CRISPR/Cas9-mediated gene deletion efficiently retards the progression of Philadelphia-positive acute lymphoblastic leukemia in a p210 BCR-ABL1^{T315I} mutation mouse model

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Figure Legends

Supplementary Figure 1: CRISPR/Cas9 strategy to make a deletion flanking the junction of *BCR-ABL1* fusion gene.

(A-B) A schematic to illustrate the rationale of targeting intron rather than the exon of CRISPR/Cas9 mediated ablation of the *BCR-ABL1* fusion gene. The black arrows indicate the CRISPR/Cas9 targeted loci. (C) Detection of *BCR-ABL1* ablation in K562 cells after the transfection of two individual CRISPR/Cas9 plasmids that respectively encode the single guide RNA 7 (sg7) targeting *BCR* intron 12 and the single guide RNA 4 (sg4) targeting *ABL1* intron 4. The positions of the designed PCR primers for detecting the *BCR-ABL1* ablation were indicated by the red arrows. Representative sequences of the PCR products derived from the co-transfected K562 cells showing the correct *BCR-ABL1* ablation were displayed at the bottom. C, non-transfected control cells, and the same meaning as in (D). (D) Structure of the 2-in-1 CRISPR/Cas9 plasmid and the detection of *BCR-ABL1* ablation in K562 cells 72 hours post-transfection. Representative sequences of the PCR products derived from the 2-in-1 plasmid-transfected K562 cells showing the correct *BCR-ABL1* ablation were displayed at the bottom. (E) The viability of LAX2 and BLQ1 cells was measured by CCK-8 assay upon 3-day treatment of various TKIs (1 μM/L) plus BCI (5 μM/L). (F) The apoptosis of LAX2 and BLQ1 cells analyzed by Annexin-V-FITC/PI dual staining assay upon 3-day treatment of various TKIs plus BCI. (G) The cell cycle of LAX2 and BLQ1 cells analyzed by BrdU staining assay upon 3-day treatment of various TKIs plus BCI.

Supplementary Figure 2: The PDX ALL model and its phenotypic reversion by the CRISPR/Cas9 mediated *in vivo* targeting.

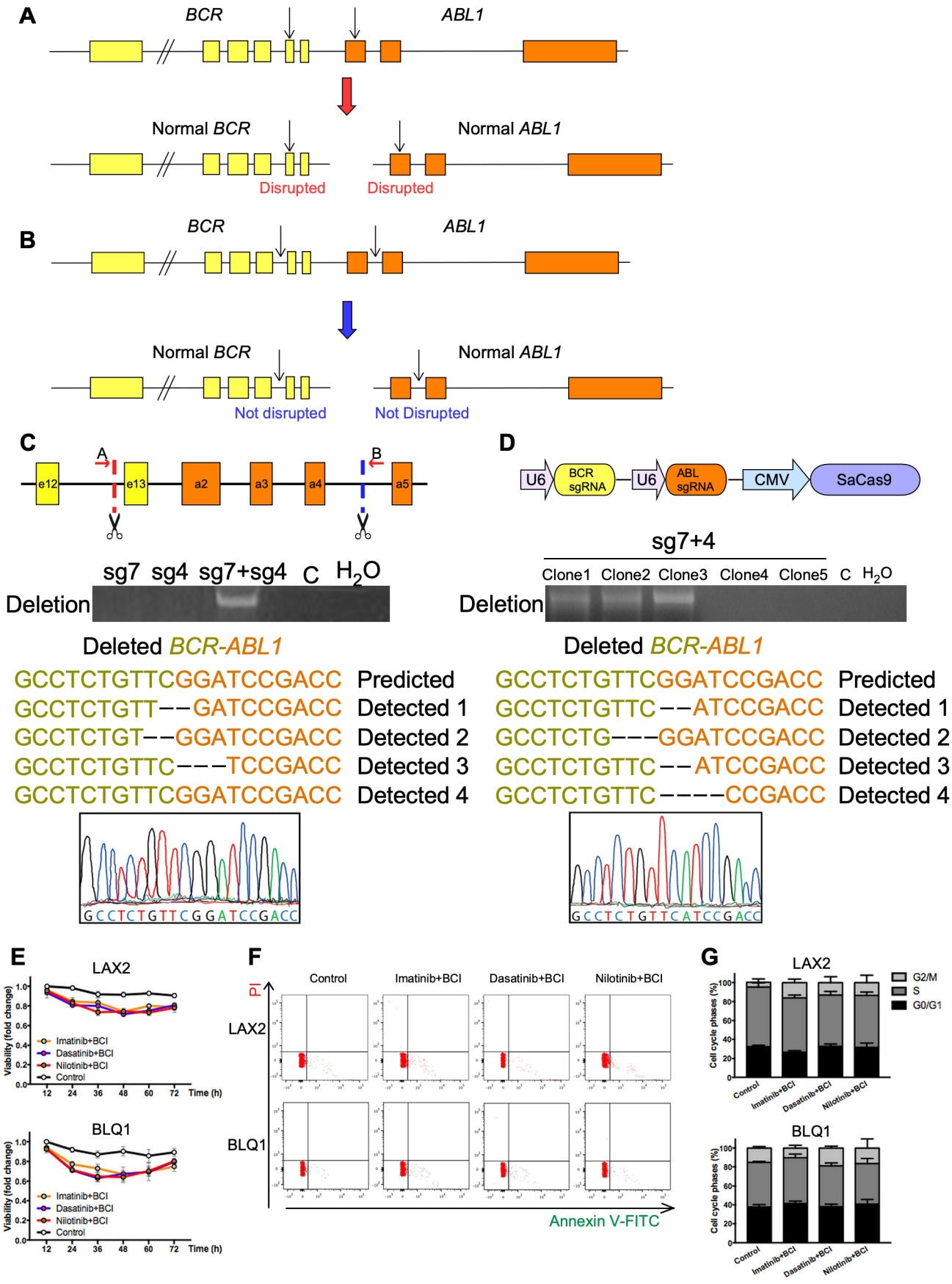
(A) The PDX model of LAX2 and BLQ1 cells with the onset of Ph+ ALL within 7 days and full-blown phenotype within 35 days. 2×10^6 LAX2 and BLQ1 cells that were pre-infected by the lentiviral vectors expressing firefly luciferase were intrafemorally injected into the sublethally irradiated NSG

mice. The leukemia burden was then measured by luciferase bioimaging, and bone marrow aspiration was performed at day 35 after transplantation for measuring the human cell chimerism (CD45-positive) and B cell proportion (CD19-positive) as shown in (B). The overall survival of the recipient mice (n=8 per group) was plotted by Kaplan-Meier analysis as shown in (C). (D) 2-D droplet fluorescence intensity plots of human *BCR-ABL1* transcript in the total bone marrow cells harvested from the CRISPR/Cas9 lentivirus-injected PDX mice at the indicated day of post-transplantation. WT, wild-type.

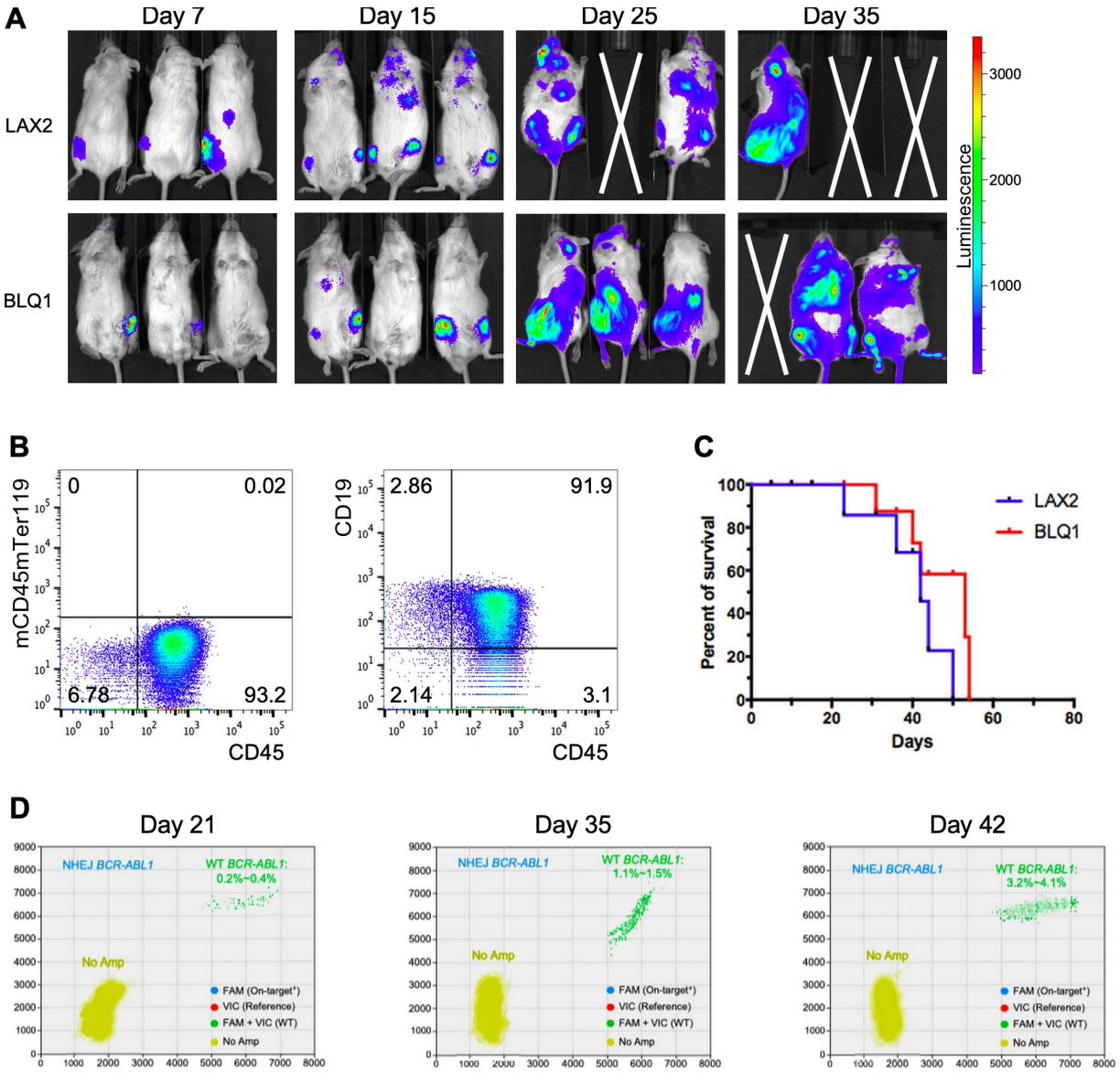
Supplementary Table 1: Off-target analysis of CRISPR/Cas9 mediated genome editing.

The off-target locus was highly ranked by the CRISPR designing tool Benchling (https://benchling.com) upon using the PAM sequence of 'NNGRRT' or 'NNGRR' for SaCas9 system. The detection of indels was determined by Sanger sequencing of the PCR products amplified by primers flanking the CRISPR/Cas9 targeting sites on *BCR* intron 12 and *ABL1* intron 4. No, no mutation was detected.

Supplementary Table 2: Primer information for this study.



Supplementary Figure 1



Supplementary Figure 2

Table S1. Off-target analysis of CRISPR/Cas9 mediated genome targeting

Target gene	Locus	On-target score	Sequence	PAM	Detection of Indel
BCR	Chr22:-23631274	100.0	GGCATAATGCTGAACAGGGAA	CAGAG	On-site target
BCR	Chr4:-22966206	2.0	ATCACAATGCTGAACAGGGAC	TTGGG	No
BCR	Chr1:-28490553	1.4	GGCCGAATGCTGCACAGGGAA	AAGGG	No
BCR	Chr14:-92314256	1.4	TACATAATGCAGAACAGAGAA	AAGGG	No
BCR	Chr2:+145638529	1.0	AGAATACTGATGAACAGGGAA	GGGGA	No
BCR	Chr8:-126162388	1.0	GCCATGAAGCAGAACAGGGAA	AAGGG	No
ABL1	Chr9:+130864286	100.0	ACTGCACTCCAGCCTAGGCAA	CAGAGT	On-site target
ABL1	Chr11:+36407380	26.8	ACTGCACTCCAGCCTGGGCAA	CAGAG	No
ABL1	Chr17:-81794900	26.8	ACTGCACTCCAGCCTGGGCAA	CAGAG	No
ABL1	Chr16:+69577830	26.8	ACTGCACTCCAGCCTGGGCAA	CAGAA	No
ABL1	Chr19:+52062192	3.3	ATTGCACTCCAGCCTGGGCAA	CAGGG	No
ABL1	Chr7:-44246294	3.0	ACAGCACTCCAGCCTAGGTAA	TGGAG	No

Supplementary Table 1

Table S2. Primer information

Primer	Sequence	Application
BCR-intron12-sg1-F	CACCGCCACTGCCCTGTGATCCCCT	For constructing sgRNA
BCR-intron12-sg1-R	AAACAGGGGATCACAGGGCAGTGGC	
BCR-intron12-sg2-F	CACCGTGCAGTGCTGGTCTGGCGG	
BCR-intron12-sg2-R	AAACCCGCCAGACCAGCACTGCAC	
BCR-intron12-sg3-F	CACCGAGAGGTGGCTCTGCATAGGT	
BCR-intron12-sg3-R	AAACACCTATGCAGAGCCACCTCTC	
BCR-intron12-sg4-F	CACCGGTGAATCCCAAACCTCCAAG	
BCR-intron12-sg4-R	AAACCTTGGAGGTTTGGGATTCACC	
BCR-intron12-sg5-F	CACCGGCAGGTCTGAGAATGAGTGG	
BCR-intron12-sg5-R	AAACCCACTCATTCTCAGACCTGCC	
BCR-intron12-sg6-F	CACCGCAGCACAGTGTGAATGCCCAA	
BCR-intron12-sg6-R	AAACTTGGGCATTCACACTGTGCTGC	
BCR-intron12-sg7-F	CACCGGCATAATGCTGAACAGGGAA	
BCR-intron12-sg7-R	AAACTTCCCTGTTCAGCATTATGCC	
BCR-intron12-sg8-F	CACCGGCTTATTTCTGGGCATAATG	
BCR-intron12-sg8-R	AAACCATTATGCCCAGAAATAAGCC	
ABL-intron4-sg1-F	CACCGTCAGCCAACAAAAATCCACAG	
ABL-intron4-sg1-R	AAACCTGTGGATTTTTGTTGGCTGAC	
ABL-intron4-sg2-F	CACCGCAGGTGCCCTGGGCATCCCCA	
ABL-intron4-sg2-R	AAACTGGGGATGCCCAGGGCACCTGC	
ABL-intron4-sg3-F	CACCGGTGAGAGCTGACAAGCATGG	
ABL-intron4-sg3-R	AAACCCATGCTTGTCAGCTCTCACC	
ABL-intron4-sg4-F	CACCGACTGCACTCCAGCCTAGGCAA	
ABL-intron4-sg4-R	AAACTTGCCTAGGCTGGAGTGCAGTC	
ABL-intron4-sg5-F	CACCGTCTTTGAGGCAGGACTGGCAC	
ABL-intron4-sg5-R	AAACGTGCCAGTCCTGCCTCAAAGAC	
ABL-intron4-sg6-F	CACCGCACTGGGGTGGGGGCATGGTT	
ABL-intron4-sg6-R	AAACAACCATGCCCCCACCCCAGTGC	
ABL-intron4-sg7-F	CACCGTTTGGGGTTACCTTAAGGACC	
ABL-intron4-sg7-R	AAACGGTCCTTAAGGTAACCCCAAAC	
ABL-intron4-sg8-F	CACCGAAGTCCTATCTGAGAAATATC	
ABL-intron4-sg8-R	AAACGATATTTCTCAGATAGGACTTC	
BCR-T7 assay-F1	AAACCTTCTTTTTTATTTCGAG	For T7 assay
BCR-T7 assay-R1	CAGACAGGAGGGAGCGCCCC	
BCR-T7 assay-F2	CTCCCTCCTGTCTGGGGCAGCC	
BCR-T7 assay-R2	TGAGGTGTGGCCCTGGAGAG	
BCR-T7 assay-F3	CTCTCGGCCCCCAATGCCAC	
BCR-T7 assay-R3	AATCTGCTCCTTGGCCCTCT	
ABL-T7 assay-F1	TTGAAGGTAGGCTGGGACTG	
ABL-T7 assay-R1	GGCGTCCTAAGGGTTTCAAA	
ABL-T7 assay-F2	GGTGAGAGCTGACAAGCATGG	
ABL-T7 assay-R2	CCAGAGGTGGATGTTTCAGTT	
ABL-T7 assay-F3	CCTTCACCCAAAGGCATAGTG	
ABL-T7 assay-R3	GAAGAACTCTGTCCCATTCA	
A-sg7+4-PCR	CTCTGCATGCCTCTGGCCTG	For PCR detection of ablated *BCR-ABL1* by BCR-sg7 and ABL1-sg4
B-sg7+4-PCR	GTGCTGGGATTATAGGCATG	
BCR-VIC	TTCCCTGTTCAGCATTATGCC	For ddPCR probe
BCR-FAM	TTCCTCACCTGGTGGAGAAA	
ABL-VIC	TTGCCTAGGCTGGAGTGCAGT	
ABL-FAM	AATTGGCGATTCTCCTTCCTC	

Supplementary Table 2