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Supplemental information

Efficient CRISPR-Cas9-mediated mutagenesis in primary human B cells for identifying plasma cell regulators

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Table S1: Amount of each plasmid in plasmid mixtures

Ratio (Main/Gag-Pol/Envelope)	Mol (picomole) [1]			DNA mass (µg) [2]			
	Main	Gag-Pol	Envelope	Main	Gag-Pol	Envelope	Total
3:1:1	0.16742	0.05581	0.05581	0.76	0.42	0.32	1.50
6:1:1	0.22262	0.03710	0.03710	1.00	0.28	0.22	1.50
12:1:1	0.26657	0.02221	0.02221	1.20	0.17	0.13	1.50
24:1:1	0.29577	0.01232	0.01232	1.33	0.10	0.07	1.50
48:1:1	0.31291	0.00652	0.00652	1.41	0.05	0.04	1.50
96:1:1	0.32224	0.00336	0.00336	1.45	0.03	0.02	1.50
192:1:1	0.32712	0.00170	0.00170	1.477	0.013	0.010	1.50
Only sgRNA (Main plasmid)	0.33215	0	0	1.50	0	0	1.50

Plasmid sizes (base pairs): Main (7,308), Gag-Pol (12,227), Envelope (9,345)

[1] Picomole calculation of each plasmid in total of 1.5 µg DNA:

$$P \text{ (picomole of } M_xG_yE_z) = \frac{1.5\ (\mu g) * 10e6}{(x*a + y*b + z*c)*617.96 + 36.04}$$

Picomole of Main plasmid = P*x
Picomole of Gag-Pol plasmid = P*y
Picomole of Envelope plasmid = P*z

$M_xG_yE_z$: Formula of plasmid mixture
M: Main plasmid
G: Gag-Pol plasmid
E: Envelope plasmid
x, y, z: Plasmid ratios (Main/Gag-Pol/Envelope)
a, b, c: Plasmid sizes (Main/Gag-Pol/Envelope) (base pairs)

[2] DNA mass calculation of each plasmid:

$$\text{DNA mass (µg)} = \frac{\text{Picomole of plasmid} * (\text{Plasmid size} * 617.96 + 36.04)}{10e6}$$

The calculations in Table S1 were adapted following formula at:
https://nebiocalculator.neb.com/#!/dsdnaamt

Table S2: Oligonucleotide and primer sequences

Name	Sequence (5'-3')	Purpose
hU6-forward	acgatacaaggctgttagagag	For sequencing of inserted gene specific targeting sequence
Furin-T2A-GFP-forward	aatgaaaagcttaggcggaagcgggggtcaggagagggcagaggaagtcttctaacatgcgg	Amplify T2A-GFP sequence from plasmid pMSCV-Cas9-2A-GFP-sgRNA (Addgene)
Furin-T2A-GFP-reverse	taacaaaagcttttacttgtacagctcgtccatgccgaga	Amplify T2A-GFP sequence from plasmid pMSCV-Cas9-2A-GFP-sgRNA (Addgene)
sg*ß2M*-forward	caccggagtagcgcgagcacagcta	Human *ß2M* targeting sequence
sg*ß2M*-reverse	aaactagctgtgctcgcgctactcc	Human *ß2M* targeting sequence
ß2M-forward	gtcctagaatgagcgcccg	Amplify human *ß2M* locus
ß2M-reverse	tgctctggagaatctcacgc	Amplify human *ß2M* locus
sg*IRF4*-forward	caccgcaagcaggactacaaccgcg	Human *IRF4* targeting sequence
sg*IRF4*-reverse	aaaccgcggttgtagtcctgcttgc	Human *IRF4* targeting sequence
IRF4-forward	actgacagagtcgcggggaag	Amplify human *IRF4* locus
IRF4-reverse	agagccgaggcctcctttcctc	Amplify human *IRF4* locus
sg*PRDM1*-forward	caccgggatggggtaaacgacccga	Human *PRDM1* targeting sequence
sg*PRDM1*-reverse	aaactcgggtcgtttaccccatccc	Human *PRDM1* targeting sequence
PRDM1-forward	tcagttctctctagccctctgtgtaatcgc	Amplify human *PRDM1* locus
PRDM1-reverse	gactgctctctctcaaggcctaccttcag	Amplify human *PRDM1* locus
sg*XBP1*-forward	caccggactgccagagatcgaaaga	Human *XBP1* targeting sequence
sg*XBP1*-reverse	aaactctttcgatctctggcagtcc	Human *XBP1* targeting sequence
XBP1-forward	aattggactgggggacggag	Amplify human *XBP1* locus
XBP1-reverse	ataggggctgaaacaacttggg	Amplify human *XBP1* locus

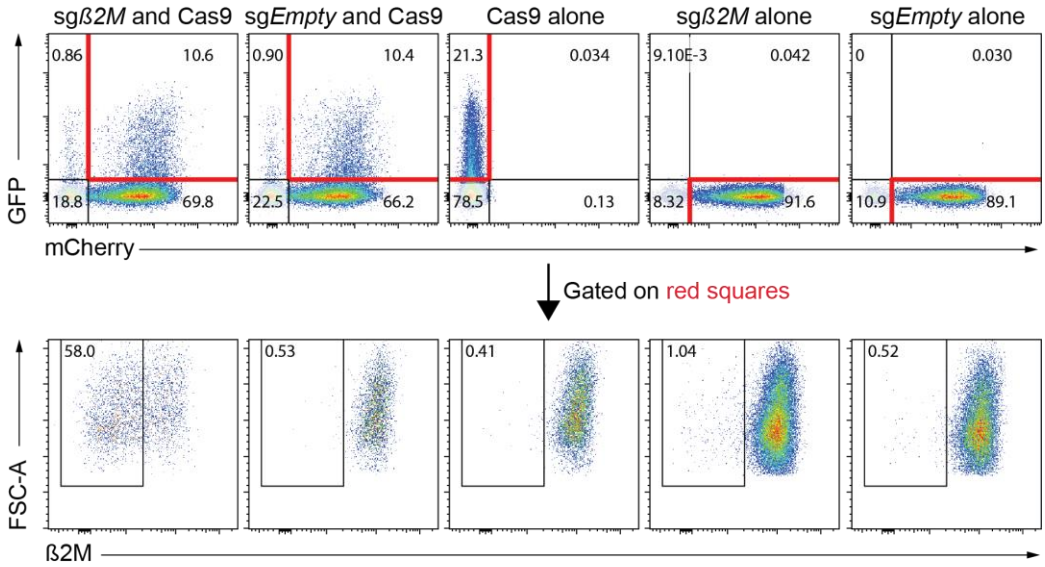

Figure S1: Efficient CRISPR/Cas9-mediated knockout of *ß2M* housekeeping gene in primary human B cells. Representative FACS plots show the frequency of β2M$^-$ B cells in sg*β2M*$^+$Cas9$^+$, sg*Empty*$^+$Cas9$^+$, Cas9$^+$, sg*β2M*$^+$ and sg*Empty*$^+$ transduced B cells at day 4 after transduction. sg*Empty* is sgRNA without the targeted sequence.

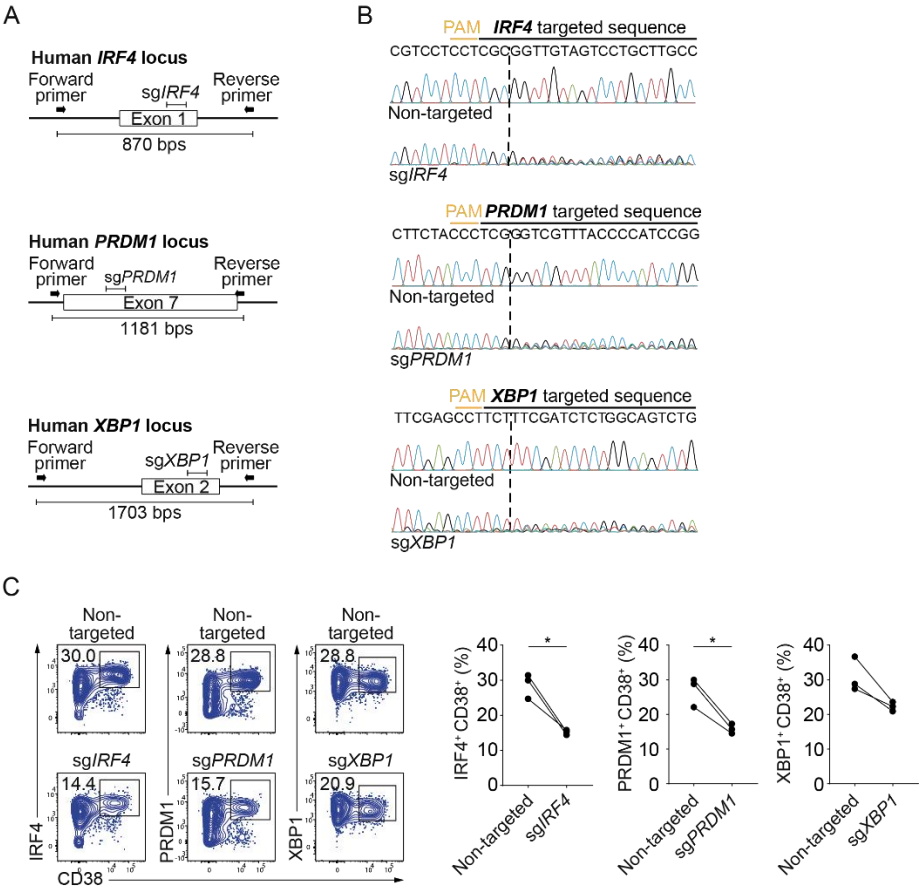

Figure S2: *IRF4*, *PRDM1*, and *XBP1* are crucial for B cell survival and PC differentiation *in vitro*. (A) The schemes show the targeted site of indicated genes and primer sites for amplifying of flanking regions of *IRF4*, *PRDM1*, and *XBP1*-targeted sites by PCR. (B) Sanger sequencing signal traces show noise peaks at *IRF4*, *PRDM1*, and *XBP1*-targeted site compared to non-targeted samples, respectively. The dashed lines indicate Cas9 cleavage sites. (C) Representative FACS plots (left) and graphs (right) show the percentage of $IRF4^{+}CD38^{+}$ $PRDM1^{+}CD38^{+}$ and $XBP1^{+}CD38^{+}$ cells at day 8 post transduction from non-targeted compared with corresponding sg*IRF4*, sg*PRDM1*, and *sgXBP1*-targeted samples. $IRF4^{+}CD38^{+}$ $PRDM1^{+}CD38^{+}$, $XBP1^{+}CD38^{+}$ cells were determined based on their isotype control stainings. Data were pooled from 3 donors. Statistical significance was calculated using paired *t*-test, only significant *p* value is shown: $*p < 0.05$. Each gene was independently targeted by three different sgRNAs. Data for one sgRNA are shown.