

YMTHE, Volume 28

## **Supplemental Information**

### **CRISPR-Cas9-Mediated *ELANE* Mutation**

### **Correction in Hematopoietic Stem and Progenitor**

### **Cells to Treat Severe Congenital Neutropenia**

**Ngoc Tung Tran, Robin Graf, Annika Wulf-Goldenberg, Maria Stecklum, Gabriele Strauß, Ralf Kühn, Christine Kocks, Klaus Rajewsky, and Van Trung Chu**

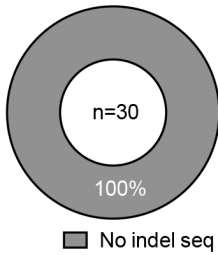


**A**

WT allele

5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG-3'  
 |||||  
 3' -TCCTCGAGTTGCACTGCCACCACTGCAGGGAGACGGCAGC-5'  
 sgELANE-L172P

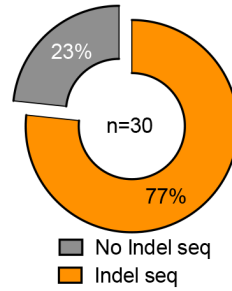
5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG

**B**

Mutant allele

5' -AGGAGCCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG-3'  
 |||||  
 3' -TCCTCGGGTTGCACTGCCACCACTGCAGGGAGACGGCAGC-5'  
 PAM sgELANE-L172P

5' -----TGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGA-----CGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGG-----GACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -----CTCTGCCGTCG  
 5' -AGGAGCCA-----ACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCA-----ACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAAC---ACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCA-----ACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAACGGTGA-GTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCA-----TGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCAAC---AC---GGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCC-----ACGGTGGTGACGTCCCTCTGCCGTCG  
 5' -AGGAGCCA-----GACGTCCCTCTGCCGTCG  
 5' -AGGAGCC-----TGACGTCCCTCTGCCGTCG



**Figure S2. Editing efficiency of the *ELANE*<sup>L172P</sup> mutation-specific sg*ELANE*-L172P in SCN-HSPCs.** Representative sequences of the *ELANE* WT (A) and mutant (B) alleles in SCN-HSPCs that were treated with sg*ELANE*-L172P/RNPs. Pie charts summarizing the indel frequencies of the targeted WT or mutant alleles. The wild-type T nucleotide is indicated in red, the mutant C nucleotide is in blue and the PAM signal is indicated in orange.

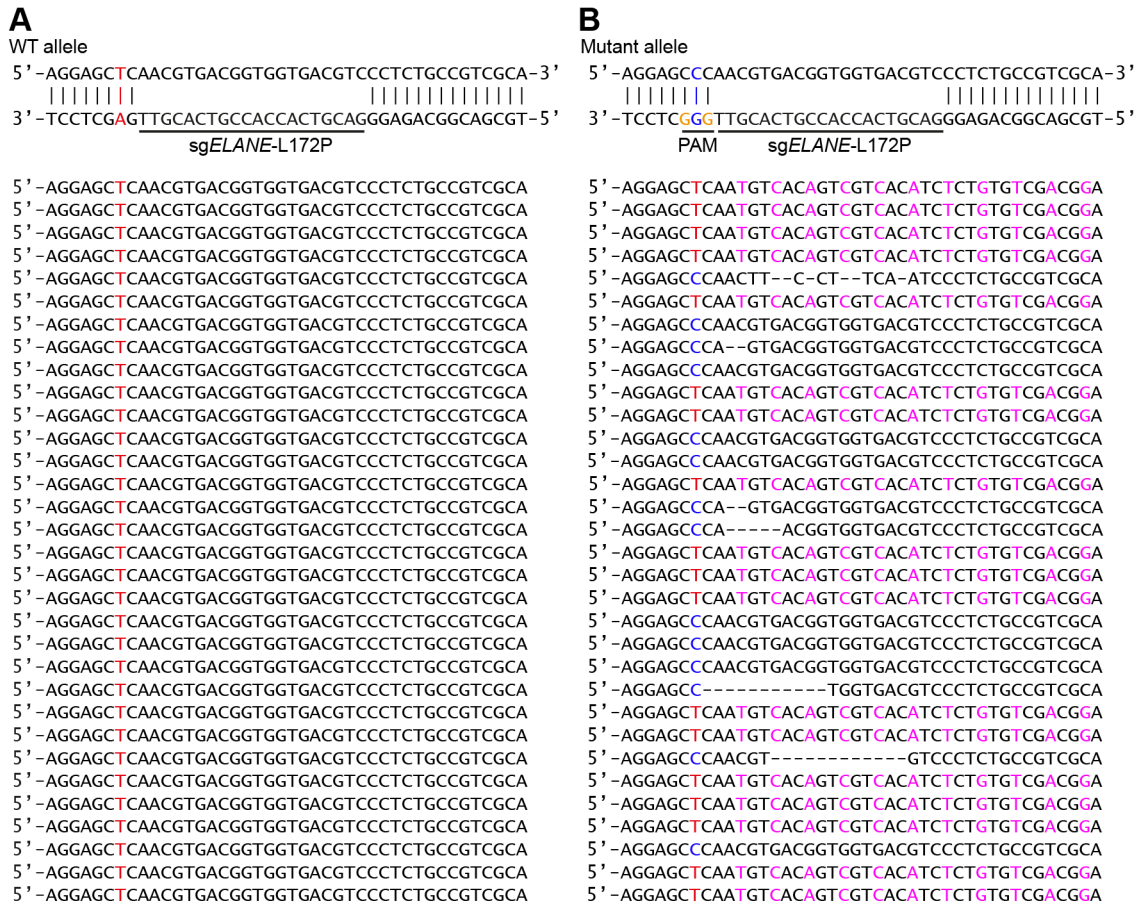


sgELANE-ex4

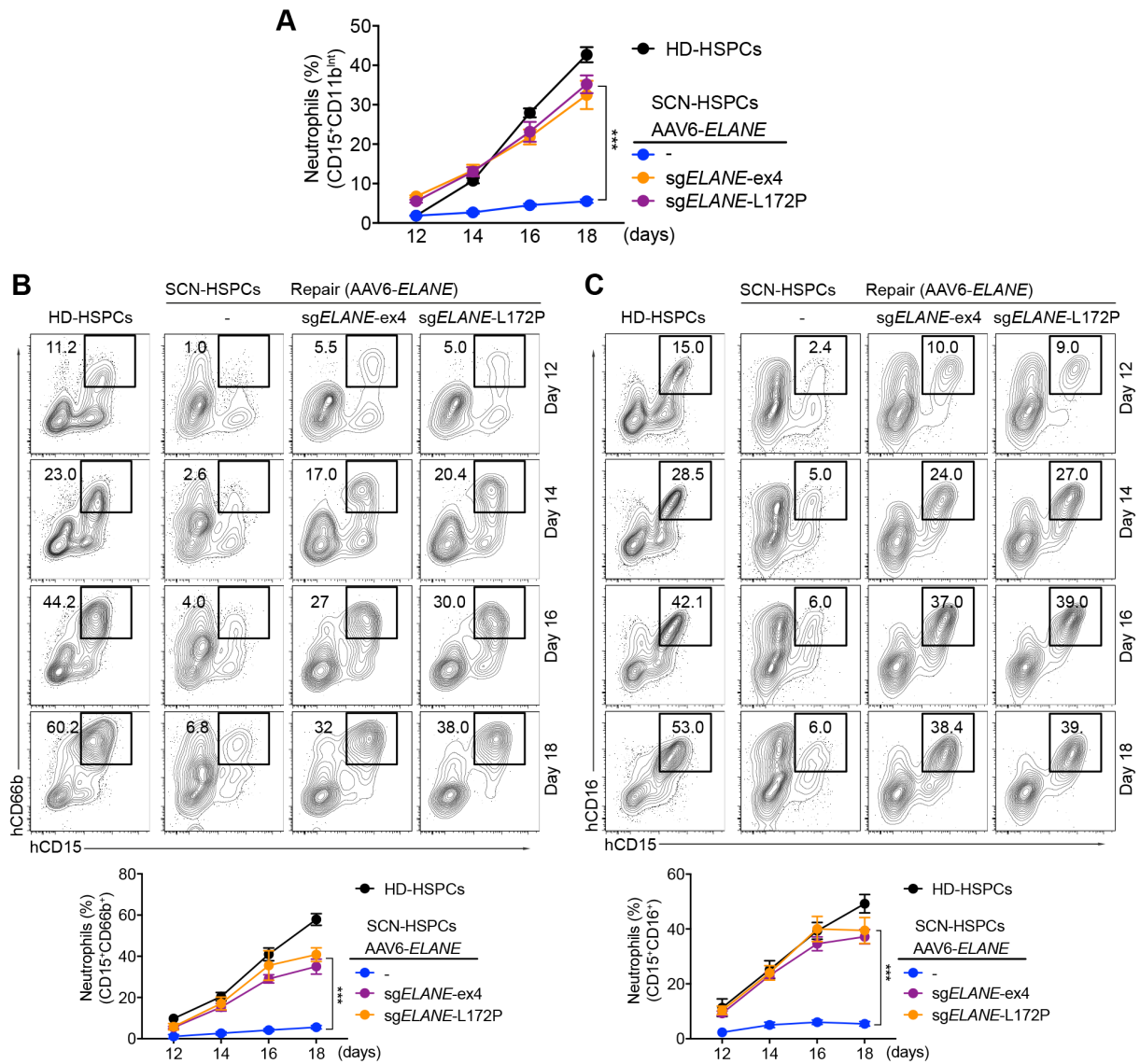
WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCGGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCGGTCGCGAGCAACGTCTGCACTCTCGT

Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTC-----GCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCG-----AGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGC-----GT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTG-----CGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCG-----GCAACGTCTGCACTCTCGT  
 Mu: 5' -AGGAGC**C**CAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT  
 WT: 5' -AGGAGCTCAACGTGACGGTGGTGACGTCCCTCTGCCGTCGCGAGCAACGTCTGCACTCTCGT  
 Re: 5' -AGGAGCTCAATGT**C**ACAGT**C**GT**C**ACATCTCTGT**G**TCG**A**CG**G**AGCAACGTCTGCACTCTCGT

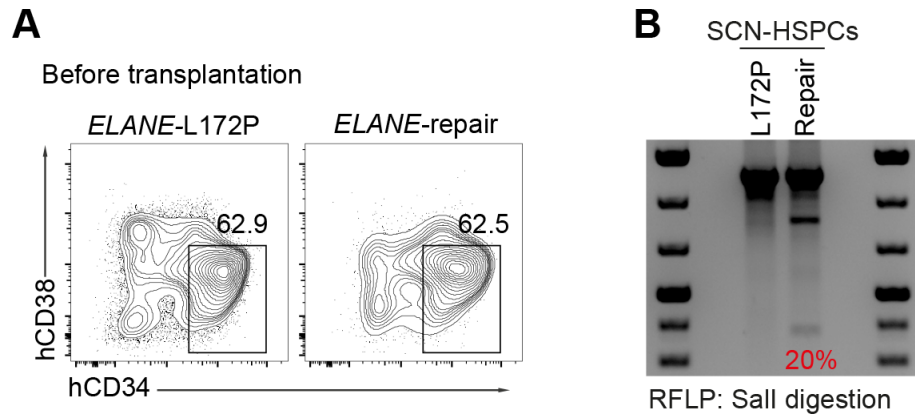
**Figure S4. Efficiency of universal exon 4-based correction in SCN-HSPCs using sgELANE-ex4.** Representative sequences of the targeted *ELANE* locus in SCN-HSPCs that received both sgELANE-ex4/RNPs and AAV6-*ELANE* donor vectors showing WT (black), repaired (Re, red), and mutant (Mu, light blue) alleles. The wild-type T nucleotide, the mutant C nucleotide and silent mutations are indicated in red, blue and magenta, respectively. The PAM sequence is indicated in orange.



**Figure S5. Efficiency of *ELANE*<sup>L172P</sup> mutation-specific correction in SCN-HSPCs using sg*ELANE*-L172P.** Representative sequences of the WT (A) and mutant (B) alleles in SCN-HSPCs which received both sg*ELANE*-L172P/RNPs and AAV6-*ELANE* donor vectors. The WT T nucleotide is shown in red, the mutant C nucleotide is in blue and the silent mutations are in magenta.

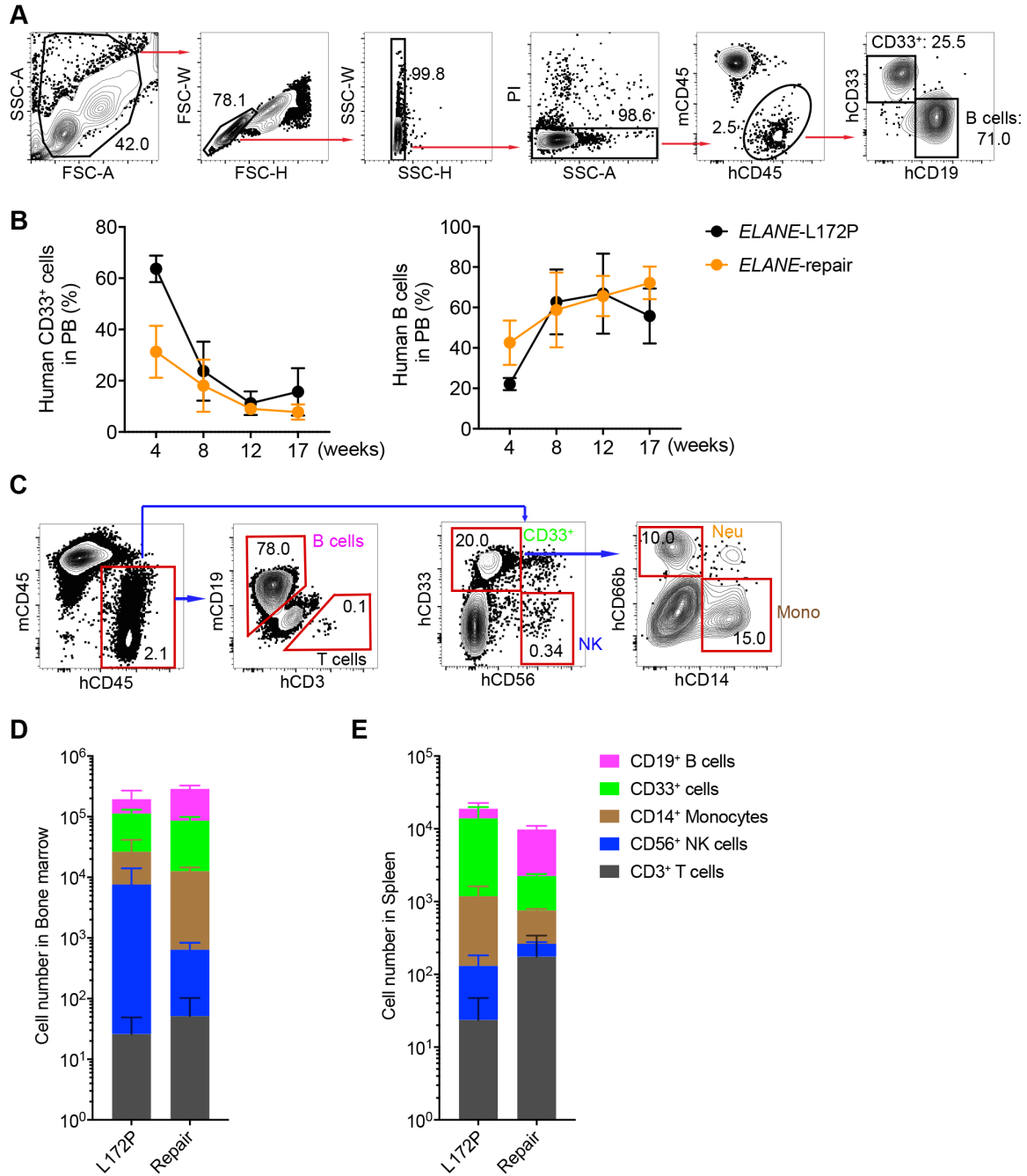


**Figure S6. Characterization of mature neutrophils differentiated from the *ELANE*-corrected SCN-HSPCs *in vitro*.** (A) Graph summarizing the percentages of human CD11b<sup>int</sup>CD15<sup>+</sup> mature neutrophils as shown in main Figure 3D. Data are represented as mean  $\pm$  SD for 3 independent experiments; \*\*\* P<0.001 (two-way ANOVA). Upper part: FACS profiles showing the percentages of mature neutrophils characterized as hCD66b<sup>+</sup>hCD15<sup>+</sup> (B) or hCD16<sup>+</sup>hCD15<sup>+</sup> (C) from the differentiation of HD, mutant or *ELANE*-corrected HSPCs that were treated with the indicated sgRNAs. Lower part: Quantification of 3 independent experiments. Data are shown as mean  $\pm$  SD; \*\*\* P<0.001 (two-way ANOVA).



**Figure S7. Efficiency of *ELANE*<sup>L172P</sup>-correction in SCN-HSPCs prior transplantation into humanized mice.** (A) FACS profile showing the percentages of CD34<sup>+</sup>CD38<sup>-</sup> cells in *ELANE*-L172P (L172P) and *ELANE*-repaired (Repair) SCN-HSPCs before transplantation. (B) Sall-RFLP assay showing the efficiency of the *ELANE*<sup>L172P</sup> correction in the SCN-HSPCs that were treated with sg*ELANE*-L172P/RNPs and AAV6-*ELANE* donor vectors. The red number indicates the correction efficiency (20%).





**Figure S8. Human immune cell lineages in bone marrow and spleen of transplanted NOG-EXL humanized mice.** (A) Gating strategy to quantify the percentages of human CD45<sup>+</sup>, CD33<sup>+</sup>, and CD19<sup>+</sup> cells in the peripheral blood (PB) of recipients mice 8 weeks post transplantation. (B) Quantification of the frequencies of human CD33<sup>+</sup> (left) and CD19<sup>+</sup> B (right) cells in the peripheral blood of *ELANE*-L172P and *ELANE*-repair recipient mice at the indicated time points post reconstitution. (C) Quantification of the percentages of human CD19<sup>+</sup> B, CD33<sup>+</sup> myeloid, CD14<sup>+</sup> monocytes/macrophages, CD3<sup>+</sup> T and CD56<sup>+</sup> NK cells in the bone marrow and spleen of recipient mice 20 weeks post transplantation. (D and E) Graphs show the absolute cell numbers for these immune cell lineages in bone marrow (D, left) and spleen (E, right) of recipient mice 20 weeks post transplantation.

**Table S1. List of oligos and primers used in this study.**

Primer name	Primer sequence
sgELANE-ex4	GAGTGCAGACGTTGCTGCGA
sgELANE-L172P	GACGTCACCACCGTCACGTT
	<b>T7EI and RFLP primers</b>
ELANE-T7-For	CTCAACGGGTCGGCCACCATCAACGCCA
ELANE-T7-Rev	TGTCCTCGGAGCGTTGGATGATAGAGTC
hELANE-5HAextern-For	CCAGGCTGGAGCGCAGTGGCACAATCTCAG
hELANE-3HA-Rev	CCTCGGAGCGTTGGATGATAGAGTCGATCC
	<b>Off-target sequencing primers</b>
OT-1-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGCTGCTGGTAGGAGACCATAACCT
OT-1-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGTATTCTGCTTACTCAAAGTCTA
OT-2-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTATGGCACTAACCAAAAACTTGC
OT-2-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAATTAAGTAAATCTTAAAGGAGGTG
OT-3-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGATGTAAACGTTTCTCGCATCGG
OT-3-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACTTGGGCCCTCAATCTATAA
OT-4-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATGTGAAAGCTATGCCTCCTGCAG
OT-4-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGAACAATCCCTTCTCCTCCTCTCA
OT-5-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTTGCCCATGCTGTGTTGGAAGTT
OT-5-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCGCAGTAAGCTTCGCAGCCTTTATG
OT-6-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTGAGCTCGACCAGGCCACGGTC
OT-6-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGGATGAGGCTATAATATGGTCAG
OT-7-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCATTCGTCTGTTATGGACACTTAGGT
OT-7-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCTACCACACAATCCAGCAATCCCA
OT-8-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATCAAGAATGACTCAACTATTTCTGC
OT-8-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATCCCGACTCCTGCGCCTTCCACT
OT-9-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGACTGTCTGGGACAGAAGGTTTG
OT-9-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAACGACTTGTTTTATGCGTCCCCT
OT-11-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCGTCATTCTGGCCAAGGGTCATGTC
OT-11-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGGAGTCCAGAGGGTGTCCATAA
OT-12-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTAAGAGGCAGATATTCCTCCTGAG
OT-12-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGAGGCCTGCTGCTCAGGGGAGTG
OT-13-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGTGGAGTTGTCATTGCAGCCTTC
OT-13-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTACAAGGAAGAAATCCTACAGCTCTT
OT-14-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTATGTCATTTAACTCGTTCAAGA
OT-14-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCGACTCTCTATCTCAACACTCTCA
OT-15-For	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCAGCTTTAGCAGCATTATGGG
OT-15-Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCGTGCACGTGTCCTGCGTGACT