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Supplementary information

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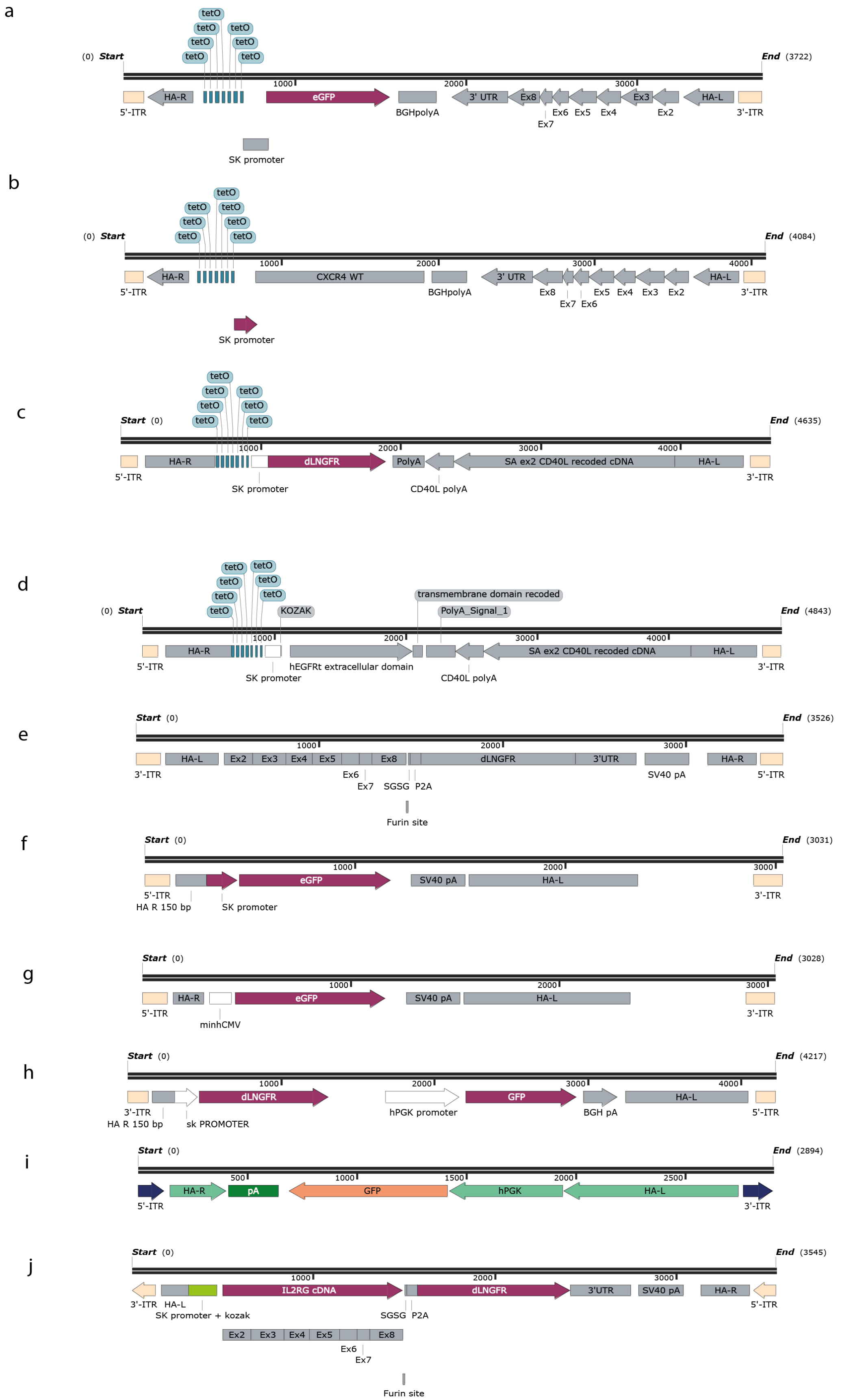
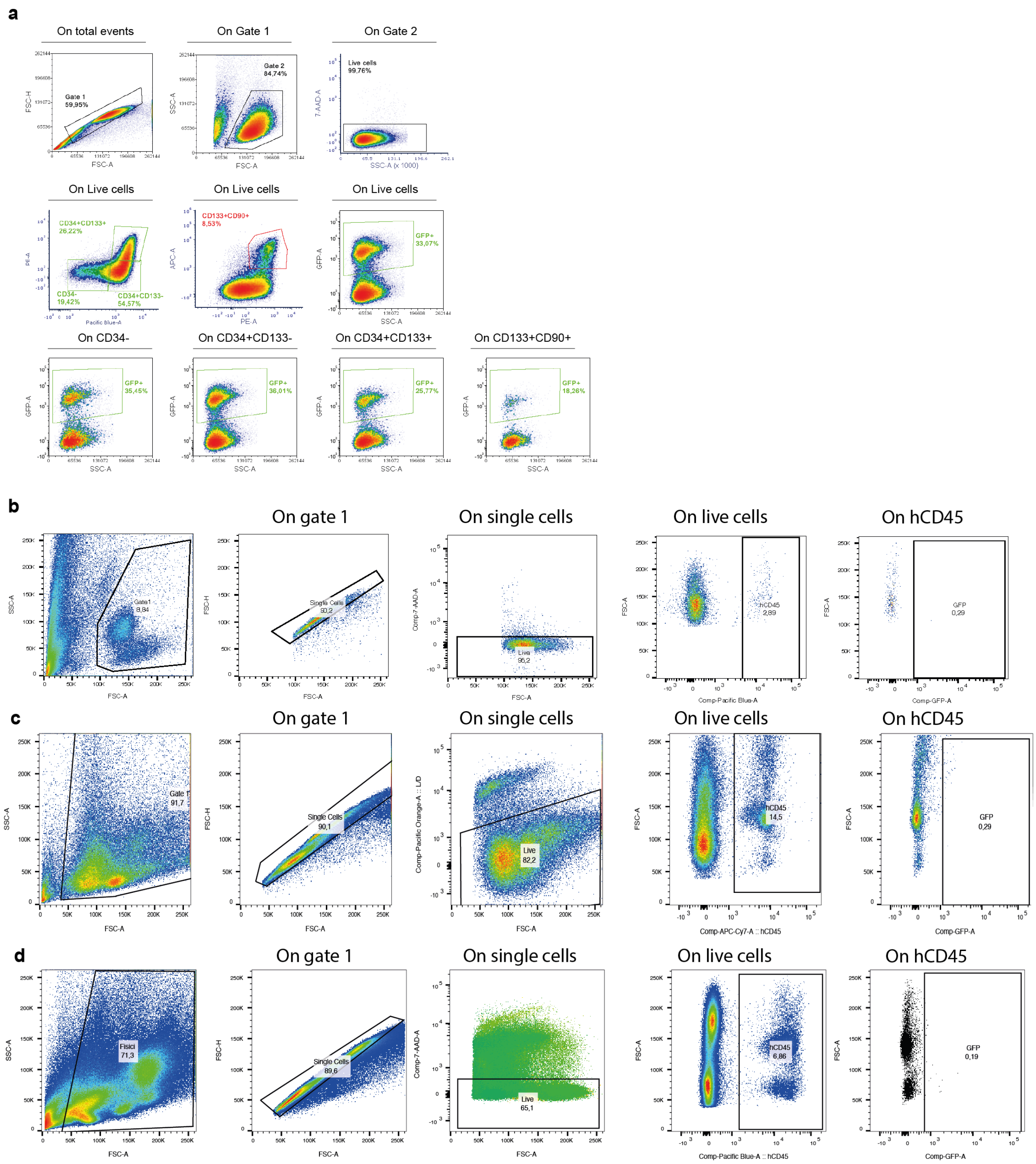
Selection of human hematopoietic stem cells bearing the intended functional edit by transient AND-gate reporters

In the format provided by the authors and unedited

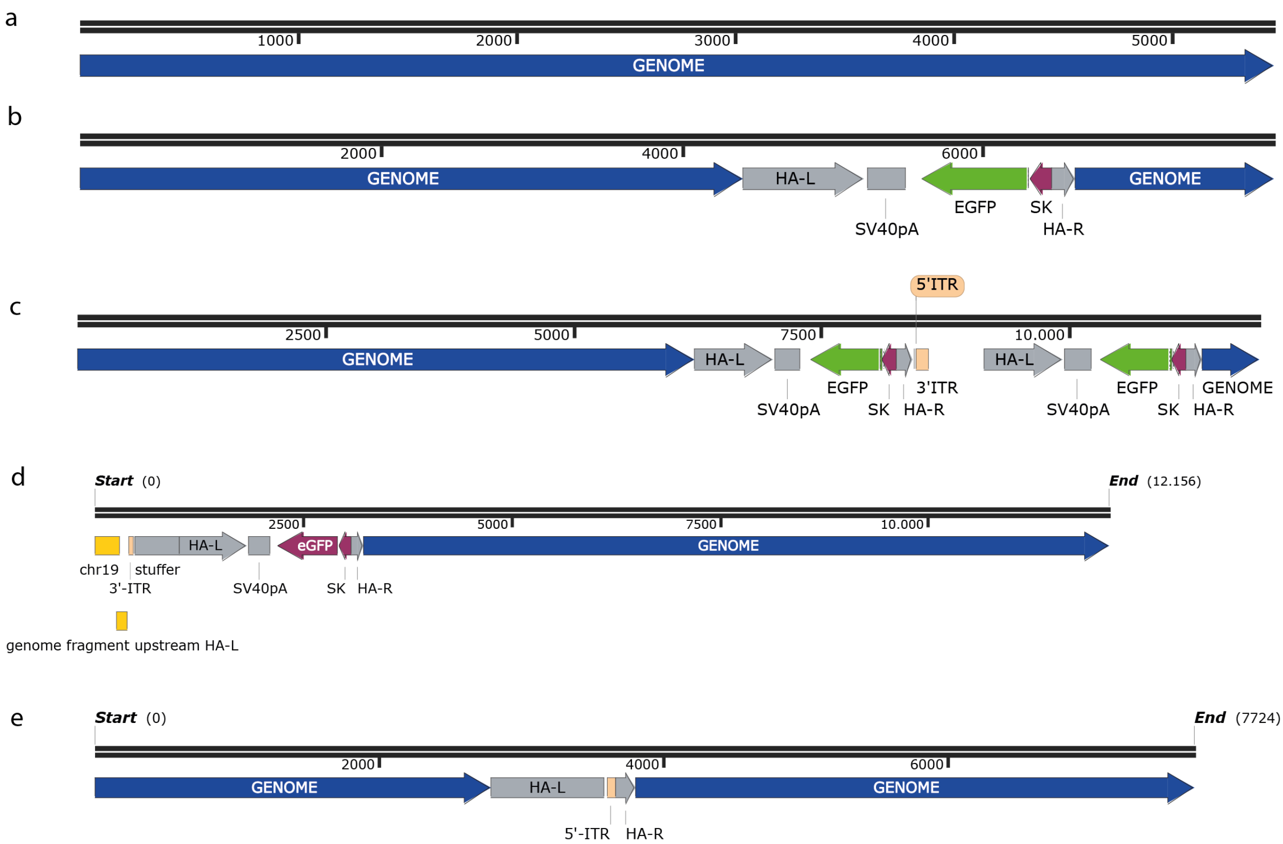
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Vector maps with features to scale. **a-d,** SMArT-1 vectors. **e,** SMArT-2 vector. **f-j,** SMArT-3 vectors.



Supplementary Figure 2. a, General gating strategies for in vitro experiments. **b-d,** General gating strategies of xenotransplantation experiments: **b,** peripheral blood; **c,** bone marrow; **d,** spleen.



Supplementary Figure 3. Exemplificative reads at *AAVS1* locus upon HDR editing with the SK-GFP donor template. **a,** Native genome. **b,** Precise HDR (classified as "functional, precise"). **c,** Concatemer (classified as "functional, imprecise"). **d,** Read comprising an HDR junction at the right homology arm, and a genomic rearrangement at the left homology arm (classified as "other"). **e,** ITR trapping at the DSB, classified as "non-functional"). Note that reads are in the same orientation but start and end at different genomic positions and are not aligned together.

Supplementary Table 1. List of gRNAs and DNA binding domains

Guide ID	sequence (5'-3')
IL2RG	ACTGGCCATTACAATCATGT
CD40LG	TGGATGATTGCACTTTATCA
AAVS1	GTCACCAATCCTGTCCCTAG
B2M	CTTACCCCACTTAACTATCT
RAG1	CGGGAAGTAAACCTCACATG

SMArT-2

TALE ID	
TALE#1	CCCAAAACAGTAGAGCT
TALE#2	TCCTTCCCAGGATCTAGGT
TALE#3	GTCACACAGCACATATTT

SMArT-3

Guide ID	
sgRNA #4	GATAAGGAATCTGCCTAAC
sgRNA #5	GTTAGGCAGATTCCTTATC
sgRNA #9	AACCTTAGAGGTTCTGGCA
sgRNA #10	AAGCAAACCTTAGAGGTTC
sgRNA #12	GGGGGATGCGTGACCTGCC
sgRNA #13	TTCTGGGAGAGGGTAGCGC
sgRNA #24	CCAGTAGCCAGCCCCGTCC
sgRNA #26	GCTACTGGCCTTATCTCAC

TALE ID	
TALE #1	TCCATCGTAAGCAAACCTT
TALE #2	TCCCACCCCCTGCCAAGCT
TALE #3	TCCAAACTGCTTCTCCTCT
TALE #4	TCCACACGGACACCCCCCT
TALE #5	TCCACCATCTCATGCCCCT
TALE #6	TCTAAGGTTTGCTTACGAT
TALE #7	TCCTCTCTGGCTCCATCGT
TALE #8	TGGTCCTGAGTTCTAACTT
TALE #9	TCCTCCGTGCGTCAGTTTT

SMArT-3 dCas9-ArT
RAG1truncated

Guide ID	sequence (5'-3')
RAG1 tgRNA 2_15nt	GTATCTTACTGCAGT
RAG1 tgRNA 3_15nt	CCAAGCTCCTTGCAG
RAG1 tgRNA 4_15nt	CAAGGATCAGCAGCA
RAG1 tgRNA 5_15nt	CGGGCTTGTCTTGCT

SMArT-3 Cas9-ArT
AAVS1truncated

Guide ID	sequence (5'-3')
AAVS1 tgRNA 9_19nt	AACCTTAGAGGTTCTGGCA
AAVS1 tgRNA 9_18nt	ACCTTAGAGGTTCTGGCA
AAVS1 tgRNA 9_17nt	CCTTAGAGGTTCTGGCA
AAVS1 tgRNA 9_16nt	CTTAGAGGTTCTGGCA
AAVS1 tgRNA 9_15nt	TTAGAGGTTCTGGCA
AAVS1 tgRNA 9_14nt	TAGAGGTTCTGGCA
AAVS1 tgRNA 9_13nt	AGAGGTTCTGGCA
AAVS1 tgRNA 9_12nt	GAGGTTCTGGCA
AAVS1 tgRNA 9_11nt	AGGTTCTGGCA
AAVS1 tgRNA 10_19nt	AAGCAAACCTTAGAGGTTC
AAVS1 tgRNA 10_18nt	AGCAAACCTTAGAGGTTC

AAVS1 tgRNA 10_17nt	GCAAACCTTAGAGGTTC
AAVS1 tgRNA 10_16nt	CAAACCTTAGAGGTTC
AAVS1 tgRNA 10_15nt	AAACCTTAGAGGTTC
AAVS1 tgRNA 10_14nt	AACCTTAGAGGTTC
AAVS1 tgRNA 10_13nt	ACCTTAGAGGTTC
AAVS1 tgRNA 10_12nt	CCTTAGAGGTTC
AAVS1 tgRNA 10_11nt	CTTAGAGGTTC

SMArT-3 Cas9-ArT
CXCR4 screening

Guide ID	sequence (5'-3')
CXCR4 -116	GGGGCAGACGCGAGGAAGGA
CXCR4 -50	ATAAAAACACGCTCCGAGCG
CXCR4 -186	ACCGACCACCCGCAAACAGC
CXCR4 -263	AGCGGGATGCGCCTGAAGAC
CXCR4 +42	CAGGTAGCAAAGTGACGCCG
CXCR4 -203	GTCGGTAGTGAGTCCGGAGC
CXCR4 -63	AGCGGCGCATGCGCCGCGCT
CXCR4 -208	TAGTGAGTCCGGAGCCGGAC
CXCR4 +96	AGAACCAGCGGTTACCATGG
CXCR4 -197	CGGGTGGTCGGTAGTGAGTC
CXCR4 +83	CGCTGGTTCTCCAGATGCGG
CXCR4 -63	AGCGCGGCGCATGCGCCGCT
CXCR4 -14	CAAACTGAAGTTTCTGGCCG
CXCR4 -18	CTGAAGTTTCTGGCCGCGGC
CXCR4 +73	GCTCCAGTAGCCACCGCATC
CXCR4 +43	AGGTAGCAAAGTGACGCCGA
CXCR4 +97	GAACCAGCGGTTACCATGGA
CXCR4 -160	TGCGCGGCTTGGGAAGCCCA
CXCR4 -277	CGCGCTTCCCACCTGTCTTC
CXCR4 -298	GCTAGGAACGCGTCTCTCTG

SMArT-3 Cas9-ArT
CXCR4 truncated

Guide ID	sequence (5'-3')
CXCR4 tgRNA -116_19nt	GGGCAGACGCGAGGAAGGA
CXCR4 tgRNA -116_18nt	GGCAGACGCGAGGAAGGA
CXCR4 tgRNA -116_17nt	GCAGACGCGAGGAAGGA
CXCR4 tgRNA -116_16nt	CAGACGCGAGGAAGGA
CXCR4 tgRNA -116_15nt	AGACGCGAGGAAGGA
CXCR4 tgRNA -116_14nt	GACGCGAGGAAGGA
CXCR4 tgRNA -116_13nt	ACGCGAGGAAGGA
CXCR4 tgRNA -116_12nt	CGCGAGGAAGGA
CXCR4 tgRNA -116_11nt	GCGAGGAAGGA
CXCR4 tgRNA -160_19nt	GCGCGGCTTGGGAAGCCCA
CXCR4 tgRNA -160_18nt	CGCGGCTTGGGAAGCCCA
CXCR4 tgRNA -160_17nt	GCGGCTTGGGAAGCCCA
CXCR4 tgRNA -160_16nt	CGGCTTGGGAAGCCCA
CXCR4 tgRNA -160_15nt	GGCTTGGGAAGCCCA
CXCR4 tgRNA -160_14nt	GCTTGGGAAGCCCA
CXCR4 tgRNA -160_13nt	CTTGGGAAGCCCA
CXCR4 tgRNA -160_12nt	TTGGGAAGCCCA
CXCR4 tgRNA -160_11nt	TGGGAAGCCCA

SMArT-3 Cas9-ArT
IL2RG truncated

Guide ID	sequence (5'-3')
IL2RG tgRNA 1_15nt	GCTGCAGGAATAAGA
IL2RG tgRNA 2_15nt	TAAGAGGGATGTGAA
IL2RG tgRNA 3_15nt	TGTGAATGGTAATGA
IL2RG tgRNA 4_15nt	ATGATGGCTTCAACA
IL2RG tgRNA 5_15nt	TGCTCTTCATTCCCT

Supplementary Table 2. ArT sequences.

ArT	Nucleotide sequence
tTA	atgcccaagaagaagaggaaggtgtctagactggacaagagcaaagtcataaactctgctctggaattactcaa tgaagtcggtatcgaaggcctgacgacaaggaaactcgctcaaaagctgggagttgagcagcctaccctgtac tggcacgtgaagaacaagcgggccctgctcgatgccctggcaatcgagatgctggacaggcatcatacccac ttctgccccctggaaggcgagtcatggcaagactttctgcggaacaacgccaagtcattccgctgtgctctcctc tcacatcgcgacggggctaaagtgcatctcggcacccgcccaacagagaaacagtacgaaaccctggaaaat cagctcgcgttcctgtgtcagcaaggcttctccctggagaacgcactgtacgctctgtccgccgtgggccacttt acactgggctgcgtattggaggatcaggagcatcaagtagcaaaagaggaaagagagacacctaccaccgat tctatgcccccacttctgagacaagcaattgagctgttcgaccatcagggagccgaacctgccttccttttcggcc tggaactaatcatatgtggcctggagaaacagctaaagtgcgaaagcggcgggccggccgacgcccttgacg attttgacttagacatgctcccagccgatgcccttgacgactttgaccttgatatgctgcctgctgacgctcttgac gattttgaccttgacatgctccccgggtaa
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Supplementary Table 3. List of antibodies used in this study.

Antibody	Conjugate	Clone	Supplier	Identifier	Dilution
Anti-human CD34	VioBlue	AC136	Miltenyi Biotec	Catalog n°130-113-182	1:50
Anti-human CD34	PE-Vio770	8G12	BD Biosciences	Catalog n° 348811	1:20
Anti-human CD133/2	PE	REA816	Miltenyi Biotec	Catalog n°130-112-157	1:50
Anti-human CD90	APC	5E10	BD Biosciences	Catalog n°559869	1:33
Anti-human CXCR4	PE-Vio770	REA649	Miltenyi Biotec	Catalog n°130-109-845	1:50
Anti-human NGFR	PE	ME20.4-1.H4	Miltenyi Biotec	Catalog n°130-113-421	1:50
Anti-human NGFR	APC	ME20.4-1.H4	Miltenyi Biotec	Catalog n°130-113-418	1:50
Anti-human EGFR	APC	AY13	BioLegend	Catalog n°352906	1:50
Annexin V	VioBlue		BioLegend	Catalong n° 640918	1:50
7AAD			BioLegend	Catalong n° 420404	1:33
Anti-human CD45	VioBlue	HI30	BioLegend	Catalog n°304029	1:50
Anti-human CD45	VioBlue	5B1	Miltenyi Biotec	Catalog n°130-113-122	1:30
Anti-human CD45	APC	2D1	BD Biosciences	Catalog n°340910	1:50
Anti-human CD45	APC-Vio770	2D1	BD Biosciences	Catalog n°348815	1:50
Anti-human CD45	PerCP Cy5	5B1	Miltenyi Biotec	Catalog n°130-113-121	1:100
Anti-human CD1a	APC	H1149	BioLegend	Catalog n°300110	1:80
Anti-human CD3	APC	UCHT1	BD Bioscience	Catalog n°555335	1:25
Anti-human CD4	PE Vio770	M-T321	Miltenyi Biotec	Catalog n°130-121-336	1:25
Anti-human CD5	PE-Vio770	REA782	Miltenyi Biotec	Catalog n°130-110-992	1:60
Anti-human CD7	APC-H7	CD7-6B7	Miltenyi Biotec	Catalog n°130-117-677	1:30
Anti-human CD8a	APC-H7	SK1	BD Biosciences	Catalog n°560179	1:100
Anti-human CD14	PE	MϕP9	BD Biosciences	Catalog n°345785	1:100
Anti-human CD56	FITC	NCAM16.2	BD Biosciences	Catalog n°345811	1:30
Anti-human TCRαβ	PerCP Cy5.5	IP26	BioLegend	Catalog n°306724	1:80
human Fc blocking			Miltenyi Biotec	Catalog n°130-059-901	1:50
mouse Fc blocking			BD Biosciences	Catalog n°553142	1:100
Anti-human CD132	APC	TUGh4	BioLegend	Catalog n°338608	1:50
Anti-human CD132	PE	AG184	BD Biosciences	Catalog n°561699	1:50
Anti-human CD90	BV421	5E10	BioLegend	Catalog n°328122	1:50
Anti-human CD3	Pecy7	HIT3a	BioLegend	Catalog n°300316	1:50
Anti-human CD40LG	PE	24-31	Invitrogen	Catalog n°12-1548-42	1:50
Anti-human CD45	PO	5B1	Miltenyi Biotec	Catalog n°130-113-124	1:50
Anti-human CD19	Pecy7	HIB19	BioLegend	Catalog n°302-216	1:50
Anti-human CD3	FITC	SK7	BD Biosciences	Catalog n°345763	1:50
Anti-human CD13	APC-H7	REA263	Miltenyi Biotec	Catalog n°130-103-671	1:50
Anti-human CD13	BV421	WM15	BD Biosciences	Catalog n°562596	1:50
Anti-human CD90	PE	5E10	BD Biosciences	Catalog n°555596	1:50
Anti-human CD40LG	APC	24-31	Invitrogen	Catalog n° 17-1548-42	5:100
Anti-mouse CD45.1	FITC	A20	BD Biosciences	Catalog n° 553775	1:100
Anti-mouse CD45.2	PB	104	BioLegend	Catalog n° 109820	1:100

Supplementary Table 4. Molecular analyses and gene expression assays.

Molecular analyses

IL2RG HDR ddPCR	FW CTAGATTGGGGAGAAAATGA RV GTGGGAAGGGGCCGTACAG PROBE (FAM) FAM-GTAGCTCCTATGCTAGGCGTAGCC
IL2RG deletions TALE ddPCR	FW AGGTGGTTGAGAATGGTGCT RV CTGTGTGACACGGGCTAAGT PROBE (FAM) CCTCAGCCCACCTAGATCCTGGG
AAVS1 HDR	FW (SK) CACCGTACACGCCTAAAGCTA FW (mCMV) CTTATATAGGCCTCCCACCGT RV TCTTGGGAAGTGTAAGGAAG PROBE (FAM) CCAGATAAGGAATCTGCCTA
AAVS1 deletions ddPCR	FW ATCCTGGGAGGGAGAGCTT RV GGATCAGTGAAACGCACCAG PROBE (FAM) AGCTGCTCTGACGCGGCCGT
PCR on K-562 with targeted integration	WT FW AACTCTGCCCTCTAACGCTGC RV AACGGGGATGCAGGGGAACG 5’ junction FW AACTCTGCCCTCTAACGCTGC RV ACCTCTACAAATGTGGTATGGCTG 3’ junction FW TCAAAACAGCGTGGATGGCGTC RV AACGGGGATGCAGGGGAACG
Human *TTC5* ddPCR (Chr.14)	PrimePCR ddPCR Copy Number Assay: TTC5, Human (Biorad), HEX
Human GAPDHddPCR (Chr.12)	PrimePCR ddPCR Copy Number Assay: GAPDH, Human (Biorad) (VIC)

CD40LG HDR assay has been previously reported (Vavassori et al 2021).

Gene expression assays.

TNNT1 hs00162848_m1 (FAM)	
HPRT hs02800695_m1 (VIC)	
AAVS1 hs01085949_m1 (FAM)	
IL2RG hs00953624_m1 (FAM)	
MED12 Hs00192801_m1	
ARHGEF6 Hs00374477_m1	
LNC00892 Hs00866930_s1	
FOXO4 Hs00172973_m1	
IL2RG recoded	FW tccccaccctgaagaacct RV cgctgaagttgccgtggta Probe FAM tcggtcaccagatctt

T7 surveryor assays - for truncated gRNA

AAVS1	FW CTTATATAGGCCTCCCACCGT
	RV CCACTGAGAACCGGGCAGGT
CXCR4	FW TCTGCACCTGACCCTAGTGA
	RV TGGAACGCTCTTTCCAGTCG

Supplementary Table 5. Primers and protocols for long-read sequencing of PCR-amplified DNA.

PRIMER	DIRECTION	SEQUENCE
AAVS1	FW	GCACTGGGGACTCTTTAAGG
	RV	CGACCTACTCTCTTCCGCAT

AAVS1	CYCLES	TIME
94°	1	30s
94°	35	30s
58.5°		1'
65°		9'
65°	1	10'
4°		hold