Supporting Information

Streamlined Human Cell-Based Recombinase-Mediated Cassette Exchange Platform Enables Multigene Expression for the Production of Therapeutic Proteins

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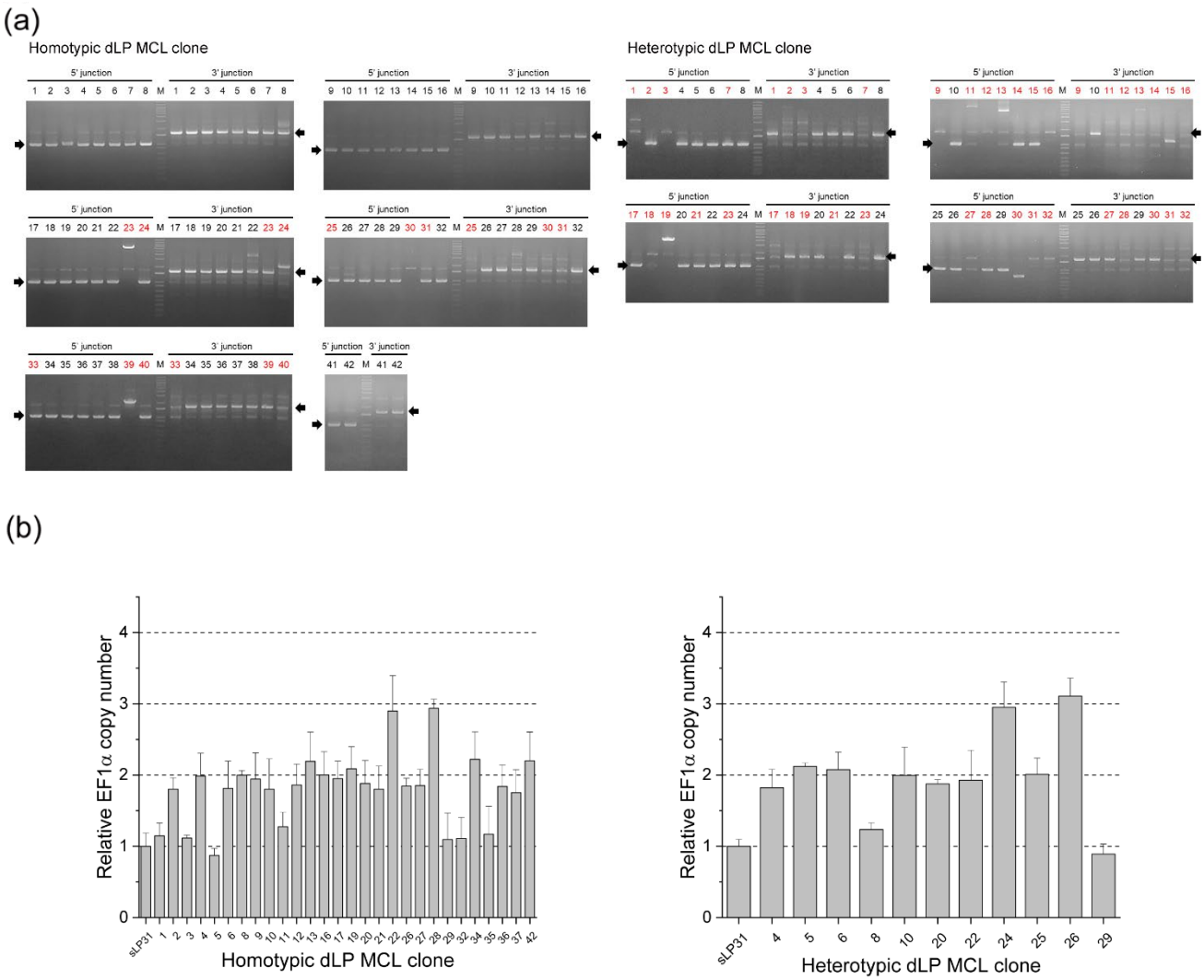
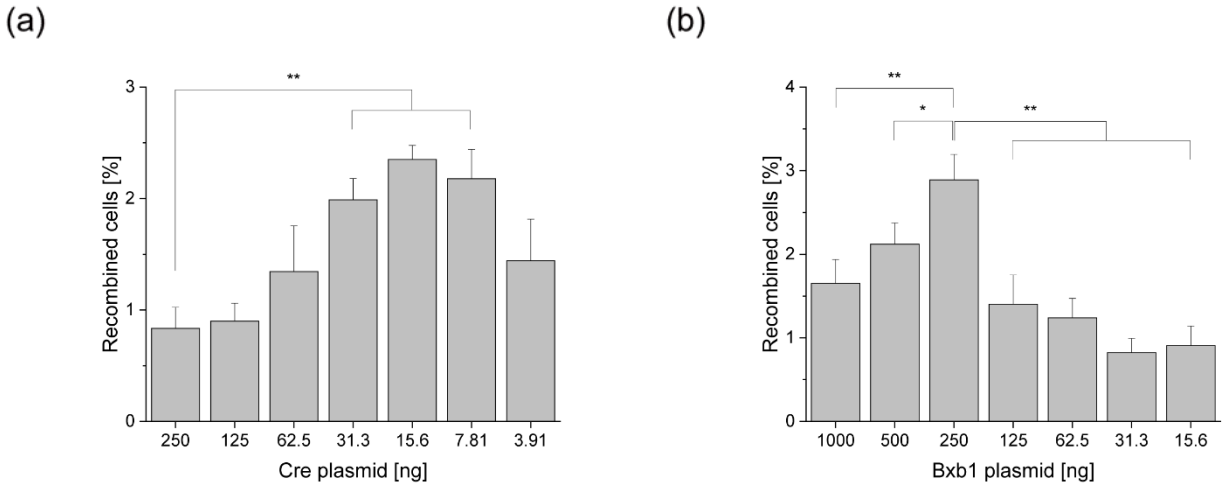
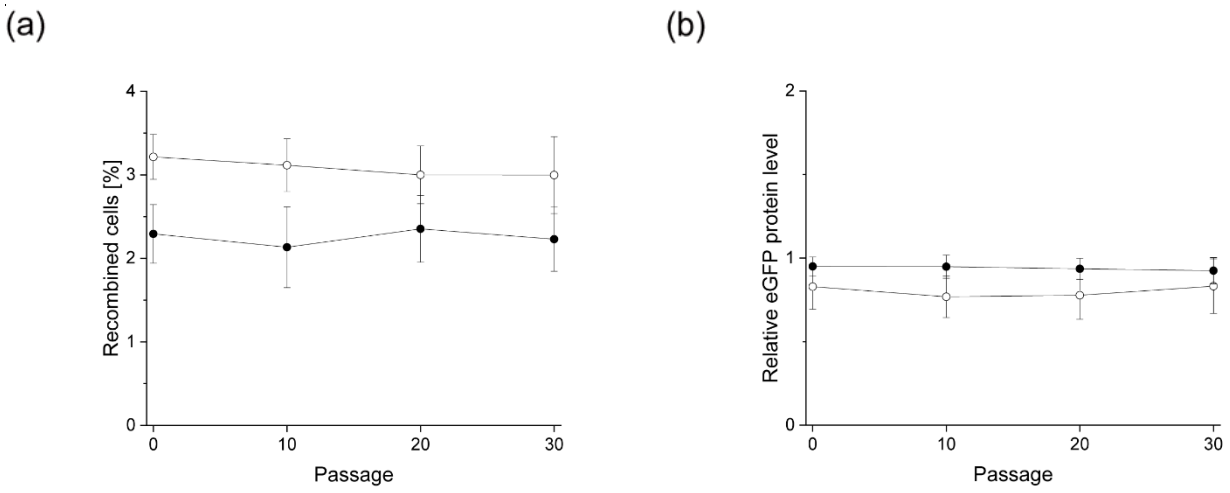


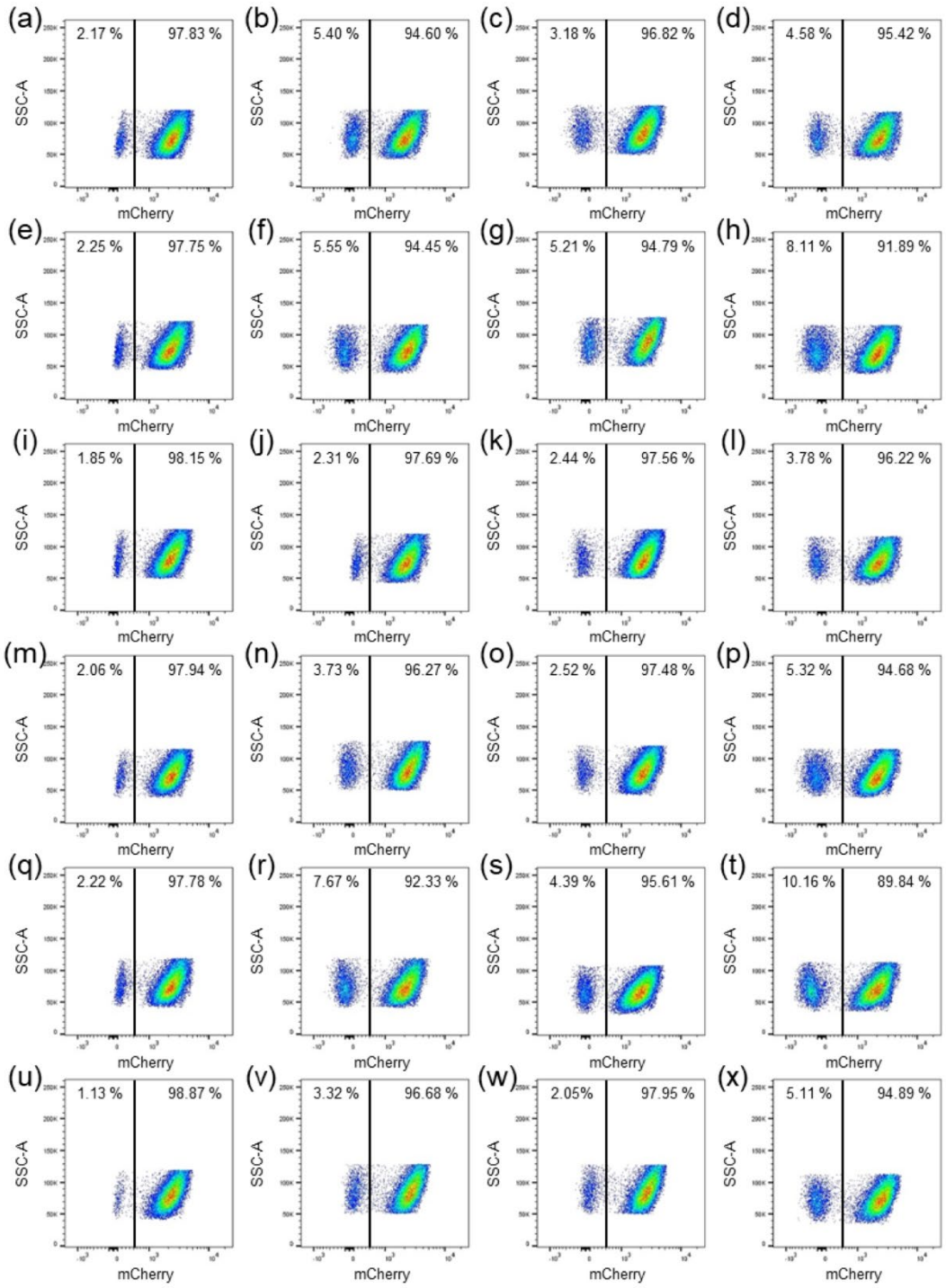
Supporting Figure S1. Generation of dual-landing pad (dLP) master cell lines (MCLs) using the CRISPR/Cas9-mediated integration. (a) Verification of targeted integration at the ROSA26 locus in a single-landing pad (sLP) 31 clone, which harbors a loxP-EF1α-mCherry-lox2272 landing pad at the AAVS1 locus, by 5'/3' junction PCR. Homotypic and heterotypic dLP MCLs harbors a second landing pad, loxP-EF1α-TagBFP-lox2272 and attP-EF1α-TagBFP-attP^{mut}, respectively, at the ROSA26 locus. Negative clones are shown in red, and black arrows indicate the expected size of the PCR amplicons. (b) Measurement of relative EF1α copy numbers in stable clones. A genomic DNA sample of the sLP31 clone was used as a reference for the measurement of EF1α copy number.[1] Error bars represent standard deviations of three independent experiments.



Supporting Figure S2. Titration of recombinase plasmid concentration. The efficiency of RMCE mediated by (a) Cre recombinase or (b) Bxb1 integrase was measured in seven different concentrations of the recombinase plasmid with a fixed amount of donor plasmid (1 μg), in sLP or heterotypic dLP MCLs, respectively. RMCE efficiency was determined by calculating the percentage of mCherry-negative and eGFP-positive cells or TagBFP-negative and eGFP-positive cells in the total number of cells. Error bars represent standard deviations of three independent experiments. The data were analyzed using a one-way analysis of variance with Tukey's *post hoc* test, *$p < 0.05$, **$p < 0.01$.

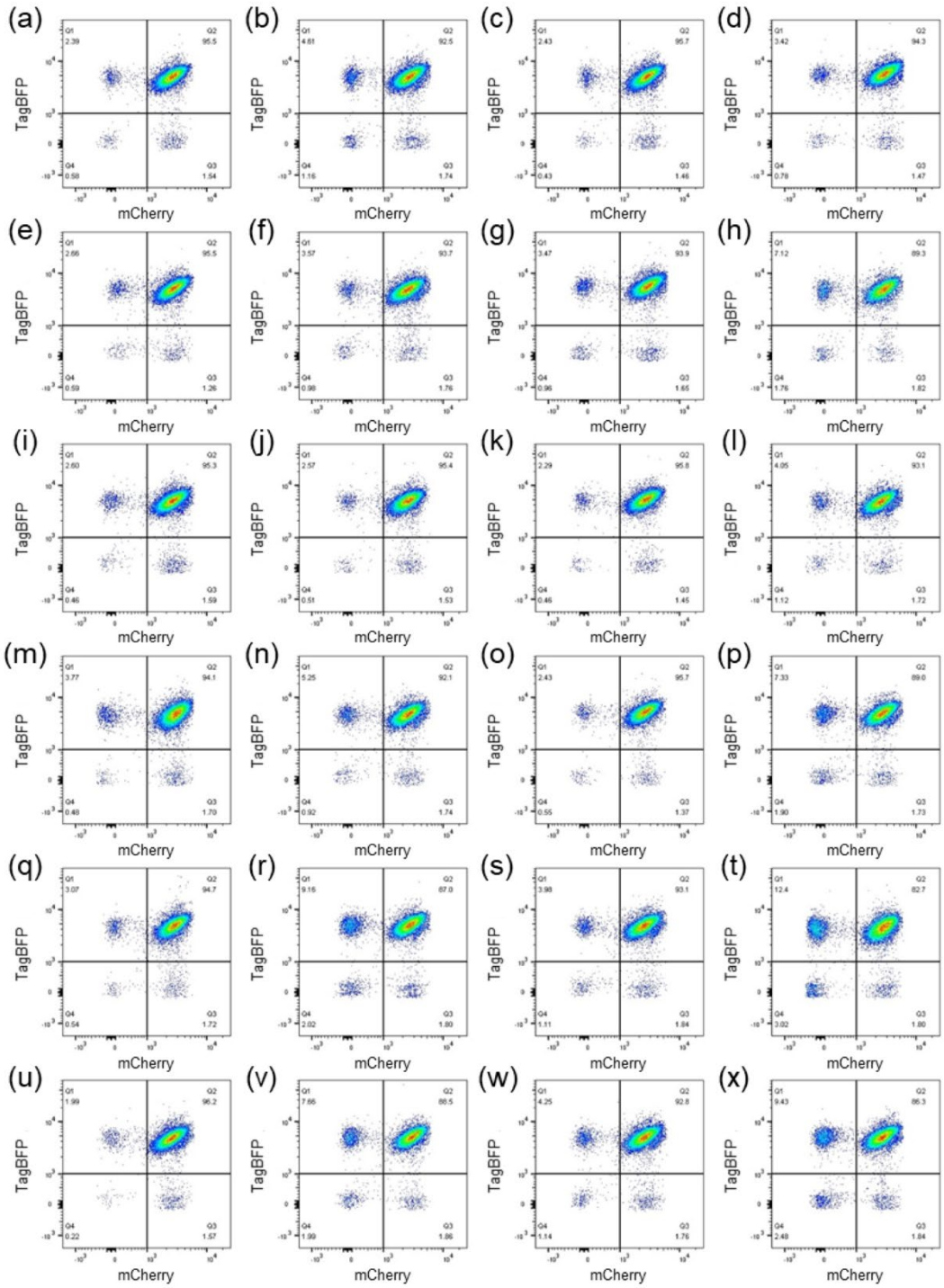


Supporting Figure S3. Stability of recombinant cell pools. (a) Change in the percentage of recombined cells in the total number of cells during long-term culture. mCherry- or TagBFP-negative cells were measured by flow cytometry at passage 0, 10, 20, and 30. (b) Change in the relative eGFP protein levels during long-term culture. eGFP protein levels were measured by flow cytometry at passage 0, 10, 20, and 30, and were normalized to the AAVS1 sample at passage 0. Cre recombinase-mediated RMCE at the AAVS1 locus (black circle) and Bxb1 integrase-mediated RMCE at the ROSA26 locus (white circle). Error bars represent standard deviations of three independent experiments.



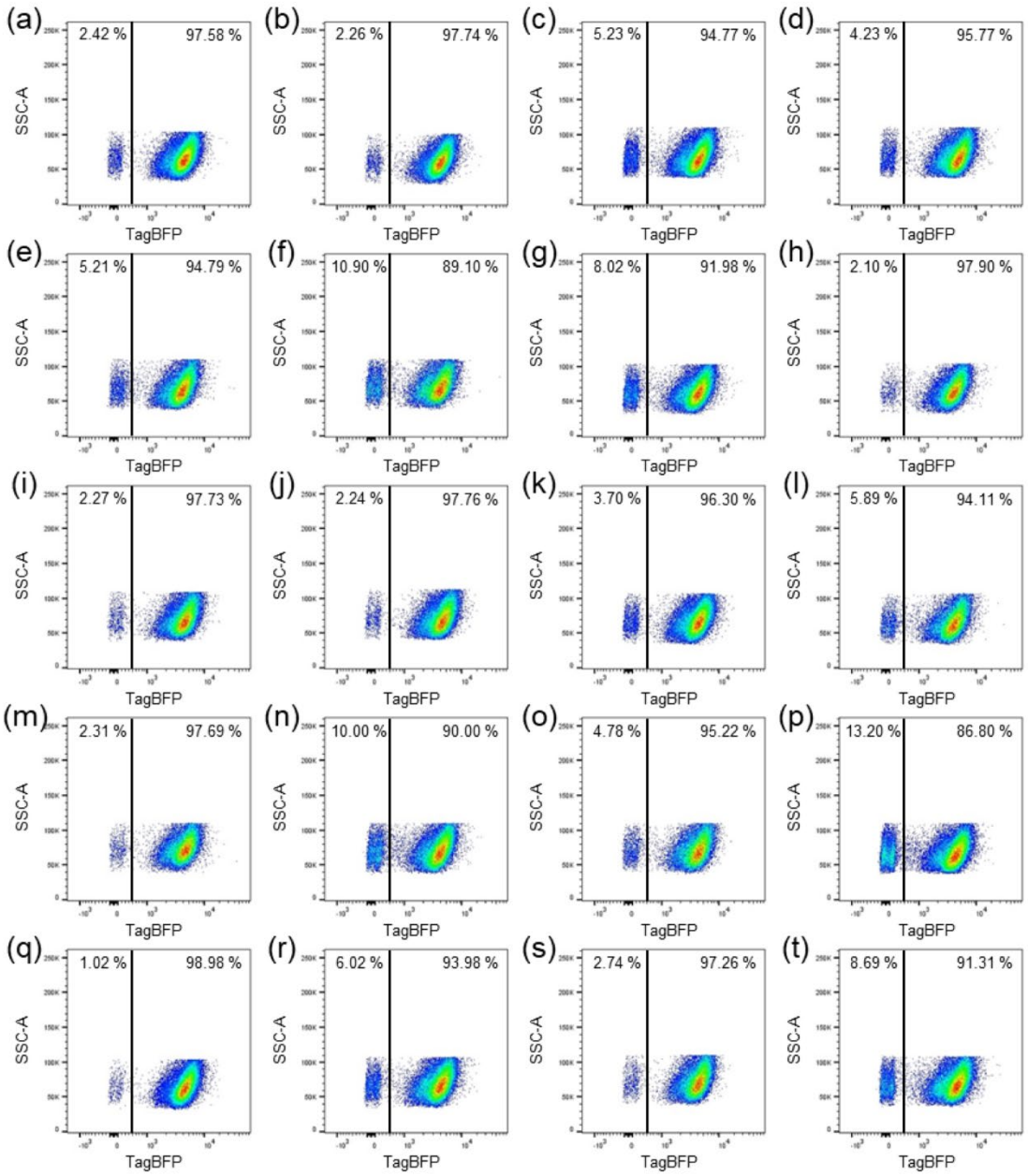
Supporting Figure S4. Representative FACS plots and gates of RMCE in the sLP MCL. eGFP loxP-donor with (a) control, (b) SV40-N, (c) SV40-C, (d) SV40-NC, (e) control, (f) SV40-N, (g) cMyc-N, or (h) NP-N NLS Cre. Control Cre with (i) control, (j) SV40-5', (k) SV40-3', (l) SV40-5'/3', (m) control,

(n) SV40-5′/3′, (o) GRE-5′/3′, or (p) NF-κB-5′/3′ DTS loxP-donor. eGFP donor in (q) control, (r) NLS, (s) DTS, or (t) NLS and DTS used. mAb donor in (u) control, (v) NLS, (w) DTS, or (x) NLS and DTS used.

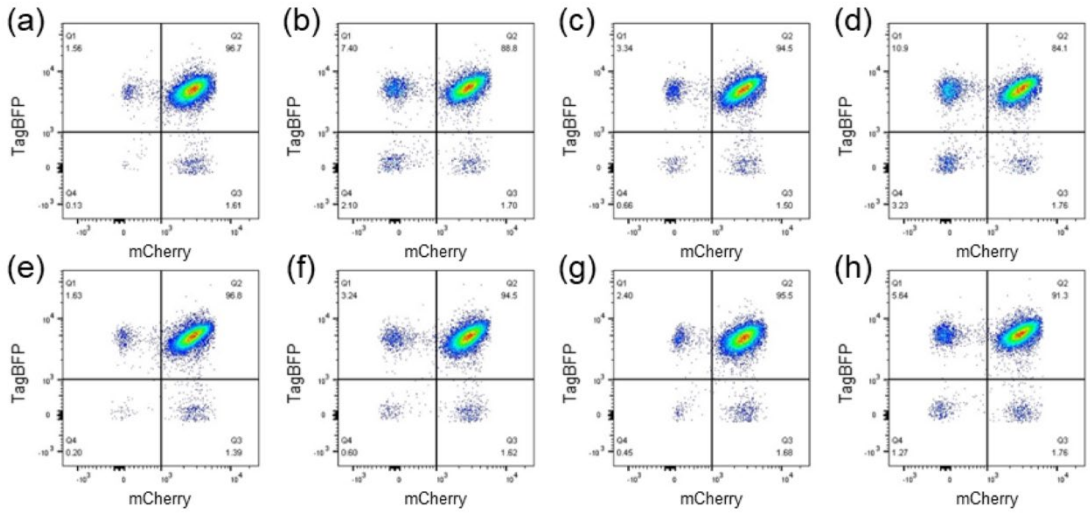


Supporting Figure S5. Representative FACS plots and gates of RMCE in the homotypic dLP MCL. eGFP loxP-donor with (a) control, (b) SV40-N, (c) SV40-C, (d) SV40-N/C, (e) control, (f) SV40-N, (g) cMyc-N, or (h) NP-N NLS Cre. Control Cre with (i) control, (j) SV40-5', (k) SV40-3', (l) SV40-

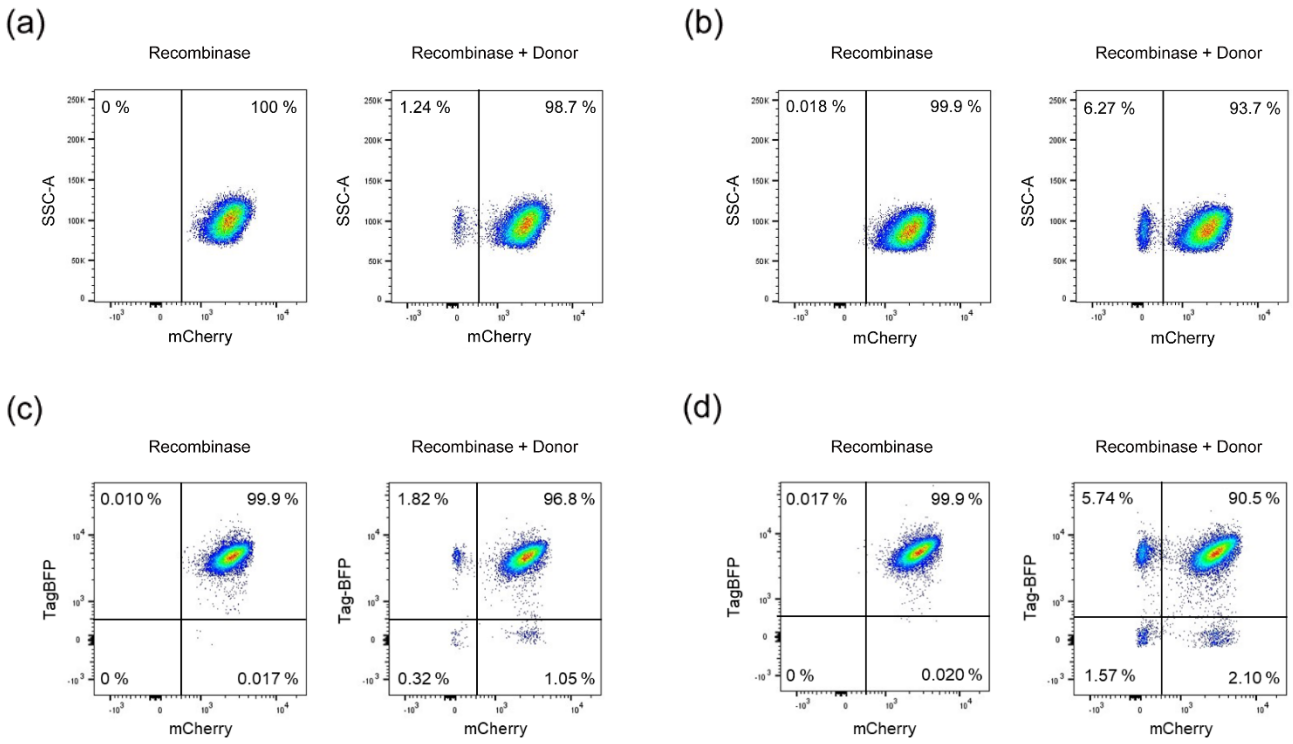
5′/3′, (m) control, (n) SV40-5′/3′, (o) GRE-5′/3′, or (p) NF-κB-5′/3′ DTS loxP-donor. eGFP donor in (q) control, (r) NLS, (s) DTS, or (t) NLS and DTS used. mAb donor in (u) control, (v) NLS, (w) DTS, or (x) NLS and DTS used.



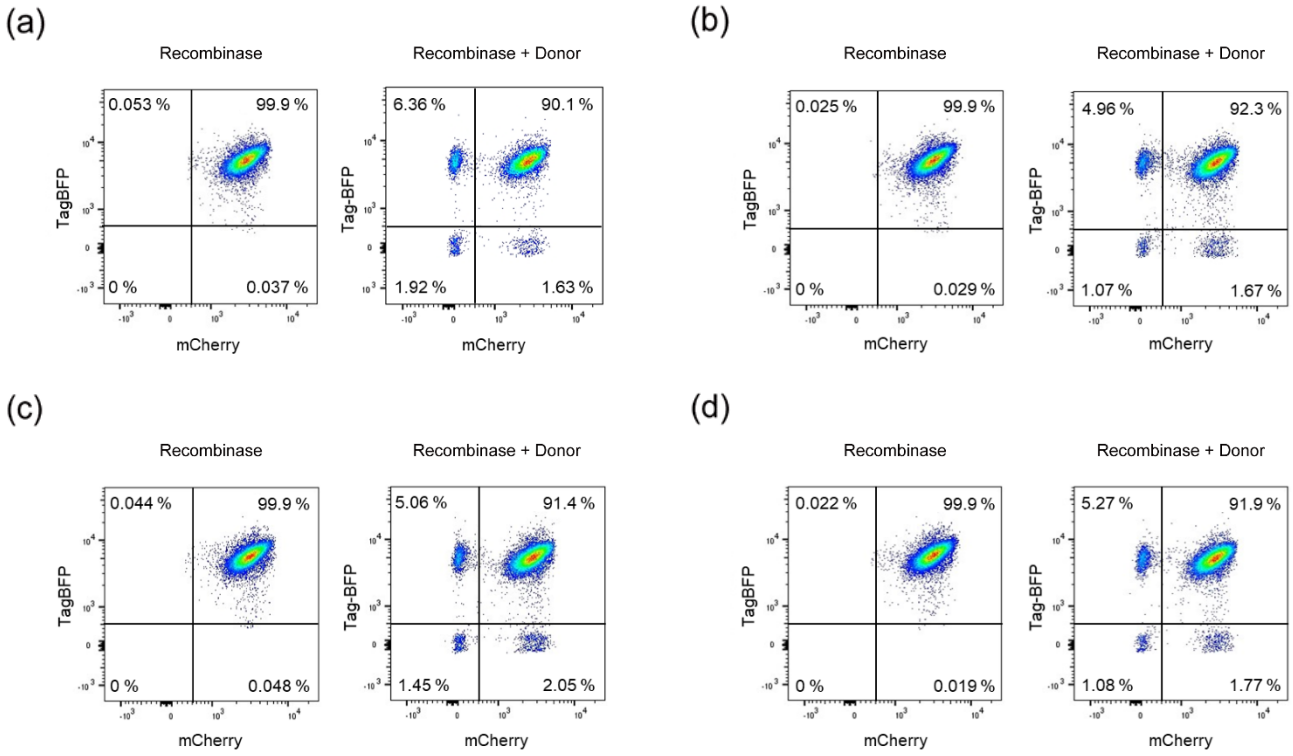
Supporting Figure S6. Representative FACS plots and gates of RMCE in the heterotypic dLP MCL. eGFP attB-donor with (a) control, (b) SV40-N, (c) SV40-C, (d) SV40-N/C, (e) NP-N, (f) NP-C, or (g) NP-N/C NLS Bxb1. Control Bxb1 with (h) control, (i) SV40-5', (j) SV40-3', (k) SV40-5'/3', or (l) NF-κB-5'/3' DTS attB-donor. eGFP donor in (m) control, (n) NLS, (o) DTS, or (p) NLS and DTS used. mAb donor in (q) control, (r) NLS, (s) DTS, or (t) NLS and DTS used.



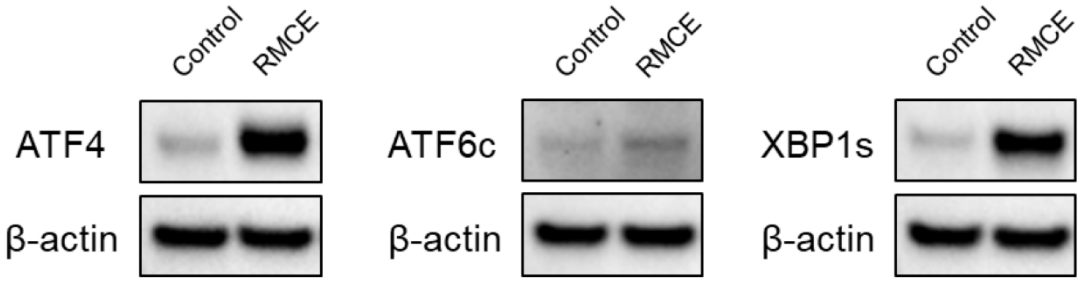
Supporting Figure S7. Representative FACS plots and gates of simultaneous RMCE in the heterotypic dLP MCL. Four plasmids, loxP-donor, Cre recombinase, attB-donor, and Bxb1 integrase, were used for simultaneous RMCE. eGFP donor in (a) control, (b) NLS, (c) DTS, or (d) NLS and DTS used. mAb donor in (e) control, (f) NLS, (g) DTS, or (h) NLS and DTS used.



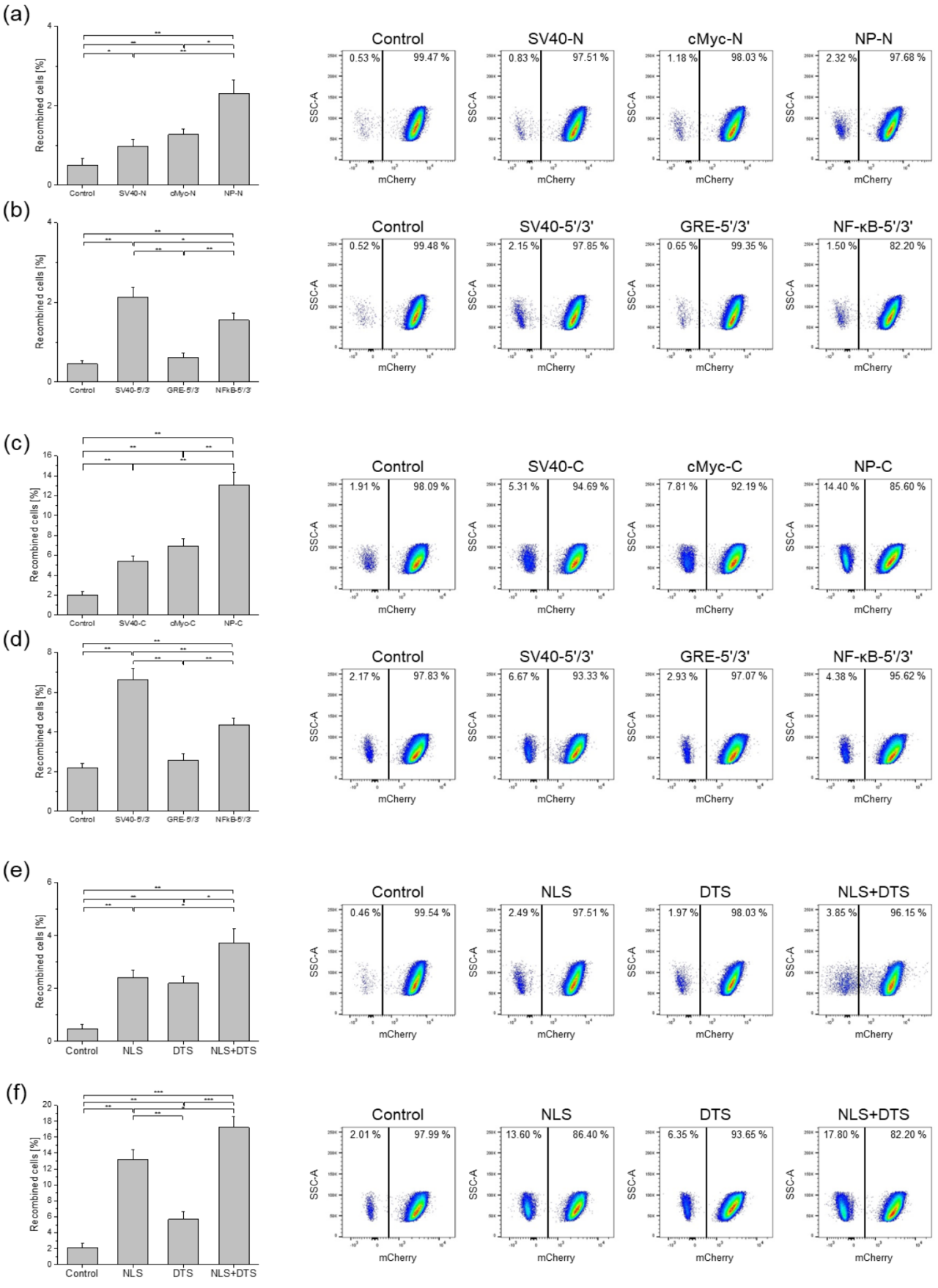
Supporting Figure S8. Generation of recombinant cell pools producing mAbs from single or dual copies. Numbers represent the percentage of cells in which the LP was replaced by mAb genes. Fluorescence of mCherry and TagBFP was measured 8 days post-transfection using flow cytometry (100000 cells). Cre-mediated RMCE was performed in sLP or homotypic dLP MCLs by transfection with Cre recombinase and mAb donor plasmid. mCherry-negative cells were gated with sLP or homotypic dLP MCLs that were transfected with Cre recombinase alone. (a) Control of sLP MCL. (b) NLS and DTS used in the sLP MCL. (c) Control of homotypic dLP MCL. (d) NLS and DTS used in the homotypic dLP MCL.



Supporting Figure S9. Simultaneous cassette exchange of mAb and effector genes related to the UPR pathway. Numbers represent the percentage of cells in which the LP was replaced by mAb and effector genes. Fluorescence of mCherry and TagBFP was measured 8 days post-transfection using flow cytometry (100000 cells). The Cre- and Bxb1-mediated dual-RMCE resulted in mCherry-negative/TagBFP-negative cells, which express mAb genes at the AAVS1 locus and effector genes at the ROSA26 locus. Cell pools expressing mAbs, and (a) eGFP, (b) ATF4, (c) ATF6c, or (d) XBP1s were sorted using heterotypic dLP MCL, which was transfected with Cre recombinase and Bxb1 integrase as a gating control.



Supporting Figure S10. Protein expression of effector genes related to the UPR pathway, *ATF4*, *ATF6c*, and *XBP1s*, in recombinant cell pools. Cells were sorted using MoFlo Astrios EQ (Beckman Coulter, Brea, CA) 8 days post-transfection, and samples were harvested 9 days post-sorting. β-actin was used as a loading control.


(a)
Recombined cells [%]
Control
SV40-N
cMyc-N
NP-N
Control
SV40-N
cMyc-N
NP-N
0.53 %
99.47 %
0.83 %
97.51 %
1.18 %
98.03 %
2.32 %
97.68 %
(b)
Recombined cells [%]
Control
SV40-5'/3'
GRE-5'/3'
NFkB-5'/3'
Control
SV40-5'/3'
GRE-5'/3'
NF-κB-5'/3'
0.52 %
99.48 %
2.15 %
97.85 %
0.65 %
99.35 %
1.50 %
82.20 %
(c)
Recombined cells [%]
Control
SV40-C
cMyc-C
NP-C
Control
SV40-C
cMyc-C
NP-C
1.91 %
98.09 %
5.31 %
94.69 %
7.81 %
92.19 %
14.40 %
85.60 %
(d)
Recombined cells [%]
Control
SV40-5'/3'
GRE-5'/3'
NFkB-5'/3'
Control
SV40-5'/3'
GRE-5'/3'
NF-κB-5'/3'
2.17 %
97.83 %
6.67 %
93.33 %
2.93 %
97.07 %
4.38 %
95.62 %
(e)
Recombined cells [%]
Control
NLS
DTS
NLS+DTS
Control
NLS
DTS
NLS+DTS
0.46 %
99.54 %
2.49 %
97.51 %
1.97 %
98.03 %
3.85 %
96.15 %
(f)
Recombined cells [%]
Control
NLS
DTS
NLS+DTS
Control
NLS
DTS
NLS+DTS
2.01 %
97.99 %
13.60 %
86.40 %
6.35 %
93.65 %
17.80 %
82.20 %
SSC-A
mCherry

Supporting Figure S11. Improvement of RMCE effciency in CHO cells. RMCE efficiency of eGFP donor in the sLP MCL, harboring a loxP-EF1α-mCherry-lox2272 landing pad, with different types of (a) N-terminal NLS in Cre recombinase and (b) 5′/3′ DTS in loxP-donor. RMCE efficiency of eGFP donor in the sLP MCL, harboring a attP-EF1α-mCherry-attP^{mut} landing pad, with different types of (c) C-terminal NLS in Bxb1 integrase and (d) 5′/3′ DTS in attB-donor. (e) RMCE efficiency of eGFP donor in the sLP MCL with a combination of NP N-terminal NLS in Cre recombinase and SV40 5′/3′ DTS in loxP-donor. (f) RMCE efficiency of eGFP donor in the sLP MCL with a combination of NP C-terminal NLS in Bxb1 integrase and SV40 5′/3′ DTS in attB-donor. Error bars represent standard deviations of three independent experiments. The data were analyzed using a one-way analysis of variance with Tukey's *post hoc* test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Supporting Table S1. Plasmid information.

Plasmid name	Description
ROSA26-sgRNA-Cas9[1]	sgRNA targeting ROSA26 locus and Cas9 expression vector
ROSA26-LP donor (loxP)	Donor plasmid targeting ROSA26 locus (GOI: SA-T2A-ZeoR-BGHpA-loxP-EF1α-TagBFP-lox2272-BGHpA)
ROSA26-LP donor (attP)	Donor plasmid targeting ROSA26 locus (GOI: SA-T2A-ZeoR-BGHpA-attP-EF1α-TagBFP-attP^{mut}-BGHpA)
RMCE-eGFP donor (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: loxP-EF1α-eGFP-lox2272)
RMCE-eGFP donor (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-EF1α-eGFP-attB^{mut})
RMCE-mAb donor (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: loxP-CMV-LC-BGHpA-CMV-HC-lox2272)
RMCE-mAb donor (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-CMV-LC-BGHpA-CMV-HC-attB^{mut})
RMCE-ATF4 donor (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-EF1α-ATF4-attB^{mut})
RMCE-ATF6c donor (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-EF1α-ATF6c-attB^{mut})
RMCE-XBP1s donor (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-EF1α-XBP1s-attB^{mut})
PSF-CMV-CRE	Cre recombinase expression vector (Sigma-Aldrich)
SV40-N-NLS-Cre	Cre recombinase with N-terminal SV40 NLS expression plasmid
SV40-C-NLS-Cre	Cre recombinase with C-terminal SV40 NLS expression plasmid
SV40-NC-NLS-Cre	Cre recombinase with N- and C-terminal SV40 NLS expression plasmid
cMyc-N-NLS-Cre	Cre recombinase with N-terminal cMyc NLS expression plasmid
NP-N-NLS-Cre	Cre recombinase with N-terminal NP NLS expression plasmid
INCTbiosyn-pUB-HspINTBxb1	Bxb1 recombinase expression vector (Addgene # 127519)[2]
SV40-N-NLS-Bxb1	Bxb1 recombinase with N-terminal SV40 NLS expression plasmid
SV40-C-NLS-Bxb1	Bxb1 recombinase with C-terminal SV40 NLS expression plasmid
SV40-NC-NLS-Bxb1	Bxb1 recombinase with N- and C-terminal SV40 NLS expression plasmid
NP-N-NLS-Bxb1	Bxb1 recombinase with N-terminal NP NLS expression plasmid
NP-C-NLS-Bxb1	Bxb1 recombinase with C-terminal NP NLS expression plasmid

NP-NC-NLS-Bxb1	Bxb1 recombinase with N- and C-terminal NP NLS expression plasmid
SV40-5'-DTS-eGFP (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: SV40 DTS-loxP-EF1α-eGFP-lox2272)
SV40-3'-DTS-eGFP (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: loxP-EF1α-eGFP-lox2272-SV40 DTS)
SV40-5'/3'-DTS-eGFP (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: SV40 DTS-loxP-EF1α-eGFP-lox2272-SV40 DTS)
GRE-5'/3'-DTS-eGFP (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: GRE DTS-loxP-EF1α-eGFP-lox2272-GRE DTS)
NF-κB-5'/3'-DTS-eGFP (loxP)	Donor plasmid for RMCE with LP (loxP) (GOI: NF-κB DTS-loxP-EF1α-eGFP-lox2272-NF-κB DTS)
SV40-5'-DTS-eGFP (attB)	Donor plasmid for RMCE with LP (attP) (GOI: SV40 DTS-attB-EF1α-eGFP-attB^{mut})
SV40-3'-DTS-eGFP (attB)	Donor plasmid for RMCE with LP (attP) (GOI: attB-EF1α-eGFP-attB^{mut}-SV40 DTS)
SV40-5'/3'-DTS-eGFP (attB)	Donor plasmid for RMCE with LP (attP) (GOI: SV40 DTS-attB-EF1α-eGFP-attB^{mut}-SV40 DTS)
NF-κB-5'/3'-DTS-eGFP (attB)	Donor plasmid for RMCE with LP (attP) (GOI: NF-κB DTS-attB-EF1α-eGFP-attB^{mut}-NF-κB DTS)
NF-κB-5'/3'-DTS-mAb (loxP)	Donor plasmid for RMCE with LP (attP) (GOI: NF-κB DTS-loxP-CMV-LC-BGHpA-CMV-HC-lox2272-NF-κB DTS)
NF-κB-5'/3'-DTS-mAb (attB)	Donor plasmid for RMCE with LP (attP) (GOI: NF-κB DTS-attB-CMV-LC-BGHpA-CMV-HC-attB^{mut}-NF-κB DTS)

Plasmid name (PCR template)	**Element**
pcDNA3.1/Zeo(+)	ZeoR (Life Technologies)
PL0626[3]	TagBFP
pAAVS1-TLR targeting vector[4]	Backbone of LP donor plasmid (Addgene plasmid #64215)
RMCE eGFP donor plasmid[5]	EF1α promoter and eGFP gene
RMCE EPO donor plasmid[5]	Backbone of RMCE donor plasmid
PSF-CMV-CRE	CMV promoter, Cre gene, SV40pA, and Backbone of recombinase plasmid
INCTbiosyn-pUB-HspINTBxb1[2]	Bxb1 gene

LP, landing pad; GOI, gene of interest; SA, splicing acceptor; mAb, monoclonal antibody; LC, light chain; HC, heavy chain; NLS, nuclear localization signal; NP, nucleoplasmin; DTS, DNA nuclear-targeting sequence; GRE, glucocorticoid responsive element

Supporting Table S2. Primer sequences.

Primer name	Description	Sequence (5'-3')
SA-T2A-ZeoR_fwd	USER primer for LP donor (loxP) plasmid	AAGCAGCGUAAGCTTCTGACCT CTTCTC
SA-T2A-ZeoR_rev	USER primer for LP donor (loxP) plasmid	ATAGGCTTUCCATAGAGCCCACC GCATC
loxP-EF1α-TagBFP-lox2272_fwd	USER primer for LP donor (loxP) plasmid	AAAGCCTAUATAACTTCGTATAG CATAC
loxP-EF1α-TagBFP-lox2272_rev	USER primer for LP donor (loxP) plasmid	ATGACGTCUATAACTTCGTATAA AGTATCCTATACGAAGTTATTTA ATTAAGCTTGTGCCCCAG
SA-T2A-ZeoR_fwd	USER primer for LP donor (attP) plasmid	AAGCAGCGUAAGCTTCTGACCT CTTCTC
SA-T2A-ZeoR- attP_rev	USER primer for LP donor (attP) plasmid	ATAGGCTTUTGGGTTTGTACCGT ACACCACTGAGACCGCGGTGGT TGACCAGACAAACCACCCATAG AGCCCACCGCATC
attP-EF1α-TagBFP-attP^{mut}_fwd	USER primer for LP donor (attP) plasmid	AAAGCCTAUGTGAGGCTCCGGT GCCCGTC
attP-EF1α-TagBFP-attP^{mut}_rev	USER primer for LP donor (attP) plasmid	ATGACGTCUTGGGTTTGTACCGT ACACCACTGAGTCCGCGGTGGTT GACCAGACAAACCACTTAATTA AGCTTGTGCCCCAG
ATF4_fwd	USER primer for ATF4 donor plasmid	AAGCAGCGUGCCACCATGACCG AAATGAG
ATF4_rev	USER primer for ATF4 donor plasmid	ACGCAAGUCTAGGGGACCCTTTT CTTCC
ATF6c_fwd	USER primer for ATF6c donor plasmid	AAGCAGCGUGCCACCATGGGGG AGCCGGC
ATF6c_rev	USER primer for ATF6c donor plasmid	ACGCAAGUCTAACTAGGGACTT TAAGCC
XBP1s_fwd	USER primer for XBP1s donor plasmid	AAGCAGCGUGCCACCATGGTGG TGGTGGC
XBP1s_rev	USER primer for XBP1s donor plasmid	ACGCAAGUTTAGACACTAATCA GCTGGG
SV40-N-NLS-Cre_fwd	USER primer for SV40-N-NLS-Cre plasmid	AAAGCCTAUGCCACCATGGCCC CAAAGAAGAAGCGGAAGGTCGG AAGCATGTCCAATTTACTGACCG
SV40-N-NLS-Cre_rev	USER primer for SV40-N-NLS-Cre plasmid	ACGCTGCTUCTAGTCGCCATCTT CCAGCAG
SV40-C-NLS-Cre_fwd	USER primer for SV40-C-NLS-Cre plasmid	AAAGCCTAUGCCACCATGGCCA TGTCCAATTTACTGACCG
SV40-C-NLS-Cre_rev	USER primer for SV40-C-NLS-Cre plasmid	ACGCTGCTUTTAACTGCCTACCT TCCTCTTCTTCTTGGGGCTGCCG TCGCCATCTTCCAGCAGGC
SV40-NC-NLS-Cre_fwd	USER primer for SV40-NC-NLS-Cre plasmid	AAAGCCTAUGCCACCATGGCCC CAAAGAAGAAGCGGAAGGTCGG AAGCATGTCCAATTTACTGACCG
SV40-NC-NLS-Cre_rev	USER primer for SV40-NC-NLS-Cre plasmid	ACGCTGCTUTTAACTGCCTACCT TCCTCTTCTTCTTGGGGCTGCCG TCGCCATCTTCCAGCAGGC
cMyc-N-NLS-Cre_fwd	USER primer for cMyc-N-NLS-Cre plasmid	AAAGCCTAUGCCACCATGGCCC CTGCTGCCAAACGCGTTAAACTA GACATGTCCAATTTACTGACCG

cMyc-N-NLS-Cre_rev	USER primer for cMyc-N-NLS-Cre plasmid	ACGCTGCTUCTAGTCGCCATCTTCCAGCAG
NP-N-NLS-Cre_fwd	USER primer for NP-N-NLS-Cre plasmid	AAAGCCTAUGCCACCATGGCCAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGGGAAGCATGTCCAATTTACTGACCG
NP-N-NLS-Cre_rev	USER primer for NP-N-NLS-Cre plasmid	ACGCTGCTUCTAGTCGCCATCTTCCAGCAG
SV40-N-NLS-Bxb1_fwd	USER primer for SV40-N-NLS-Bxb1 plasmid	AAAGCCTAUGCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGAAGCATGCGGGCACTGGTGGTAATTAG
SV40-N-NLS-Bxb1_rev	USER primer for SV40-N-NLS-Bxb1 plasmid	ACGCTGCTUCTAAGACATCCCTGTATGCAG
SV40-C-NLS-Bxb1_fwd	USER primer for SV40-C-NLS-Bxb1 plasmid	AAAGCCTAUGCCACCATGGCCATGCGGGCACTGGTGGTAATTAG
SV40-C-NLS-Bxb1_rev	USER primer for SV40-C-NLS-Bxb1 plasmid	ACGCTGCTUTTAACTGCCTACCTTCCTCTTCTTCTTGGGGCTGCCAGACATCCCTGTATGCAGTC
SV40-NC-NLS-Bxb1_fwd	USER primer for SV40-NC-NLS-Bxb1 plasmid	AAAGCCTAUGCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGAAGCATGCGGGCACTGGTGGTAATTAG
SV40-NC-NLS-Bxb1_rev	USER primer for SV40-NC-NLS-Bxb1 plasmid	ACGCTGCTUTTAACTGCCTACCTTCCTCTTCTTCTTGGGGCTGCCAGACATCCCTGTATGCAGTC
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NP-N-NLS-Bxb1_rev	USER primer for NP-N-NLS-Bxb1 plasmid	ACGCTGCTUCTAAGACATCCCTGTATGCAG
NP-C-NLS-Bxb1_fwd	USER primer for NP-C-NLS-Bxb1 plasmid	AAAGCCTAUGCCACCATGGCCATGCGGGCACTGGTGGTAATTAG
NP-C-NLS-Bxb1_rev	USER primer for NP-C-NLS-Bxb1 plasmid	ACGCTGCTUTTAACTGCCCTTTTTCTTTTTTGCCTGGCCGGCCTTTTTCGTGGCCGCCGGCCTTTTGCTGCCAGACATCCCTGTATGCAGTC
NP-NC-NLS-Bxb1_fwd	USER primer for NP-NC-NLS-Bxb1 plasmid	AAAGCCTAUGCCACCATGGCCAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGGGAAGCATGCGGGCACTGGTGGTAATTAG
NP-NC-NLS-Bxb1_rev	USER primer for NP-NC-NLS-Bxb1 plasmid	ACGCTGCTUTTAACTGCCCTTTTTCTTTTTTGCCTGGCCGGCCTTTTTCGTGGCCGCCGGCCTTTTGCTGCCAGACATCCCTGTATGCAGTC
SV40-5'-DTS-eGFP_fwd	USER primer for SV40-5'-DTS-eGFP donor plasmid	AGTCGGTGUGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCACGATGGTAGTGTGGGGACTC
SV40-5'-DTS-eGFP_rev	USER primer for SV40-5'-DTS-eGFP donor plasmid	ACGCAAGUGTGTCGCCCTTATTCGACTC

SV40-3'-DTS-eGFP_fwd	USER primer for SV40-3'-DTS-eGFP donor plasmid	AGTCGGTGUCGATGGTAGTGTGGGGACTC
SV40-3'-DTS-eGFP_rev	USER primer for SV40-3'-DTS-eGFP donor plasmid	ACGCAAGUTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCGTGTCGCCCTTATTCGACTC
SV40-5'/3'-DTS-eGFP_fwd	USER primer for SV40-5'/3'-DTS-eGFP donor plasmid	AGTCGGTGUGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCACGATGGTAGTGTGGGGACTC
SV40-5'/3'-DTS-eGFP_rev	USER primer for SV40-5'/3'-DTS-eGFP donor plasmid	ACGCAAGUTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCGTGTCGCCCTTATTCGACTC
GRE-5'/3'-DTS-eGFP_fwd	USER primer for GRE-5'/3'-DTS-eGFP donor plasmid	AGTCGGTGUACGGGTGGTACAGAATGTTCTTTTGGCGTCGACACGGGTGGTACAGAATGTTCTTTTGGCCGATGGTAGTGTGGGGACTC
GRE-5'/3'-DTS-eGFP_rev	USER primer for GRE-5'/3'-DTS-eGFP donor plasmid	ACGCAAGUGCCAAAAGAACATTCTGTACCACCCGTGTCGACGCCAAAAGAACATTCTGTACCACCCGTCCTTATTCGACTCACTACGC
NF-κB-5'/3'-DTS-eGFP_fwd	USER primer for NF-κB-5'/3'-DTS-eGFP donor plasmid	AGTCGGTGUCTGGGGACTTTCCAGCTGGGGACTTTCCAGCTGGGGACTTTCCAGCTGGGGACTTTCCAGGCGATGGTAGTGTGGGGACTC
NF-κB-5'/3'-DTS-eGFP_rev	USER primer for NF-κB-5'/3'-DTS-eGFP donor plasmid	ACGCAAGUCCTGGAAAGTCCCCAGCTGGAAAGTCCCCAGCTGGAAAGTCCCCAGCTGGAAAGTCCCCAGCCTTATTCGACTCACTACGC
CMV_fwd	USER primer for Cre and Bxb1 recombinase plasmid	AGTCGGTGUTGCTGATCGAGTGTAGCCAG
CMV_rev	USER primer for Cre and Bxb1 recombinase plasmid	ATAGGCTTUGATCTGACGGTTCACTAAAC
SV40pA_fwd	USER primer for Cre and Bxb1 recombinase plasmid	AAGCAGCGUCTGACTGAGATACAGCGTAC
SV40pA_rev	USER primer for Cre and Bxb1 recombinase plasmid	ACGCAAGUTAGAGATGGGCCAATACCAC
Backbone-Recombinase_fwd	USER primer for Cre and Bxb1 recombinase plasmid	ACTTGCGUGGGCCGGATTGCTATCTACC
Backbone-Recombinase_rev	USER primer for Cre and Bxb1 recombinase plasmid	ACACCGACUTCGCTATCCATCGAAGATGG
Backbone-NF-κB_fwd	USER primer for NF-κB-5'/3'-DTS donor plasmid	ACTTGCGUCTGGGGACTTTCCAGCTGGGGACTTTCCAGCTGGGGACTTTCCAGCTGGGGACTTTCCAGGAGTGAGTCGAATAAGGGCGAC
Backbone-NF-κB_rev	USER primer for NF-κB-5'/3'-DTS donor plasmid	ACACCGACUCCTGGAAAGTCCCCAGCTGGAAAGTCCCCAGCTGGAAAGTCCCCAGGCGACACCCCATAATTAGC

ROSA26_5' junction_fwd	ROSA26 amplicon for 5' junction PCR	CTAGCCTCTTGTCGCCGATT
ROSA26_5' junction_rev	ROSA26 amplicon for 5' junction PCR	CGGGATTCTCCTCCACGTCACCGCA
ROSA26_3' junction_fwd	ROSA26 amplicon for 3' junction PCR	AGAAAACACTCGGCTGGGAG
ROSA26_3' junction_rev	ROSA26 amplicon for 3' junction PCR	GTTGGGTCGCATTCAAAGCTGTCCTG
EF1α_fwd	EF1α amplicon for qRT-PCR	CAGCTTGGCACTTGATGTAATTC
EF1α_rev	EF1α amplicon for qRT-PCR	CACGACACCTGAAATGGAAGA
ACTB_fwd	ACTB amplicon for qRT-PCR	CTGGAACGGTGAAGGTGACA
ACTB_rev	ACTB amplicon for qRT-PCR	AAGGGACTTCCTGTAACAACGCA

fwd, forward primer; rev, reverse primer; LP, landing pad; SA, splicing acceptor; NLS, nuclear localization signal; NP, nucleoplasmin; DTS, DNA nuclear-targeting sequence; GRE, glucocorticoid responsive element

Supporting Table S3. Sequence information.

GOI	Sequence (5'-3')
ZeoR	ATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGG TCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGC CGGTGTGGTCCGGGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTG CCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGTACGCCGAG TGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCGAG ATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGC GTGCACTTCGTGGCCGAGGAGCAGGACTGA
TagBFP	ATGAGCGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTG GACAACCATCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACC CAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCC TGGCTACTAGCTTCCTCTACGGCAGCAAGACCTTCATCAACCACACCCAGGGCATCCC CGACTTCTTCAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATAC GAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTC ATCTACAACGTCAAGATCAGAGGGGTGAACTTCACATCCAACGGCCCTGTGATGCAG AAGAAAACACTCGGCTGGGAGGCCTTCACCGAGACGCTGTACCCCGCTGACGGCGGC CTGGAAGGCAGAAACGACATGGCCCTGAAGCTCGTGGGCGGGAGCCATCTGATCGCA AACATCAAGACCACATATAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCTGGC GTCTACTATGTGGACTACAGACTGGAAAGAATCAAGGAGGCCAACAACGAGACCTAC GTCGAGCAGCACGAGGTGGCAGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGG CACAAGCTTAATTAA
loxP	ATAACTTCGTATAGCATACATTATACGAAGTTAT
lox2272	ATAACTTCGTATAGGATACTTTATACGAAGTTAT
attP	GTGGTTTGTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGGTACAAACCCA
attP^{mut}	GTGGTTTGTCTGGTCAACCACCGCGGACTCAGTGGTGTACGGTACAAACCCA
attB	GGCCGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGG
attB^{mut}	GGCCGGCTTGTCGACGACGGCGGACTCCGTCGTCAGGATCATCCGG
ATF4	ATGACGGAGATGAGCTTTTTGTCTTCCGAGGTCTTGGTAGGTGATCTGATGTCACCCTT TGATCAGTCTGGGCTGGGTGCGGAAGAAAGTCTGGGACTTCTGGACGATTACCTGGAA GTAGCGAAGCACTTCAAGCCGCACGGGTTTTCAAGTGATAAGGCGAAAGCGGGATCT AGTGAGTGGCTTGCAGTCGATGGCCTTGTTTCTCCGTCAAATAATTCCAAGGAGGATG CCTTTTCCGGGACCGACTGGATGCTCGAGAAAATGGATCTCAAAGAGTTTGACCTTGA TGCGCTCCTTGGCATAGACGATCTTGAAACGATGCCGGACGATCTGCTTACTACTTTG GATGACACATGTGATCTGTTCGCGCCACTTGTTCAGGAGACCAATAAACAACCGCCTC AAACGGTCAACCCCATTGGACACCTGCCAGAGAGTTTGACGAAGCCTGACCAGGTAG CCCCCTTCACATTCCTTCAGCCATTGCCACTCAGCCCCGGCGTCCTTTCATCCACGCCT GATCACAGTTTCTCCCTCGAACTGGGAAGTGAAGTCGACATCACTGAGGGAGATAGG AAGCCGGATTATACAGCATATGTGGCGATGATCCCACAATGCATCAAAGAAGAGGAC ACGCCAAGTGACAACGATAGTGGCATTTGTATGAGCCCTGAGAGTTACTTGGGCTCTC CACAGCATTCCCCCAGTACCAGAGGTAGTCCTAACCGCAGCCTTCCATCACCAGGGGT TCTTTGTGGGAGTGCCCGGCCCAAACCATACGACCCACCGGGAGAAAAGATGGTTGC GGCTAAGGTTAAGGGCGAGAAATTGGATAAGAAGCTCAAAAAAATGGAGCAAAATA AAACGGCTGCGACTCGCTATCGACAAAAGAAGCGGGCGGAGCAGGAAGCCCTCACAG GGGAGTGTAAAGAACTGGAGAAGAAAAACGAAGCTCTTAAAGAGCGGGCAGACTCTT TGGCCAAGGAGATTCAGTACCTCAAAGATCTCATCGAAGAGGTGAGGAAGGCGAGGG GGAAAAAGCGGGTGCCGTAG
ATF6c	ATGGGAGAACCGGCCGGTGTAGCCGGTACTATGGAAAGTCCCTTCTCACCCGGCCTCT TCCACAGATTGGACGAAGACTGGGATAGTGCGCTGTTTGCAGAGTTGGGATACTTTAC AGATACTGATGAGCTCCAGCTTGAGGCTGCCAATGAGACATACGAGAATAACTTTGAC

	AACCTTGACTTCGACCTTGACCTTATGCCTTGGGAATCTGACATATGGGATATCAACA ACCAGATATGTACCGTTAAGGATATAAAGGCAGAACCTCAACCGTTGTCCCCTGCATC ATCCAGTTACTCAGTGAGTTCCCCCCGATCAGTTGACTCTTACAGTAGTACACAGCAC GTTCCGGAGGAATTGGACCTTTCCTCCTCTAGTCAGATGAGCCCACTTAGCCTGTACG GGGAGAACTCCAATAGCTTGAGCAGTGCTGAACCGCTGAAGGAGGATAAACCCGTCA CTGGACCCCGGAACAAAACGGAGAACGGACTTACACCAAAAAAGAAAATACAGGTC AACTCAAAACCTAGTATACAACCTAAACCTCTTCTGCTTCCAGCGGCTCCAAAAACGC AGACTAATTCAAGCGTTCCCGCCAAAACCATCATTATTCAAACAGTTCCAACGCTTAT GCCACTCGCCAAGCAGCAACCCATCATTAGTTTGCAGCCGGCCCCCACGAAGGGCCA AACTGTACTTTTGTCTCAACCGACAGTTGTCCAACTCCAAGCGCCTGGCGTTTTGCCGT CAGCACAACCGGTCCTGGCTGTTGCTGGAGGAGTTACACAACTGCCTAACCATGTTGT TAATGTGGTCCCAGCACCATCAGCCAATTCTCCGGTCAACGGGAAGCTGAGCGTAACT AAACCGGTGCTCCAAAGCACGATGCGCAACGTCGGATCCGACATAGCTGTCCTCCGA AGGCAACAGCGGATGATAAAGAATAGGGAAAGCGCGTGCCAGTCCAGAAAAAAGAA AAAGGAGTATATGCTTGGGCTGGAGGCACGGCTCAAGGCGGCGCTGTCTGAAAACGA ACAGCTCAAGAAGGAAAACGGGACTCTGAAAAGACAACTGGATGAAGTAGTCTCAGA AAACCAACGCTTGAAAGTCCCGAGCTAG
XBP1s	ATGGTTGTCGTAGCTGCAGCCCCAAACCCTGCTGACGGCACTCCGAAAGTGTTGCTGC TTTCAGGACAACCTGCCTCTGCCGCCGGTGCACCCGCAGGGCAAGCCCTGCCCTTGAT GGTACCTGCTCAAAGGGGCGCTTCTCCCGAGGCTGCTAGTGGCGGGCTTCCACAAGCC CGGAAACGCCAACGCCTTACCCATCTTTCCCCAGAAGAAAAGGCTCTCCGGAGGAAA CTCAAGAATAGAGTAGCCGCTCAGACAGCACGAGACCGGAAGAAGGCCCGGATGTCC GAACTTGAGCAGCAGGTGGTTGACTTGGAGGAGGAAAACCAAAAATTGTTGTTGGAA AATCAACTCTTGCGAGAGAAAACCCACGGATTGGTGGTAGAAAACCAAGAACTGAGA CAGCGACTCGGGATGGACGCGCTTGTAGCAGAAGAAGAAGCTGAAGCTAAGGGCAAC GAGGTGCGGCCGGTTGCCGGCAGTGCAGAATCAGCGGCCGGAGCAGGACCAGTTGTC ACTCCTCCGGAGCACTTGCCTATGGACAGTGGCGGGATAGACTCTAGTGACAGTGAAT CAGATATACTCCTCGGCATCTTGGATAACCTTGACCCCGTAATGTTTTTTAAGTGTCCT TCCCCCGAGCCGGCGTCCCTGGAAGAGTTGCCAGAGGTCTATCCGGAAGGTCCCTCTT CCCTTCCCGCGTCCCTGAGTCTGTCAGTTGGTACTTCTTCCGCTAAATTGGAGGCGATT AACGAACTGATTCGATTTGATCATATCTATACAAAACCACTTGTCTTGGAGATCCCCA GTGAGACCGAGTCCCAAGCCAATGTGGTGGTGAAGATTGAAGAAGCTCCTTTGTCACC CTCAGAAAACGATCATCCCGAATTTATAGTATCAGTCAAGGAGGAACCCGTAGAGGA TGATCTTGTTCCTGAGCTGGGGATAAGTAATCTCCTGAGCTCTAGCCACTGCCCGAAG CCAAGCTCATGCCTGCTCGACGCATATTCTGACTGTGGATACGGGGGTTCCCTTAGCC CGTTTAGCGACATGAGCTCTCTTTTGGGTGTCAATCATTCCTGGGAAGATACCTTTGCA AACGAGCTCTTCCCCCAACTCATAAGCGTCTAG
NLS	**Sequence**
SV40	CCCAAGAAGAAGAGGAAGGTA
cMyc	CCTGCTGCCAAACGCGTTAAACTAGAC
NP	AAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAG
DTS	**Sequence** (linker sequences are underlined)
SV40	GGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCA ATTAGTCAGCAACCA
GRE	ACGGGTGGTACAGAATGTTCTTTTGGC<u>GTCGAC</u>ACGGGTGGTACAGAATGTTCTTTTG GC
NF-κB	<u>CTG</u>GGGACTTTCC<u>AGCTG</u>GGGACTTTCC<u>AGCTG</u>GGGACTTTCC<u>AGCTG</u>GGGACTTTCC <u>AGG</u>

GOI, gene of interest; NLS, nuclear localization signal; NP, nucleoplasmin; DTS, DNA nuclear-targeting sequence; GRE, glucocorticoid responsive element

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