro Kit (Qiagen) and cDNA was prepared from 1 µg of total RNA with Omniscript RT Kit (Qiagen). qPCR was performed by means of SYBR Green qPCR master mix (Roche) on Light Cycler 480 (Roche). Target genes were normalized to GAPDH. Primer sequences are presented in **Supplementary Table 2**.

Screening of single iPSCs clones

Genomic DNA was isolated from single iPSC clones using the QuickExtract DNA extraction kit (Lucigen, #QE09050). PCR was carried out using the GoTaq Hot Start Polymerase Kit (Promega, #M5006) and *ELANE*-specific primers (F: 5'-GGCTCCTTGGCAGGCACTCA-3', R: 5'- CACCTCACAGACCGGGACGC-3'). *In vitro* cleavage assay of PCR products was performed using 1 µM Cas9 RNP assembled with crRNA used for *ELANE* knock out experiments. The reaction mix was incubated at 37°C for 60 minutes and subsequently, run on 1% agarose gel. The PCR products from iPSC clones that showed no cleavage were purified by ExoSAP, which is a master mix of one-part Exonuclease I 20 U/µl (Thermo Fisher Scientific, #EN0581) and two parts of FastAP thermosensitive alkaline phosphatase 1U/µl (Thermo Fisher Scientific, #EF0651). Sanger sequencing of purified PCR products was performed by Eurofins Genomics. The tracking of

Indels by Decomposition (TIDE) webtool[20] was used to estimate CRISPR/Cas9 modification efficiency.

EB-based hematopoietic differentiation of iPSCs

iPSCs were cultured on SNL feeder cells for 24 hours and then dissociated using PBS/EDTA (0.02 %) for 5 min. EB generation was performed by centrifugation of 2 x 10^4 cells per EB in 96-well plates using APEL serum-free differentiation medium (Stemcell Technologies) supplemented with bFGF (20 ng/µl) and ROCK Inhibitor (R&D). On day one, BMP4 (40 ng/µl) was added to the culture medium to induce mesodermal differentiation. On day four, EBs were plated on Matrigel-coated 6-well plates (10 EBs/well) in APEL medium supplemented with VEGF (40 ng/µl), SCF (50 ng/µl) and IL-3 (50 ng/µl). For neutrophilic differentiation, the medium was changed 3 days later to fresh APEL medium supplemented with IL3 (50 ng/µl) and G-CSF (50 ng/µl). All cytokines were purchased from R&D Systems if nothing else is indicated. First hematopoietic suspension cells were appeared on between day 12 and 14. Suspension cells were harvested every 3 - 4 days and analyzed starting from day 14 to day 32. For flow cytometry analysis, cells were incubated in PBS/1% BSA containing 0.05 % sodium azide and stained with mouse monoclonal anti-human antibodies. Two multicolor FACS antibody panels were applied: 'early-stage' hematopoietic differentiation panel using CD33 (BioLegend, #303416), CD34 (BD, #348811), CD309 (BioLegend, #359910), CD43 (BD, #560199), CD41a (BD, #557296), CD235a (BD, #559943), CD45 (BioLegend, #304036), 7-AAD (BD, #559925); and 'late-stage' myeloid/hematopoietic differentiation using CD15 (BD, #555402), CD45 (BioLegend, #304012), CD33 (BioLegend, #303416), 7-AAD (BD, #559925). For iPSCs characterization, antibodies against human TRA1-60 (BD, #560380), mouse TRA-1-85/CD147 (R&D, #FAB3195A) and human SSEA4 (BD, #560126) were used. Anti-Mouse Ig κ/Negative Control Compensation Particles (BD Biosciences) were applied for compensation. Samples were analyzed on FACSCanto II (BD) and FlowJo V10 (FlowJo LLC).

Liquid culture differentiation of CD34$^+$ cells

CD34$^+$ cells were seeded in a 24-well plate at a density of 2 x 10^5 cells/ml. Cells were incubated for 7 days in RPMI 1640 GlutaMAX supplemented with 10 % FBS, 1 % penicillin/streptomycin, 5 ng/ml SCF, 5 ng/ml IL-3, 5 ng/ml GM-CSF and 10 ng/ml G-CSF. Medium was exchanged every second day. On day 7, cells were plated in RPMI 1640 GlutaMAX supplemented with 10 % FBS, 1 % penicillin/streptomycin and 10 ng/ml G-CSF. On day 14, cells were analyzed by FACS using following mouse anti-human antibodies: CD34 (BD, #348811), CD33 (BioLegend, #303416), CD45 (BioLegend, #304036), CD11b (BD, #557754), CD15 (BD, #555402).

Assessment of phagocytosis kinetics using the IncuCyte ZOOM system

Granulocytes from day 14 of liquid culture differentiation were cultured in RPMI 1640 medium supplemented with 0.5 % BSA and pHrodo Green E. coli Bioparticles Conjugate (#4616 Essen Bio) according to manufacturer's protocol in IncuCyte ZOOM system (Essen Bio) at 37°C, 5 % CO_2. Briefly, Briefly, 10^4 cells were seeded in 90 µl medium and 10 µg of Bioparticles were added to a final volume of 100 µl. The cells were monitored for 8 hours. The analysis was conducted in IncuCyte S3 Software.

Determination of chemotactic activity of WT and *ELANE* KO granulocytes *in vitro*

Granulocytes from day 14 of liquid culture differentiation were resuspended at 1 x 10^6 cells/ml in RPMI 1640 medium supplemented with 0.5 % BSA (Sigma). Two hundred microliters of cell suspension were placed into inserts of the polycarbonate transwell chemotaxis chamber inserts have 6.5 mm diameter, 5-µm pore size (#3421 Costar Corning, Corning, NY). Bottom well of the transwell chemotaxis chamber was filled with RPMI 1640 medium supplemented with 0.5 % BSA (medium control) or the same medium supplemented with 1 nM *N-formyl-Met-Leu-Phe* (*f*MLP) (#F3506, Sigma). The inserts were added to the lower chambers and incubated at 37°C, 5 % CO_2 for 2 hours. After that, 70 µL of 50 mM EDTA was added to the lower chambers to release the migrated granulocytes from the bottom

of the insert membrane and the bottom of the well. Plates were incubated at 4° C for 30 min. Inserts where removed, cells rigorously resuspended and counted with FACSCanto II for 3 min at 120 µl/min with gating on forward and sideward scatter.

***In vitro* reactive oxygen species (ROS) assay**

Cells were seeded at the density of $1x10^5$ with or without *f*MLP (Sigma, #F3506) at a final concentration 10 nM and incubated for 30 minutes at 37 °C, 5% CO_2. The level of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), was measured by ROS-Glo H_2O_2 Assay kit (Promega, #G8820) according to the manufacturer's protocol.

Electron Microscopy

Granulocytes from day 14 of liquid culture differentiation were fixed in 2.5% glutaraldehyde/4% formaldehyde (Sigma, # G5882), (Thermo Fischer Scientific, # 50-980-487) in PBS for 2 hours at room temperature. For scanning electron microscopy (SEM) analysis, cells were post-fixed with 1% osmium tetroxide for 45 minutes on ice. Subsequently, samples were dehydrated in a graded ethanol series followed by critical point drying (Polaron) with CO_2. Finally, the cells were sputter-coated with a 5 nm thick layer of platinum (CCU-010, Safematic) and examined with a field emission scanning electron microscope (Regulus 8230, Hitachi High Technologies) at an accelerating voltage of 5 kV. For transmission electron microscopy (TEM), cells were embedded in gelatin, post-fixed with 1% osmium tetroxide for 1 hour on ice, dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and analyzed with a Tecnai Spirit (Thermo Fisher Scientific) operated at 120 kV.

Transplantation of human *ELANE* KO PMNs into zebrafish embryos

Zebrafish were maintained according to standard protocols and handled in accordance with European Union animal protection Directive 2010/63/EU. Wild-type TE embryos at 48 hours post-fertilization (hpf) were used for xenotransplantation experiment. To visualize engrafted human PMNs, they were labeled with Vybrant CFDA SE Cell Tracer Kit

(Invitrogen) according to the manufacturer's protocol. Approximately 200 fluorescently labeled *ELANE* KO PMNs were injected per embryo into the duct of Cuvier over the yolk sac. After one hour, 2-5µl of Alexa-594-conjugated *Staphylococcus aureus* BioParticles (Invitrogen, #S23372) diluted in PBS were injected into the tailfin close to the caudal vein. Xenotransplanted embryos were kept at 35°C during the entire experimental procedure. Live imaging of embryos was performed with a Zeiss LSM 710 NLO confocal microscope using a 40x water-immersion objective. Images were analyzed with Imaris software as described previously[21].

Western blotting

$1x10^6$ cells were lysed in 200 µl 3 x Laemmli buffer and protein was denatured for 10 min at 95°C. 5 µl of cell lysate in Laemmli buffer were loaded per lane. Proteins were separated on a 12 % polyacrylamide gel and transferred on a nitrocellulose membrane (GE Healthcare) (1 hour, 100V, 4°C). The membrane was blocked for 1 hour in 5 % BSA/TBST and incubated with primary anti-neutrophil elastase (Santa Cruz #sc-9520 or #sc-55549) or ß-actin (Cell Signaling, #4970) antibody overnight (at 4°C). After that, membranes were washed and incubated with secondary HRP-conjugated antibody (Santa-Cruz, #sc-2004) for 1 hour at room temperature. Pierce ECL solution (Thermo Fisher) and Amersham Hyperfilms were used to detect chemiluminescence signal of proteins.

Alkaline phosphatase assay

iPSCs colonies cultured on SNL feeder cells for 10 days were washed with PBS, fixed in 4 % PFA /10 % Sucrose in water and stained with NBT/BCIP staining dye (Sigma) for 20 min at room temperature.

Off-target analysis of *ELANE* specific CRISPR/Cas9-gRNA RNP

Prediction of possible off-target activity of ELANE-specific CRISPR/Cas9-sgRNA RNP was performed in CN patients-derived iPSC knock out clones using http://crispr.mit.edu

website. The predicted sites are shown in **Supplementary Table 1**. Sanger sequencing histograms of highlighted sites are depicted in **Supplemental Figure 4**.

Supplemental Table 1. Off-target prediction sites of the guide RNA that used for the *ELANE* knockout

Sequence	PAM	Score*	Gene	Chromosome	Position	Mismatches
CTGCGCGGAGGCCACTTCTG	CGG	100	*ELANE* - ENSG00000197561	chr19	852970	0
CAACGCTGAGGCCACTTCTG	**GGG**	**1.74086**		**chr12**	**24306644**	**3**
CTCGGCGGCGGCCACTTCTG	**GAG**	**1.609912**	***OR2W4P* - ENSG00000216629**	**chr6**	**27977653**	**3**
CAAGGCGGAAGCCACTTCTG	AGG	1.37665		chr22	26781774	4
GCTCACGGAGGCCACTTCTG	GAG	1.356226		chr6	168880349	4
GGGCCCTGAGGCCACTTCTG	TAG	1.001312		chr6	110344397	4
TTCCCCAGAGGCCACTTCTG	**TAG**	**0.979234**	***GPR107* - ENSG00000148358**	**chr9**	**130139434**	**4**
TAACGCTGAGGCCACTTCTG	CGG	0.971305		chr20	47239318	4
CCCTGCTGAGGCCACTTCTG	AAG	0.94827		chr1	53554854	4
CTCTCCCGAGGCCACTTCTG	CAG	0.926302		chr15	73042681	4
CTGCACTTAAGCCACTTCTG	GGG	0.885757		chr2	191383953	4
GTGCAGGGATGCCACTTCTG	CAG	0.866831		chrX	155207552	4
ATTCATGGAGGCCACTTCTG	CAG	0.846669		chr2	20922707	4
TCACGAGGAGGCCACTTCTG	CAG	0.839976		chr10	13237774	4
CCTCCAGGAGGCCACTTCTG	CAG	0.826902		chr22	31964196	4
CTGAGGGGAGGCCACTGCTG	GAG	0.815023		chr6	25056185	3
ATGGGCAGAGTCCACTTCTG	TGG	0.61663		chr5	181038358	4
CTGTTCTGTGGCCACTTCTG	TAG	0.592302		chr10	7204351	4
CTGCTCCAGGGCCACTTCTG	GAG	0.574007		chr22	42645189	4
ATGAGCAGAGGTCACTTCTG	GGG	0.562032		chr18	48858809	4
CTGGGCTCAGGCCACTTCTA	AGG	0.542588		chr17	33658165	4
ATGAGCAGAGGCCACTGCTG	AAG	0.5119		chr2	104940101	4

CGGCGCAGAAGGCACTTCTG	TGG	0.50345		chr20	62767850	4
CTGAGTGGCAGCCACTTCTG	TGG	0.49912		chr1	162708586	4
CTGCTCAGAAGCCACTTCTA	GGG	0.494435	*GS1-393A7.1* - ENSG00000248522	chr8	55453007	4
CAGCTCCGAGGCCACTGCTG	GAG	0.490459		chr11	119513936	4
CTGGGCAGAGGCCACTTTTG	AAG	0.490076		chr3	63531713	3
CTGCAGGCAGTCCACTTCTG	AAG	0.488244		chr13	66147593	4
ATGTGCGGGGGCCACTGCTG	TAG	0.46817		chr1	11068959	4
CTGTGCCCAGGCCACTGCTG	TGG	0.456964		chr2	235543980	4
CTGCTCTCAGGCCACTGCTG	TGG	0.44397		chr13	102854199	4
CTGCTGGTAGGTCACTTCTG	AGG	0.443688	*GREB1* - ENSG00000196208	chr2	11597937	4
CTGGGCAGATGCCACTGCTG	TGG	0.42924		chr8	96005905	4
CTCCCCAGAGGCTACTTCTG	CAG	0.420118		chr1	8728052	4
CTGCAGGGAAGGCACTTCTG	GAG	0.415592		chrX	17816371	4
CTCCATGGAGGCCACTGCTG	AAG	0.411145		chr8	131061497	4
CTCCCCTGAGGCCACTTCAG	TGG	0.408499		chr1	48034806	4
CTGCCCGGATGCCCCTTCTG	CAG	0.408036	*RP11-465B22.3* - ENSG00000217801	chr1	1064258	3
CTGGCAGGAGGCCACTGCTG	GGG	0.404778		chr10	559812	4
CTGGGCTGAGGCCACATCTG	CAG	0.393659		chr19	28454919	3
CTGGCCGGAGCCCACTCCTG	GAG	0.390368		chr10	11389286	4
CTGCCCAGATGCCACTTCAG	AGG	0.362002		chr7	133820875	4
CTGTGAGCAGGCCACTTCAG	GGG	0.355457		chr5	143029316	4
CTGGGCAGTGCCCACTTCTG	GGG	0.345233	*SLC35C1* - ENSG00000181830	chr11	45811038	4
GTGTGCGGAGGAGACTTCTG	GAG	0.344322		chr10	116849877	4
CTGCCCAGCGCCCACTTCTG	GAG	0.336778	*ATM* - ENSG00000149311	chr11	108223020	4
CAGGGCGGAGGAGACTTCTG	GAG	0.334145		chr7	149638674	4
CAGGGCGGAGGGGACTTCTG	AAG	0.334145		chr15	40859614	4
CTGTGGAGAGGCCACTTCTT	AAG	0.328266		chr3	63862706	4

CTGTGCTGTGGACACTTCTG	AGG	0.313925		chr15	73832815	4

* using crispr.mit.edu

Supplemental Table 2. Primer sequences

Target	FW Primer Sequence (5‘-3‘)	RV Primer Sequence (5’-3’)
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG
PRE	CTGGGCTACACTGAGCACC	TGACAGGTGGTGGCAATGCC
OCT4	CCTCACTTCACTGCACTGTA	CAGGTTTTCTTTCCCTAGCT
SOX2	TTCACATGTCCCAGCACTACCAGA	TCACATGTGTGAGAGGGGCAGTGTGC
NANOG	TCACACGGAGACTGTCTCTC	GAACACAGTTCTGGTCTTCTG
ABCG2	TACCTGTATAGTGTACTTCAT	GGTCATGAGAAGTGTTGCTA
chr12 position (24306644)	TCTTCTTCAGGCTTTGCTTGCAGG	GGTAGGAGTAGAAGGGTGGC
OR2W4P	GTGGGTTTCTCTGATCGTCCCA	GGAAGAACTGTTTCCTGGCTGC
GPR107	CTGCCAAGCTGCTGTACTTCAA	TCTTACGTGCTCCAAAGGCTGA
BiP	AGGACAAGAAGGAGGACGTGG	GGTTGGAGGTGAGCTGGTTC
Bcl-XL	GGGTTCCCTTTCCTTCCATC	AGTGGCCCTAAATGGCTCT

Supplementary figure legends

Supplementary Figure 1. Granulocytic differentiation of gene edited HL60 cells

A, Representative Sanger sequencing results from two HL60 cell clones expressing *ELANE* mutations; **B**, representative WB images of NE and ß-actin of respective HL-60 cell lysates; **C**, representative FACS dot plot images of the respective HL60 cell clones differentiated with ATRA.

Supplementary Figure 2. Undetectable expression of NE in *ELANE* KO THP1 cell clones

A and **B**, Single cell clones of *ELANE* KO THP1 cells were generated, as described in Material and Methods. Original WB images of NE (A) and α-tubulin (B) expression in respective THP1 cell clones are depicted.

Supplementary Figure 3. Analysis of pluripotency of the gene edited iPSC clones

A, qRT-PCR analysis of mRNA expression of pluripotency-specific genes as well as PRE. mRNA was isolated from indicated iPSC clones. Target gene mRNA expression is normalized to GAPDH and shown relative to $CD34^+$ cells. Data represent means ± SD from two independent experiments. **B**, Representative images of the alkaline phosphatase activity analysis in iPSC colonies, indicated by purple staining. **C**, Representative FACS histograms of pluripotency-specific surface markers Tra1-60 and SSEA4 of indicated iPSC clones.

Supplementary Figure 4. Assessment of genome editing efficiency and of off-target effects of *ELANE* specific CRISPR/Cas9 RNP in *ELANE* KO iPSC clones

A, Gene editing efficiency of healthy control *ELANE* KO iPSC was assessed by Sanger sequencing and sequence trace decomposition algorithm (TIDE) after 4 days post electroporation. R^2 is calculated to assess the goodness of fit by TIDE algorithm and R2 > 0.9 is considered as reliable prediction**. B,** Possible off-target sites were predicted using Feng Zhang method, as indicated in Material and Methods. Based on the predicted scores, three genomic sites [2 genes: (*OR2W4P* and *GPR107*) and one intergenic site)] with the highest off-target scores were chosen for further analysis. Sanger sequencing of *ELANE* knockout iPSC clones revealed no mutations in the predicted off-target regions.

Supplementary Figure 5. Representative FACS dot plot images of differentiated iPSC clones

A, Representative FACS dot plots for granulocytic $CD45^+CD15^+CD16^+$cell subsets derived from EB-based differentiation of respective iPSC clones.

Supplementary Figure 6. Evaluation of early stages of hematopoietic differentiation of iPSCs

A, Flow cytometry analysis of suspension cells harvested from EBs hematopoietic culture of iPSCs on day 14 of differentiation. Data represent means ± SD from two independent experiments. *P < 0.05, **P < 0.01.

Supplementary Figure 7. Assessment of genome editing efficiency of *ELANE* KO primary HSPCs by TIDE

Gene editing efficiency of *ELANE* KO primary HSPCs assessed by sequence trace decomposition algorithm (TIDE) after 7 days post electroporation. R^2 is calculated to assess the goodness of fit by TIDE algorithm and $R^2 > 0.9$ is considered reliable prediction.

Supplementary Figure 8. Reduced NE levels in gene edited CD34$^+$ cells

A, and **B** HSPCs of two healthy controls (**A**) or two CN patients (**B**) were electroporated with *ELANE*-specific CRISPR/Cas9 RNP, on day 14 of culture, cells were lysed in Laemmli buffer and WB analysis using anti-NE antibody was performed, staining with α-tubulin antibody was used as loading control. Original WB images of two independent experiments are depicted.

Supplementary Figure 9. Representative FACS dot plot images of differentiated primary CD34$^+$ cells

A and **B**, Representative FACS dot plots for granulocytic CD45$^+$CD11b$^+$CD15$^+$ cell subsets of primary CD34$^+$ cells isolated from healthy controls (**A**) or CN patients (**B**).

Supplementary Figure 10. Evaluation of early stages of *in vitro* myeloid differentiation of *ELANE* KO primary HSPCs

A, Differentiation capacity of *ELANE* KO CD34$^+$ cells was assessed by liquid culture differentiation for 7 days by investigating hematopoietic surface marker expression. Data represent means ± SD from triplicates. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Figure 11. Evaluation of the effects of *ELANE* KO on the expression of UPR gene BiP and anti-apoptotic factor Bcl-xl

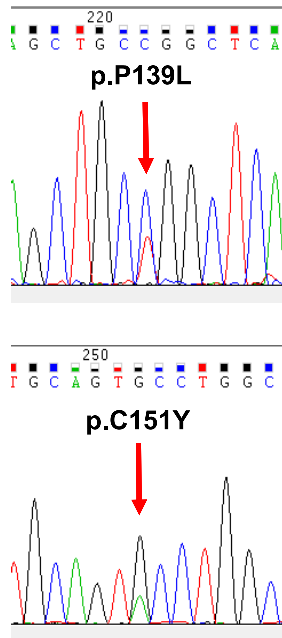
A, qRT-PCR analysis of mRNA expression of Bcl-xl in HSPCs collected at day 14 of iPSC culture or of BiP and ELANE in neutrophils collected at day 28-32 of iPSC culture. Data represent means ± SD from two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

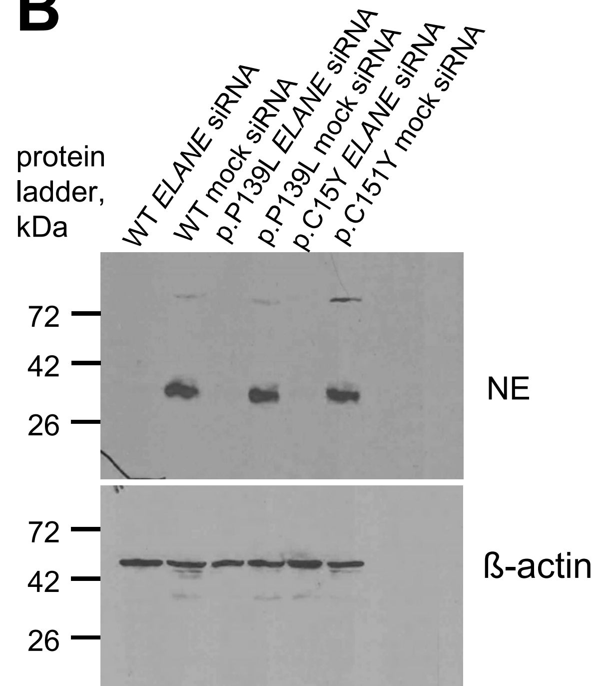
Supplementary video figure legends

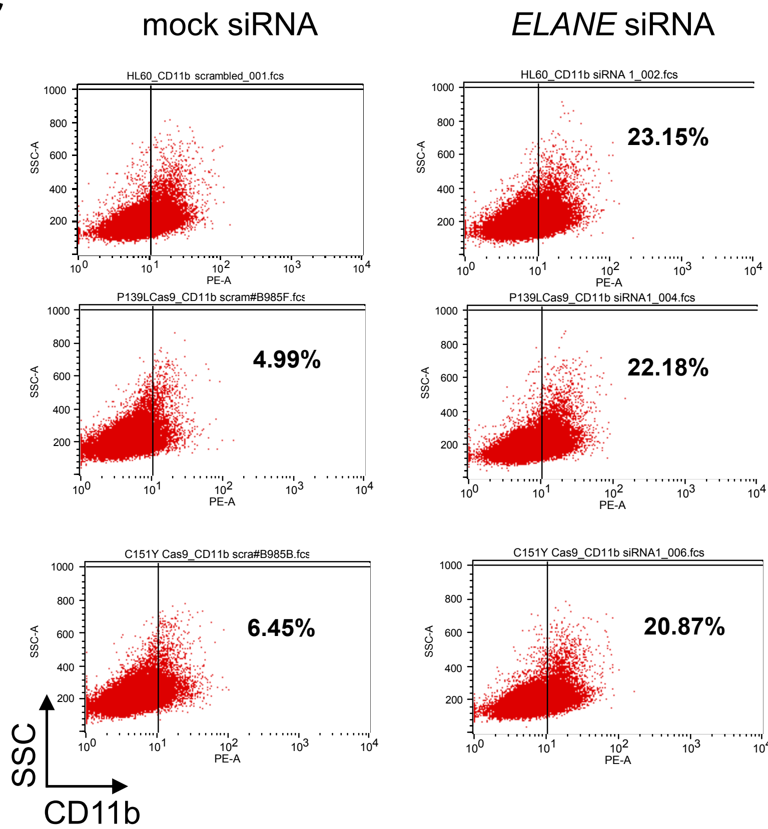
Supplementary video 1. In vivo phagocytosis of human ELANE KO PMN's.

Three-dimensional rendering of a z-stacks of 12µm illustrating three human ELANE KO cells (green), which one of them has engulfed Alexta-594-conjugated bacteria (red).

Supplementary video 2. Phagocytosis of human ELANE KO PMN's in zebrafish embryos. Time-lapse imaging of transplanted human ELANE KO cells (green) in the caudal hematopoietic site of a zebrafish embryo. Some human cells have engulfed Alexta-594-conjugated bacteria (red). Time is shown in minutes.

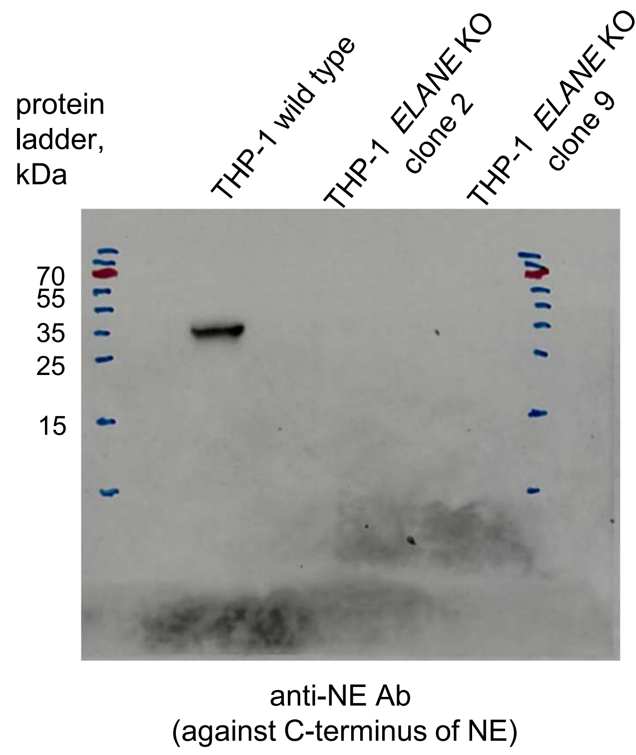






Supplementary Figure 1

A



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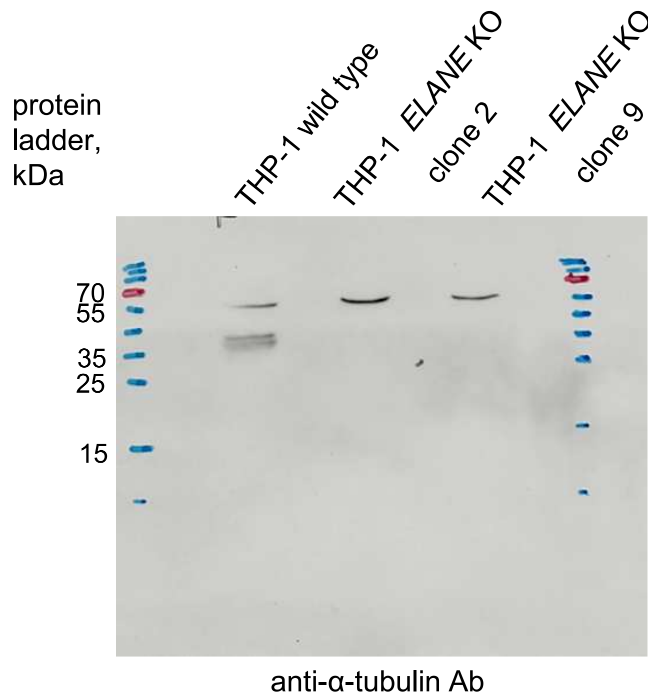
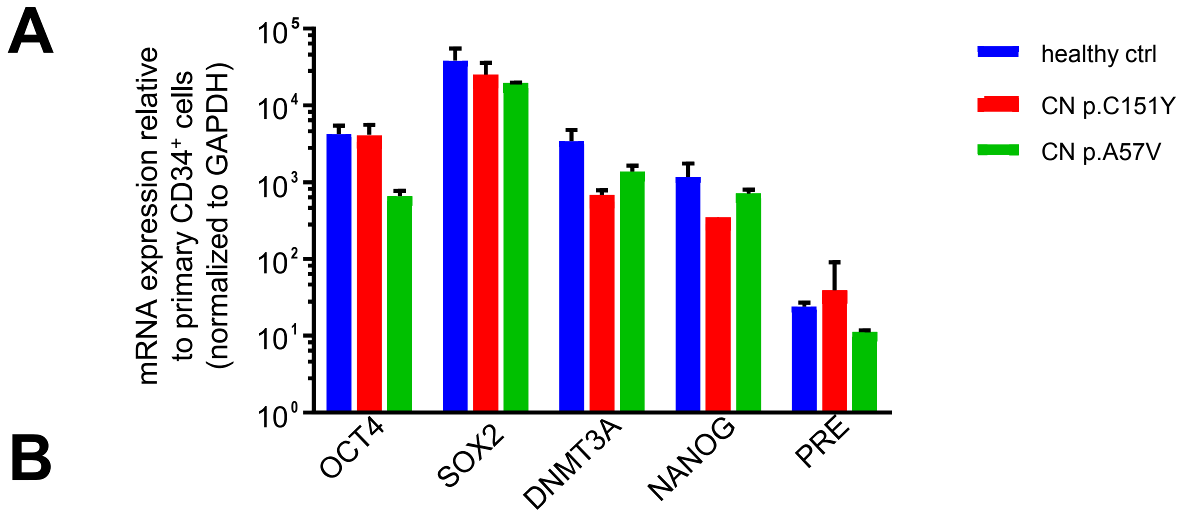
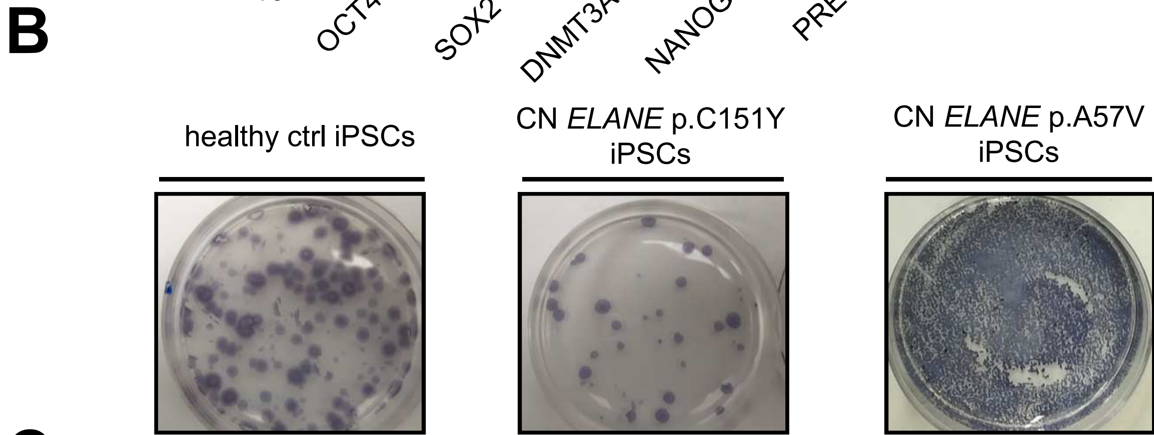
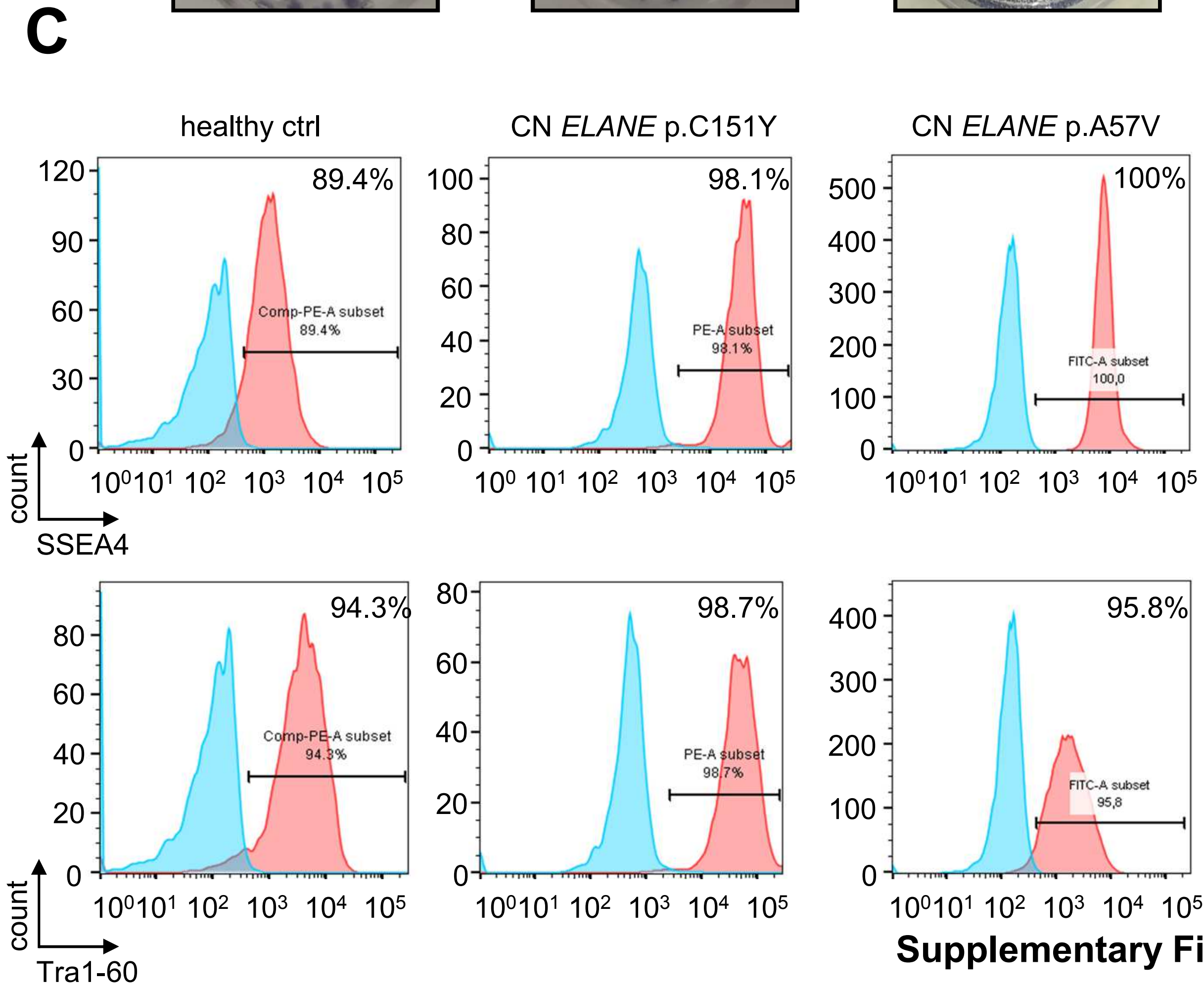


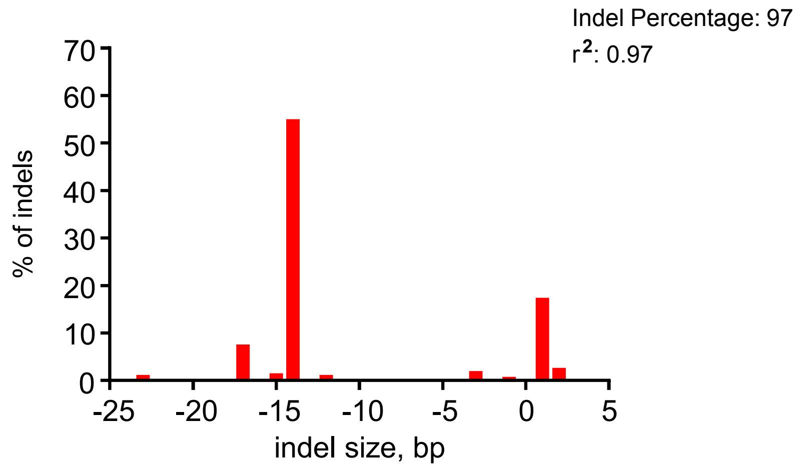
Supplementary Figure 2

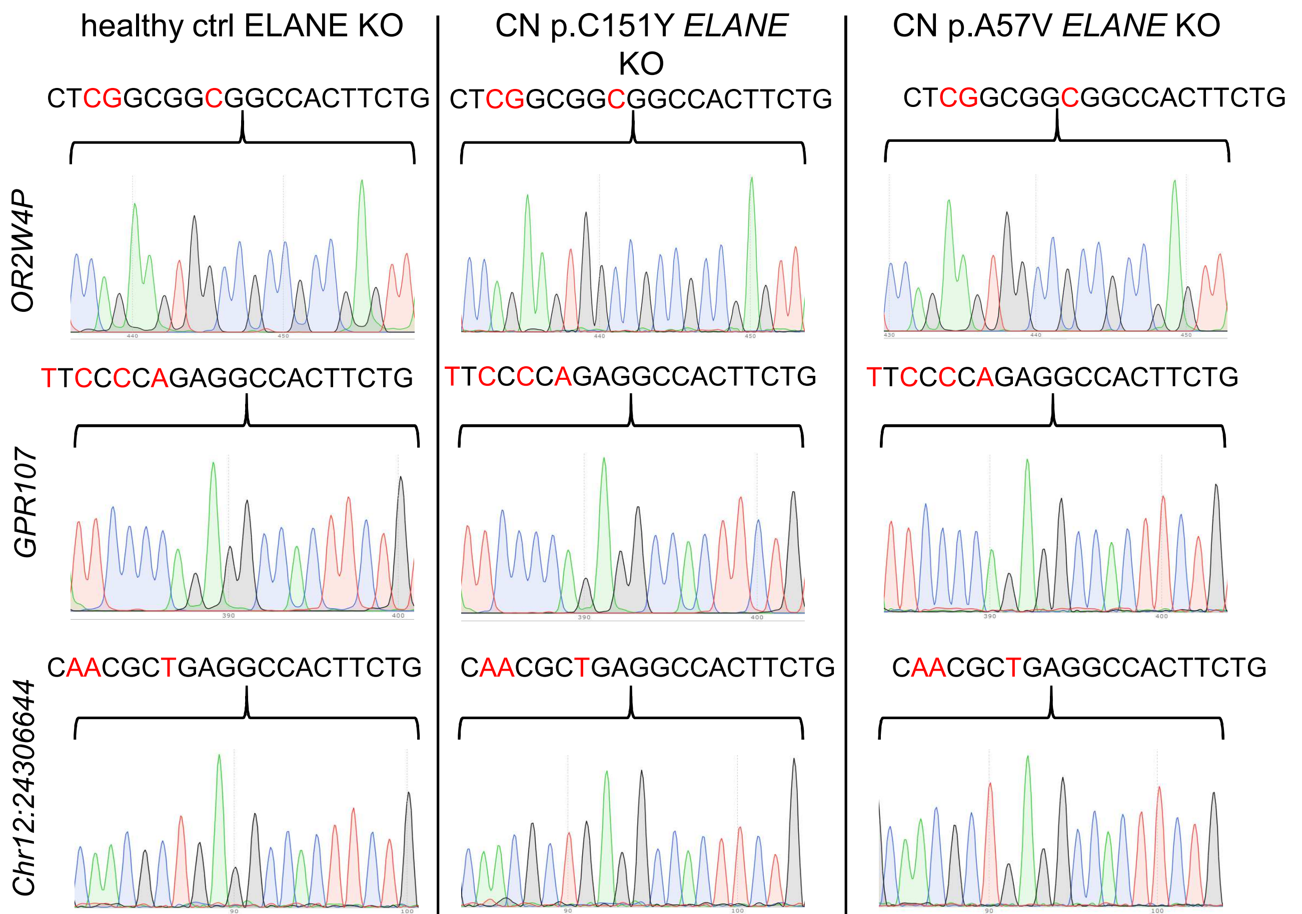






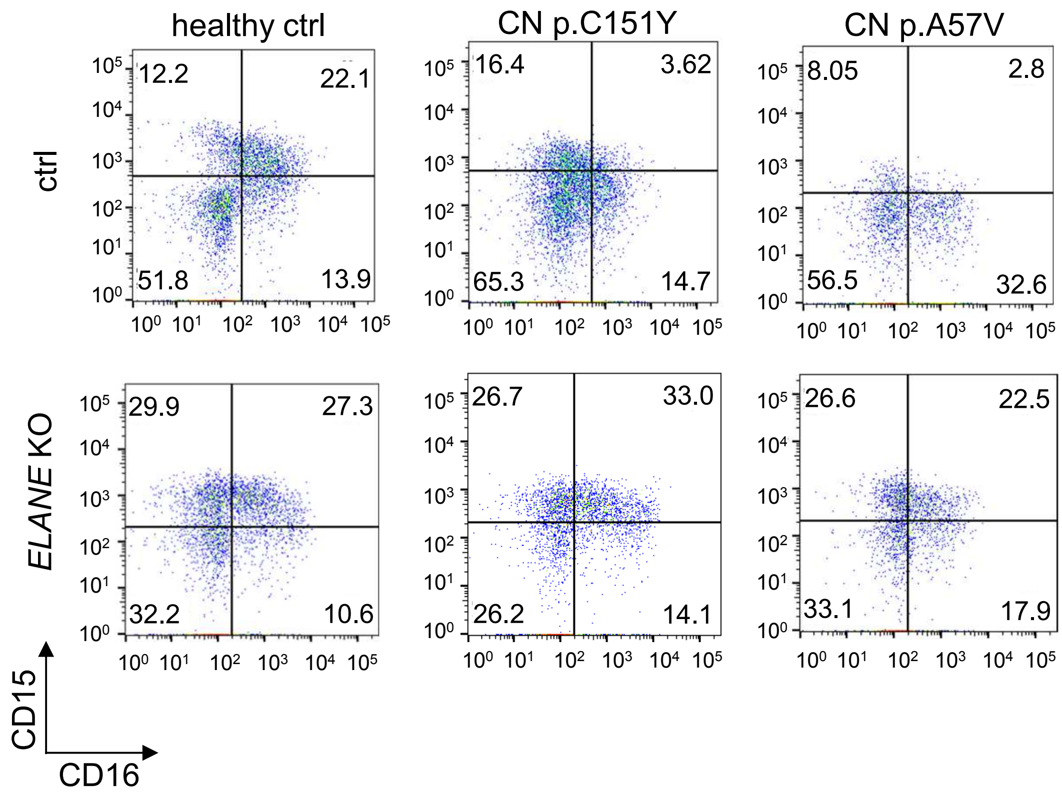
Supplementary Figure 3





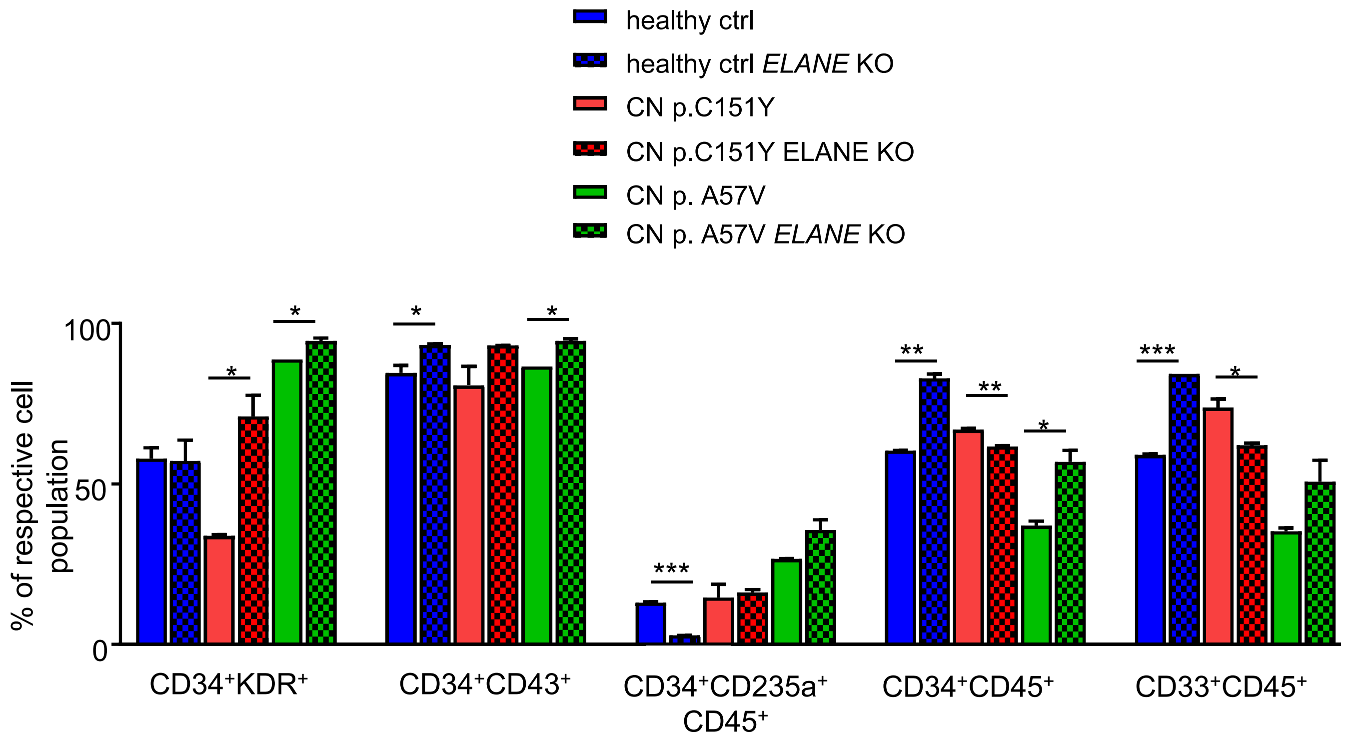
Supplementary Figure 4

A

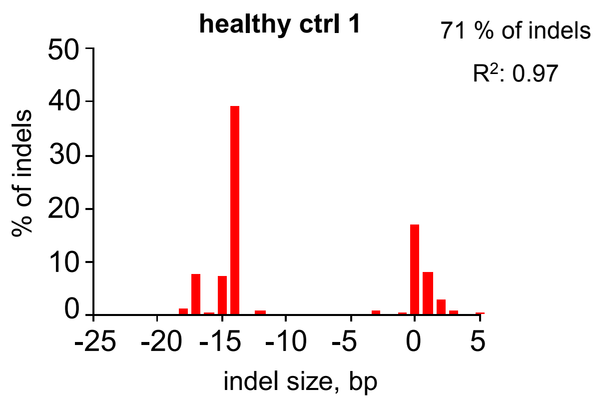


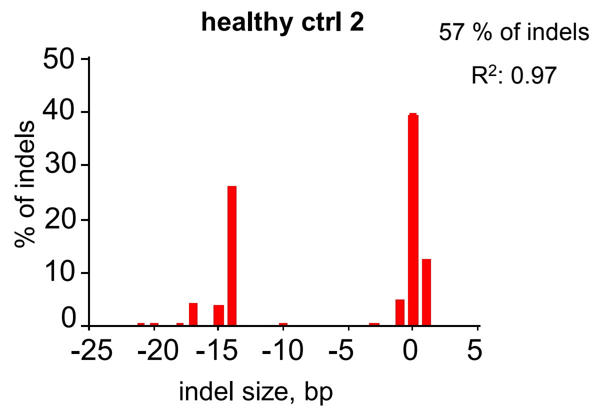
Supplementary Figure 5

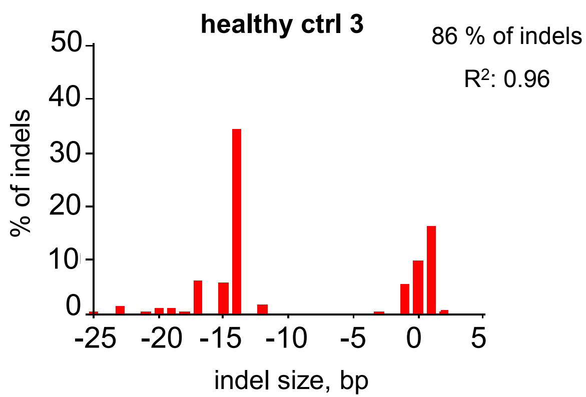
A

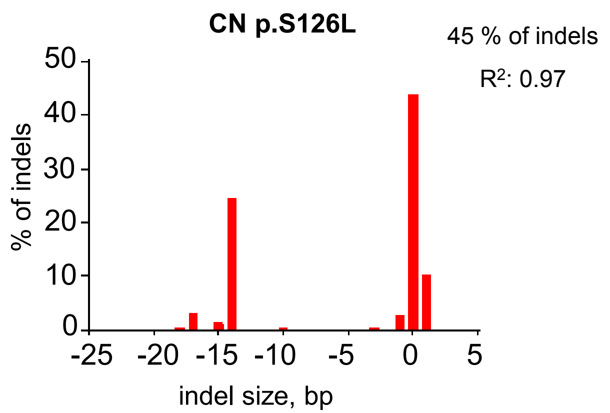


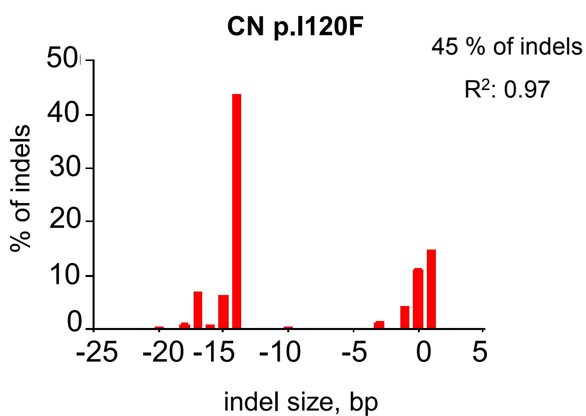
Supplementary Figure 6

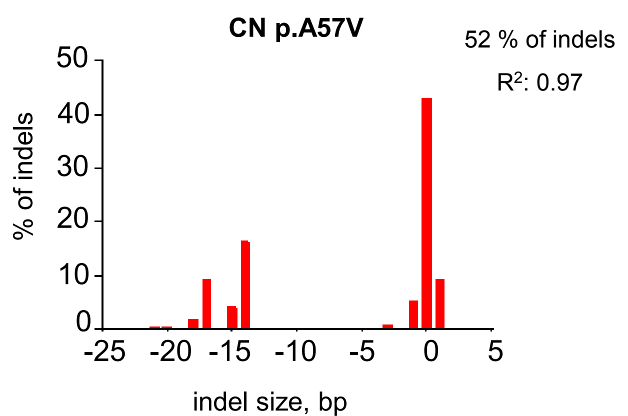


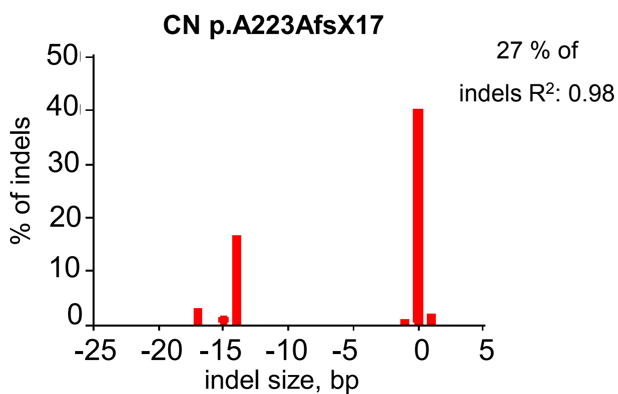




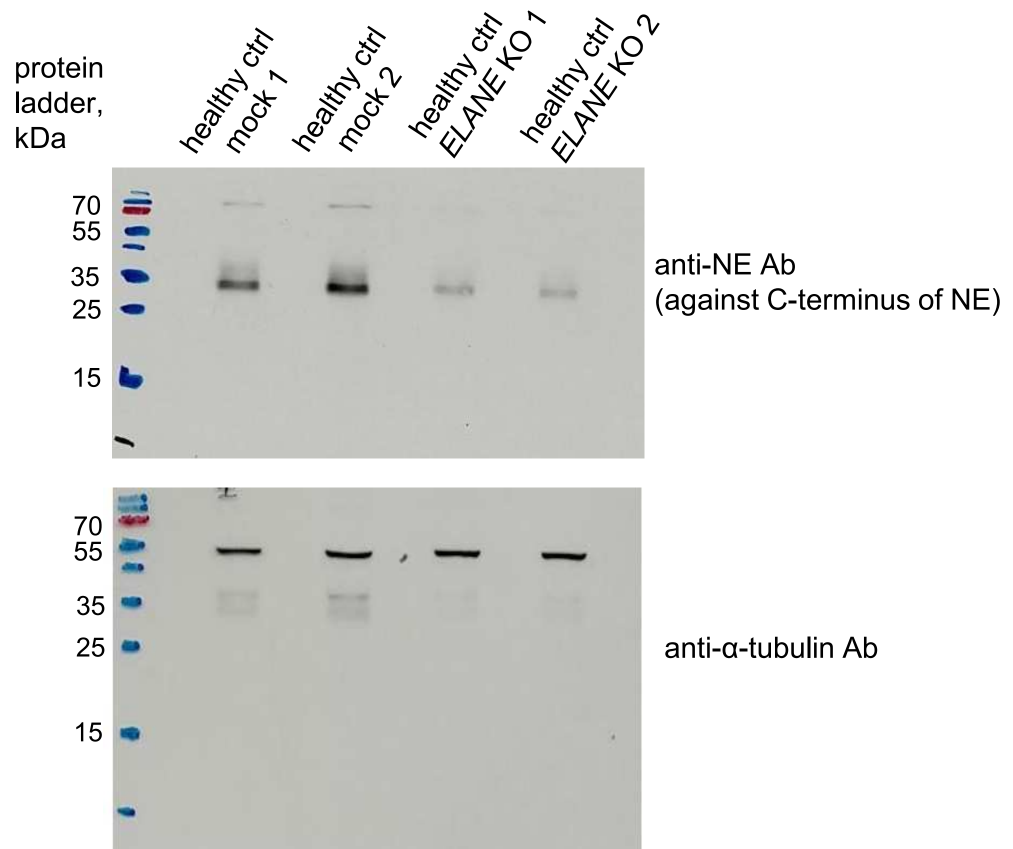


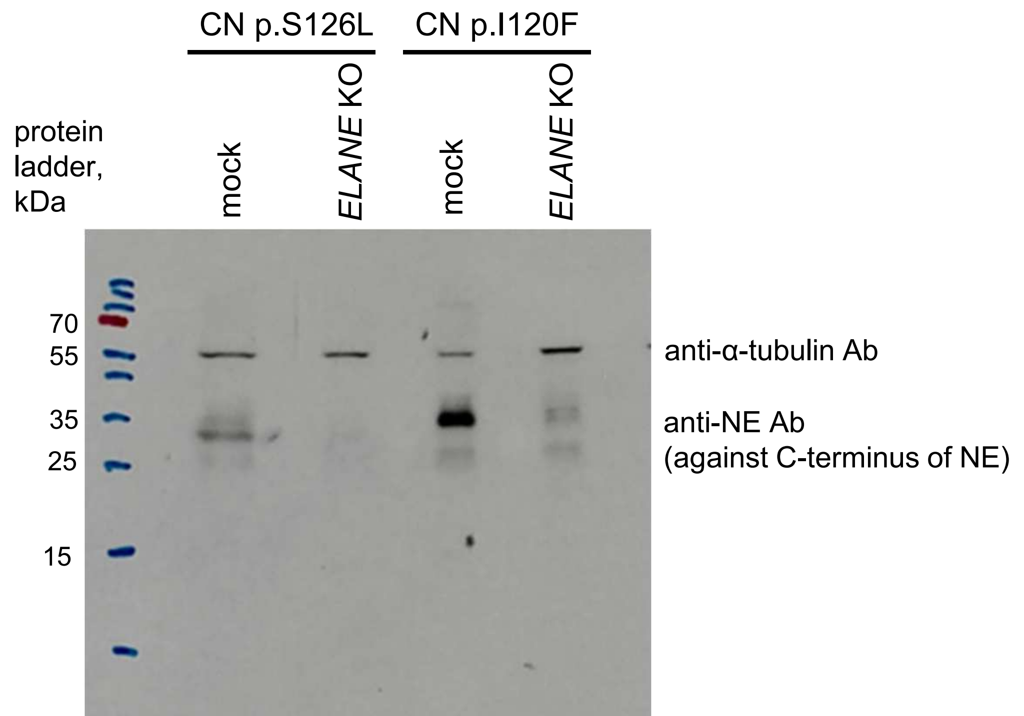






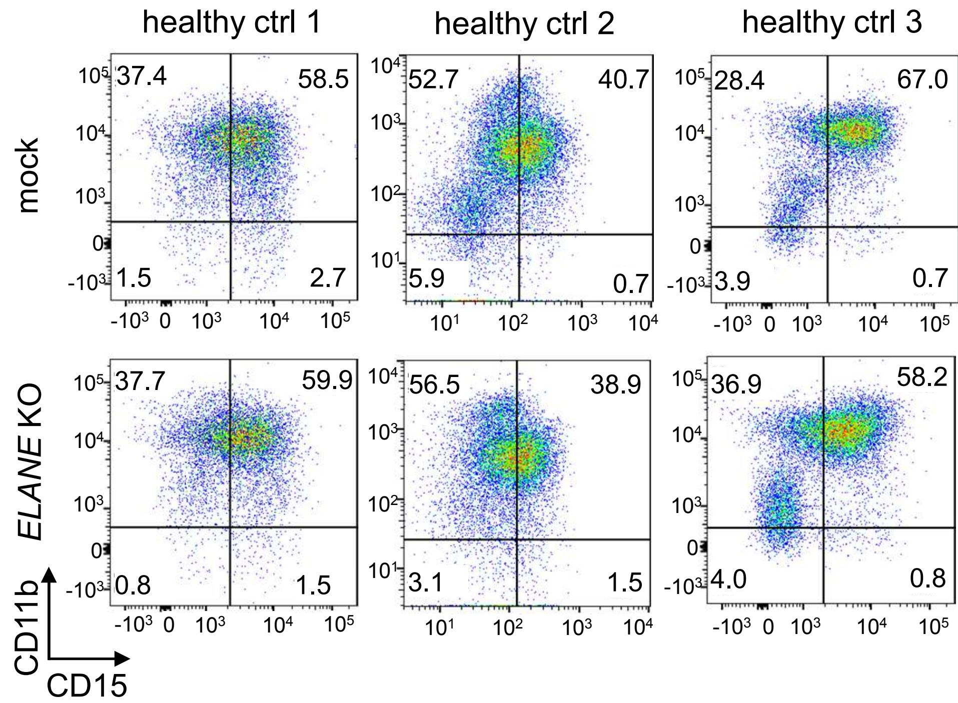
Supplementary Figure 7



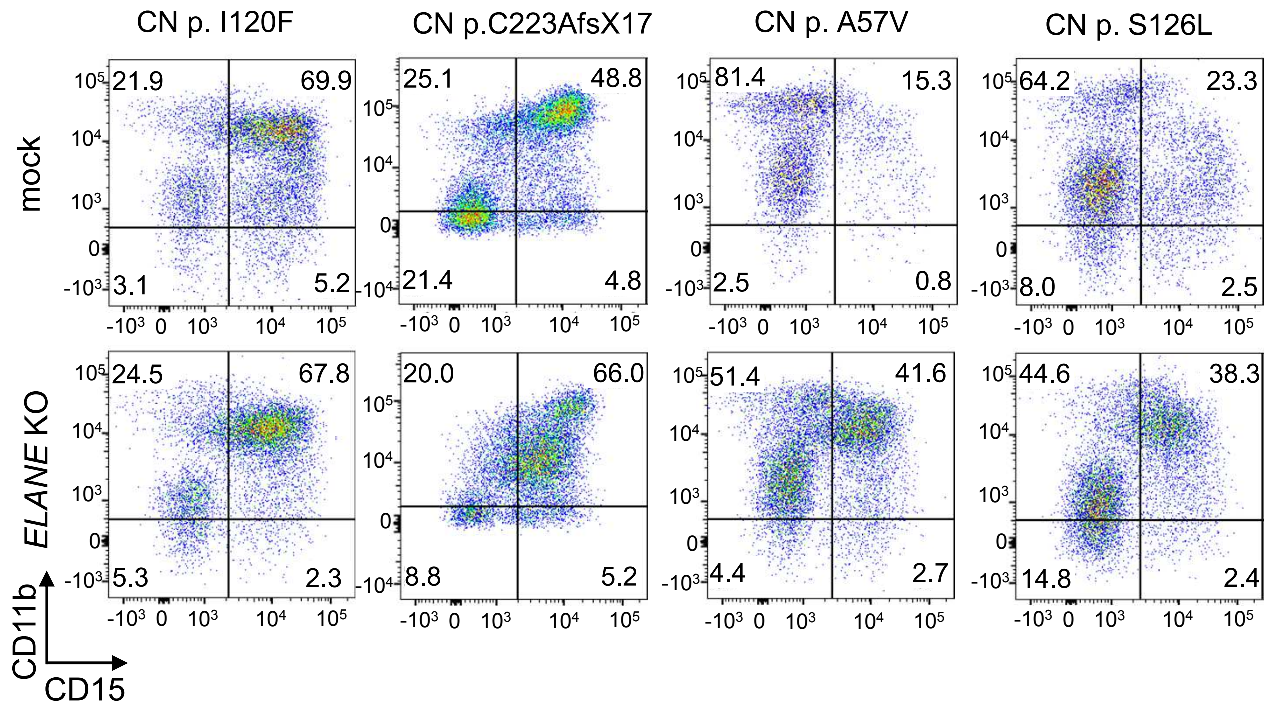


Supplementary Figure 8

A

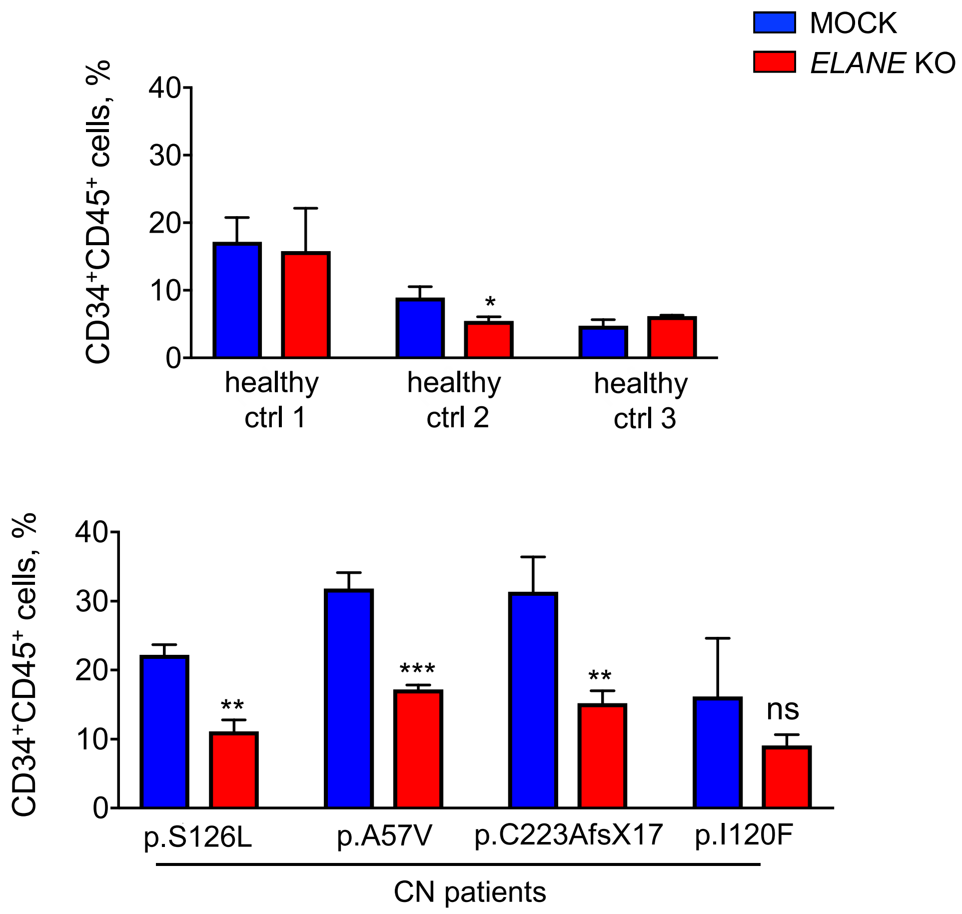


B



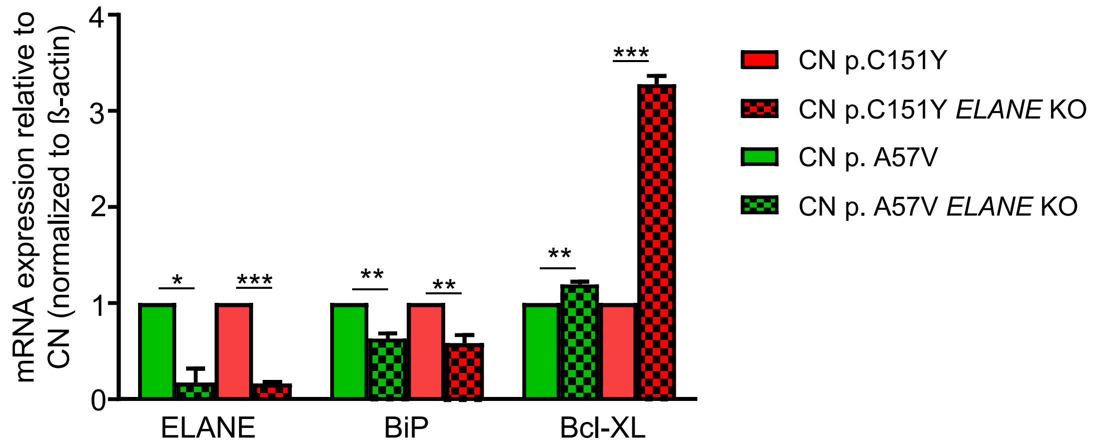
Supplementary Figure 9

A



Supplementary Figure 10

A



Supplementary Figure 11

Supplemental Table 3. CN patients information.

Patient ID	*ELANE* mutation (NP_001963.1)	Age, years	Gender	G-CSF dose, µg/kg/day
CN p.C223AfsX17	p.C223AfsX17	18	female	N.A.
CN p.S126L	p.S126L	13	female	8
CN p.A57V[§]	p.A57V	1	female	non-responder
CN p.I120F	p.I120F	29	female	5
CN p.C151Y[&]	p.C151Y	19	male	13.5

[§]primary HSPCs and iPSCs-generated $CD34^+$ cells of this patent were analysed

[&]iPSCs-generated $CD34^+$ cells of this patent were studied, this patent overt AML at the age of 20 and received BMT.

N.A.: not available