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Supplemental Information

High Efficiency Gene Correction in Hematopoietic Cells by Donor-Template-Free CRISPR/Cas9 Genome Editing

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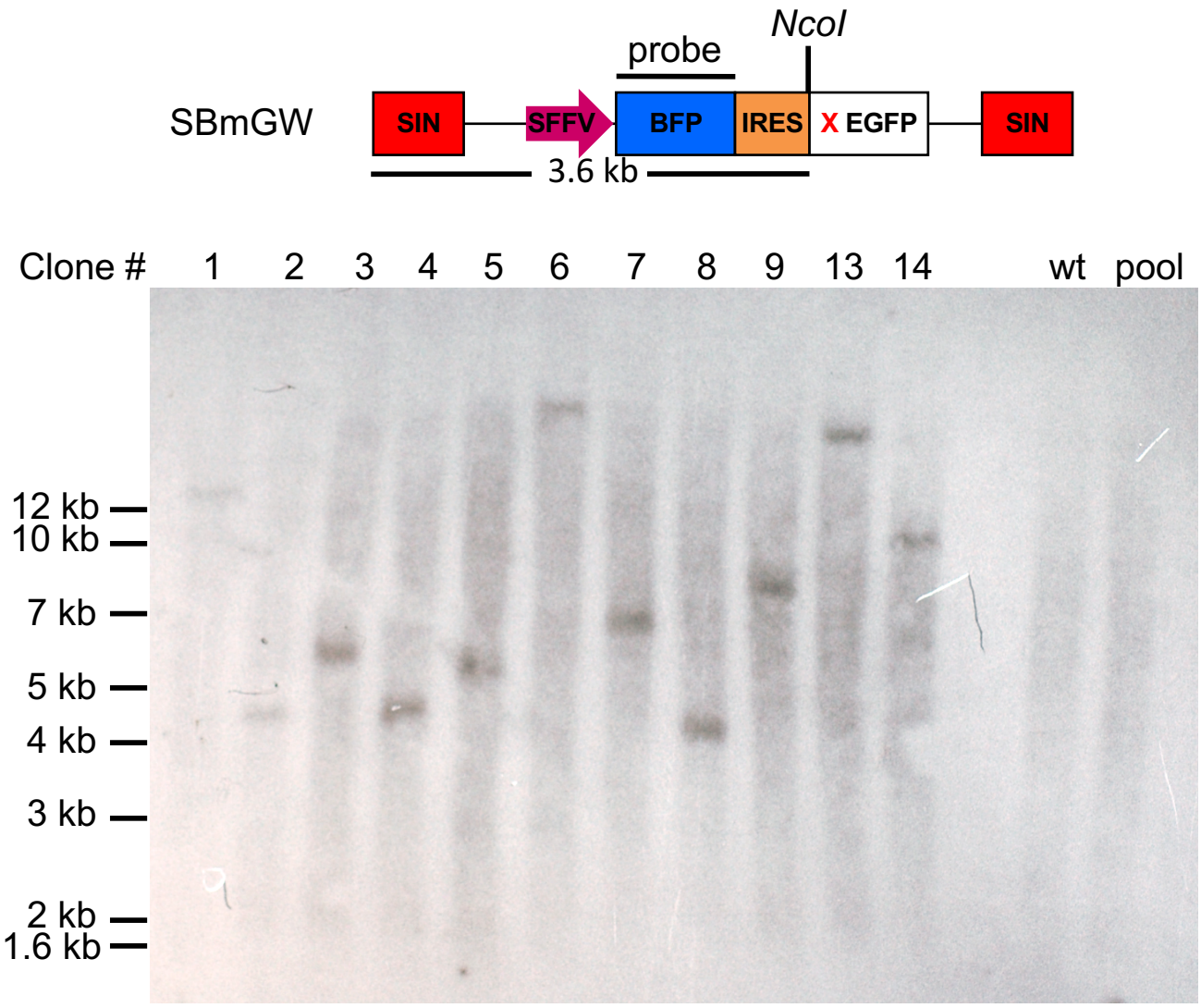
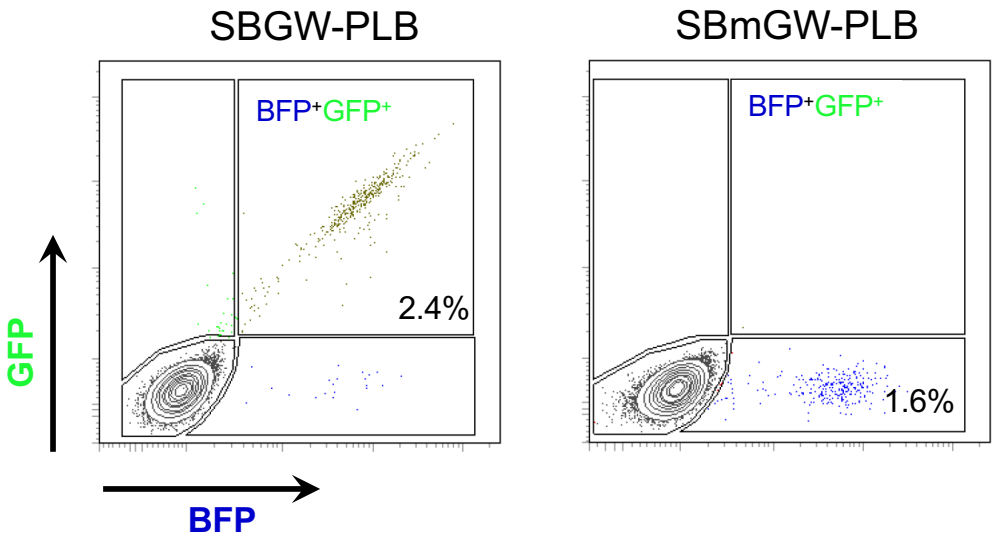
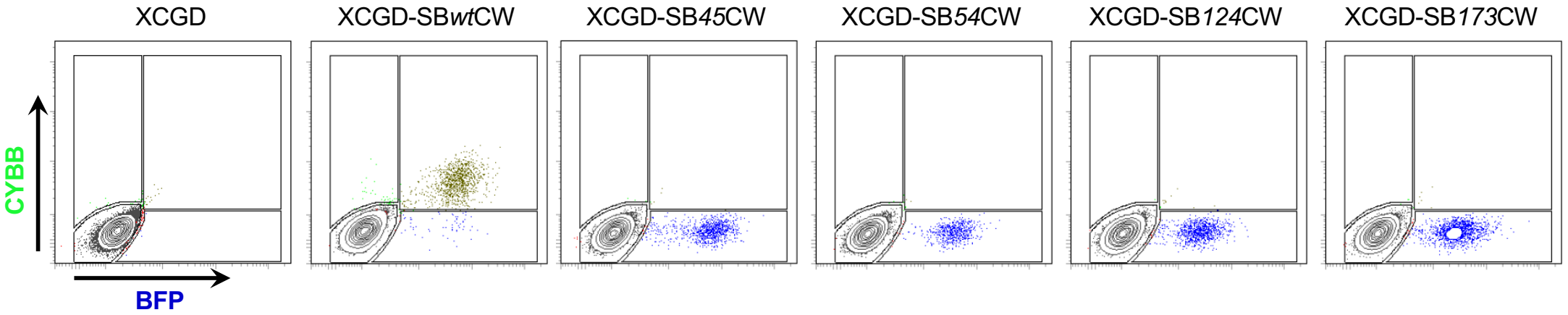


Supplemental Figure 1: Single copy integration of SBmGW lentiviruses in PLB cells. BFP+ SBmGW-PLB clones were isolated by limiting dilution and subjected to Southern blot analysis. *NcoI* restriction fragments of genomic DNAs of individual clones were resolved by agarose gel electrophoresis, transferred onto nylon mebranes and hybridized to a ^{32}P-labeld BFP probe.



Supplemental Figure 2: FACS profiles of SBGW- and SBmGW-PLB reporter cells



Supplemental Figure 3: FACS profiles of XCGD cells expressing wild-type and mutant CYBB cDNAs. Note that only XCGD-SB*wt*CW cells expressing wild type *CYBB* are double positive for BFP and CYBB.

		Genotype
SB*54*CW	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGGCCCAGGGCCCCTGCCGCCTGCCTGAAC	
K1	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K2	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K3	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K4	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K5	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGGCCCAGGGCCCCTGCCGCCTGCCTGAAC	0
K6	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CC**G**GGGCCCCTGCCGCCTGCCTGAAC	-1(A>G)
K7	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K8	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K9	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K10	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K11	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K12	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K13	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K14	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K15	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K16	CCCGGAAGCTGCTGGGCTC---------------CCAGGGCCCCTGCCGCCTGCCTGAAC	-15
K17	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K18	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K19	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K20	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K21	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTG-CCCAGGGCCCCTGCCGCCTGCCTGAAC	-1
K22	CCCGGAAGCTGCTGGGCTCAGCACTGGCACTGG-CCAGGGCCCCTGCCGCCTGCCTGAAC	-1

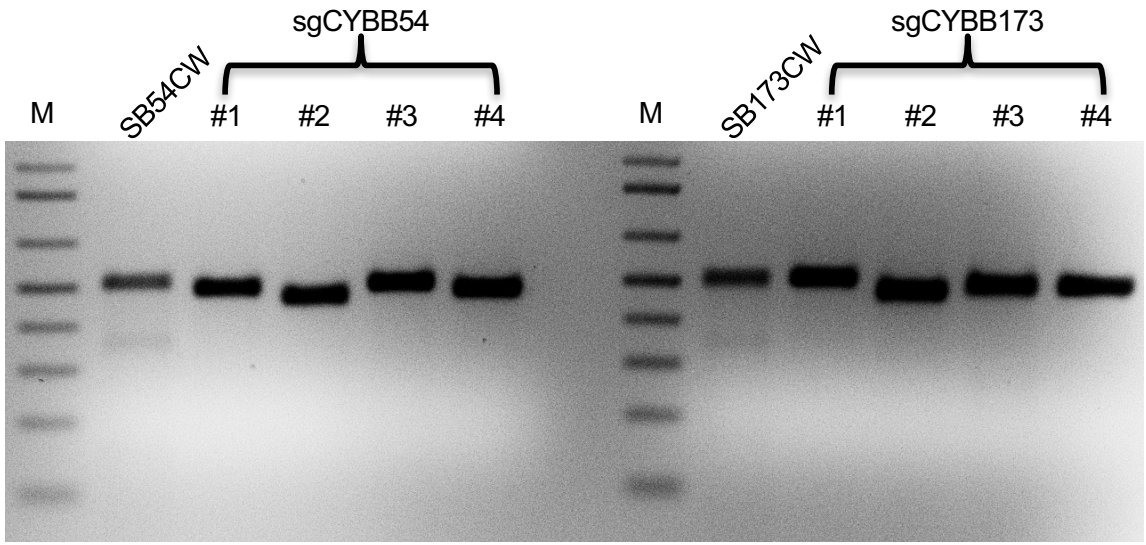
		Genotype
SB*173*CW	CCCCGAGGGCGGCCTGTACCTGGCTGTGACC----TGTTGGCCGGCATCACCGGCGTGGT	
K1	CCCCGAGGGCGGCCTGTACCTGGCTGTGACC----TGTTGGCCGGCATCACCGGCGTGGT	0
K2	CCCCGAGGGCGGCCTGTACCTG----------GCCGGCATCACCGGCGTGGT	-14
K3	CCCCGAGGGCGGCCTGTACCTG----------GCCGGCATCACCGGCGTGGT	-14
K4	CCCCGAAGGCGGCCTGTACCTGGCTGTGA----------CCAGCATCACCGGCGTGGT	-8
K5	CCCCGAGGGCGGCCTGTACCTGGCTGTGA------TGTTGGCCGGCATCACCGGCGTGGT	-6
K6	CCCCGAGGGCGGCCTGTACCTGGCTG----------TGGCCGGCATCACCGGCGTGGT	-8
K7	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCC---TGTTGGCCGGCATCACCGGCGTGGT	+1
K8	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCC---TGTTGGCCGGCATCACCGGCGTGGT	+1
K9	CCCCGAGGGCGGCCTGTACCTGGCTGTGA----------CCGGCATCACCGGCGTGGT	-8
K10	CCCCGAGGGCGGCCTATACCTG----------TGTTGGCCGGCATCACCGGCGTGGT	-9
K11	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCAGGCTGTTGGCCGGCATCACCGGCGTGGT	+4
K13	CCCCGAGGGCGGCCTGTACCTGGCTGTGACC----TGTTGGCCGGCATCACCGGCGTGGT	0
K14	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCC---TGTTGGCCGGCATCACCGGCGTGGT	+1
K15	CCCCGAGGGCGGCCTGTACCTGGCTGTGACC----TGTTGGCCGGCATCACCGGCGTGGT	0
K16	CCCCGAGGGCGGCCTGTACCTGGCTGTGA------TGTTGGCCGGCATCACCGGCGTGGT	-2
K17	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCC---TGTTGGCCGGCATCACCGGCGTGGT	+1
K18	CCCCGAGGGCGGCCTGTACCTGGCTGTGAC----------CACCGGCGTGGT	-14
K19	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCT---TGTTGGCCGGCATCACCGGCGTGGT	+1
K20	CCCCGAGGGCGGCCTGTACCTG----------GCCGGCATCACCGGCGTGGT	-14
K21	CCCCGAGGGCGGCCTGTACCTGGCTGTGACC----TGTTGGCCGGCATCACCGGCGTGGT	0
K22	CCCCGAGGGCGGCCTGTACCTGGCTGTGACCC---TGTTGGCCGGCATCACCGGCGTGGT	+1

Supplementary Figure 4 : Indel sequences recovered by shot gun cloning from XCGD-SB*54*CW and XCGD-SB*173*CW cells.

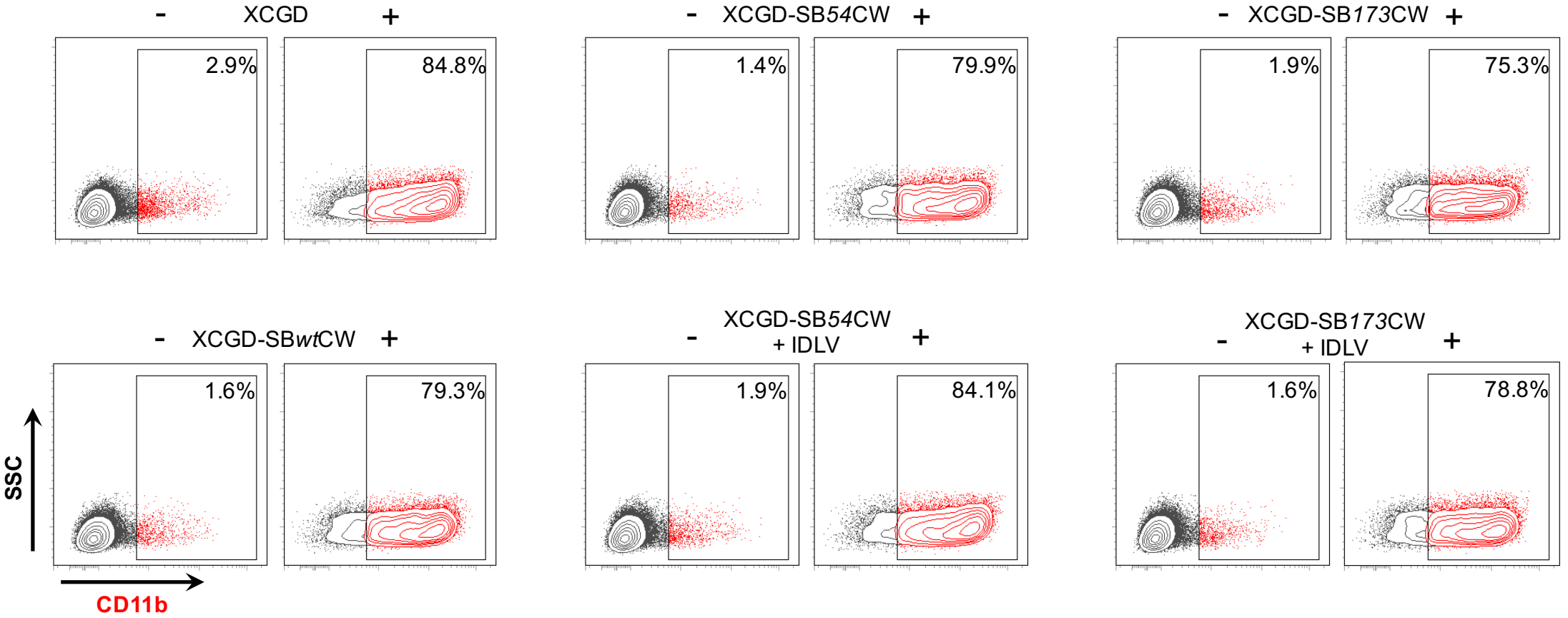
A

GTACCTGGCTGTGACCTGT**TGG**	sgCYBB54
CAGCACAGACACTGGCCCA**AAG**	chr14:+32749541
CATCACTGGCTCTGGCCCA**TAG**	chr12:-103703290
CAACAGGGGCACTGGCCCA**TGG**	chr1:+204799512
TGGCAGTGGCACTGGCCCA**GGG**	chr19:-46999128
CAGCACTGGCACTGGCCCA**GGG**	sgCYBB173
TTACCTTGCTGTGACCTGT**TAG**	chr2:+186140924
GTTCCTGGCTGTGATCTGT**GAG**	chr7:+73675051
AAACCTAGCTGTGACCTGT**AAG**	chrX:-128220322
CCAACTTGCTGTGACCTGT**GGG**	chr18:+24746359

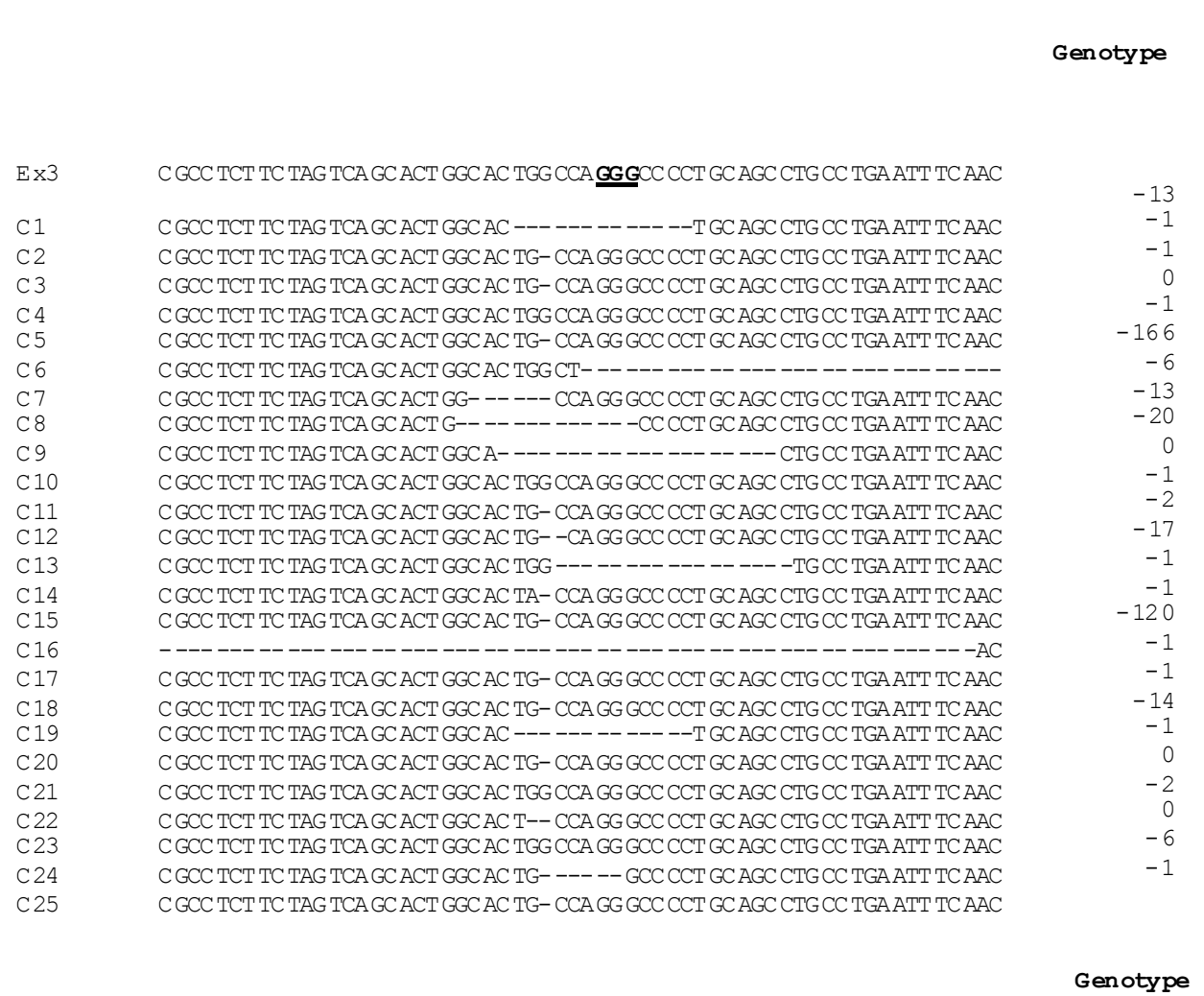
B



Supplemental Figure 5: Off target analysis in IDLV treated XCGD-54CW and XCGD-173CW cells. **A.** Top 4 off targets for sgCYBB54 and sgCYBB173 RNAs selected from the benchling.com website. **B.** Surveyor assay



Supplemental Figure 6: Differentiation of XCGD cells after exposure to DMSO. Cells were incubated for 7 days with DMSO (+), stained with anti-CD11b antibody and analyzed by FACS.



Supplemental Figure 7: Indel sequences recovered by shot gun cloning from LC-sgEx3- and LC-sgEx6-LV transduced cells.

Supplemental Table 1: Oligo nucleotides used in this study

Oligo		Sequence (5′-3′)
Cloning of EGFP sgRNAs		
BH001	g32DG1 s	CACCGGAGGAACTGTTCACCGGCG
BH002	g32DG1 as	AAACGTGAACAGTTCCTCGCCCTC
BH003	g32DG2 s	CACCGGAGGAACTGTTCACCGGCG
BH004	g32DG2 as	AAACCGCCGGTGAACAGTTCCTCC
BH037	g32DG1.4 s	CACCGGGGCGAGGAACTGTTTCAC
BH038	g32DG1.4 as	AAACGTGAAACAGTTCCTCGCCCC
BH039	g32DG1.7 s	CACCGAAGGGCGAGGAACTGTCAC
BH040	g32DG1.7 as	AAACGTGACAGTTCCTCGCCCTTC
BH041	g32DG2.3 s/ sgEGFP2.3 s	CACCGGGAACTGTTCACCGCGGCG
BH042	g32DG2.3 as/ sgEGFP2.3 as	AAACCGCCGCGGTGAACAGTTCCC
BH043	g32DG2.5 s	CACCGCGAGGAACTGTTCACGGCG
BH044	g32DG2.5 as	AAACCGCCGTGAACAGTTCCTCGC
Cloning of CYBB sgRNAs		
BH137	sgCYBB54 s	CACCGCAGCACTGGCACTGGCCCA
BH138	sgCYBB54 as	AAACTGGGCCAGTGCCAGTGCTGC
BH139	sgCYBB173 s	CACCGGTACCTGGCTGTGACCTGT
BH140	sgCYBB173 as	AAACACAGGTCACAGCCAGGTACC
BH141	sgCYBB45 s	CACCGTTTACACAAGAAAACGTCT
BH142	sgCYBB45 as	AAACAGACGTTTTCTTGTGTAAAC
BH143	sgCYBB124 s	CACCGACATCTATTTAATGTGTAA
BH144	sgCYBB124 as	AAACTTACACATTAAATAGATGTC
Generation of EGFP mutant vectors		
SFHR037	SBmGW s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGCGGCGTGGTGCCCATCCTG
SFHR038	SBmGW as	ATGGGCACCACGCCGCGGTGAACAGTTCCTCGCCCTTGGACACCATGGTT
SFHR046	SBmGW-K6 s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGTGGTGCCCATCCTG
SFHR047	SBmGW-K6 as	ATGGGCACCACGGTGAACAGTTCCTCGCCCTTGGACACCATGGTT
SFHR048	SBmGW-K16 s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGCGAGCGTGGTGCCCATCCTG
SFHR049	SBmGW-K16 as	ATGGGCACCACGCTCGCGGTGAACAGTTCCTCGCCCTTGGACACCATGGTT
SFHR050	SBmGW-K18 s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGCGGGCGTGGTGCCCATCCTG
SFHR051	SBmGW-K18 as	ATGGGCACCACGCCCGCGGTGAACAGTTCCTCGCCCTTGGACACCATGGTT
SFHR052	SBmGW-K29 s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGCGGTGCCCATCCTG
SFHR053	SBmGW-K29 as	ATGGGCACCGCGGTGAACAGTTCCTCGCCCTTGGACACCATGGTT
SFHR054	SBmGW-K33 s	ATGGTGTCCAAGGGCGAGGAACTGTTCACCGGCGCGGTGCCCATCCTG
SFHR055	SBmGW-K33 as	ATGGGCACCGCGCCGGTGAACAGTTCCTCGCCCTTGGACACCATGGGT
Generation of CYBB mutant vectors		
BH111	SB173CW s	GGGCGGCCTGTACCTGGCTGTGACCTGTTGGCCGGCATCACCGGCGTG
BH112	SB173CW as	CACGCCGGTGATGCCGGCCAACAGGTCACAGCCAGGTACAGGCCGCCC
BH113	SB54CW s	GCTGCTGGGCTCAGCACTGGCACTGGCCCAGGGCCCCTGCCGCCTGCCTGAA
BH114	SB54CW as	TTCAGGCAGGCGGCAGGGGCCCTGGGCCAGTGCCAGTGCTGAGCCCAGCAGC
BH121	SB124CW s	GCGCCATCCACACCATCGCACATCTATTTAATGTGTAATGGTGCGTGAACGCCCGGGTG
BH122	SB124CW as	CACCCGGGCGTTCACGCACCATTACACATTAAATAGATGTGCGATGGTGTGGATGGCGC
BH123	SB45CW s	CGACATCCCCCCCAAGTTCTTTTACACAAGAAAACGTCTTGGCAGCGCCCTGGCCCTGG
BH124	SB45CW as	CCAGGGCCAGGGCGCTGCCAAGACGTTTTCTTGTGTAAAAGAACTTGGGGGGGATGTCG
Shotgun cloning		
AR36	Shotg HPRT s	TCCCTTCATAGAGACAAGGAATG
AR163	Shotg HPRT as	CCTGGGTTCTACCCCAGCACAG
AR102	Shotg EGFP as	TCATGTGGTCGGGGTATCTG
SFHR20	Shotg EGFP as	CTTGTACAGCTCGTCCATGC
SFHR43	Shotg IRES s	TCTGTAGCGACCCTTTGCAG
BH234	Shotg CYBB as	AGGATCAGGCACAGGGTGAT
Surveyor assay		
BH229	Svyr45/54 s	TGGCTCTCCTCAAGCGTATT
BH230	Svyr45/54 as	CCATCTTGTGGAAGGTCAGG
BH231	Svyr173 s	CACCTGTTCAACGTGGAGTG
BH232	Svyr173 as	CTCGCACAGGTACAGGAACA
BH233	Svyr124 s	AACGTGTTCCTGTTCGTGTG
BH234	Svyr124 as	AGGATCAGGCACAGGGTGAT
SFHR 135	Svyr54_off1 s	ATTCCTTTCAACGCTAGCCTTC
SFHR 136	Svyr54 off1 as	GTACATTTGTTTGCCATCACCG
SFHR 137	Svyr54_off2 s	AAGCCATGGTCCAAAATCCTG
SFHR 138	Svyr54_off2 as	CCTGTTGTTCACAGCTCTATGT

SFHR141	Svyr54_off3 s	TGCAGGATCTGGTCCCTAATC
SFHR142	Svyr54_off3 as	GGAATCTCCCATCCTAGCCTAG
SFHR143	Svyr54_off4 s	CGGTCGTTGATTTCTGAGGAG
SFHR144	Svyr54_off5 as	ACAGAGCCTTGGTCTCATGA
SFHR145	Svyr173_off1 s	ACCCACTACGCACATATATATGC
SFHR146	Svyr173_off1 as	TAATGACCTGCAAACAAACCTAC
SFHR147	Svyr173_off2 s	CTCTGTGTTCAGCTCCAGC
SFHR148	Svyr173_off2 as	GTGATTCACAAACAAGGAGGTG
SFHR151	Svyr173_off3 s	CTTTGGCTCTGATGTTATATCTGTAG
SFHR152	Svyr173_off3 as	TCATCAGCATATCGTCTTGCC
SFHR153	Svyr173_off4 s	ACTTCTGCTTCAGGAATTGCC
SFHR154	Svyr173_off4 as	CTACTGGTGCCAGCAAAGAC

Mutation	DNA change	Protein Alteration	Accesion Number	sgRNA Sequence
SB*54*CW	c.159dupC	p.R54fs	A53X102	CAGCACTGGCACTGG**C**CCA**GGG**
SB*173*CW	c.517delC	p.L173fs	L173X188	GTACCTGGCTGTGACC-TGT**TGG**
SB*124*CW	c.370G>T	p.E124X	E124X	ACATCTATTTAATGTG**T**AA**TGG**
SB*45*CW	c.134T>G	p.L45R	L45R	TTTACACAAGAAAAC**G**TCT**TGG**

Supplemental Table 2: Selected *CYBB* disease mutations and nucleotide sequences of the *CYBB* targeting sgRNAs.