**OMTM, Volume *26***

## Supplemental information

# Mutant allele knockout with novel CRISPR nuclease promotes myelopoiesis in ELANE neutropenia

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**Supplemental Information**

**Supplemental Figures**

Figure S1.

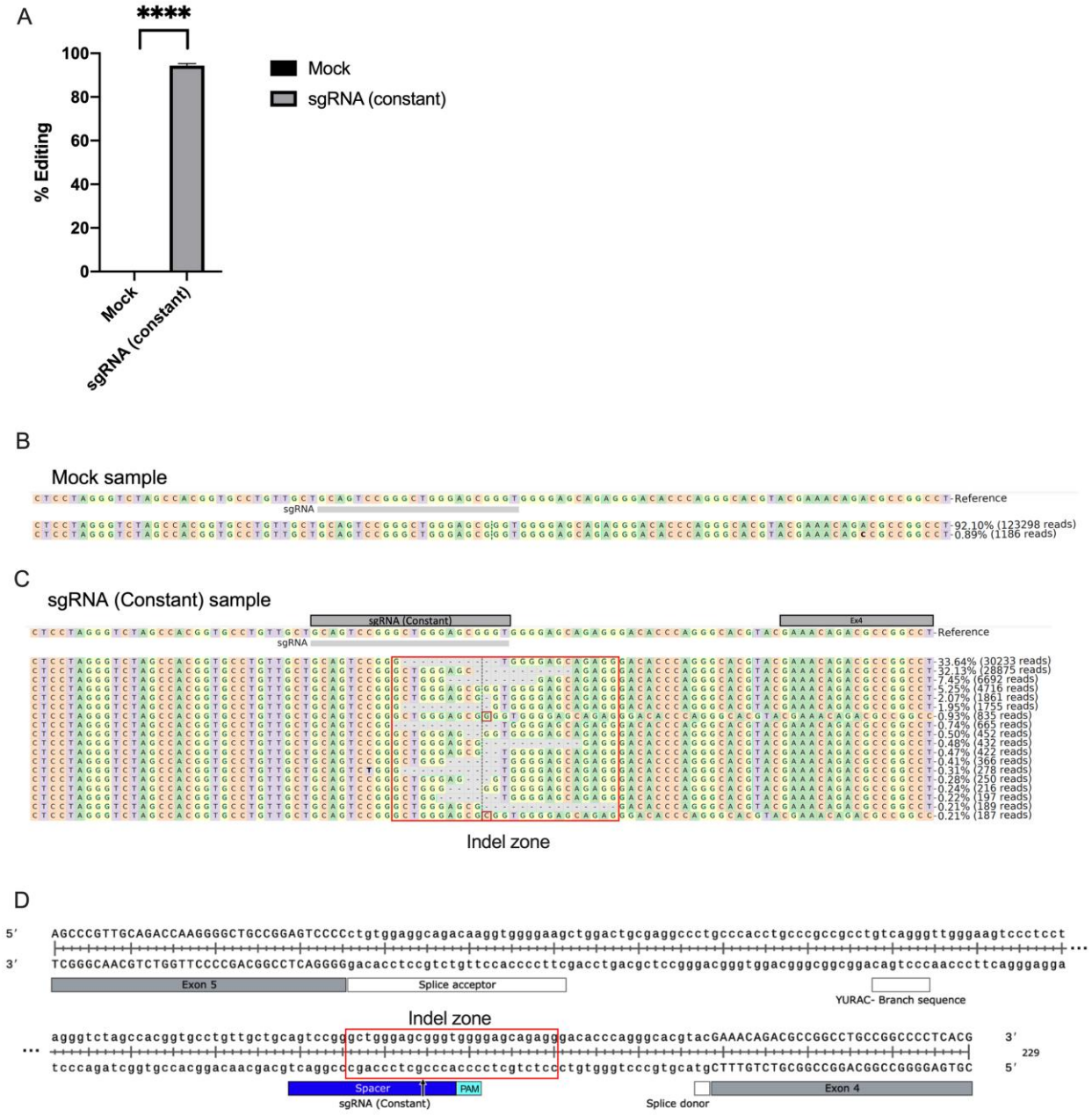



Figure S1. Editing by sgRNA (constant). (A) Bar graphs representing percentages of editing in HSCs taken from a healthy donor that were either electroporated without nuclease and guides (Mock, black) or treated with OMNI-A1 V10 nuclease and only sgRNA (constant) (sgRNA (constant), gray) as measured by ddPCR. (n=3 groups of cells). Statistical significance is indicated as ****P<.0001. Bars represent mean values with standard deviation. (B and C) NGS outputs of Mock-treated (Mock sample, B) and sgRNA (constant)-treated cells (C). The area where indels were detected is lineated by a red square (Indel zone). sgRNA (constant) and exon 4 (EX4) locations are depicted in gray. Only reads above 0.2% are presented. (D) Schematic of a section of *ELANE* gene (spanning from exon 5 in the upper row to exon 4 in the lower row) depicting exon 4, exon 5, sgRNA(constant) and regulatory elements: Splice

acceptor, branch sequence and splice donor. The area where indels were detected following editing with sgRNA(constant) is lineated by a red square (Indel zone).

Figure S2.

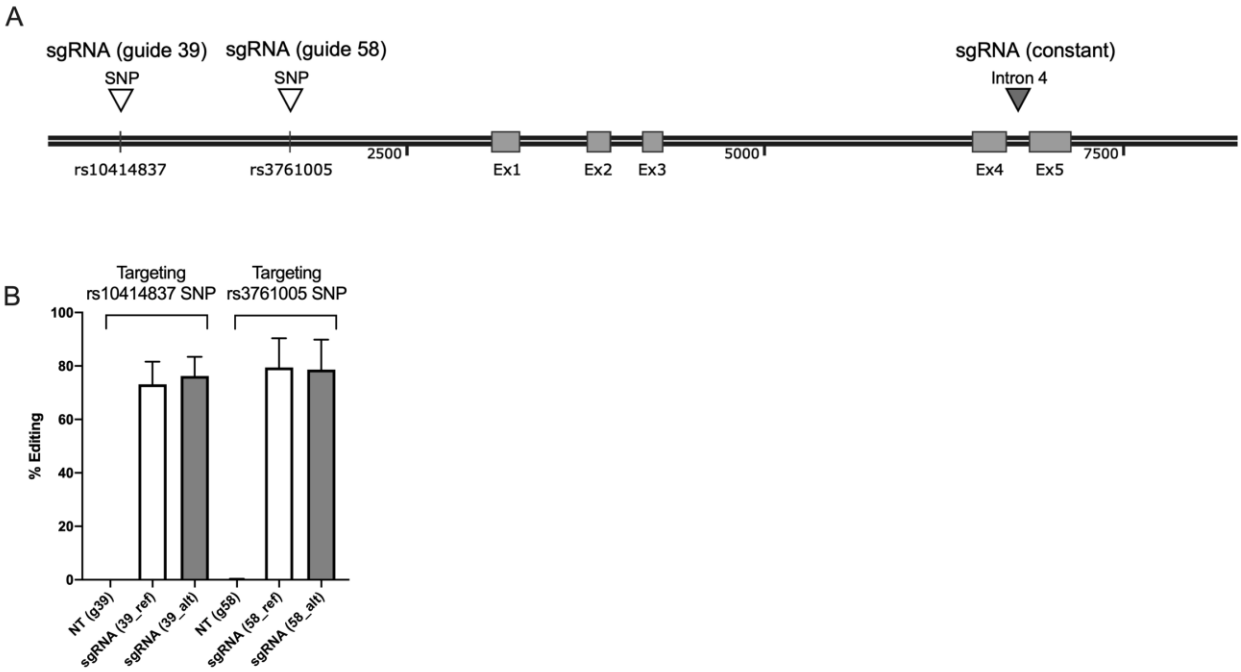



Figure S2. Targeting upstream SNPs. (A) Schematic of ELANE gene depicting rs10414837 and rs3761005 SNPs and their corresponding sgRNAs, guides 39 and 58, respectively. (B) Bar graphs representing percentages of editing in U2OS cells that were either not-treated (NT, black) or electroporated with the non-engineered nuclease, OMNI-A1, and sgRNA 39 or sgRNA 58 targeting the reference (white) or alternative (gray) forms of the SNPs as measured by ddPCR. (n=3 groups of cells). Bars represent mean values with standard deviation.

Figure S3.

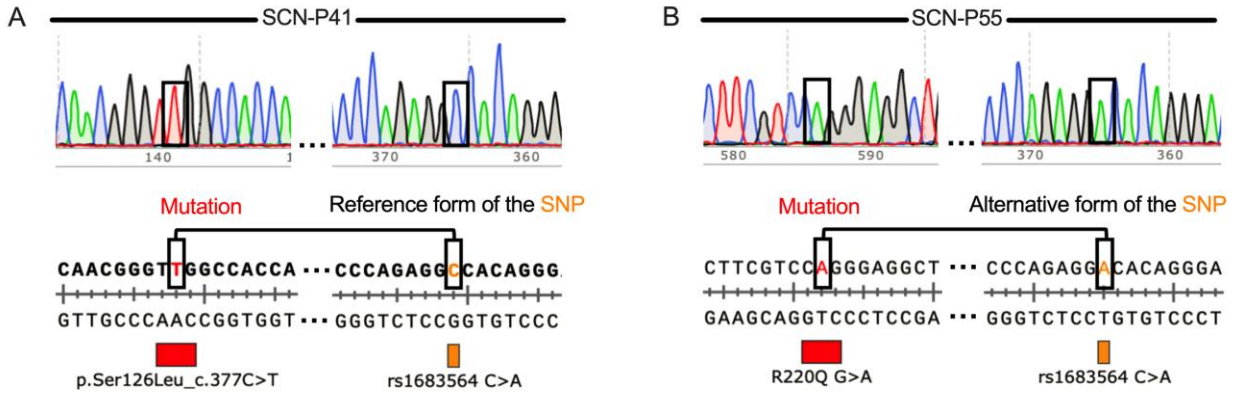



Figure S3. Mutation-SNP linkage determination in SCN-P41 and SCN-P55 patients. Electropherograms of sequencing analyses of the mutation site and the rs1683564 SNP. (A) SCN-P41 patient harbors a mutation (red) on the same allele as the reference form of the SNP (C, cytosine, orange), whereas (B) SCN-P55 patient harbors a mutation (red) on the same allele as the alternative form of the SNP (A, adenosine, orange).

Figure S4.

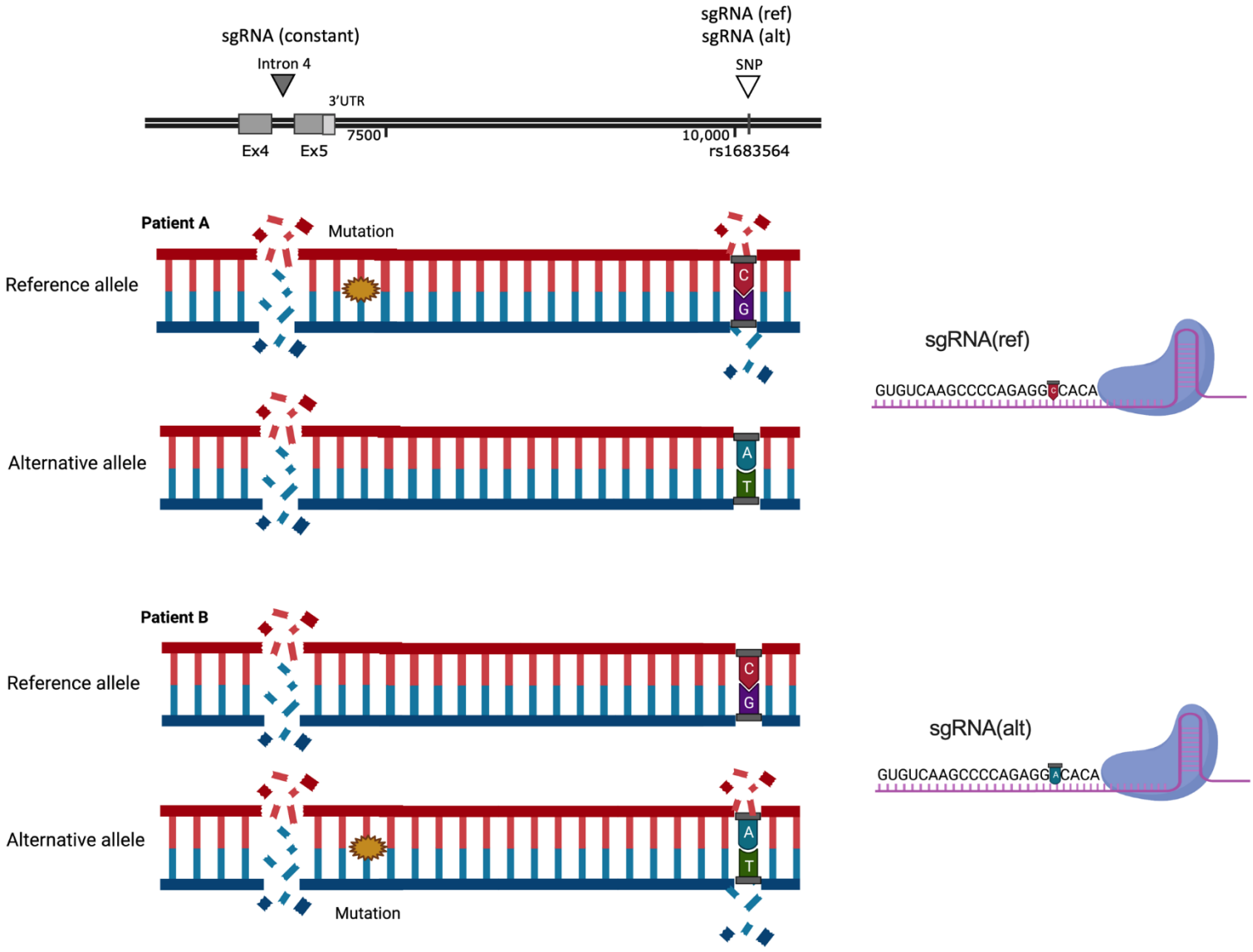



Figure S4. Same editing outcomes with RNP(ref) and RNP(alt) compositions. The *ELANE* gene is cleaved in two locations: 1) intron 4, a biallelic site guided by sgRNA(constant) guide and 2) a heterozygous SNP site, rs1683564, a single allelic site guided by either sgRNA(ref) or sgRNA(alt) depending on the linkage to the mutation site. If the mutation is located at the allele harboring the reference form of the SNP (C, cytosine), RNP(ref) composition, including a nuclease, sgRNA(ref) and sgRNA(constant), is chosen and a section of the reference mutated allele is cleaved (Patient A, upper panel). If the mutation is located at the allele harboring the alternative form of the SNP (A, adenosine), RNP(alt) composition, including a nuclease, sgRNA(alt) and sgRNA(constant), is chosen and a section of the alternative mutated allele is cleaved (Patient B, lower panel). Illustration created with BioRender.com.

Figure S5.

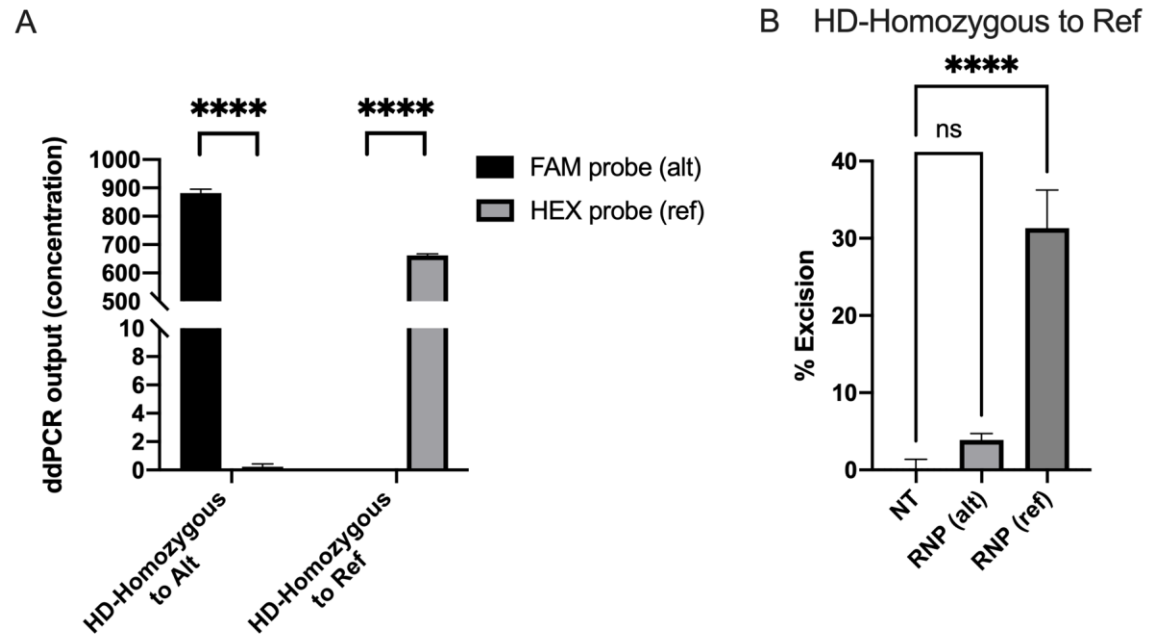



Figure S5. Probes' and guides' specificity. (A) The binding of each probe (FAM, black; HEX, gray) to DNA extracted from healthy donor (HD) cells that are homozygous to either the reference or alternative forms of the SNP, was measured by ddPCR. Bar graphs representing concentration of positive events. (n=3 groups of cells). Statistical significance is indicated as ****P<.0001. (B) Bar graphs representing percentages of excision in a healthy donor HSCs that are homozygous to the reference form of the rs1683564 SNP and were either not-treated (NT), treated with RNP(alt) or RNP(ref) as measured by ddPCR. (n=3 groups of cells). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. Bars represent mean values with standard deviation.

Figure S6.

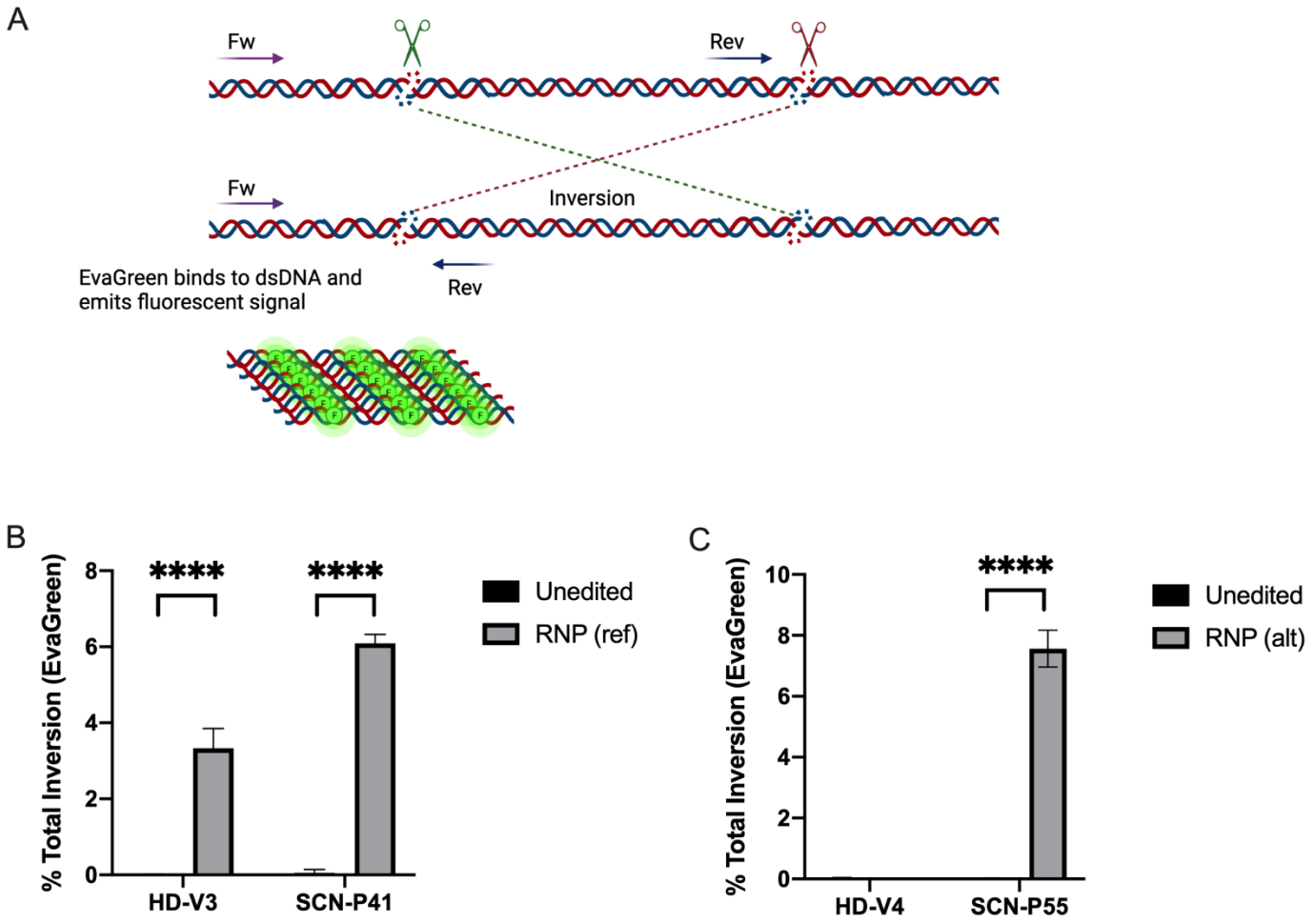



Figure S6. Inversion events following excision. (A) Schematic of detection of inversion events: Specific primers were designed to amplify inverted variations of the excised fragment. EvaGreen dye, a fluorescent DNA-binding dye that binds dsDNA, was used in a ddPCR assay to measure all inversion events. Illustration created with BioRender.com. (B) Quantification of total inversion events measured by EvaGreen-based ddPCR assay in unedited (black) and RNP(ref)-treated (gray) HD-V3 healthy donor and SCN-P41 patient derived differentiated HSCs. Statistical significance is indicated as ****P<.0001. (C) Quantification of total inversion events measured by EvaGreen-based ddPCR assay in unedited HD-V4 healthy donor and SCN-P55 patient-derived differentiated HSCs (black) and in RNP(alt)-treated (gray) SCN-P55 patient-derived differentiated HSCs. Statistical significance is indicated as ****P<.0001. Bars represent mean values with standard deviation. (n=3-4 groups of cells from HD-V3 or HD-V4 healthy /SCN-P41 or SCN-P55 patient donors).

Figure S7.

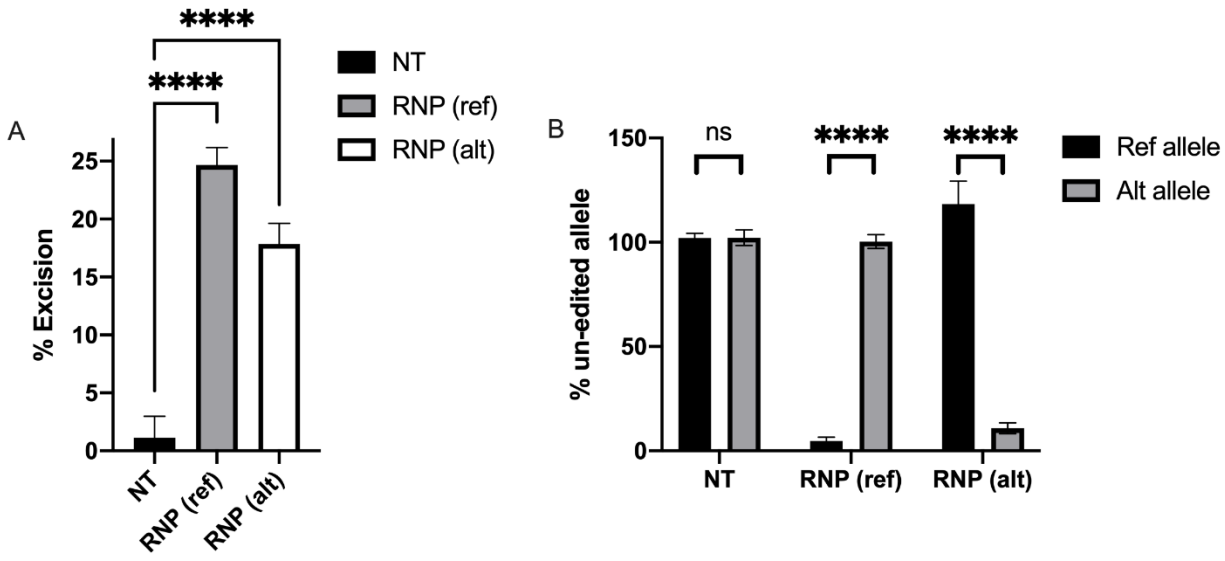



Figure S7. Excision levels and allele specificity in additional healthy donors. (A) Bar graphs representing percentages of excision in HSCs taken from healthy donors that were either non-treated (NT, black), RNP (ref)-treated (gray) or RNP(alt)-treated (white) as measured by ddPCR. (n=4 groups of cells from 2 healthy donors in each group). Statistical significance is indicated as ****P<.0001. (B) Bar graphs representing percentages of un-edited reference (black) and alternative (gray) alleles in HSCs taken from healthy donors that were non-treated (NT), treated with RNP(ref) or RNP(alt), as measured by ddPCR. Average of each allele concentration was normalized to endogenous gene control RPP30 and STAT1 and presented relatively to non-treated cells. (n=4 groups of cells from 2 healthy donors in each group). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. Bars represent mean values with standard deviation.

Figure S8.

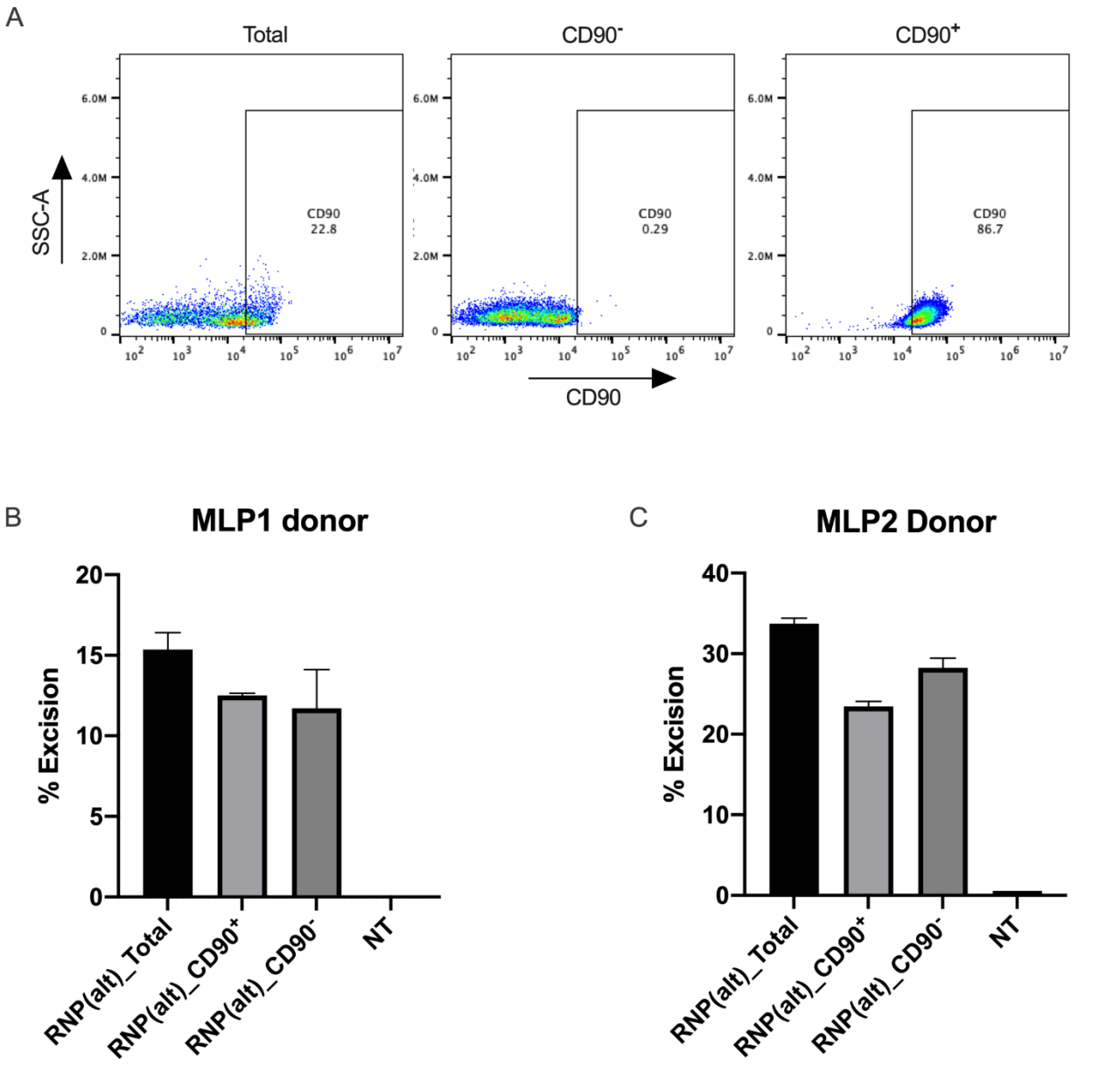
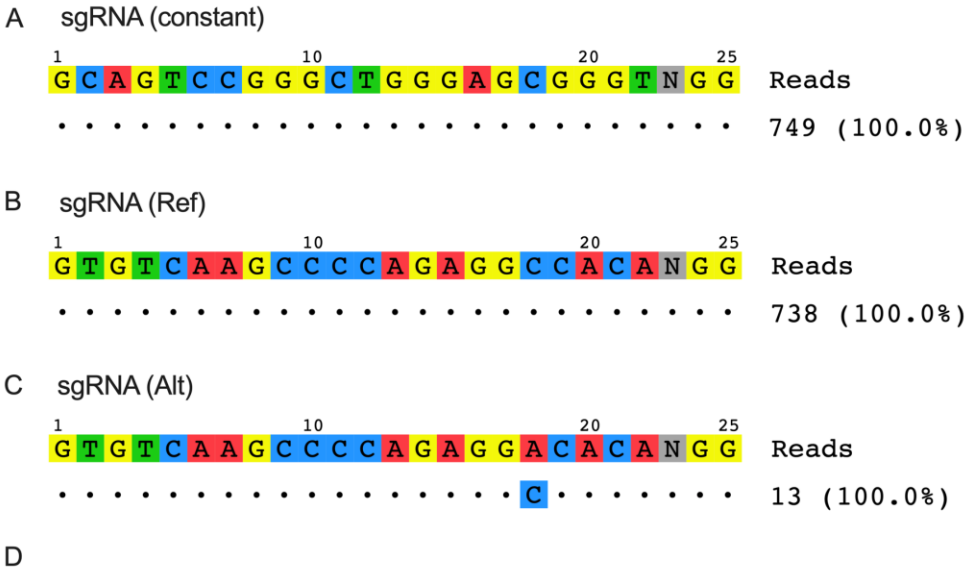



Figure S8. Excision levels in Long Term HSC population. (A) Representative FACS plots of healthy donor-derived $CD34^+$ HSCs prior to sorting (Total, left panel) and following sorting to $CD90^-$ (middle panel) and $CD90^+$ (right panel) populations. (B and C) Bar graphs representing percentages of excision from HSCs taken from two healthy donors (MLP1; B - heterozygous to the alternative form of the SNP and MLP2, C - homozygous to the alternative form of the SNP) prior to sorting (Total, black) and following sorting to $CD90^+$ (light gray) and $CD90^-$ (dark gray) populations as measured by ddPCR. Non-treated HSCs prior to sorting served as control (NT) (n=2 groups of cells from each healthy donor in each group). Bars represent mean values with standard deviation.

Figure S9.




D

| Chromosome | Position | Gene | Type | Sequence | Mismatches | Reads SCN-P41 | Reads SCN-P55 |
|---|---|---|---|---|---|---|---|
| sgRNA-Int4-const | | | | | | | |
| chr15 | 90957975 | RCCD1 | intron | aCAGTCCtGGCTGGGAGCaGGTGGG | 3 | 0 | 0.03±0 |
| chr17 | 74081352 | LINC02074 | intron | aCAGTCCGGGCTGGGAGCtGGgAGG | 3 | 0 | 0.03±0 |
| *sgRNA-564DS-alt* | | | | | | | |
| chr22 | 28908576 | ZNRF3 | intron | GTGaCAtGCCCCAtAGGACACAGGG | 3 | | 0 |
| chr2 | 26950233 | DPYSL5 | exon | GTGTCAAcCCCaAGAcGACACATGG | 3 | | 0.01±0.01 |
| *sgRNA-564DS-ref* | | | | | | | |
| chr12 | 58121865 | NA | intergenic | GTGTgAAGgCCCAGAGGCCAaAGGG | 3 | 0.07±0.02 | |
| chr12 | 116915161 | FBXW8 | intron | GTtgCAAGCCCCAGAGcCCACAGGG | 3 | 0.03±0.03 | |

Figure S9. No detected off targets following editing of OMNI-A1 V10 nuclease and each of the sgRNAs. (A-C) An unbiased survey (GUIDE-seq) of whole-genome off-target cleavage using OMNI A1 V10 nuclease and each of the constant guide (A, SgRNA(constant)), reference guide (B, SgRNA(ref)) and alternative guide (C, SgRNA(alt)), showing all reads are of the target sequence and no off targets detected (≤4 mismatches) (for raw data see Table S2). Note, analysis was done in U2OS cells that are homozygous to the reference form of rs1683564 SNP. Since OMNI-A1 V10 nuclease is highly allele discriminatory, when using sgRNA(alt) there is only minor on-target editing of the reference allele (13 reads of the reference cytosine genotype) and no detectable off targets. (D) A table summarizing the results of an *in-silico* off target analysis for constant, alternative and reference guides depicting a few potential off-targets. None of these off targets were validated by rhAmpSeq analysis performed on HSCs derived from SCN-P41 and SCN-P55 patients edited with RNP(ref) and RNP(alt), respectively, see two right columns. rhAmpSeq validation threshold was set to editing ≥0.2%.

Figure S10.

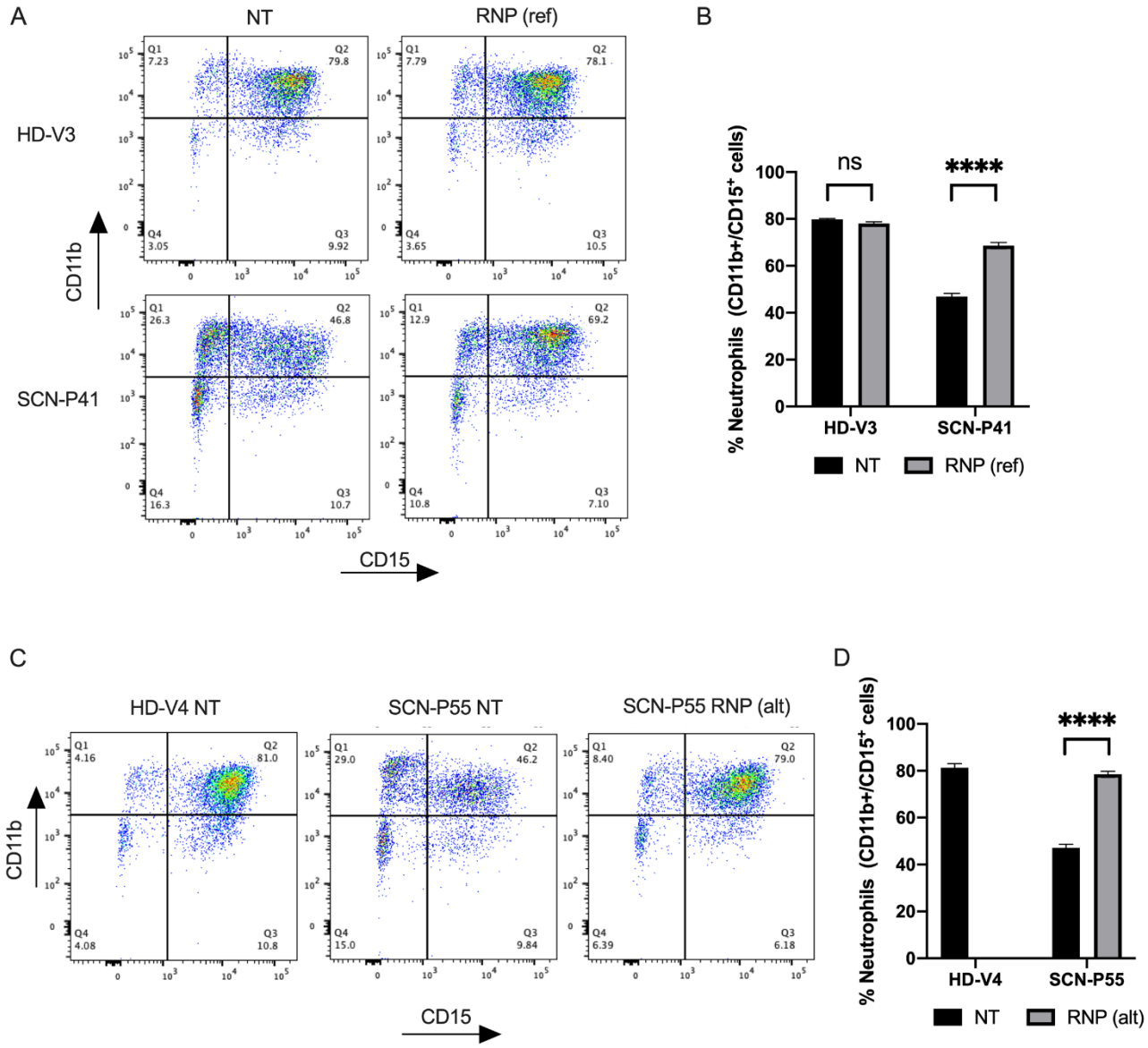



Figure S10. Differentiation into $CD11b^{+}/CD15^{+}$ neutrophils. (A) Representative FACS plots of non-treated (NT, left panel) and RNP(ref)-treated (right panel) healthy donor (HD-V3, upper panel) and SCN-P41 patient (lower panel) differentiated HSCs, analyzed for neutrophilic ($CD11b^{+}/CD15^{+}$) subset. (B) Quantitative analysis of respective FACS data for percentages of neutrophils ($CD11b^{+}/CD15^{+}$ cells) in healthy (HD-V3) and SCN patient (SCN-P41) differentiated HSCs that were non-treated (NT, black) or treated with RNP(ref) (gray). (n=3 groups of cells from HD-V3 healthy /SCN-P41 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (C) Representative FACS plots of non-treated healthy donor (HD-V4 NT, left panel), non-treated SCN patient (SCN-P55 NT, middle panel) and RNP(alt)-treated SCN patient (right panel) differentiated HSCs, analyzed for neutrophilic ($CD11b^{+}/CD15^{+}$) subset. (D) Quantitative analysis of respective FACS data for percentages of neutrophils ($CD11b^{+}/CD15^{+}$ cells) in differentiated HSCs from non-treated healthy donor (HD-V4, NT; black) and SCN patient (SCN-P55) either non-treated (NT, black) or treated with RNP(alt) (gray). (n=3 groups of cells from HD-V4 healthy /SCN-P55 patient donors). Statistical significance is indicated as ****P<.0001. Bars represent mean values with standard deviation.

Figure S11.

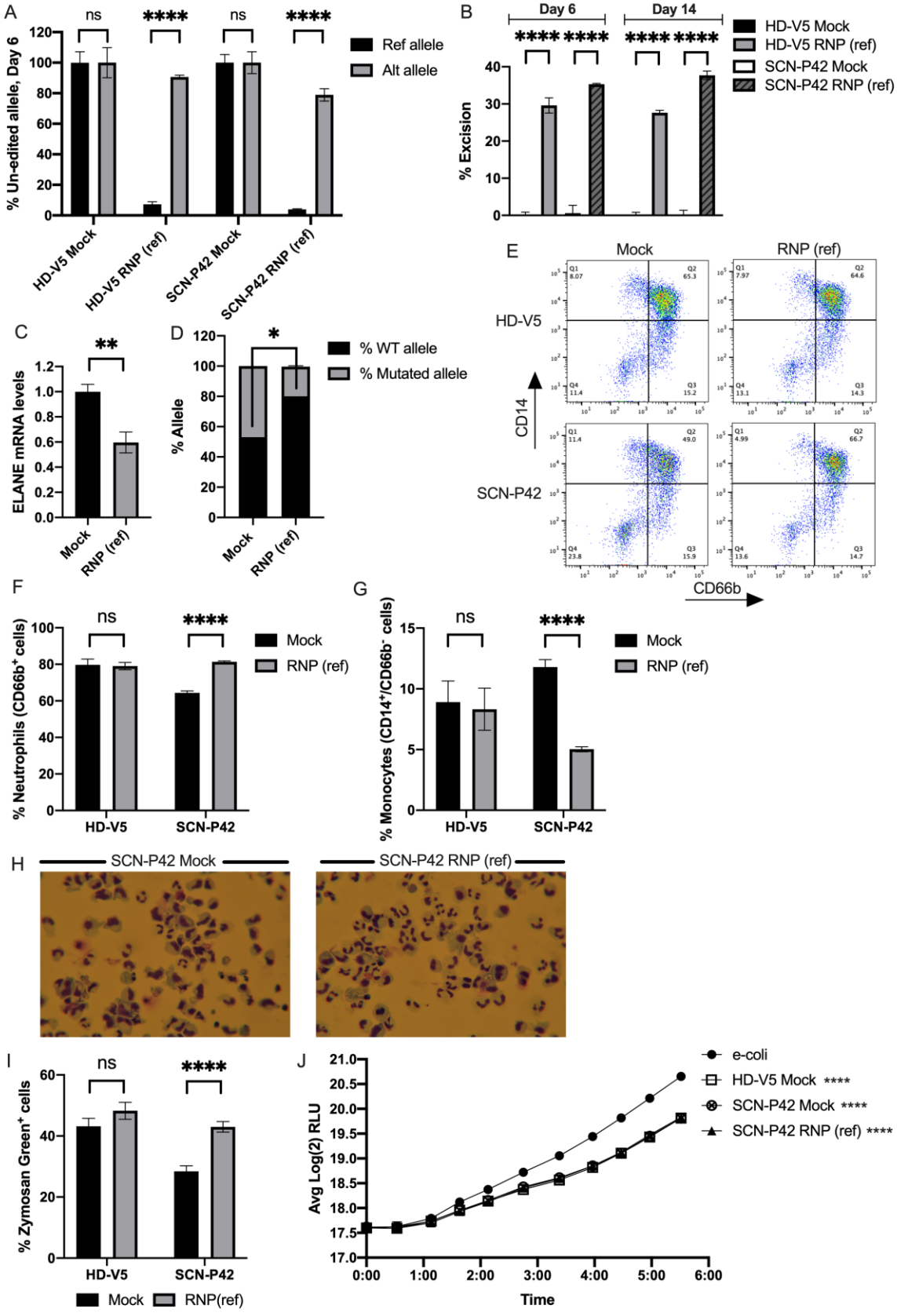

A
ns
****
ns
****
% Un-edited allele, Day 6
Ref allele
Alt allele
HD-V5 Mock
HD-V5 RNP (ref)
SCN-P42 Mock
SCN-P42 RNP (ref)
B
Day 6
Day 14
**** **** **** ****
% Excision
HD-V5 Mock
HD-V5 RNP (ref)
SCN-P42 Mock
SCN-P42 RNP (ref)
C
**
ELANE mRNA levels
Mock
RNP (ref)
D
*
% Allele
% WT allele
% Mutated allele
Mock
RNP (ref)
E
Mock
RNP (ref)
HD-V5
SCN-P42
CD14
CD66b
F
ns
****
% Neutrophils (CD66b+ cells)
Mock
RNP (ref)
HD-V5
SCN-P42
G
ns
****
% Monocytes (CD14+/CD66b- cells)
Mock
RNP (ref)
HD-V5
SCN-P42
H
SCN-P42 Mock
SCN-P42 RNP (ref)
I
ns
****
% Zymosan Green+ cells
HD-V5
SCN-P42
Mock
RNP(ref)
J
Avg Log(2) RLU
Time
e-coli
HD-V5 Mock ****
SCN-P42 Mock ****
SCN-P42 RNP (ref) ****

Figure S11. Excision using RNP(ref) in SCN-P42 and HD-V5. (A) Bar graphs representing percentages of un-edited reference (black) and alternative (gray) alleles at day 6 of differentiation in HSCs taken from either healthy donor (HD-V5) or SCN patient (SCN-P42) treated with RNP (ref) composition or electroporated without a nuclease composition (Mock), as measured by ddPCR. (n=3 groups of cells from HD-V5 healthy/SCN-P42 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (B) Bar graphs representing percentages of excision at days 6 and 14 of differentiation in HSCs taken from either healthy donor (HD-V5): Mock-treated (Mock, black) or RNP (ref)-treated (gray), or SCN patient (SCN-P42): Mock-treated (Mock, white) or RNP (ref)-treated (dark oblique lines), as measured by ddPCR. (n=3 groups of cells from HD-V5 healthy /SCN-P42 patient donors). Statistical significance is indicated as ****P<.0001. (C) Bar graphs representing *ELANE* mRNA levels in day 6 differentiated HSCs of SCN-P42 patient that were either Mock-treated (Black) or RNP(ref)-treated (Gray). Data is presented relatively to the mock group. (n=3 groups of cells from SCN-P42 patient). Statistical significance is indicated as **P<.01. (D) Bar graphs representing percentages of wild-type (black) and mutated (gray) alleles in cDNA taken from SCN-P42 patient HSCs that were either RNP (ref)-treated or Mock-treated (Mock), as measured by NGS targeting the mutation site. (n=3 groups of cells from SCN-P42 patient). Statistical significance is indicated as *P<.05. (E) Representative FACS plots of mock-treated (Mock, left panel) and RNP(ref)-treated (right panel) healthy donor (HD-V5, upper panel) and SCN-P42 patient (lower panel) differentiated HSCs, analyzed for neutrophilic ($CD66b^+$) and monocytic ($CD14^+/CD66b^-$) subsets. (F) Quantitative analysis of respective FACS data for percentages of neutrophils ($CD66b^+$ cells) in healthy (HD-V5) and SCN patient (SCN-P42) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(ref) (gray). (n=3 groups of cells from HD-V5 healthy /SCN-P42 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (G) Quantitative analysis of respective FACS data for percentages of monocytes ($CD14^+/CD66b^-$ cells) in healthy (HD-V5) and SCN patient (SCN-P42) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(ref) (gray). (n=3 groups of cells from HD-V5 healthy /SCN-P42 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (H) Diff-Quik staining of P42 SCN patient-derived differentiated HSCs treated with RNP(ref) or electroporated without a nuclease composition (SCN-P42 Mock). Microphotographs were taken on LEITZ LABORLUX S polarizing light microscope at 400X magnification using Nikon DSLR digital camera. (I) Quantification of percentages of Zymosan Green uptake by healthy (HD-V5) and SCN patient (SCN-P42) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(ref) (gray). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (J) Graph depicts real time change in light emission, relative light units (RLUs), from 200,000 Luciferase expressing bacterial cells incubated with differentiated neutrophils from healthy mock-treated (HD-V5, Mock; white square), patient mock-treated (SCN-42 Mock; crossed circle) or patient RNP(ref)-treated (SCN-P42 RNP(ref); triangle) HSCs compared to bacterial cells only control (e-coli, circle). Statistical significance for each one of the groups versus e-coli control at the last time point presented, when RLU levels reached plateau, is indicated as ****P<.0001. Bars represent mean values with standard deviation.

Figure S12.

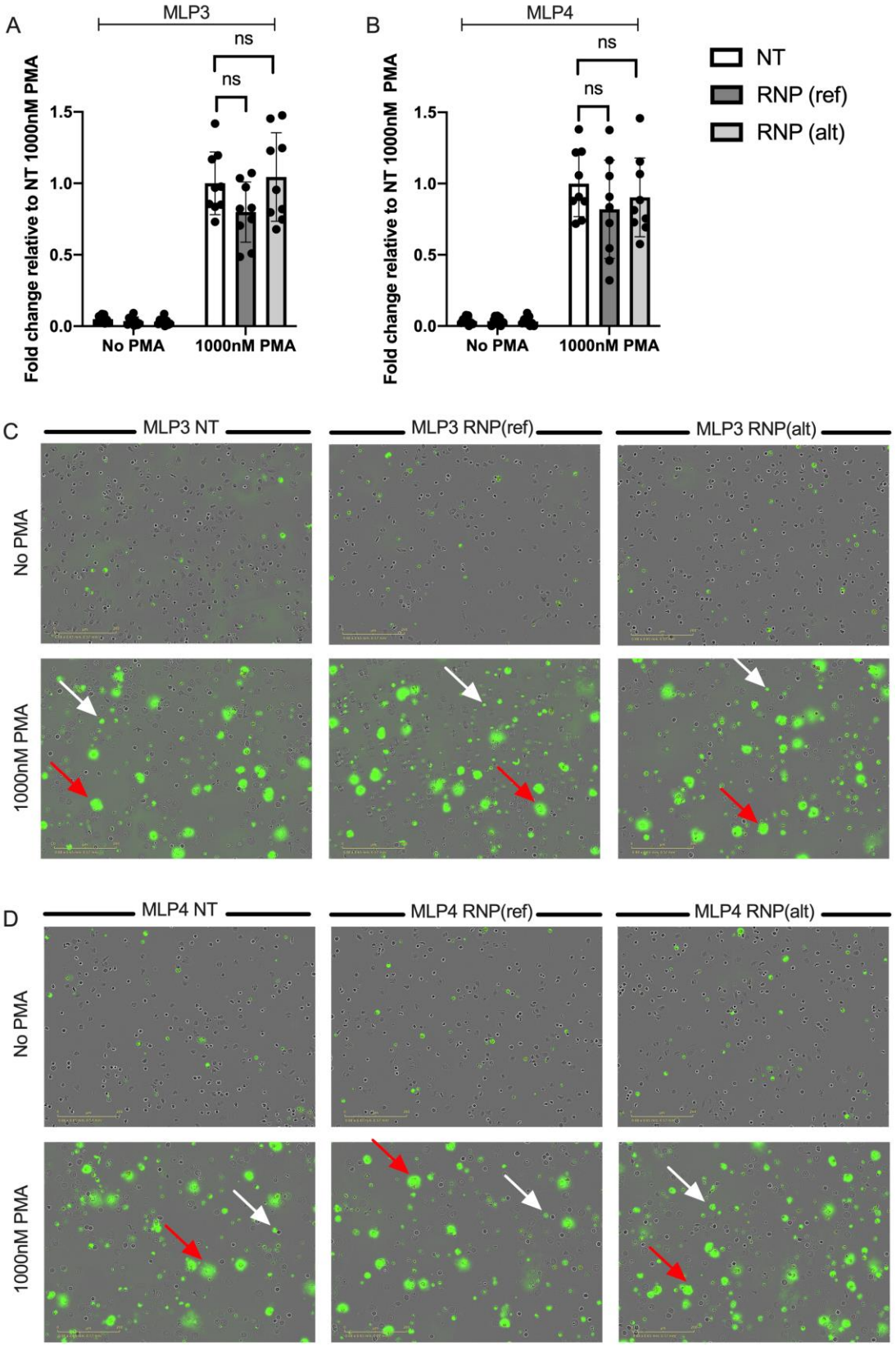

A
MLP3
ns
ns
Fold change relative to NT 1000nM PMA
1.5
1.0
0.5
0.0
No PMA
1000nM PMA
B
MLP4
ns
ns
Fold change relative to NT 1000nM PMA
1.5
1.0
0.5
0.0
No PMA
1000nM PMA
NT
RNP (ref)
RNP (alt)
C
MLP3 NT
MLP3 RNP(ref)
MLP3 RNP(alt)
No PMA
1000nM PMA
D
MLP4 NT
MLP4 RNP(ref)
MLP4 RNP(alt)
No PMA
1000nM PMA

Figure S12. Excision did not compromise NETosis capacity. (A and B) Bar graphs representing fold of change in NETosis levels in differentiated HSCs taken from healthy donors (MLP3 (A) and MLP4 (B)). HSCs were either not-treated (NT), RNP(ref)-treated or RNP(alt)-treated and were stimulated, following differentiation, with PMA (1000nM PMA) or added with 0.1% DMSO (No PMA control) for 10 hours. Cells were incubated with SYTOX Green dye for detection of membrane-damaged cells and imaged by Incucyte® S3 System. Data is presented relatively to averaged NT 1000nM PMA group. (n=9 wells of cells for each condition from 3 independent experiments). Statistical significance is indicated as ns = Not statistically significant. (C and D) Incucyte® images of differentiated HSCs from donors MLP3 (C) and MLP4 (D) that were either not-treated (NT, left panel), RNP(ref)-treated (middle panel) or RNP(alt)-treated (right panel) and were added with 0.1% DMSO (No PMA) or stimulated with PMA (1000nM PMA) in the presence of SYTOX Green dye. Enlarged green cells represent cells undergoing NETosis. Images were acquired with a scale bar of 200 μm. Red and white arrows depict representative NETotic and apoptotic cells, respectively.

Figure S13.

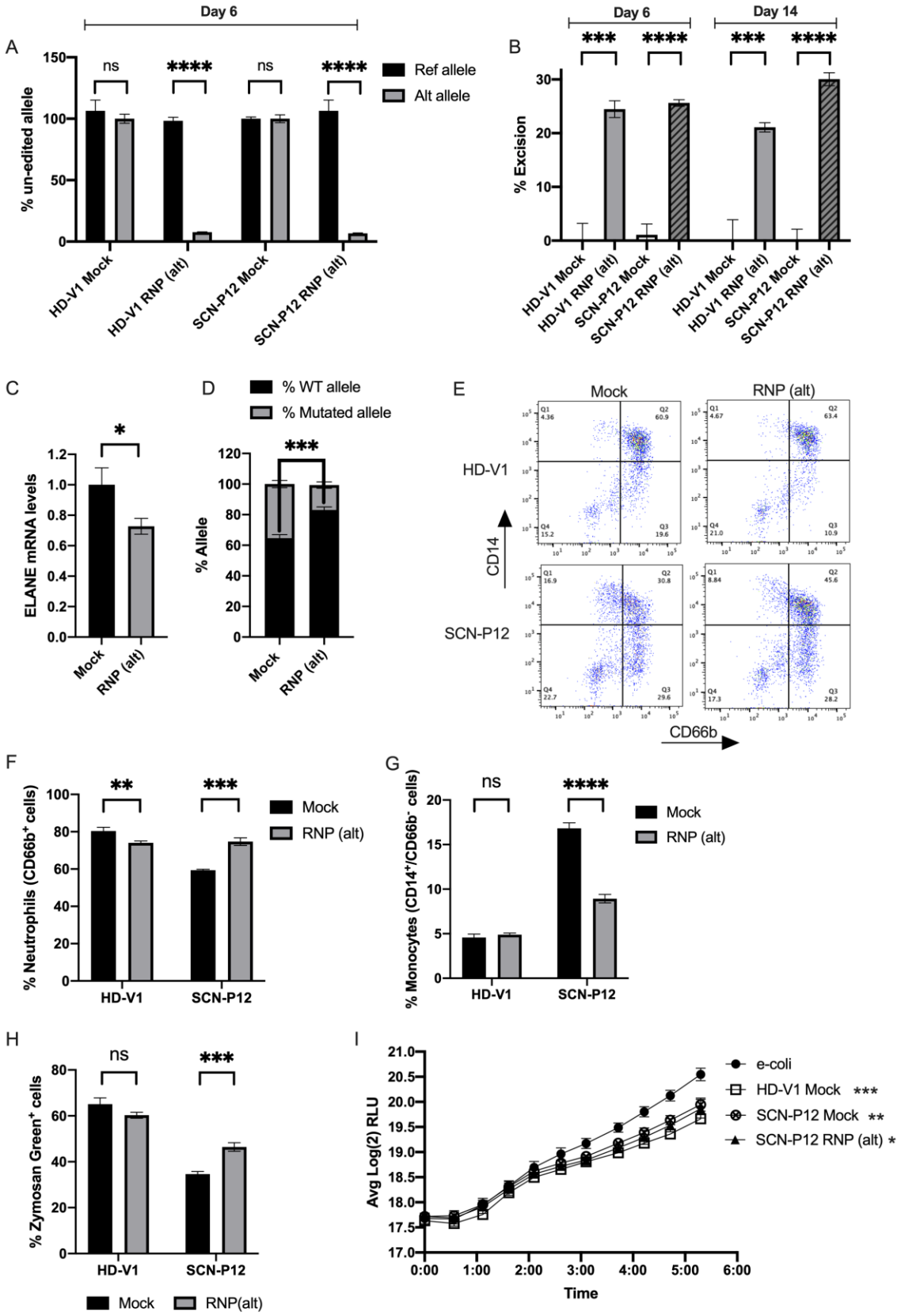

A
Day 6
% un-edited allele
Ref allele
Alt allele
ns
****
ns
****
HD-V1 Mock
HD-V1 RNP (alt)
SCN-P12 Mock
SCN-P12 RNP (alt)
B
Day 6
Day 14
***
****
***
****
% Excision
C
*
ELANE mRNA levels
D
% WT allele
% Mutated allele
***
% Allele
E
Mock
RNP (alt)
HD-V1
SCN-P12
CD14
CD66b
F
**
***
Mock
RNP (alt)
% Neutrophils (CD66b+ cells)
HD-V1
SCN-P12
G
ns
****
% Monocytes (CD14+/CD66b- cells)
H
ns
***
% Zymosan Green+ cells
I
e-coli
HD-V1 Mock ***
SCN-P12 Mock **
SCN-P12 RNP (alt) *
Avg Log(2) RLU
Time

Figure S13. Excision using RNP(alt) in SCN-P12 and HD-V1. (A) Bar graphs representing percentages of un-edited reference (black) and alternative (gray) alleles at day 6 of differentiation in HSCs taken from either healthy donor (HD-V1) or SCN patient (SCN-P12) treated with RNP (alt) composition or electroporated without a nuclease composition (Mock), as measured by ddPCR. (n=3 groups of cells from HD-V1 healthy/SCN-P12 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (B) Bar graphs representing percentages of excision at days 6 and 14 of differentiation in HSCs taken from either healthy donor (HD-V1): Mock-treated (Mock, black) or RNP (alt)-treated (gray), or SCN patient (SCN-P12): Mock-treated (Mock, white) or RNP (alt)-treated (dark oblique lines), as measured by ddPCR. (n=3 groups of cells from HD-V1 healthy /SCN-P12 patient donors). Statistical significance is indicated as ***P<.001, ****P<.0001. (C) Bar graphs representing *ELANE* mRNA levels in day 6 differentiated HSCs of SCN-P12 patient that were either Mock-treated (Black) or RNP(alt)-treated (Gray). Data is presented relatively to the mock group. (n=3 groups of cells from SCN-P12 patient). Statistical significance is indicated as *P<.05. (D) Bar graphs representing percentages of wild-type (black) and mutated (gray) alleles in cDNA taken from SCN-P12 patient HSCs that were either RNP (alt)-treated or Mock-treated (Mock), as measured by NGS targeting the mutation site. (n=3 groups of cells from SCN-P12 patient). Statistical significance is indicated as ***P<.001. (E) Representative FACS plots of mock-treated (Mock, left panel) and RNP(alt)-treated (right panel) healthy donor (HD-V1, upper panel) and SCN-P12 patient (lower panel) differentiated HSCs, analyzed for neutrophilic ($CD66b^+$) and monocytic ($CD14^+/CD66b^-$) subsets. (F) Quantitative analysis of respective FACS data for percentages of neutrophils ($CD66b^+$ cells) in healthy (HD-V1) and SCN patient (SCN-P12) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). (n=3 groups of cells from HD-V1 healthy /SCN-P12 patient donors). Statistical significance is indicated as **P<.01, ***P<.001. (G) Quantitative analysis of respective FACS data for percentages of monocytes ($CD14^+/CD66b^-$ cells) in healthy (HD-V1) and SCN patient (SCN-P12) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). (n=3 groups of cells from HD-V1 healthy /SCN-P12 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (H) Quantification of percentages of Zymosan Green uptake by healthy (HD-V1) and SCN patient (SCN-P12) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). Statistical significance is indicated as ***P<.001, ns = Not statistically significant. (I) Graph depicts real time change in light emission, relative light units (RLUs), from 200,000 Luciferase expressing bacterial cells incubated with differentiated neutrophils from healthy mock-treated (HD-V1, Mock; white square), patient mock-treated (SCN-12 Mock; crossed circle) or patient RNP(alt)-treated (SCN-P12 RNP(alt); triangle) HSCs compared to bacterial cells only control (e-coli, circle). Statistical significance for each one of the groups versus e-coli control at the last time point presented, when RLU levels reached plateau, is indicated as *P<.05, **P<.01, ***P<.001. Bars represent mean values with standard deviation.

Figure S14.

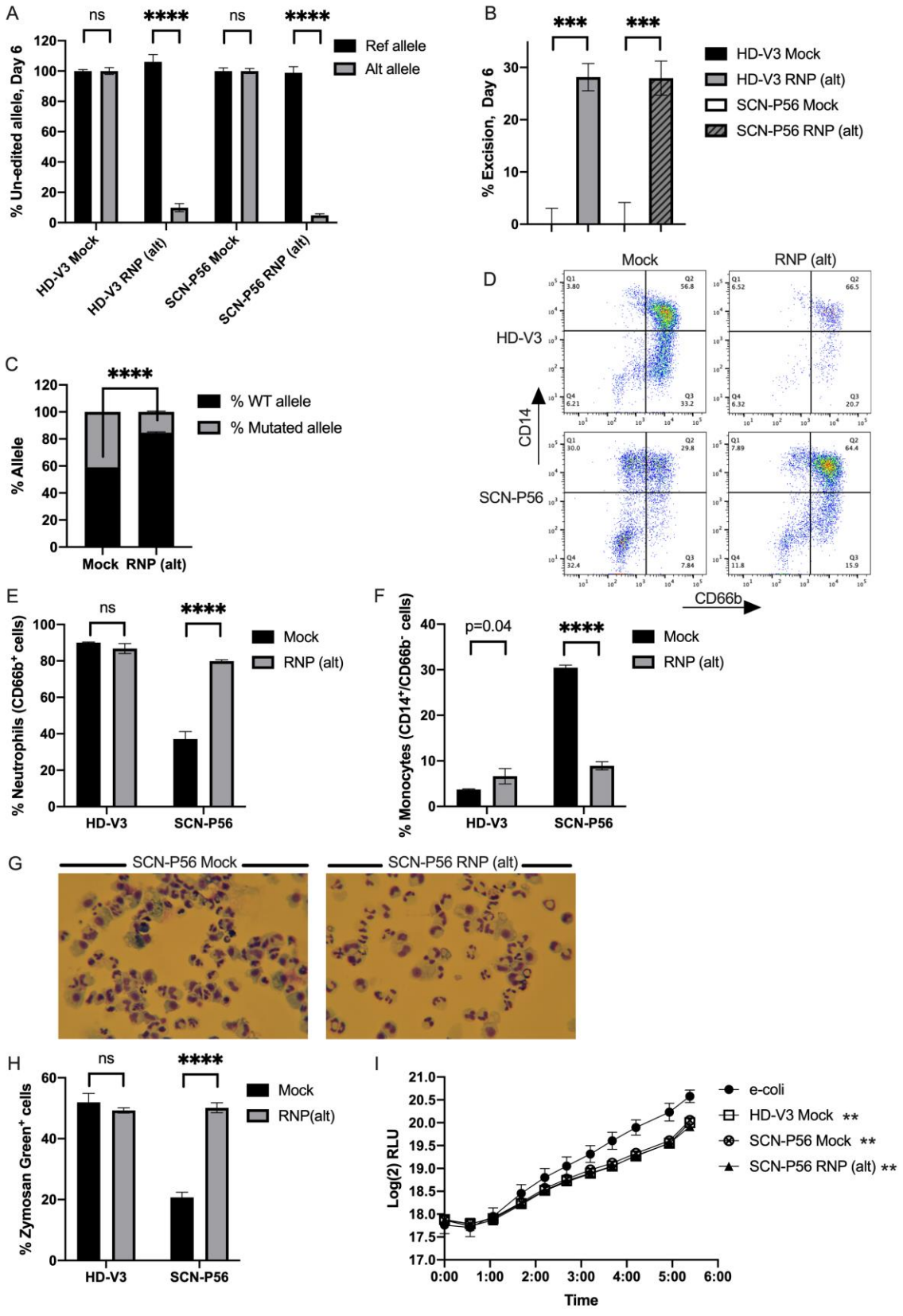

A
ns
****
ns
****
% Un-edited allele, Day 6
Ref allele
Alt allele
HD-V3 Mock
HD-V3 RNP (alt)
SCN-P56 Mock
SCN-P56 RNP (alt)
B
***
***
% Excision, Day 6
HD-V3 Mock
HD-V3 RNP (alt)
SCN-P56 Mock
SCN-P56 RNP (alt)
C
****
% Allele
% WT allele
% Mutated allele
Mock
RNP (alt)
D
Mock
RNP (alt)
HD-V3
SCN-P56
CD14
CD66b
E
ns
****
% Neutrophils (CD66b+ cells)
Mock
RNP (alt)
HD-V3
SCN-P56
F
p=0.04
****
% Monocytes (CD14+/CD66b- cells)
Mock
RNP (alt)
HD-V3
SCN-P56
G
SCN-P56 Mock
SCN-P56 RNP (alt)
H
ns
****
% Zymosan Green+ cells
Mock
RNP(alt)
HD-V3
SCN-P56
I
Log(2) RLU
e-coli
HD-V3 Mock **
SCN-P56 Mock **
SCN-P56 RNP (alt) **
Time

Figure S14. Excision using RNP(alt) in SCN-P56 and HD-V3. (A) Bar graphs representing percentages of un-edited reference (black) and alternative (gray) alleles at day 6 of differentiation in HSCs taken from either healthy donor (HD-V3) or SCN patient (SCN-P56) treated with RNP (alt) composition or electroporated without a nuclease composition (Mock), as measured by ddPCR. (n=3 groups of cells from HD-V3 healthy/SCN-P56 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (B) Bar graphs representing percentages of excision at day 6 of differentiation in HSCs taken from either healthy donor (HD-V3): Mock-treated (Mock, black) or RNP (alt)-treated (gray), or SCN patient (SCN-P56): Mock-treated (Mock, white) or RNP (alt)-treated (dark oblique lines), as measured by ddPCR. (n=3 groups of cells from HD-V3 healthy /SCN-P56 patient donors). Statistical significance is indicated as ***P<.001. (C) Bar graphs representing percentages of wild-type (black) and mutated (gray) alleles in cDNA taken from SCN-P56 patient HSCs that were either RNP (alt)-treated or Mock-treated (Mock), as measured by NGS targeting the mutation site. (n=3 groups of cells from SCN-P56 patient). Statistical significance is indicated as ****P<.0001. (D) Representative FACS plots of mock-treated (Mock, left panel) and RNP(alt)-treated (right panel) healthy donor (HD-V3, upper panel) and SCN-P56 patient (lower panel) differentiated HSCs, analyzed for neutrophilic ($CD66b^+$) and monocytic ($CD14^+/CD66b^-$) subsets. (E) Quantitative analysis of respective FACS data for percentages of neutrophils ($CD66b^+$ cells) in healthy (HD-V3) and SCN patient (SCN-P56) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). (n=3 groups of cells from HD-V3 healthy /SCN-P56 patient donors). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (F) Quantitative analysis of respective FACS data for percentages of monocytes ($CD14^+/CD66b^-$ cells) in healthy (HD-V3) and SCN patient (SCN-P56) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). (n=3 groups of cells from HD-V3 healthy /SCN-P56 patient donors). Statistical significance is indicated as P=0.04, ****P<.0001. (G) Diff-Quik staining of P56 SCN patient-derived differentiated HSCs treated with RNP(alt) or electroporated without a nuclease composition (SCN-P56 Mock). Microphotographs were taken on LEITZ LABORLUX S polarizing light microscope at 400X magnification using Nikon DSLR digital camera. (H) Quantification of percentages of Zymosan Green uptake by healthy (HD-V3) and SCN patient (SCN-P56) differentiated HSCs that were mock-treated (Mock, black) or treated with RNP(alt) (gray). Statistical significance is indicated as ****P<.0001, ns = Not statistically significant. (I) Graph depicts real time change in light emission, relative light units (RLUs), from 200,000 Luciferase expressing bacterial cells incubated with differentiated neutrophils from healthy mock-treated (HD-V3, Mock; white square), patient mock-treated (SCN-56 Mock; crossed circle) or patient RNP(alt)-treated (SCN-P56 RNP(alt); triangle) HSCs compared to bacterial cells only control (e-coli, circle). Statistical significance for each one of the groups versus e-coli control at the last time point presented, when RLU levels reached plateau, is indicated as **P<.01. Bars represent mean values with standard deviation.

Figure S15.

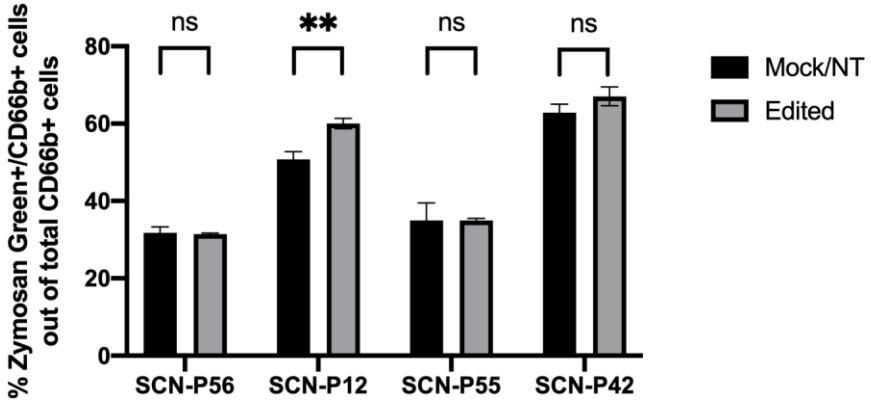



Figure S15. Phagocytosis out of total CD66b$^+$ cells. Quantification of percentages of Zymosan Green$^+$/CD66b$^+$ out of the total CD66b$^+$ neutrophils in patient-derived (SCN-P56, SCN-P12, SCN-P55 and SCN-P42) differentiated HSCs that were either mock/not-treated (black) or edited with RNP(alt)/(ref) (gray). Statistical significance is indicated as **P<.01, ns = Not statistically significant.

Table S1. Coverage of the three SNPs by the patient population.

| Patient # | Diagnosis | Source | ELANE mutation | Location of mutation | rs10414837 | rs3761005 | rs1683564 |
|---|---|---|---|---|---|---|---|
| 1 | SCN | Bone Marrow | S126L | 4 | CC | TA | CC |
| 2 | SCN | Bone Marrow | G210V | 5 | CT | TT | CC |
| 3 | SCN | Bone Marrow | C55S | 2 | CC | TA | CC |
| 4 | SCN | Bone Marrow | G221ter | 5 | CC | TA | AA |
| 5 | SCN | Bone Marrow | D230fs | 5 | CC | AA | CC |
| 6 | SCN | Bone Marrow | A57T | 2 | CT | TA | AA |
| 7 | SCN | Bone Marrow | R103P | 3 | CC | TT | AA |
| 8 | SCN | Bone Marrow | T128del | 4 | CT | AA | CA |
| 9 | SCN | Bone Marrow | V83D | 3 | CT | TT | AA |
| 10 | SCN | Bone Marrow | L84P | 3 | CT | TA | CA |
| 11 | SCN | Bone Marrow | L84P | 3 | CT | TT | CA |
| 12 | SCN | Bone Marrow | M154R | 4 | CC | TT | CA |
| 13 | SCN | Bone Marrow | G221ter | 5 | CT | TT | AA |
| 14 | SCN | Bone Marrow | G203R | 5 | CT | TA | CA |
| 15 | SCN | Bone Marrow | G214R | 5 | CC | AA | CA |
| 16 | SCN | Bone Marrow | P139L | 4 | CC | TA | CC |
| 17 | SCN | Bone Marrow | Y228ter | 5 | CT | TT | AA |
| 18 | SCN | Bone Marrow | P139L | 4 | CC | TA | CA |
| 19 | SCN | Bone Marrow | W156R | 4 | CT | TA | CC |
| 20 | SCN | Bone Marrow | P139L | 4 | CC | AA | CA |
| 21 | CyN | iPSC | P139L | 4 | CC | TT | CC |
| 22 | SCN | iPSC | I120N | 3 | CT | TT | AA |
| 23 | SCN | iPSC | G214R | 5 | CC | TA | CC |
| 24 | CyN | iPSC | S46F | 2 | CC | TA | CA |
| 25 | SCN | Bone Marrow | IVS4 +5G>A | INT4 | CC | TT | CA |
| 26 | SCN | Bone Marrow | S126L | 4 | CC | TA | CA |
| 27 | SCN | Bone Marrow | R103L | 3 | CC | TA | CA |
| 28 | SCN | Bone Marrow | M66R | 2 | TT | TT | CC |
| 29 | SCN | Bone Marrow | C208X | 5 | CT | TA | CC |
| 30 | SCN | Bone Marrow | M1R | 1 | CC | TT | CA |
| 31 | SCN | Bone Marrow | P234fs | 5 | CC | AA | CC |
| 32 | SCN | Bone Marrow | I60T | 2 | CC | TT | CA |
| 33 | CyN | iPSC | IVS4 +1sd | INT4 | CT | TT | CC |
| 34 | SCN | iPSC | P139L | 4 | CT | TT | AA |
| 35 | SCN | iPSC | R191S | 4 | TT | TT | CC |
| 41 | SCN | Bone Marrow | S126L | 4 | CC | AA | CA |
| 42 | SCN | Bone Marrow | S126L | 4 | CC | AA | CA |
| 43 | CyN | Bone Marrow | IVS4 +5 G>A | INT4 | CC | TT | CC |
| 44 | CyN | Bone Marrow | IVS4 +1 G>A | INT4 | CT | TA | CA |
| 45 | CyN | Bone Marrow | R220Q | 5 | CT | TT | CC |
| 46 | CyN | Bone Marrow | IVS4 +3 A>T | INT4 | CC | TA | CC |
| 47 | CyN | Bone Marrow | W241ter | 5 | CT | AA | CC |
| 48 | SCN | Bone Marrow | S126L | 4 | CT | TA | CC |
| 49 | SCN | Bone Marrow | S126L | 4 | CT | TA | CC |
| 50 | SCN | Bone Marrow | P139L | 4 | CT | TA | CC |
| 51 | SCN | Bone Marrow | S46F | 2 | CC | TA | CC |
| 52 | SCN | Bone Marrow | IVS3 -8 | INT3 | CT | TA | CC |
| 53 | SCN | Bone Marrow | P139L | 4 | CC | TA | CA |
| 54 | SCN | Bone Marrow | R220Q | 5 | CC | TA | CA |
| 55 | SCN | Bone Marrow | R220Q | 5 | CT | TA | CA |
| 56 | SCN | Bone Marrow | A57V | 2 | CT | AA | CA |
| 57 | SCN | Bone Marrow | R191S | 4 | TT | TT | CC |
| 58 | CyN | Bone Marrow | S126L | 4 | CC | AA | CA |

Sequencing results of the three SNPs in ELANE gene in samples obtained from SCN and Cyclic Neutropenia (cyN) patients. The pathogenic mutations in ELANE gene were also verified by sequencing. Green cells depict heterozygous SNPs.

Table S2. GUIDE-seq raw data.
Raw data from an unbiased survey (GUIDE-seq) of whole-genome off-target cleavage using OMNI A1 V10 nuclease and each of the constant guide (SgRNA(constant)), reference guide (SgRNA(ref)) and alternative guide (SgRNA(alt)).

**Supplemental Methods**

**Human cells**

Study approval was attained from Institutional Review Board of the University of Washington. Informed written consent was obtained from all the subjects of this study. Bone marrow samples were collected in association with an annual follow-up as recommended by the Severe Chronic Neutropenia International Registry.

**Human SCN Patient HSC isolation**

Three to 6 mls of freshly collected bone marrow was shipped overnight at ambient temperature. Hematopoietic stem and progenitor cells, HSPC's, were initially enriched using RosetteSep Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail, (Cat. No.15027) and Lymphoprep (Cat.no. 07801) according to manufacturer's protocol. The HSC enriched cell population was expanded by culturing for 4 days in $CD34^+$ expansion media (StemSpan SFEMII media (Cat.no. 09655) supplemented with 1% Penn Strep (Cat.no 03-031-1B, Biological Industries), 1x StemSpan CD34 Expansion Supplement(10x) (Cat.no. 02691), and 1.0µM UM729 (Cat.no.72332), at 37°C 5% $CO_2$. After expansion, $CD34^+$ cells were further enriched using EasySep Human CD34 Positive Selection Kit II (Cat.no. 17856) according to manufacturer's protocol. Enriched $CD34^+$ cells were cryopreserved at $1x10^6$ cells/ml in Cryostor CS10 (cat.no. 07931). Cells were stored in liquid nitrogen, vapor phase. All catalog numbers refer to materials from StemCell Technologies unless indicated otherwise. Patients used in this study were: SCN-P41 and SCN-P42, harboring the S126L mutation in exon 4 and SCN-P12 harboring the M154R mutation in exon 4, both mutations are linked to the reference form of the rs1683564 SNP, and SCN-P55 harboring the R220Q mutation in exon 5 and SCN-P56 harboring the A57V mutation in exon 2, both mutations are linked to the alternative form of the rs1683564 SNP.

**Human healthy donor HSC isolation**

Cryopreserved healthy human $CD34^+$ progenitor cells from mobilized peripheral blood were obtained from Lonza (Cat no. 4Y-101C). Cells were suspended in $CD34^+$ expansion media at 50,000 cells/ml and expanded for 4 days at 37°C, 5% $CO_2$ prior to electroporation.

**Heterozygosity frequency of SNPs in the healthy and patient populations**

Variant call files encompassing the *ELANE* gene region (± 3 kb of *ELANE* gene) were downloaded from the 1000 Genomes Project Consortium (phase 3) using the Data Slicer tool and analyzed in the R statistical computing environment. 3501 genotypes were available from 3501 individuals. Familial relationship was omitted from the analysis, which resulted in 2407 genotypes from unrelated individuals. The allele frequency for all common polymorphism (>1% MAF) was calculated. Three SNPs were chosen (rs3761005, rs1683564, and rs10414837 polymorphisms) to optimize the population coverage for allele-specific *ELANE* knock-out. The percentage of the population being heterozygous for at least one of the three chosen SNPs was calculated.

53 patients' samples were sequenced for the pathogenic mutation and the three chosen SNPs. A total of 46 bone marrow samples and 7 iPSC lines were used. 44 of the samples were from SCN patients and nine of them were from patients with Cyclic Neutropenia. Heterozygosity frequency of each of the three chosen SNPs and the percentage of the population being heterozygous for at least one of them was calculated.

**CRISPR-associated OMNI A1 V10 *ELANE* gene editing**

Editing of HSCs was carried out using a ribonucleic protein (RNP) system at a molar ratio of 1:2.5 (nuclease: sgRNA), including 17µg nuclease and 262pmol of each guide. Nuclease and sgRNA complex were incubate at 25°C for 10 minutes. Human $CD34^+$ cells were washed once with PBS. $2x10^5$ $CD34^+$ cells were suspended into 20µl of P3 electroporation buffer (Lonza P3 kit S) and were added to RNPs mix. After electroporation, using the CA-137 program (Lonza 4D, Nucleofector™), the cells were transferred to pre-warmed $CD34^+$ expansion media at a concentration of $1.25\times 10^5$ cells/ml. Guides were manufactured by Agilent. Guide sequences are summarized in table S3.

Table S3. Guide sequences.

| sgRNA name | Guide sequence (scaffold + spacer) |
|---|---|
| sgRef | GUGUCAAGCCCCAGAGGCCACAgUUUGAGAGUUAUGAAAAUGACGAGUUCAAA UAAAAAUUUAUUCAAACCGCCUAUUUAUAGGCCGCAGAUGUUCUGCUUU |
| sgAlt | GUGUCAAGCCCCAGAGGACACAgUUUGAGAGUUAUGAAAAUGACGAGUUCAAA UAAAAAUUUAUUCAAACCGCCUAUUUAUAGGCCGCAGAUGUUCUGCUUU |

| | |
|---|---|
| sgConstant | GCAGUCCGGGCUGGGAGCGGGUgUUUGAGAGUUAUGAAAAUGACGAGUUCAAA UAAAAAUUUAUUCAAACCGCCUAUUUAUAGGCCGCAGAUGUUCUGCUUU |

OMNI A1 V10 is an engineered form of the newly discovered OMNI A1, a novel CRISPR nuclease of 1370 amino acids and an NGG PAM. OMNI-A1 was subjected to iterative rounds of mutagenesis followed by positive and negative selections (as described in Chen Z and Zhao H.[1] and Kleinstiver BP, et al.[2]) The resultant V10 nuclease, used in the current research, showed superior allele and target specificity, and had four amino acid substitutions relative to the original OMNI A1. One of the mutations is located at the REC1 domain and another one at the REC3 domain, suggesting potential interactions with the sgRNA and target DNA.

**Digital Droplet PCR for percentage excision and allele specificity**

Percentage excision and allele specificity were measured using Digital Droplet PCR™ (ddPCR™, Bio-Rad, Hercules, CA, USA) on genomic DNA that was extracted using QIAamp DNA Micro Kit, Qiagen (Cat no. 56304). According to manufacturer's protocol.

ddPCR reaction contained 1× ddPCR Supermix for probe without dUTP (#1863024), 25-100ng of digested DNA using HindIII (diluted in X1 Cutsmart Buffer to 4U/μL) and suitable primers/probes. For excision reaction, amplification of two regions in *ELANE* gene, exon 1 and exon 5 was performed, using two different probes labeled with FAM (X1) and HEX (X1), respectively. The ratio between the HEX and the FAM signals was translated to excision efficiency. The location of the probes is presented in Figure S16. The same probes can be used for the strategies based on the upstream SNPs (rs10414837 and rs3761005).

For allele specificity, two competitive probes: a FAM probe, which binds the alternative allele, and a HEX probe, which binds the reference allele were used (FAM+HEX). The ratio between the concentrations of the two in heterozygote non-treated cell is 1, which was normalized to the endogenous genes RPP30 and STAT1 for each gDNA sample. Reaction total volume was 22μL. The binding of each probe to DNA extracted from healthy donor cells that were homozygous to either the reference or alternative forms of the SNP was measured by ddPCR, confirming the probes do not cross react, (**Figure S5A**). Moreover, healthy donor cells homozygous to the reference form of the SNP depicted efficient excision when treated with RNP(ref) composition, compared to treatment with RNP(alt) composition that resulted in excision levels comparable to non-treated cells. This further demonstrates the specific targeting of the sgRNAs (**Figure S5B**).

Genomic DNA in the ddPCR mixture was partitioned into individual droplets using QX100 Droplet Generator, transferred to a 96-deep well PCR plate and amplified in a Bio-Rad PCR thermocycler. Bio-Rad Droplet Reader and QuantaSoft Software were used to read and analyzed the experiment following manufacturer's guidelines (Bio-Rad). The primers and probes were manufactured by Bio-Rad and are detailed in table S4.

Table S4. Primers and probes for excision and allele specificity measurements.

| Probe name | Catalog number |
|---|---|
| *ELANE* ddPCR assay Exon1 (FAM) | dHsaCNS328407057 |
| *ELANE* ddPCR assay Exon5 (HEX) | qHsaCEP0055470 |
| rs1683564 Editing ddPCR assay (FAM+HEX) | dHsaMDS873573221 |
| RPP30 | dHsaMDS117591774 |
| STAT1 | dHsaCNS850507320 |

Figure S16.

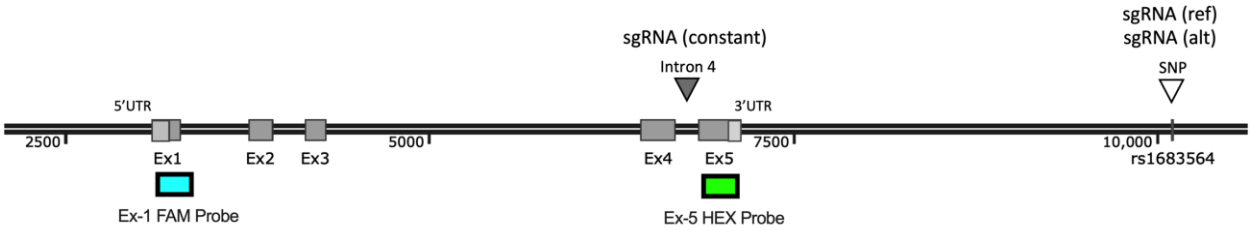



Figure S16. Location of FAM and HEX probes used to determine excision.

**Assessment of mutated/wild-type allele ratio**

cDNAs from HSCs treated with either RNP(ref) or RNP(alt), mock-treated or not-treated were mapped using next-generation sequencing (NGS) targeting exon 4 (for patients 41 and 42 (harboring the S126L mutation) and patient 12 harboring the M154R mutation), exon 5 (for patient 55 harboring the R220Q mutation) and exon 2 (for patient 56 harboring the A57V mutation). The raw FASTQ files were analyzed, and BAM files (text-based format for storing biological sequences) were generated using FASTQ to BAM script. The relative ratio of the mutated allele to the wild-type allele was calculated and compared to the non-treated or mock-treated cells in all patients. This assay was used as a robust approach targeting the exons that harbor the mutations, instead of addressing each mutation individually (as SCN is associated with more than 200 different mutations in *ELANE*). The primers used in this assay are suitable for several patients as detailed in table S5.

Table S5. Primers used for assessment of mutated/wild-type allele ratio.

| Mutation | Sequence |
|---|---|
| S126L (P41, P42); M154R, (P12) | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTACGACCCCGTAAACTTGCT<br>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCGGAGCGTTGGATGATAGAG |
| R220Q (P55) | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTCGCAGTCCAGCTTCCCCAC<br>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNACAGCCAAGGAGCATCAAAC |
| A57V (P56) | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNCCCTTCATGGTGTCCCTGC<br>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCCGTCACGTTGAGCTCCTG |

**ddPCR *ELANE* expression assay**

RNA purification.

Pellets of fresh, day 6 differentiated HSCs centrifuged at 300 g for 5 min and kept on ice were used. Total RNA was extracted using RNeasy® Mini Kit according to the manufacturer's instructions (QIAGEN #74104) with 600 µl RTL supplemented with β-Mercaptoethanol and on-column DNAseI treatment for 30 min. Total RNA concentration was determined by NanoDrop and RNA integrity number equivalent (RINe) of samples was determined by TapeStation according to Agilent RNA ScreenTape Quick Guide for TapeStation Systems (Agilent, Publication Part Number: G2991-90021). RNA samples had RINe > 8.0.

ELANE mRNA expression levels estimate

RNA was purified from patient HSCs excised with either RNP(ref) or RNP (alt), mock-treated or not-treated, as described. cDNA was prepared using High-Capacity RNA-to-cDNA kit according to the manufacturer's instructions (Applied Biosystems, #4387406), normalized to 10 pg/µl initial RNA and stored at -20°C until use. For each sample, a no reverse transcriptase control was also prepared with ultra-pure water instead of reverse transcriptase to ensure there is no gDNA contamination. For each of the cDNA samples, a PCR master mix of 2x QX200 ddPCR EvaGreen® Supermix (10 µl per reaction, Bio-Rad, 1864033) and cDNA (6 µl per reaction, 60 pg initial RNA) was prepared according to the number of reactions needed (2 technical repeats per each target plus 1 extra reaction to account for pipetting errors). For each PCR reaction, the master mix (16 µl) was dispensed into reaction tubes prior to adding forward and reverse primers mix (500 nM, 4 µl) specific to the target. For each of the targets, a no template control reaction with ultra-pure water instead of cDNA was included to ensure there is no primer dimer

formation or extraneous nucleic acid contamination. Primers used are detailed in table S6.

Table S6. Primer sequences for measuring *ELANE* expression levels by ddPCR.

| Gene | Forward primer sequence | Reverse Primer sequence |
|---|---|---|
| *ELANE* | CTACGACCCCGTAAACTTGCT | CCGACCCGTTGAGCTGGAG |
| *GAPDH* | CATCACCATCTTCCAGGAGCGAG | CCCCTGCAAATGAGCCCCAG |

PCR-ready samples were loaded into DG8 cartridges and droplets were generated according to the QX200 Droplet Generator Instruction Manual (Bio-Rad, Bulletin# 10031907). The droplets were transferred into a 96-well ddPCR plate according to the experiment pre-designed plate layout. The plate was sealed with a pierceable foil heat seal according to the PX1TM PCR Plate Sealer Instruction Manual (Bio-Rad, Bulletin# 10023997). The PCR-ready plate was placed into Bio-Rad's C1000 Touch Cycler for PCR amplification according to Table 2. Thermal Cycling Protocol in the ddPCR Gene Expression EvaGreen® Assays Product Insert (Bio-Rad, Bulletin# D107737) with annealing/extension at 59°C. The post-PCR plate was placed into the QX200 Droplet Reader. Setup, Run, and analyze were preformed according to the QX200 Droplet Reader and QuantaSoft Software Instruction Manual (Bio-Rad, Bulletin# 10031906). For Setup, ABS (Absolute Quantification) option for Experiment and the QX200 ddPCR EvaGreen Supermix option for Supermix were used. Run followed as described in the manual. For Analyze, the concentration data, number of events, and the thresholds were reviewed for each of the wells in the QuantaSoft Software. Thresholds were adjusted, if needed, and the results exported to Excel. For each well, the average concentration and standard deviation of the 2-technical repeats of each of the targets was calculated. Wells with anomalous values, such as too few droplets or high standard deviation between the technical repeats, were omitted from the analysis. For each biological sample, ELANE mRNA expression levels were normalized to the GAPDH mRNA levels. Then, the ELANE normalized levels were normalized to the average of ELANE normalized levels of the mock or NT samples, as follows:

$$\frac{ELANE\ average\ copies/\mu l}{GAPDH\ average\ copies/\mu l} = ELANE\ normalized\ levels$$

$$\frac{ELANE\ normalized\ levels}{Average\ of\ ELANE\ normalized\ levels\ of\ the\ MOCK/NT\ samples} = ELANE\ mRNA\ ratio$$

**Differentiation assay**

Edited and non-treated or mock-treated HSCs were allowed to recover for 3 days in $CD34^+$ expansion media and were subjected to a differentiation protocol adopted from Nasri et al.[3] In brief, HSCs were cultured for 7 days in RPMI (Cat.no 11875093, Gibco™) supplemented with 1% Glutamax (Cat.no 35050061, Gibco™), 10% FBS (Cat.no 04-001-1A, Biological Industries), 5ng/ml IL-3 (Cat.no 200-03), SCF (Cat.no 300-07), GM-CSF (Cat.no 300-03) & 10ng/ml G-CSF (Cat.no 300-23), all from PeproTech, for proliferation and myeloid progenitor differentiation followed by a 7-day culture in RPMI , 1% Glutamax, 10% FBS, 1% Penn Strep (Cat.no 03-031-1B, Biological Industries), 10ng/ml G-CSF for neutrophil differentiation and maturation. On day 14 cells were analyzed by flow cytometry for monocytic ($CD14^+/CD66b^-$) and neutrophilic ($CD66b^+$ or $CD11b^+/CD15^+$) subsets. CD66b anti-human; Pacific Blue (Cat No. 305112, Biolegend), CD14 anti-human; APC (Cat No. 130-110-520), CD11b anti-human; APC (Cat No. 130-110-554) and CD15 anti-human; Pacific Blue (Cat No. 130-113-488) all from Miltenyi Biotec, unless indicated otherwise, were used.

**Cytospin staining**

$8x10^4$ HSCs at day 15 of differentiation were spun onto Cytoslide microscope slides (ThermoFisher) using Cytospin 4 low speed cytocentrifuge (Thermo Scientific) and stained with Diff-Quick staining system (MilliporeSigma) according to manufacturer's recommendations. Microphotographs were taken on LEITZ LABORLUX S polarizing light microscope at 400X magnification using Nikon DSLR digital camera.

**Bacterial killing assay**

Day 13 differentiated HSCs (subjected to a differentiation protocol adopted from Nasri et al.[3]) from healthy donors and SCN patients (edited with either RNP(ref), RNP(alt), mock-treated or non-treated), were evaluated for their bacterial killing capacity as described in J. T. Atosuo.[4] Briefly, 100,000 differentiated HSCs were incubated in the

presence of 200,000 Luciferase expressing bacterial cells, pAKLUX2, per well (Addgene, Cat No. 14080)[5]. Cells were cultured in 200 µl HBSS++, 10% FBS at 37°C. At 30-minute intervals luminescence was measured by transferring the plate to a Luminometer (Berthold CentroXS3 LB960) and measuring luminescence for 0.5 sec per well). A real time change in light emission, relative light units (RLUs), was measured over 5 hrs. Last time point presented is when RLU levels reached plateau. Wells without differentiated HSCs (E.coli only) and with 10ng/ml phagocytosis inhibitor (data not shown), Cytochalasin D (Santa Cruz Biotec, Cat No. sc-20144) served as controls. pAKlux2 was a gift from Attila Karsi (Addgene plasmid # 14080; http://n2t.net/addgene:14080 ; RRID:Addgene_14080).

**Phagocytosis assay**

Phagocytosis capacity was evaluated using the EZCell™ Phagocytosis Assay Kit (Green Zymosan), (BioVision, Cat no. K397 according to manufacturer's protocol). Day 14 differentiated HSCs (subjected to a differentiation protocol adopted from Nasri et al.[3]) from healthy donors and SCN patients (edited with either RNP(ref), RNP(alt), mock-treated or non-treated), were resuspended in HBSS++/10% FBS ($0.5X10^6$ cells/ml) and incubated for 1.5 hours at 37°C in the presence of 5ml opsonized Alexa Fluor 488-conjugated zymosan particles per 200ml suspended cells. As a negative control, cells were incubated with 10ng/ml of the phagocytosis inhibitor, Cytochalasin D (Santa Cruz Biotec, Cat No. sc-201442) 1 hour prior and during incubation with Zymosan Green reagent. Cells were then washed and incubated in a quencher solution, based on kit's instructions, to remove fluorescence from particles that were not internalized. Cells were then analyzed by flow cytometry for internalization of opsonized fluorescent Zymosan Green particles.

**Editing at upstream SNPs**

Genomic DNAs from U2OS cells (ATCC), treated with non-engineered OMNI-A1 and either sgRNA 39 (targeting rs10414837 SNP) or sgRNA 58 (targeting rs3761005 SNP), were mapped using next-generation sequencing (NGS) with PCR amplicons spanning the sgRNA genomic target sites (SNPs rs10414837 or rs3761005) to measurer genetic variation due to editing. For gene specific PCR, we used NEBNext® Ultra™ II Q5® Master Mix (NEB, #M0544) with the primers listed in table S7.

Table S7. Primers used to measure editing at upstream SNPs.

| | |
|---|---|
| ELANE_g39 rs10414837 | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGGTGGGTCCTCAGTGACTCT RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNGGGAATTCCAGCCTGACCAA |
| ELANE_g58 rs3761005 | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGAGTGAGGACCAAGCCTGAG RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNAGGGCCATTGTCTCCCTAAC |

The indexing PCR was performed with illumina Nextera index sets. The generated library was sequenced with illumina NextSeq SR150 and the FASTQ files were analyzed with CRISPRESSO2 pipeline.[6]

**Inversions**

Inversion events were detected and quantified by a Droplet Digital PCR (ddPCR) mutation assay. First, a perfect inversion was mimicked using a SnapGene software and verified by NGS.
Then, total inversion events were quantified by ddPCR using EvaGreen dye, a fluorescent DNA-binding dye that binds dsDNA (BIO-RAD, catalog number 186-4034, according to manufacturer's protocol). Specific primers were designed to amplify inverted variations of the excised fragment (See illustration in Figure S6). Fluorescent signals were normalized to amplification of *ELANE* exon 1 region that was not affected by excision (performed by two different sets of primers. Averaged normalized data is presented). Primers are detailed in table S8.

Table S8. Primers used to detect and quantify inversion events.

| Primer name | Sequence |
|---|---|
| Exon 4 primer | ACGTCTGCACTCTCGTGAGG |
| Exon 5 primer (inverted) | TTCAGGCTCCACCCAGTTTGTC |

| | |
|---|---|
| Exon 1 primers | Mix3:<br>GCACAGGGCTATAAGAGGAGC<br>GCGGGAGGTTGGACTCAAAA |
| | Mix6:<br>GGGAGAGGAAGTGGAGGGC<br>GAGGGTCATGGTGGGGCT |

**Excision levels in Long Term HSC population**

HSCs of two healthy donors (MLP1 (3055934); heterozygous to the alternative form of the SNP and MLP2 (3055940), homozygous to the alternative form of the SNP), isolated from leukopaks purchased from AllCells, were edited a day after thaw according to 'CRISPR-associated OMNI A1 V10 *ELANE* gene editing' section above, with minor changes. Cell number was 2M cells/electroporation. Upscale of guides and nuclease was performed accordingly. A molar ratio of 1:2.5 (nuclease: sgRNA) was used including 85µg nuclease and 1310 pmol of each guide. Nucleofection was performed in P3 nucleofection solution (Lonza) and Lonza 4D-Nucleofector™ X Kit L (program CA-137). Three days after editing, cells were sorted in a FACS ARIA™ II SORP Flow Cytometer Cell (BD), using CD90-APC-Vio770, human (130-114-863 Milteny). Sorted cells were incubated for 7 days in a proliferation medium according to Nasri et al.[3] Excision levels of $CD90^+$, $CD90^-$ and total population were evaluated using ddPCR as described in ddPCR section.

**Mutation-SNP linkage**

First, *ELANE* mutation and possible SNPs were identified in cells from SCN patients by targeted short read NGS. Then, a part of the gene encompassing both the mutation and the SNP was amplified by a PCR reaction with linkage primers and cloned into bacteria. Each clone bore an amplicon from one allele. A plasmid of multiple clones was Sanger sequenced (with T7 and SP6 primers) for the mutation and the SNP regions. If the mutation and the SNP were in cis, they were found at the same clone, if in trans, the mutation and SNP were found in different clones. Primers are detailed in table S9.

Table S9. Primers used for Mutation-SNP linkage assay.

| Primer name | Sequence |
|---|---|
| rs10414837 | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGGTGGGTCCTCAGTGACTCT |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNGGGAATTCCAGCCTGACCAA |
| rs3761005 | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGAGTGAGGACCAAGCCTGAG |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNAGGGCCATTGTCTCCCTAAC |
| rs1683564 | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTCCTGCTACCCTCCCTTCTT |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNTTTTAGGAGGGGCCCACTGA |
| Linkage | FP: GAGGGTCATCATCACTGCCC |
| | RP: GCCAGACTCACACCAGAGTCGACAAGT |
| T7 | TAATACGACTCACTATAGGG |
| SP6 | ATTTAGGTGACACTATAG |
| R220Q mutation (SCN-P55) | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTCGCAGTCCAGCTTCCCCAC |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNACAGCCAAGGAGCATCAAAC |
| S126L mutation (SCN-P41, SCN-P42) | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGAGGGTCATCATCACTGCCC |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNAGTCCGGGCTGGGAGCGGGT |
| A57V mutation (SCN-P56) | FP: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNCGCACACTCCCGGCTACTCA |
| | RP: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNCTCAGTTTCCTCATCTGAACAACAG |

**Analysis of off-targets**

GUIDE-seq analysis was performed as described in Tsai et al[7] using 50pmol 46bp dsODN (ATATCGCGTCCGTTATTAACATATGACAACTCAATTAAACGCGAAC). NGS library preparation was done as in Palani's protocol[8]. For *in-silico* analysis, *Cas-Offinder* was used to identify potential off-targets of up to 3 mismatches for each of the guides. The off-target sites retrieved from *Cas-Offinder* were tested by rhAmpSeq analysis. The rhAmpSeq multiplex amplicon sequencing technology (IDT, Coralville, IA) was used to quantify off-target editing activity, the rhAmpSeq library for the targeted amplicons sequencing was prepared according to IDT's protocol.[9] The accuracy of the multiplex rhAmpSeq technology is based on blockage primers containing RNA bases at the 3′ end of the primer, causing DNA/RNA hybridization. A perfect DNA/RNA alignment is cleaved by RNase H2 enzyme, allowing continuance amplification. rhAmpSeq primers were designed using the IDT rhAmpseq design website[10] to flank each off-target cut-sites identified by Cas-Offinder and pooled together for multiplex assay amplification (IDT, Coralville, IA). The off-target panels were tested on HSCs derived from patients edited with RNP(ref) or RNP(alt). Each experiment was performed with three independent repeats. 100 ng DNA from each sample was submitted to a two-round rhAmpSeq PCR according to the manufacturing protocol. rhAmpSeq amplicons were purified and sequenced using the Illumina NextSeq platform (150-bp paired-end reads) and analyzed with IDT pipeline.

**NETosis assay**

HSCs taken from two healthy donors were either not-treated (NT), RNP(ref)-treated or RNP(alt)-treated and were subjected to a differentiation protocol as described above. 20,000 differentiated HSCs were seeded in a 96 well plate, in F-12 medium (Sartorius), a low auto-fluorescence media. Cells were stimulated with PMA (Sigma-Aldrich) (1000 nM) to induce NETosis or added with 0.1% DMSO (No PMA control). 250μM SYTOX Green dye (Sartorius), a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes was added for detection of membrane-damaged cells. Cells were imaged using phase contrast and green (300-ms exposure) channels in the Incucyte® S3 System, which was housed in a cell incubator at 37˚C with 5% $CO_2$. Three image sets from distinct regions per well were taken every 30 minutes for up to 10 hours. Each condition was run in triplicate. Cells undergoing NETosis were identified by green staining following membrane damage. For the green channel, edge sensitivity was set to -20, and hole fill was set to 30$\mu m^2$. A minimum area of 400$\mu m^2$ was set in the processing definition for exclusion of apoptotic cells that are also stained by the SYTOX Green dye, but to a much smaller area compared to NETotic cells. To determine the number of neutrophils per well, total number of

neutrophils were calculated according to phase images using adherent cell-by-cell analysis. To determine the percentage of cells undergoing NETosis, the green object count after 10-hour stimulus was divided by the total cell count at the starting time point. Data is presented as fold change relative to NT 1000nM PMA.

**Statistical methods**

The two-sample T-test for independent samples or the Anova model, as appropriate, was applied for testing the statistical significance of the difference in continuous variables between treatment groups. The two-ways Analysis of Variance with repeated measurements was used to analyze killing assays. Chi-square test or Fisher's Exact test, as appropriate, was applied to test the statistical significance of the difference in heterozygosity between healthy and patient populations. All tests were two-tailed, and a p-value of 5% or less was considered statistically significant. The data was analyzed using Prism software (GraphPad version 9.0.2).

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