

Supplementary Materials

High-Level Production of scFv-Fc Antibody Using an Artificial Promoter System with Transcriptional Positive Feedback Loop of Transactivator in CHO Cells

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Supplementary Figure Legends

Figure S1. Flow cytometry analysis of GFP expression in CHO/aTF_GFP cells. (a) During cultivation at normal temperature (37°C) (turned “on” state). (b) After 8 days of Dox treatment. (c) After transient transfection of pCMV/aTF plasmid into Dox-treated cells.

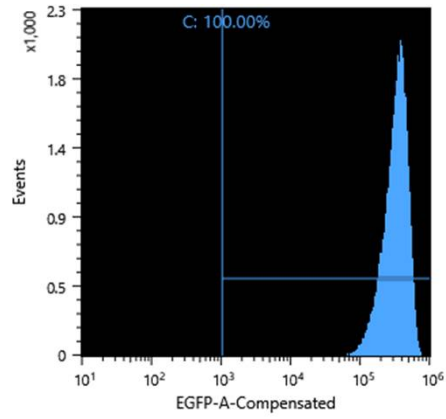
Figure S2. Average values of scFv-Fc, glucose, and lactate in the culture supernatant obtained from semi-continuous culture of CHO/aTF_scFv-Fc2 cells. (a) scFv-Fc concentration. (b) scFv-Fc-specific production rate. (c) Glucose concentration. (d) Lactate concentration.

Figure S3. Scatter plots from DNA microarray analysis of CHO/aTF_scFv-Fc2 cells in semi-continuous culture. (a) Global gene expression signal values between each seeding cell density condition. (b) Signal values for genes that were upregulated with Z-scores of 5 or higher during low-temperature culture under all seeding cell density conditions.

Figure S4. Genes obtained from characteristic functional clustering analysis in DNA microarray analysis of CHO/aTF_scFv-Fc2 cells in semi-continuous culture. List of upregulated genes with the most significant differences in “Biological process” and “Cellular component.”

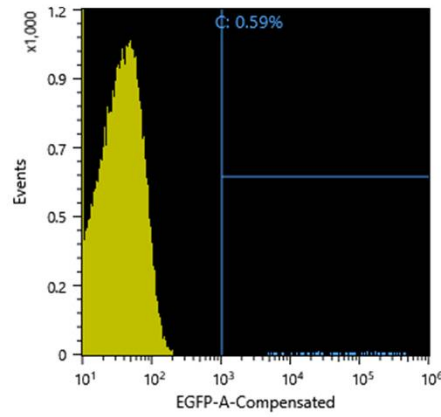
a

Turn “on” state
in CHO/aTF_GFP



b

Turn “off”
by Dox addition



c

Turn “on” again by
transient transfection
of pCMV/aTF

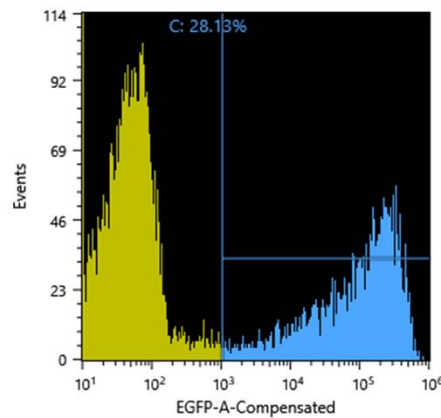


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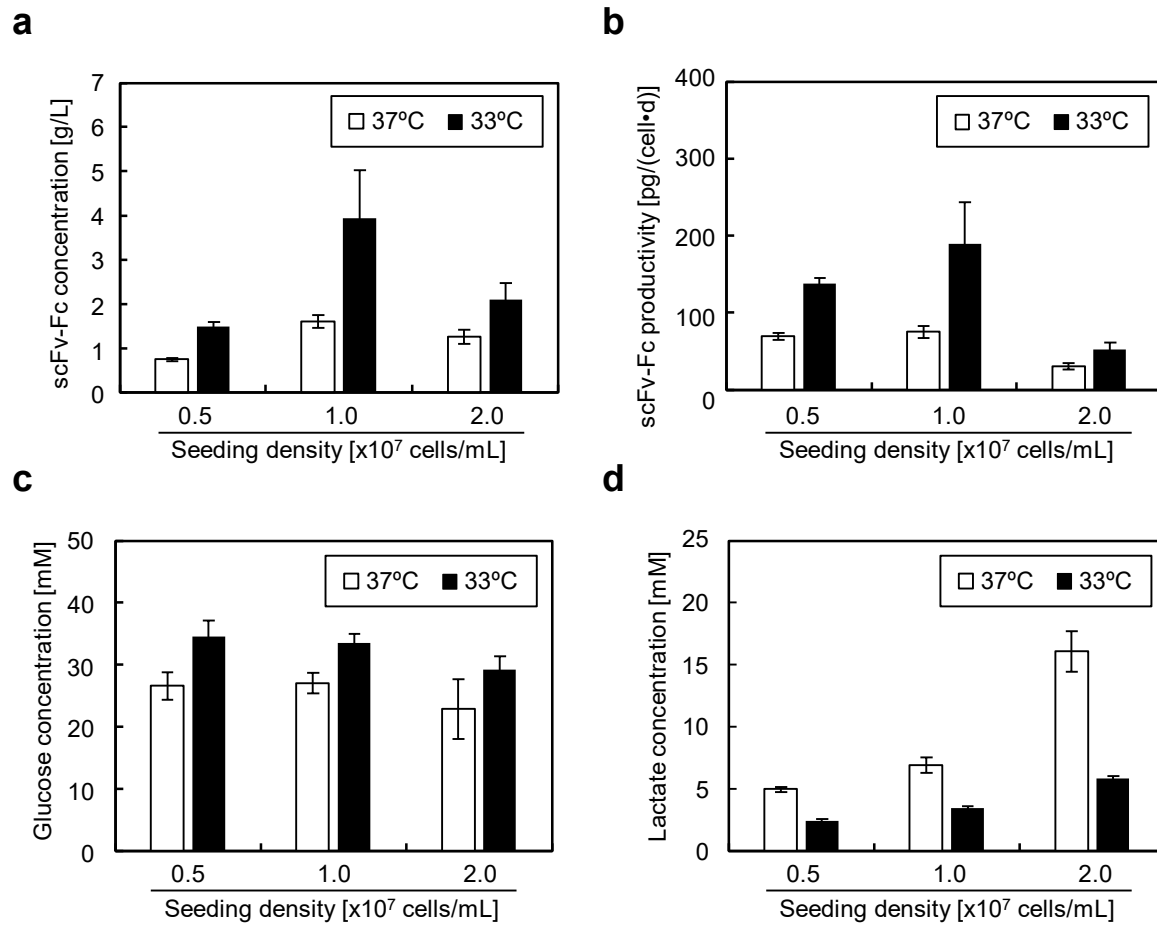


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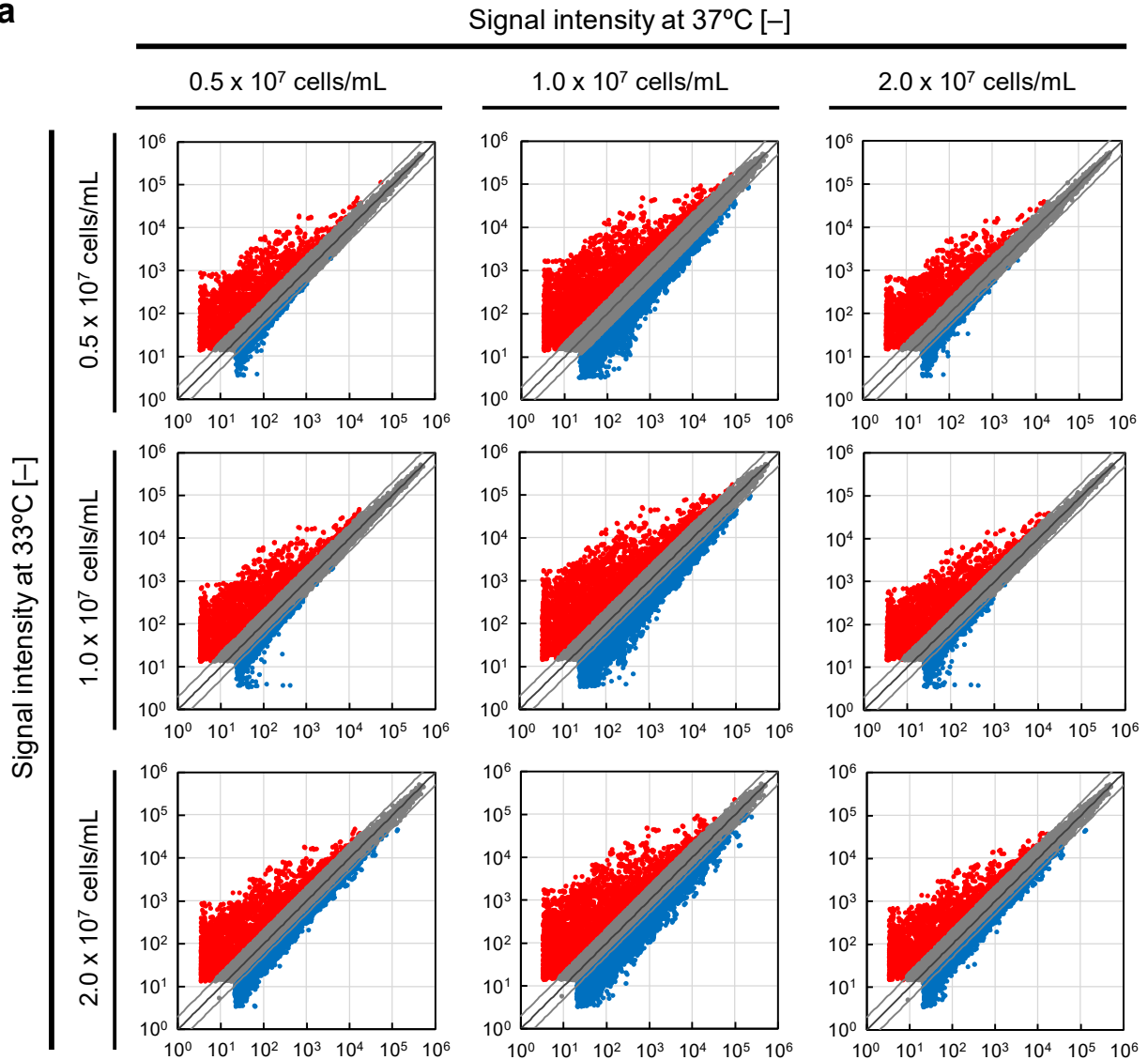
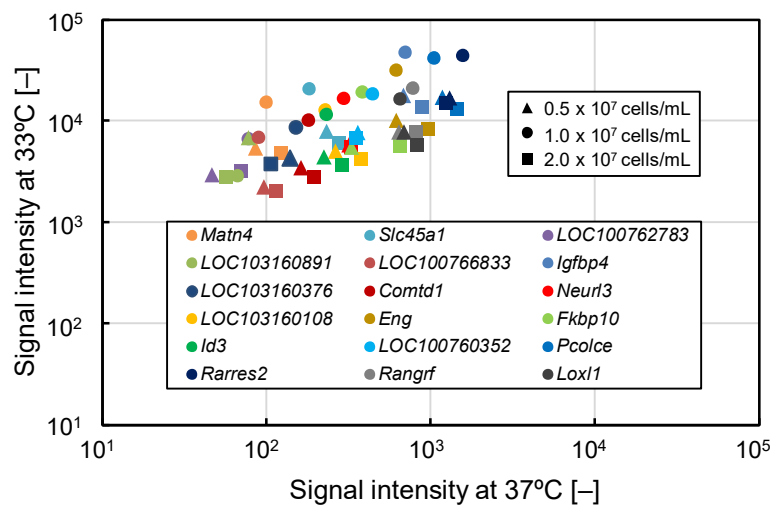
a**b**

Figure S3. Scatter plots from DNA microarray analysis of CHO/aTF_scFv-Fc2 cells in semi-continuous culture. (a) Global gene expression signal values between each seeding cell density condition. (b) Signal values for genes that were upregulated with Z-scores of 5 or higher during low-temperature culture under all seeding cell density conditions.

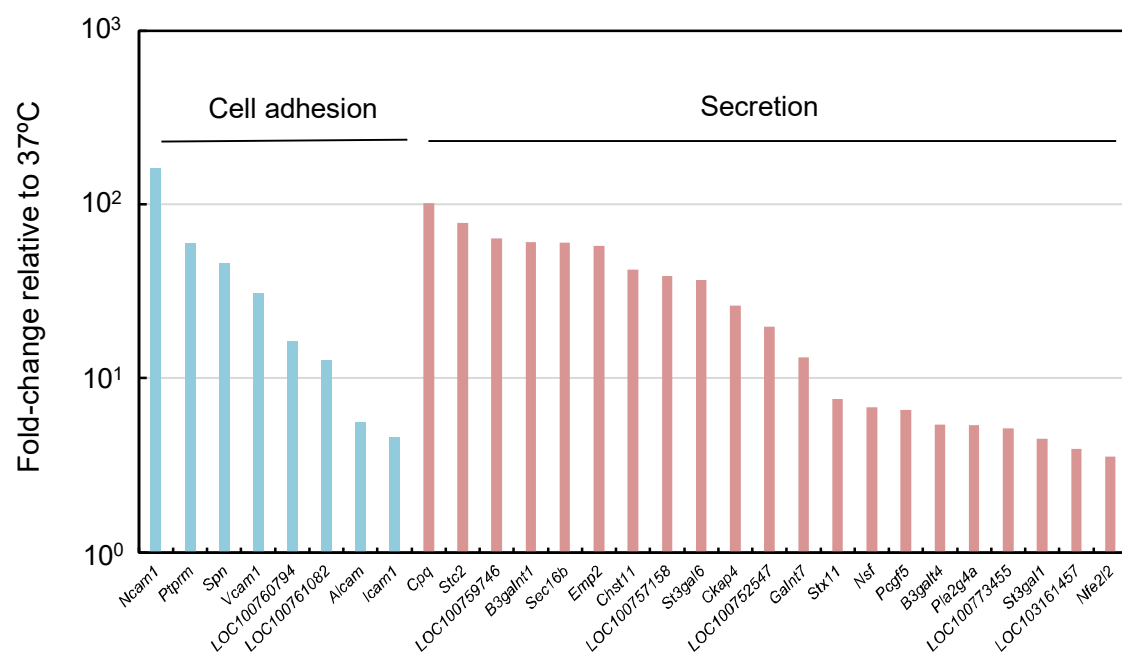


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Table S1 Primer and probe lists for evaluation of transgene copy number by quantitative PCR

Target	Oligo type [#]	Sequence (5'→3')
GFP	F	CGAGGACAGCGTGATCTTC
	R	CCACGGTGGCGTTGCT
	P	CCGACAAGATCATCC
scFv-Fc [§]	F	AGTGCTGCACCAGGATTGG
	R	CTTGTTGGACACCTTGCACTTG
	P	TGAACGGCAAAGAGT
scFv-Fc	F	TGGAGTGGGAGAGCAATGG
	R	CGGGAGGCGTGGTCTTG
	P	AGCCGGAGAACAAC

[#]F, Forward primer; R, Reverse primer; P, Taqman probe. The probes were labeled with FAM dye 5' end and minor groove binder as a quencher at 3' end.

[§]scFv-Fc gene in donor vector plasmid using Cre-RMCE was codon-optimized based on human codon usage.

Table S2. Up-regulated genes in low temperature culture at 33°C

Gene symbol	Gene description	Fold-change (Z-score)		
		0.5 x 10 ⁷ cells/mL	1.0 x 10 ⁷ cells/mL	2.0 x 10 ⁷ cells/mL
<i>Matn4</i>	matrilin 4	61.78 (9.73)	154.23 (6.45)	39.16 (7.64)
<i>Slc45a1</i>	solute carrier family 45, member 1	33.46 (8.29)	114.80 (8.47)	21.65 (6.41)
<i>LOC100762783</i>	synaptonemal complex protein 3-like	45.42 (9.01)	86.76 (5.71)	61.83 (5.59)
<i>LOC103160891</i>	synaptonemal complex protein 3-like	43.64 (5.22)	85.26 (5.69)	48.57 (5.27)
<i>LOC100766833</i>	cytochrome b-245 light chain	22.81 (7.38)	77.54 (5.57)	17.41 (5.95)
<i>Igfbp4</i>	insulin-like growth factor binding protein 4	25.50 (12.92)	68.03 (7.54)	15.45 (7.39)
<i>LOC103160376</i>	late cornified envelope protein 1C-like	31.22 (8.12)	57.14 (5.18)	35.44 (7.43)
<i>Comtd1</i>	catechol-O-methyltransferase domain containing 1	20.78 (7.16)	57.04 (5.17)	14.29 (5.54)
<i>Neurl3</i>	neuralized E3 ubiquitin protein ligase 3	17.89 (6.81)	56.84 (7.22)	16.67 (5.86)
<i>LOC103160108</i>	multivesicular body subunit 12B	18.72 (6.92)	56.54 (5.16)	11.04 (5.01)
<i>Eng</i>	endoglin	16.20 (11.11)	51.49 (7.04)	8.64 (5.82)
<i>Fkbp10</i>	FK506 binding protein 10, 65 kDa	16.29 (6.59)	51.14 (7.03)	8.76 (5.86)
<i>Id3</i>	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	19.20 (6.98)	49.98 (5.01)	12.61 (5.28)
<i>LOC100760352</i>	serpin B6	20.79 (7.17)	42.12 (6.69)	19.25 (6.16)
<i>Pcolce</i>	procollagen C-endopeptidase enhancer	14.19 (10.58)	39.88 (6.59)	8.88 (5.90)
<i>Rarres2</i>	retinoic acid receptor responder (tazarotene induced) 2	12.83 (10.18)	28.49 (7.18)	12.11 (6.74)
<i>Rangrf</i>	RAN guanine nucleotide release factor	11.99 (9.91)	27.22 (5.91)	9.44 (6.06)
<i>Loxl1</i>	lysyl oxidase-like 1	11.14 (9.62)	25.43 (5.79)	6.95 (5.24)

Table S2