

Supplementary Material

Endogenous p21-Dependent Transgene Control for CHO Cell Engineering

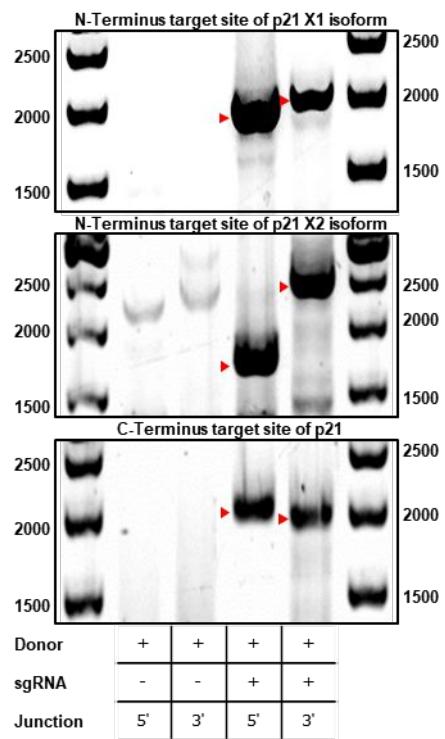
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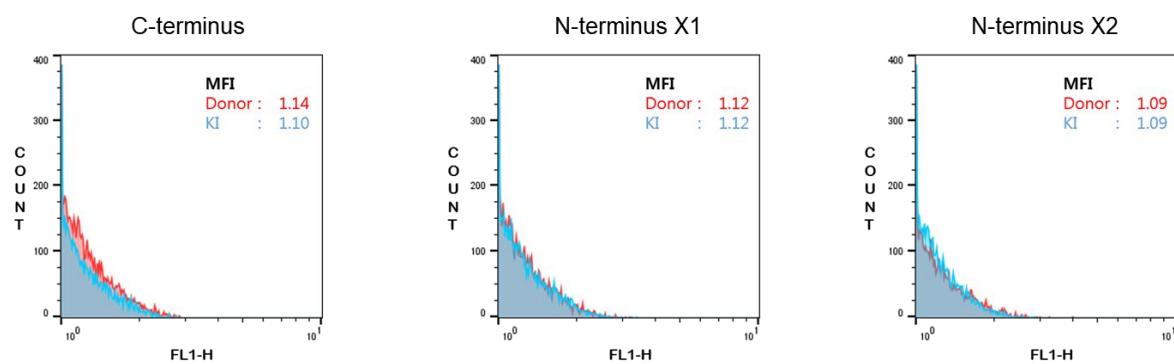
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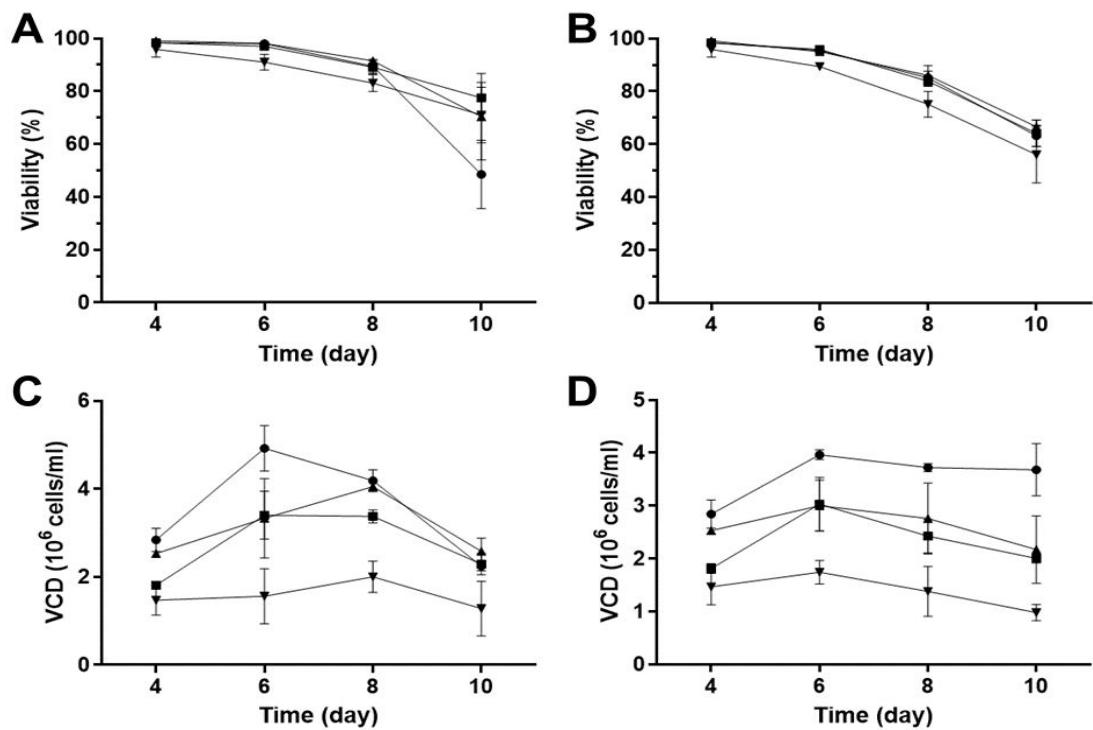
FIGURES



Supplementary Figure S1. Validation of the targeted integration by junction PCR analysis in transfected mixtures of control and knock-in populations. The expected size of 5' and 3' junction PCR amplicons in knock-in populations was 1.9 kb and 2 kb in N-terminus X1, 1.7 kb and 2.5 kb in N-terminus X2, and 2.1 kb and 2 kb in C-terminus (red arrowheads).



Supplementary Figure S2. Flow cytometry analysis of EGFP-hBcl-2 expression in the p21 targeting cells 3 days after transfection with donor plasmids (Donor) or donor plasmids and Cas9/sgRNA expression vectors (KI). Mean fluorescence intensity (MFI) of each sample was shown.



Supplementary Figure S3. Growth profiles during six-well plate cultures with 0.125 mM hydroxyurea. Viability in the presence of (A) vehicle control (DMSO) or (B) 0.125 mM hydroxyurea. Cell growth in the presence of (C) DMSO or (D) 0.125 mM hydroxyurea. GSR (closed circle), C-term tagging clone (closed square), random sorting pool (closed triangle), and random clone (inverted triangle).

TABLES

Supplementary Table S1. Plasmids used in this study

| Plasmid name | Description | Reference |
|-----------------------------------|---|-----------------|
| Cas9-2A-mCherry | Cas9 2A peptide-linked mCherry without specific targeting sgRNA expression control (Addgene #64324) | Chu et al. 2015 |
| pEGFP-C1-hBcl-2 expression vector | GFP-hBcl-2 fusion vector having neomycin selection marker | Clontech |
| Cas9-p21_C-term sgRNA | sgRNA/Cas9 vector [Target genomic sequence: CTATCATTCTAACGCGCCGGCTGG] | This study |
| Cas9-p21_N-term sgRNA_X1 | sgRNA/Cas9 vector [Target genomic sequence: TTCTTCGGCCTCCTCAACGGAGG] | This study |
| Cas9-p21_N-term sgRNA_X2 | sgRNA/Cas9 vector [Target genomic sequence: GACGTCATCGGGACCCGACATGG] | This study |
| p21 donor_C-term | Circular donor targeting C-terminus of p21 [Target GOI: T2A-EGFP-hBcl-2] | This study |
| p21 donor_N term-X1 | Circular donor targeting N- terminus of p21 X1 isoform [Target GOI: EGFP-hBcl-2- T2A] | This study |
| p21 donor_N term-X2 | Circular donor targeting N- terminus of p21 X2 isoform [Target GOI: EGFP-hBcl-2- T2A] | This study |

Supplementary Table S2. Primer sequences

| Primer name | Purpose | Sequence (5'-3') |
|--------------------------|--------------------------------------|---------------------------------|
| Donor plasmid | | |
| pcDNA3.1 backbone_O5_fwd | USER PCR primer for donor plasmids | ACTTGCAGTTTCGGGGAAATGTGCG |
| pcDNA3.1 backbone_LA_rev | USER PCR primer for donor plasmids | ACACCGACUCAGTCGGAAACCTGTCGTG |
| CDKN1A 5' arm_LA_fwd | USER PCR primer for p21 donor_C-term | AGTCGGTGUACCTCTCAGGGTCGAAA |
| CDKN1A 5' arm_LB_rev | USER PCR primer for | ATGACGTCUCCGTGGCCCAGATAGGCACCCA |

| | | |
|-----------------------------|--|---|
| | p21 donor_ C-term | |
| CDKN1A 3' arm_LC_fwd | USER PCR primer for p21 donor_ C-term | AGACGTCAUTAATGTGCCACAGGAGGCCT |
| CDKN1A 3' arm_O5_rev | USER PCR primer for p21 donor_ C-term | ACGCAAGUACCTTCCAGGTGCCAGAGAT |
| 2A-hBcl2- pEGFPC1_LB_fwd | USER PCR primer for p21 donor_ C-term | AAGCAGCGU GGCAGTGGAGAGGGCAGAGGAAGTCTGCTAAC ATGCGGTGACGTCGAGGAGAAATCCTGGCCCAA TGGTGAGCAAGGGCGAG |
| 2A-hBcl2- pEGFPC1_LC_rev | USER PCR primer for p21 donor_ C-term | ATGACGTCU CTTGTGGCCCAGATAGGCACCCA |
| NX1_5'Arm_LA_Fwd | USER PCR primer for p21 donor_ N term-X1 | AGTCGGTGU CCTGGAAAGTAAGTCCCAT |
| NX1_5'Arm_LB_Rev | USER PCR primer for p21 donor_ N term-X1 | ACGCTGCTU TCCTCAATGGAGGCTTCTCGGCCTCCTCAACG GAGGCTC |
| N-Term GOI fwd | USER PCR primer for p21 donor_ N-term | AAGCAGCGUATGGTGAGCAAGGGCGAGGA |
| N-Term GOI Rev | USER PCR primer for p21 donor_ N-term | ATGACGTCUTGGGCCAGGATTCTCCTCGACGT CACCGCATGTTAGCAGACTCCTCTGCCCTCTC CACTGCCCTTGTGGCCAGATAGGC |
| NX1_3' arm_LC_fwd | USER PCR primer for p21 donor_ N term-X1 | AGACGTCAUATGGCCTCCAAAGGTTGG |
| NX1_3' arm_O5_rev | USER PCR primer for p21 donor_ N term-X1 | ACGCAAGUGAACATAGCCAGCCTCCCCAGT |
| NX2_5'Arm_LA_Fwd | USER PCR primer for p21 donor_ N term-X2 | AGTCGGTGUATATTGGGGTCACCCAGCA |
| NX2_5'Arm_LB_Rev | USER PCR primer for p21 donor_ N term-X2 | ACGCTGCTUGGTGGCGCCTGCGGCTGAAA |
| NX2_CDKN1A 3' arm_LC_fwd | USER PCR primer for p21 donor_ N term-X2 | AGACGTCAU ATGTCGGGTCCCGATGAC |
| NX2_CDKN1A | USER PCR primer for p21 donor_ N term-X2 | ACGCAAGU TGGGTTCTAGGTCAGTGCTCTAC |

| | | |
|--------------------|--|---------------------------------|
| 3' arm_05_rev | | |
| BglII-hBcl-2_fwd | PCR primer for fusing hBcl-2 coding sequence with pEGFP-C1 | CTTAGATCTATGGCGCACGCTGGGAGAACAA |
| hBcl-2-HindIII_rev | PCR primer for fusing hBcl-2 coding sequence with pEGFP C1 | AGTAAGCTTCACTTGTGGCCCAGATAGGC |

5'/3' junction PCR

| | | |
|------------------------|--|----------------------|
| 5'Arm_Junction_fwd | 5' junction PCR primer for C-terminus of p21 | GTCTTGTGTTCAGCCGCAG |
| hBclII_5'Arm_Rev2 | 5' junction PCR primer for N/C-terminus of p21 | CCAGCGTGCGCCATAGATCT |
| hBclII_3'Arm_Fwd | 3' junction PCR primer for N/C-terminus of p21 | AGCAAAGACCCCAACGAGAA |
| 3' arm_Junction rev | 3' junction PCR primer for C-terminus of p21 | GGCTAGGGGCAAACAGTGTA |
| 5'Arm_NX1_fwd_K2 | 5' junction PCR primer for N- terminus of p21 X1 isoform | CCTGCACGCCTGTGATAAGA |
| 3' arm_Junction rev_K2 | 3' junction PCR primer for N- terminus of p21 X1 isoform | AGAAAGCTAGTCCCGACCCA |
| NX2_5'Arm_Fwd_5 | 5' junction PCR primer for N- terminus of p21 X2 isoform | AGGAGGAGATGCACACTTGC |
| NX2_3'Arm_Rev_5 | 3' junction PCR primer for N- terminus of p21 X2 isoform | AGGTGACAAGGAGACCCCAA |

sgRNA expression vector

| | | |
|-----------------------|--|---------------------------|
| CDKN1A_sgRNA1_fwd | Cas9-p21_C-term sgRNA expression vector | CACCGCTATCATTCTAACGCCGGC |
| CDKN1A_sgRNA1_rev | Cas9-p21_C-term sgRNA expression vector | AAAACGCCGGCGCTTAGAATGATAG |
| CDKN1A_N-sgRNA_X1_fwd | Cas9-p21_N-term sgRNA_X1 expression vector | CACCGTTCTCGGCCTCCTAACCGG |
| CDKN1A_N-sgRNA_X1_rev | Cas9-p21_N-term sgRNA_X1 expression vector | AAACCCGTTGAGGAGGCCGAAGAA |
| CDKN1A_N- | Cas9-p21_N-term sgRNA_X2 | CACCGGACGTCATCGGGACCCGACA |

| | | |
|-----------------------|---|--------------------------|
| sgRNA_X2_fwd | expression vector | |
| CDKN1A_N-sgRNA_X2_rev | Cas9-p21_N-term sgRNA_X2 expression vector | AAACTGTCGGGTCCCGATGACGTC |
| qRT-PCR | | |
| GFP_1_fwd | GFP amplicon for qRT-PCR [Amplicon size: 112 bp] | GAACCGCATCGAGCTGAA |
| GFP_1_rev | GFP amplicon for qRT-PCR [Amplicon size: 112 bp] | TGCTTGTGCCATGATATAG |
| hBcl-2_fwd | hBcl-2 amplicon for qRT-PCR [Amplicon size: 86 bp] | GGATGCCTTGTGGAAGTGTA |
| hBcl-2_rev | hBcl-2 amplicon for qRT-PCR [Amplicon size: 86 bp] | CCAAACTGAGCAGAGTCTTCAG |
| GAPDH_fwd | GAPDH amplicon for qRT-PCR [Amplicon size: 109 bp] | TTGTCATCAACGGGAAGG |
| GAPDH_Rev | GAPDH amplicon for qRT-PCR [Amplicon size: 109 bp] | GTGAAGACGCCAGTAGATT |
| P21_Fwd | P21 amplicon for qRT-PCR [Amplicon size: 117 bp] | TTGAGCCGCGATTGTGAT |
| P21_Rev | P21 amplicon for qRT-PCR [Amplicon size: 117 bp] | AACGCCCTCCAAACATA |