Supporting Information

Enhancing Protein Production Yield from CHO Cells by CRISPR Interference (CRISPRi)

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Running Title: CRISPRi to enhance protein yield in CHO cells

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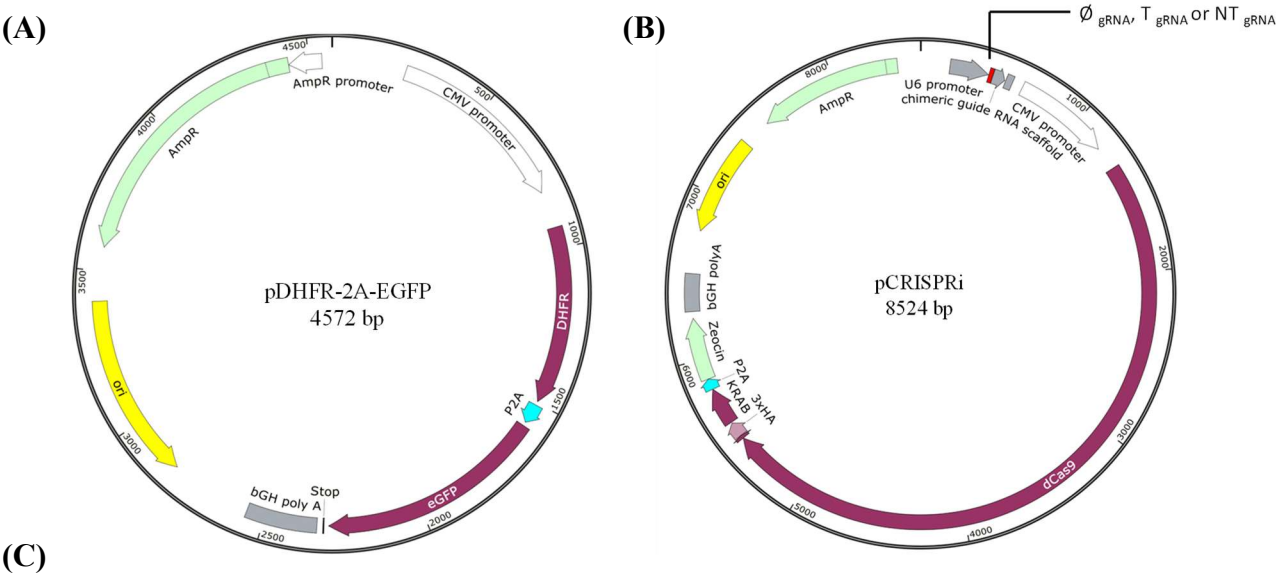

(C)

DHFR gene sequence

Transcription start site(+1)

...TAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTC

CMV promoter

TGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTT

Translation start site

GGTACCGAATTCGCCACCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGA

PAM NT gRNA

TTGGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGA

T gRNA PAM

CCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCTCCAT
TCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATAGTTCTCAGTAGAGAACTCAAAGAACCA
CCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGACTTATTGAACAACCGGAATT
GGCAAGTAAAGTAGACATGGTCTGGATAGTCGGAGGCAGTTCTGTTTACCAGGAAGCCATGAATCA
ACCAGGCCACCTCAGACTCTTTGTGACAAGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCCCA
GAAATTGATTTGGGGAAATATAAACTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGG
AAAAAGGCATCAAGTATAAGTTTGAAGTCTACGAGAAGAAAGGCGGATCCACCGGT

Spacer sequence in Ø $_{gRNA}$:CGGGTCTTCGAGAAGACCTG
Spacer sequence in T $_{gRNA}$: GCAAGAACGGAGACCTACCC
Spacer sequence in NT $_{gRNA}$: ACGGCGACGATGCAGTTCAA

Fig. S1. Vector and gRNA design. (A) Map of pDHFR-2A-EGFP. (B) Map of pCRISPRi plasmids. The plasmids harbored two cassettes: one co-expressing dCas9-KRAB fusion protein and Zeocin^R while the other expressing different gRNA ($Ø_{gRNA}$, T_{gRNA} or NT_{gRNA}). (C) DHFR sequence, gRNA targeting sites and spacer sequence in the gRNA. PAM, protospacer adjacent motif. T_{gRNA} targeted +158~177 (from the transcription start site) of the template strand while NT_{gRNA} targeted +137~118 of the non-template strand.

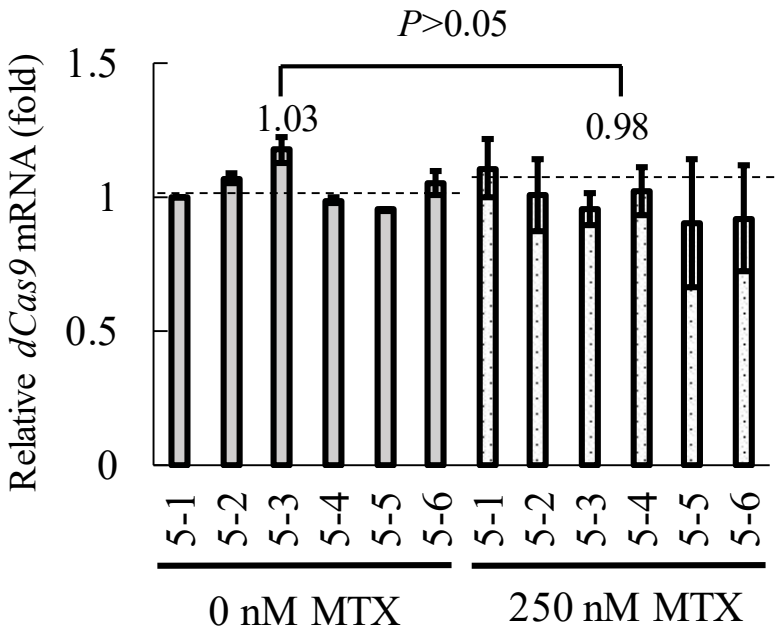

Fig. S2. dCas9 mRNA level after 250 MTX treatment. To confirm the dCas9 stability during the MTX selection and gene amplification process, *dCas9* mRNA levels of all 6 clones in the G-NT group were analyzed by qRT-PCR. The *dCas9* mRNA levels for each clone were measured 3 times and normalized to that in clone 5-1 at 0 nM MTX. The average dCas9 mRNA levels were statistically similar (p>0.05) at 0 nM MTX (1.03) and 250 nM MTX (0.98), indicating that the CRISPRi system remained functional during the MTX selection process.

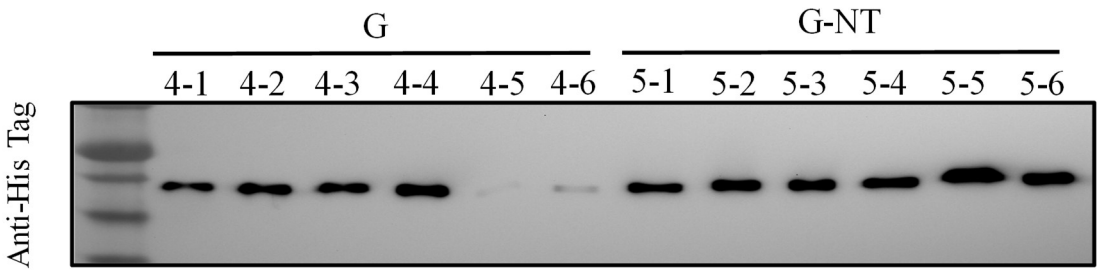

Fig. S3. G-CSF protein expression level after 250 nM MTX treatment. After gene amplification process, the culture supernatant from each clone was analyzed by Western blot using anti-His antibody because G-CSF was fused with the His_6 tag.

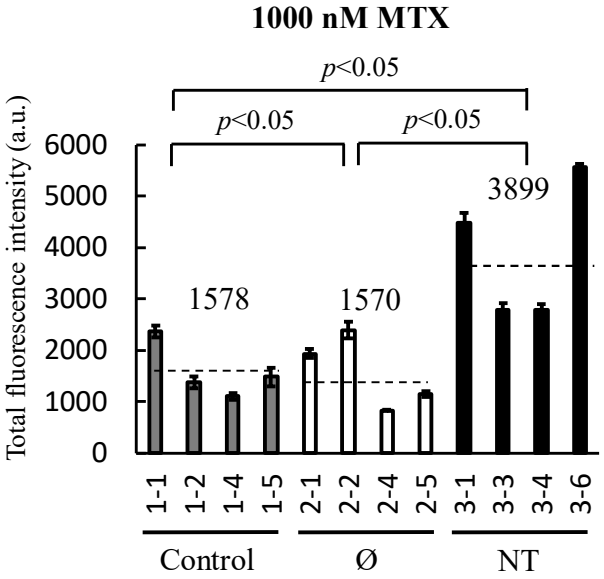

Fig. S4. EGFP expression after 1000 nM MTX selection. The cell clones after 250 nM MTX selection as shown in Fig. 3 continued to be selected using 1000 nM MTX. The average total FI for the Control, Ø and NT groups were 1578, 1570 and 3899 a.u., respectively. The extent of increase from 250 nM to 1000 nM MTX was ≈43%, probably because the excessive accumulation of intracellular EGFP provoked cellular stress and toxicity.

Primer Name	Primer sequence
Q CHO β-actin F	ATGACGATATCGCTGCGCTC
Q CHO β-actin R	ATCACGCCCTGGTGCCTA
Q mDHFR F	GAATCAACCAGGCCACCTCA
Q mDHFR R	ACTTGATGCCTTTTTCCTCCTG
Q EGFP F	TGAACTTCAAGATCCGCCACA
Q EGFP R	TTCTCGTTGGGGTCTTTGCT
Q dCas9 F	AATGGCATCCGAGACAAGCA
Q dCas9 R	TGTGCTCGTGAAGACTGTCC
Q G-CSF F	CACTCCGGCCTGTTTCTGTA
Q G-CSF R	CCAGATGGTGGTGGCGAAAT

Table. S1. Primer sequences of qRT-PCR and genomic DNA Q-PCR. The qRT-PCR and Q-PCR (for genomic DNA quantification) shared the same primers for DHFR and EGFP.