LETTER TO THE EDITOR

CXCR2 deficiency with myelokathexis caused by a novel variant: correction via CRISPR/Cas9

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Supplementary information for

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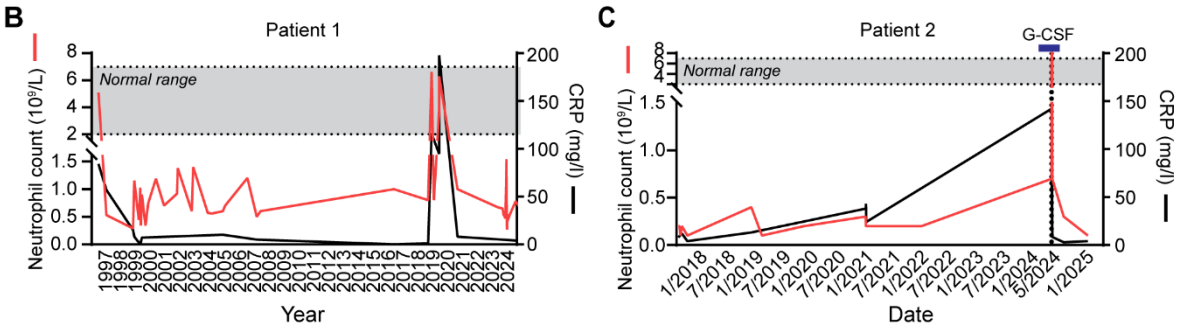
Supplementary Table 1. Comparison of genetic characteristics of *CXCR2* variants and clinical presentation of patients from different publications, including the patients described in this paper (P1 and P2) and patients from Auer et al.[5], Marin-Esteban et al.[6], and Klimiankou et al.[7].

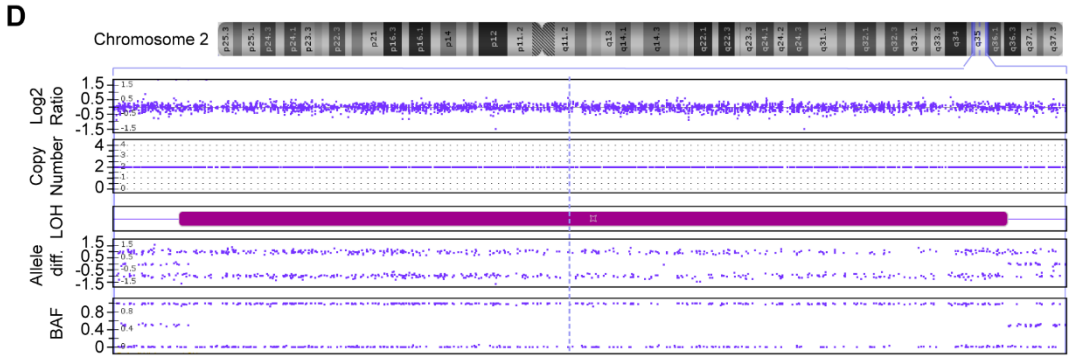
Publication, pt	Hinke et al., P1	Hinke et al., P2	Marin-Esteban et al., P1[6]	Marin-Esteban et al., P2[6]	Marin-Esteban et al., P3[6]	Marin-Esteban et al., P4[6]	Auer et al., P1[5]	Auer et al.,P2[5]	Klimiankou et al., P1[7]	Klimiankou et al., P2[7]	Klimiankou et al., P3[7]
Sex	M	M	F	F	F	M	F	F	F	F	F
Variant in *CXCR2*	c.865C>T (p.R289C)	c.865C>T (p.R289C)	CXCR2 del	c.430C>T, p.R144C	c.634C>T, pR212W	c.550C>T/c 865C>T (p.R184*/p .R289C)	c.986delA (p.H323fs6*)	c.986delA (p.H323fs6*)	c.431G>A, p.(R144H)	c.431G>A/c.720_727del p.(R144H)/p.(Lys240AsnfsTer45)	c.431G>A/WT p.(R144H)/ WT
GnomAD frequency	0.00008984	0.00008984	NA	0.00002292	0.000006814	NA	NA	NA	0.0000124	0.0000124/ NA	0.0000124
CADD score	23.5‡	23.5‡	NA	31§	23.1§	23§	NA	NA	25.4‡	25.4‡	25.4‡
Baseline neutrophil levels (x 10^9/L)	0.5-1.2†	0.1-0.4	0.28-1.8	0.1-0.85	0.1-10.8†	0.3-1	0.1 – 0.2	0.5-1.2	0.3	0.5	1.2
Other abnormal lab value?	Elevated IgG and IgA	Elevated IgG	Elevated IgG and IgM	Elevated IgG	Elevated IgM	Elevated B cells and NK cells	NA	NA	Anti-Fcg-RIIIb auto-antibodies	No	No
Myeloka-thexis	Yes	Yes (33%)	Yes (35%)	No	No	No	Yes	Yes	No	No	No
Clinical manifesta-tions	Oral ulcerations recurrent upper respiratory tract infections	Ulcerations lips, oral cavity, and nose	Oral lesions	Oral lesions, cellulitis episode	Oral lesions, pneumoni-tis episode	Oral lesions	Recurrent bacterial infections, septic thrombo-phlebitis, subacute bacterial endocarditis	Few infectious episodes	Frequent upper airway- and skin infections	Recurrent pharyngitis, bronchitis, and oral ulcerations	No history of recurrent infections

†Neutrophil counts increased spontaneously during infectious episodes. ‡ CADD score from gnomAD v4.1.0 database. § CADD score reported by in publication; NA = information not available

A

Characteristic	Normal range	Values P1	Values P2
Clinical profile			
Age at diagnosis (years)		3	16
Oral lesions		Yes	Yes
Infections		Yes	Yes, mild
G-CSF therapy (dose, period)		Yes (15 MU and 30 MU, 3 weeks)	Yes (48 MU, one administration)
Age at last follow-up (years)		29	23
CRP	<8.0	<4.0	4
Hematologic values			
Total leukocytes (10^9/L)	3.5 - 10.0	2.8	1.8
Neutrophils (10^9/L)	1.8 - 6.9	0.78	0.1
Monocytes (10^9/L)	0.20 - 0.70	0.54	0.38
Lymphocytes (10^9/L)	1.30 - 3.50	1.28	1.1
Basophils (10^9/L)	<0.1	0.04	0
Platelets (10^9/L)	145 - 350	305	376
Hemoglobin (mmol/L)	8.3 - 10.5	8.8	13.6
Immunoglobulin levels			
IgA (g/L)	0.80 - 3.90	4.96	
IgG (g/L)	6.1 - 14.9	17.1	
IgM (g/L)	0.39 - 2.08	0.80	





E

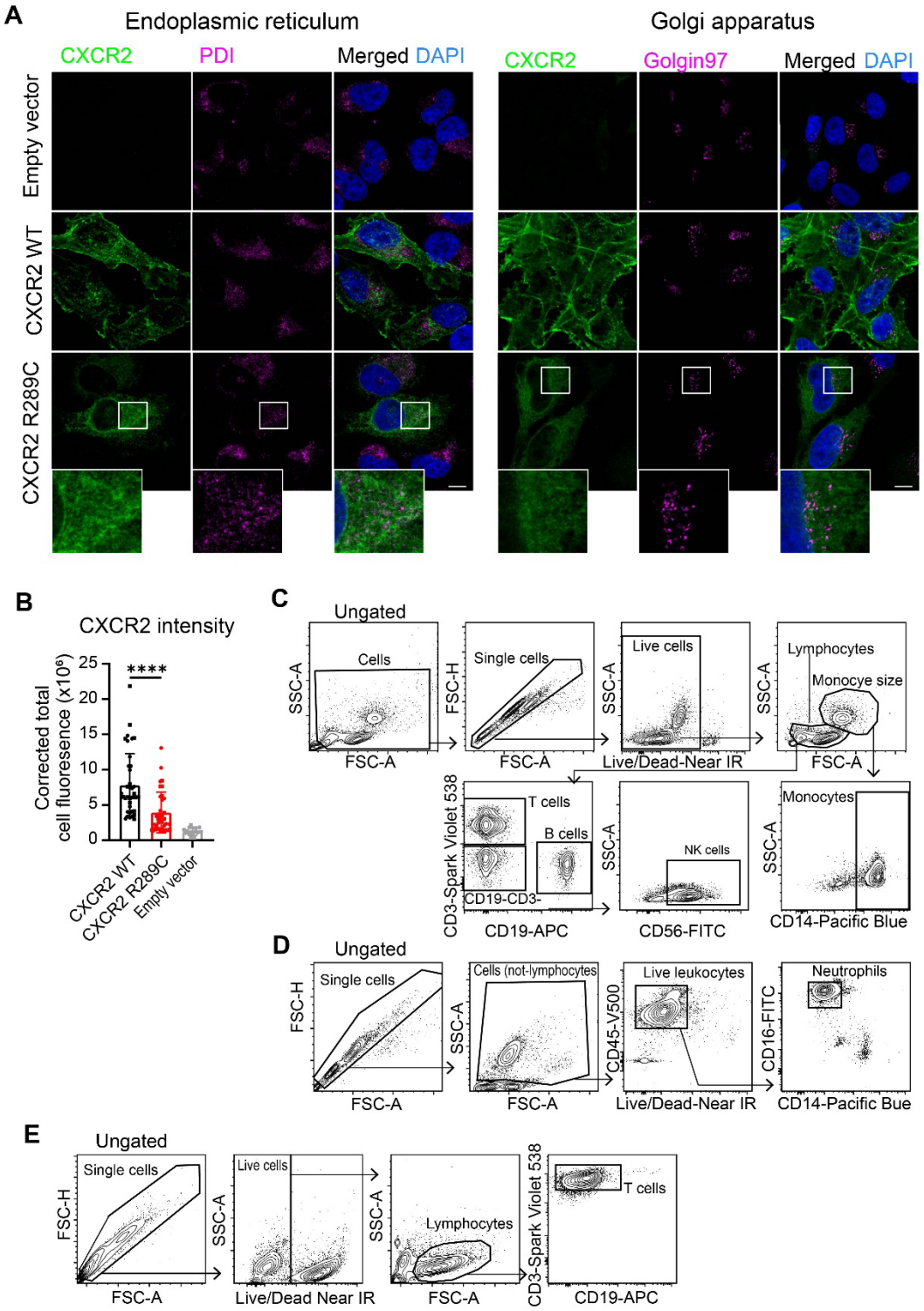
Classification of CXCR2 R289C variant according to ACMG/AMP

Supporting	
PP1	Cosegregation with neutropenia in two families represented herein and in ref. 6
PP2	Low allele frequency of <0.01
PP3	Various computational algorithms support a deleterious effect (CADD>MSC, SIFT 0.01, PolyPhen2 1.0)
Moderate	
PM3	The variant was reported in trans with pathogenic variant p.Arg184Ter in ref. 6
Strong	
PS3	Functional studies herein and in ref. 6 support a damaging effect of the variant on the gene product

Sum: 1 strong + 1 moderate + 3 supporting criteria → Classifies R289C variant as likely pathogenic

Supplementary Figure 1. Clinical blood values and neutrophil count for P1 and P2 plus SNP array and pathogenic evaluation of patient variant. (A) Clinical and immunological blood values for P1 and P2

during the last follow-up visits. Red indicates that the value is lower, and underlined indicates that the value is higher than the normal range (defined by Danish medical standard). **(B)** Extended blood neutrophil levels and CRP for P1 in the period from 1996-2025. **(C)** Blood neutrophil levels and CRP for P2 in the period from 2017-2025. Date for G-CSF treatment is indicated. Abbreviations: G-CSF: granulocyte colony-stimulating factor; CRP: C-reactive protein; MU: million units. **(D)** Results of a genome wide SNP array. Emphasized is a 3.3 kb region of a long continuous stretch of homozygosity encompassing the CXCR2 locus in chromosome 2q35: arr[GRCh37] 2q35(217451281_220734778). The CXCR2 locus is marked in the figure by an interrupted blue line. LOH: Loss of heterozygosity. Allele diff: Allele difference; BAF: B-allele frequency. **(E)** The CXCR2 R289C variant was classified as likely pathogenic according to guidelines from the American college of Medical Genetics (ACMG)/ Genomics and Association for Molecular Pathology (AMP)[13] supplemented with recommendations from ClinGen (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation) and the UK Association for Clinical Genomic Science (ACGS) (https://www.acgs.uk.com/media/12533/uk-practice-guidelines-for-variant-classification-v12-2024.pdf).



Supplementary Figure 2. Confocal and flow cytometry data. (A-B) HeLa cells were transfected with pcDNA3.1 vectors encoding either CXCR2 WT, CXCR2 R289C variant, or empty vector as negative control. Two days post transfection, cells were stained for CXCR2 (green), DNA (DAPI, blue), and ER (PDI, magenta) or Golgi (golgin97, magenta). (A) Immunofluorescence images. The scale bar (white) indicates 10 µm. (B) Quantification of the corrected cell fluorescence of CXCR2. Two experiments are

shown. Values for each experiment are shown with different symbols. Shown are individual values plus mean ±SD. **** p<0.0001, unpaired two-tailed t-test. **(C)** Gating strategy for Figure 3D for identification of live monocytes ($CD14^+$), B cells ($CD19^+CD3^-$), T cells ($CD19^-CD3^+$) and NK cells ($CD19^-CD3^-CD56^+$) from PBMCs. **(D)** Gating strategy for Figure 3F-H for identification of live neutrophils ($CD45^+CD14^{lo}CD16^{hi}$) from whole blood for analysis of CXCR2 expression and neutrophil migration in chemotaxis assay. **(E)** T cells were expanded from PBMCs using anti-CD3/anti-CD28 activation beads three days before editing with CRISPR/Cas9 RNP, ssODN DNA template for HDR, and AZD7648 HDR enhancer. On day 10, T cells were treated with expansion beads a second time. On day 13, CXCR2 transcription was induced using CRISPRa. CXCR2 surface expression on T cells was analyzed on day 14. Shown here is the gating strategy for the identification of T cells ($CD3^+CD19^-$) for Figure 3K-L.