Supporting Information

Enhanced secretion of recombinant proteins via signal recognition particle (SRP)-dependent secretion pathway by deletion of *rrsE* in *Escherichia coli*

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SDS-PAGE analysis and western blotting. The expression level of recombinant proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue (Bio-Rad) staining. After cultivation, cell densities were normalized by OD_{600} of 2, and 1 ml of the normalized cells were harvested by centrifugation at 6,000 rpm for 5 min at 4°C. For the preparation of total protein samples, the cells were disrupted by using BugBuster® Master Mix (Novagen), and then the lysates were mixed with SDS-PAGE sample buffer followed by boiling for 10 min. For the periplasmic fraction samples, the harvested cells were osmotically fractionized by using periplasting buffer (200 g/L of sucrose, 300 mg/L of lysozyme, 1 mM of EDTA, and 200 mM of Tris-HCl; pH 7.5). The periplasmic fractions were mixed with SDS-PAGE sample buffer followed by boiling for 10 min. The protein samples were then analyzed on 8 to 15% SDS-PAGE according to the standard procedures[31]. To analyze the expression of IgG, Western blot analysis was performed after SDS-PAGE running. In addition, non-reducing sample buffer (without β-mercaptoethanol) was used for analyzing the correct assembly of IgG. After the gel electrophoresis, proteins were transferred to polyvinylidene fluoride (PVDF; Roche) membranes, and then those were blocked with 5% (w/v) skim milk solution in Tris-buffered saline containing Tween-20 (TBS-T; 10 mM of Tris, 150 mM of NaCl, and 0.05% of Tween-20; pH 8.0) for 1 h. The membranes were then incubated with a 1:5000 diluted horseradish peroxidase (HRP)-conjugated goat anti-human IgG (H+L) antibody (Jackson ImmunoResearch Labs) dissolved in TBS-T containing 5% skim milk for 2 h. After four times washing with TBS-T, immunoreactive bands were detected by using Amersham™ ECL™ Prime (GE Healthcare) and visualized on X-ray film (AGFA). To analyze the periplasmic expression of endogenous DsbA, same Western blot procedures were performed with normal sample buffer (with β-mercaptoethanol). In addition, different antibodies—mouse anti-*E. coli* DsbA (GeneTex, Inc.; San Antonio, TX, US; primary

antibody) and rabbit anti-mouse IgG-HRP (Abcam; Cambridge, MA, US; secondary antibody)—were used for detection of immunoreactive bands.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). For the quantification of several transcripts, reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR) was performed. The primer sets for performing RT-qPCR were designed by using Primer3web (http://primer3.ut.ee/), and the resulting primer sequences were listed in Table S2. Total RNA extracts of *E. coli* were prepared by using RNeasy Mini Kit (Qiagen), and it was subjected to perform the RT-qPCR. One-step RT-qPCR was carried out by using One Step SYBR® PrimeScript™ RT-PCR Kit (Takara Bio) according to the manufacture's protocol. Thermal cycling and real time monitoring of DNA synthesis were carried out using LightCycler® 96 System (Roche).

Table S1. *E. coli* strains and plasmids used in this study

E. coli or plasmid	Genotype or description	Source
E. coli strains		
MG1655	*F⁻ lambda⁻ ilvG⁻ rfb⁻⁵⁰ rph⁻¹ endA1 gyrA96(nal^R) thi⁻¹ recA1 relA1 lac glnV44*	Blattner, F. R. *et al.*[a]
XL1-Blue	*F'[::Tn10 proAB⁺ lacI^q Δ(lacZ)M15] hsdR17(r_K⁻ m_K⁺)*	Stratagene[b]
YJ001	FACS-isolated *rrsE*-deficient mutant of MG1655	this study
YJ002	Deleterious *rrsE*-deficient mutant of MG1655	this study
YJ003	Deleterious *rrsE*-deficient mutant of XL1-Blue	this study
Plasmids		
pMoPac16	*lac* promoter, ColE1 origin of replication, Amp^R, PelB signal peptide, Skp	Hayhurst, A. *et al.*[c]
pMBPF	FlAsH tag (and 6X His tag)-fused MBP (MBPF) coding gene in pMoPac16	this study
pMMBPF	MBP signal peptide-fused MBPF coding gene in pMoPac16	this study
pDMBPF	DsbA signal peptide-fused MBPF coding gene in pMoPac16	this study
pDsbA	6X His tag-fused *dsbA* gene in pMoPac16	this study
pTorT	6X His tag-fused *torT* gene in pMoPac16	this study
pTolB	6X His tag-fused *tolB* gene in pMoPac16	this study
pDM18	DsbA signal peptide-fused M18 scFv coding gene in pMoPac16	Lee and Jeong 2013
pDLPH	YMF10 IgG coding gene in pMAZ360, DsbA signal peptide-fused light chain, PelB signal peptide-fused heavy chain, Amp^R	Lee et al. 2013
pDLDH	YMF10 IgG coding gene in pMAZ360, DsbA signal peptide-fused light and heavy chains, Amp^R	Lee et al. 2013
pBAD33*ffh/dsbC*	Ffh and DsbC coding genes in pBAD33, Cm^R	Lee et al. 2013
pBAD33*yidC*	YidC coding genes in pBAD33, Cm^R	Lee and Jeong 2013
pDrNTR1	DsbA signal peptide-fused rat NTR1 D03^{25} coding gene in pMoPac16	this study
pJET1.2	T7 promoter, pMB1 origin of replication, Amp^R	Thermo Fisher[d]

[a]Blattner, F. R. *et al*. *Science* **277,** 1453-1462 (1997).
[b]Thermo Fisher Scientific, Waltham, MA, USA.
[c]Hayhurst, A. *et al*. *J. Immunol. Methods* **276,** 185-196 (2003).
[d]Stratagene Cloning Systems, La Jolla, CA, USA.

Table S2. Oligonucleotide primers used in this study

Primer names	Sequences (5' to 3')
MBPF F	gcatcagtcatatgaaaatcgaagaaggtaaactggtaatctggattaacg
MMBPF F	gcattctagattgaactttaagaaggagatatacatatgaaaataaaaacaggtgcacgc
DMBPF F	gcatctgacatatgaaaaagatttggctggcgctggctggtttagttttagcgtttagcgcatcggcgaaa atcgaagaaggtaaactggtaatctgg
MBPF R	atgcaagcttatcacggttccatacagcagcccggacagcaattcagaaagtgatgatggtgatgatgc ttggtgatacgagtctgc
DsbA F	gcattctagattgaactttaagaaggagatatacatatgaaaaagatttggctggcg
DsbA R	gcataagcttatcaatggtgatggtgatgatgttttttctcggacagatatttcactgtatc
TorT F	gcattctagattgaactttaagaaggagatatacatatgcgcgtactgctatttta
TorT R	gcataagcttatcaatggtgatggtgatgatgtttcttagccgctgatgtg
TolB F	gcattctagattgaactttaagaaggagatatacatatgaagcaggcattacgag
TolB R	gcataagcttatcaatggtgatggtgatgatgcagatacggcgaccaggc
RrsE KO F	tacagagagcgttagctgaatttttcgcgaaaaactcagctaacgcccctaacggggcatccttatttttgaa cgcggccgccagctgaa
RrsE KO R	aagggcggtgtcctgggcctctagacgaaggggacgtatcagtctgcttcgcaagacgccttgcttttcac cgcataggccactagtgga
RrsE F	catccatcatctccgggaaa
KAN-2 F	acctacaacaaagctctcatcaacc
KAN-2 R	gcaatgtaacatcagagattttgag
16S F	actcaaatgaattgacgggg
16S R	caacatttcacaacacgagc
23S F	agtaccccgaggaaaagaaa
23S R	atttagccttggaggatggt
GyrB F	gacaaagaaggctacagcaa
GyrB R	agttcgttcatctgctgttc
Ffs F	tctgttggttctcccg
Ffs R	cctgccagctacatc
Ffh F	tcttcccttctgatgttggt
Ffh R	gaatgcttttgccgtattgg

Table S3. Comparison of fed-batch cultivations for the production of human full-length IgG

Strain	Max. cell density (OD_{600})		Max. production yield (mg/L)	Max. volumetric productivity (mg/L/h)	Ref.
XL1-Blue[a]	127.5	periplasm	62	2.21	Lee et al. 2013
		extracellular	n.d.[c]	n.d.[c]	
YJ003[b]	157.2	periplasm	165.4	5.85	this study
		extracellular	236.5	7.63	

[a]*E. coli* XL1-Blue wild type.
[b]*rrsE*-deficient mutant of XL1-Blue.
[c]Not determined.

Table S4. Statistical analysis for the result of RT-qPCR

Target gene	*P* value[a]
gyrB	> 0.5
ffs	0.0085
ffh	0.2354

[a]Paired two-tailed *t*-test

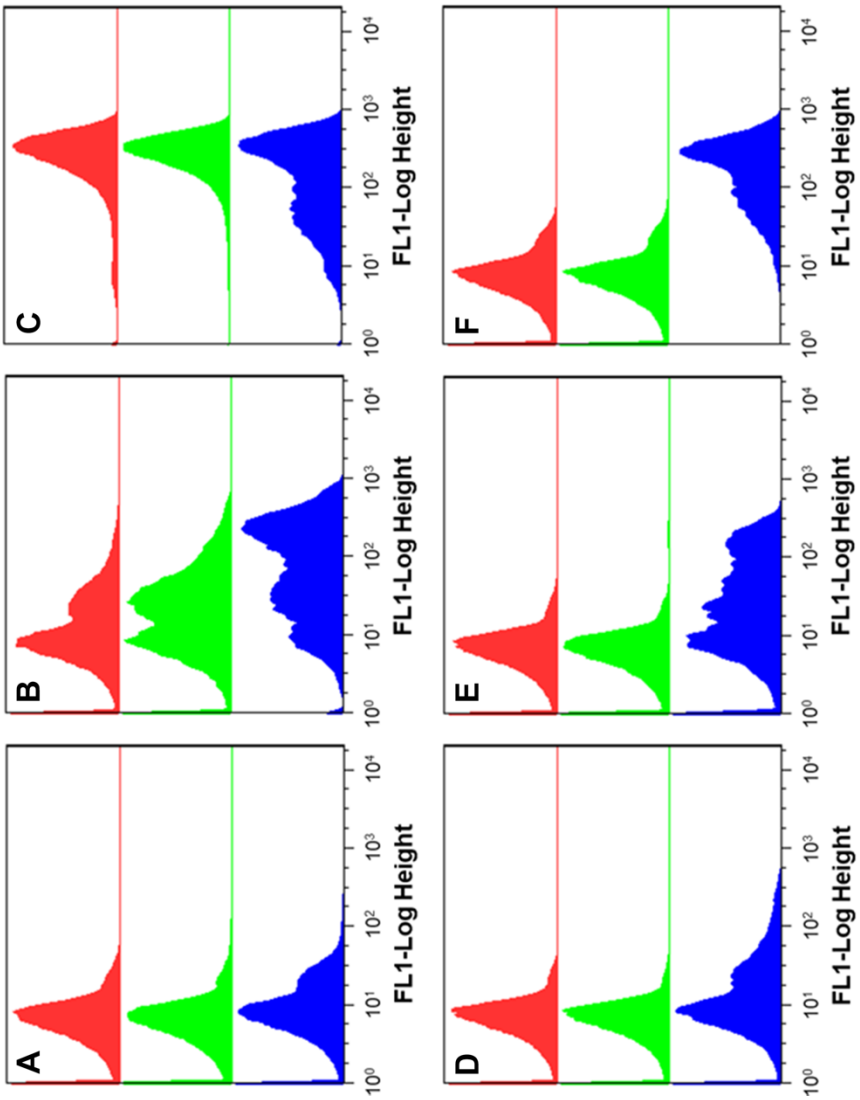

Figure S1. Cytometric analysis for the optimization of FlAsH-PECS assay. The analysis was performed with different conditions such as: (**A**) 0.7X PBS, 25 µM FlAsH-EDT_2; (**B**) 2X PBS, 25 µM FlAsH-EDT_2; (**C**) 5X PBS, 25 µM FlAsH-EDT_2; (**D**) 1X PBS, 5 µM FlAsH-EDT_2; (**E**) 1X PBS, 10 µM FlAsH-EDT_2; and (**F**) 1X PBS, 25 µM FlAsH-EDT_2. Red, green and blue histograms represent the signals from *E. coli* harboring pMoPac16, pMBPF and pDMBPF, respectively.

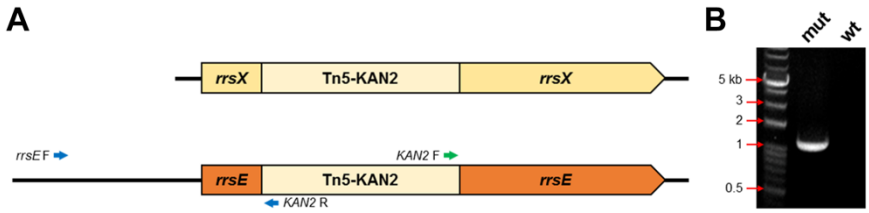

Figure S2. Genotypic analysis of isolated mutant. (**A**) Schematic figure of genomic scar and binding locations of the primers for genetic analysis. (**B**) Colony PCR analysis for identifying which copy of 16S rRNA coding gene was knocked out. The primer set of '*rrsE* F/*KAN2* R' was used for this analysis. The 'mut' and 'wt' represent *E. coli* YJ001 and MG1655 wild type, respectively.

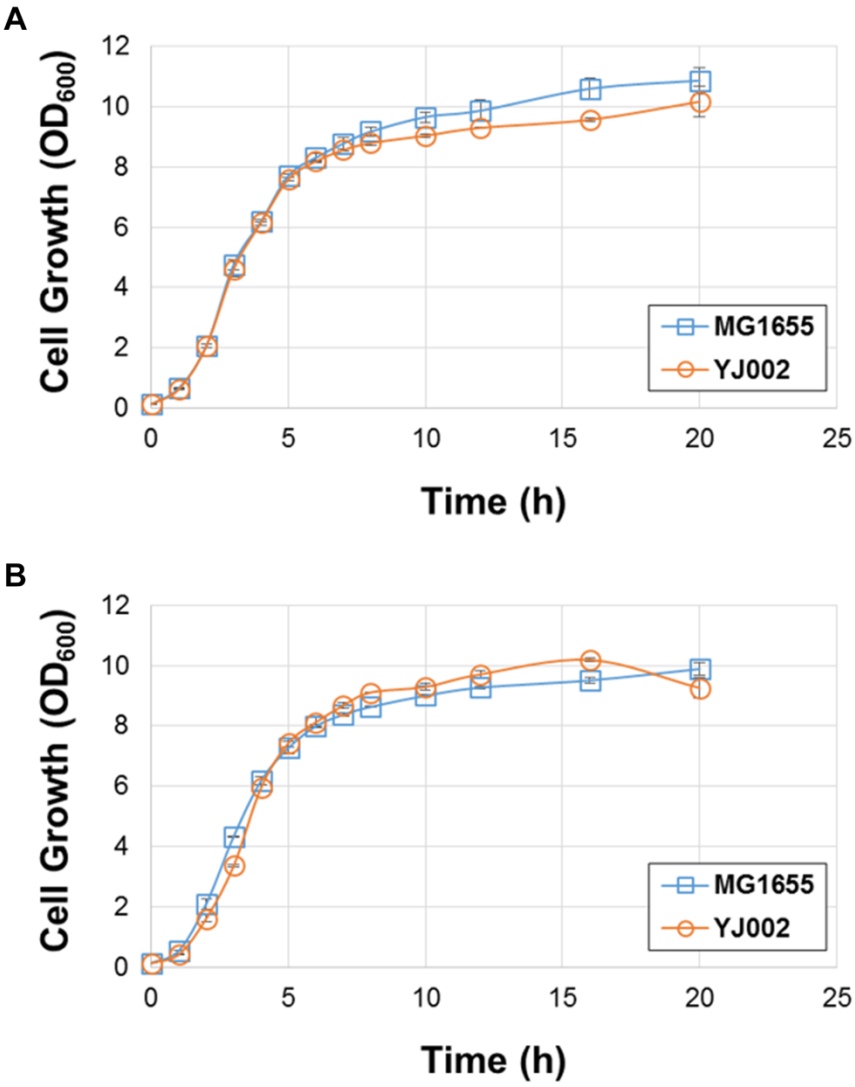

Figure S3. Comparison of the growth profile of wild type *E. coli* MG1655 and *rrsE*-deficient mutant (YJ002). Growth curves were obtained from (**A**) the cells without plasmids; and (**B**) the cells harboring plasmid expressing model protein (pDMBPF), respectively.

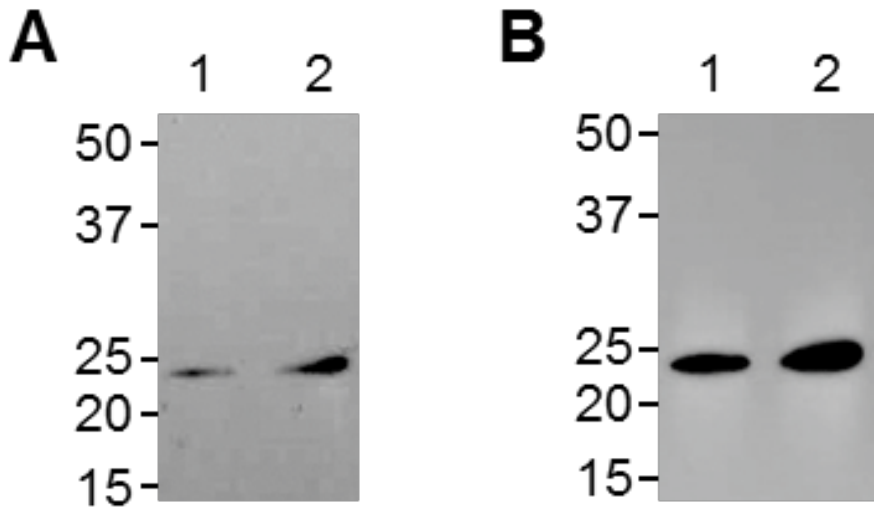

Figure S4. Comparison of the periplasmic levels of endogenous DsbA in *E. coli* MG1655 and YJ002. Western blot was performed with *E. coli* DsbA-specific antibody. After immunoblotting, chemiluminescence signals were visualized on the X-ray film with different exposure times of (**A**) 3 minutes; and (**B**) 10 minutes, respectively. Lanes 1 and 2, MG1655 and YJ002, respectively.

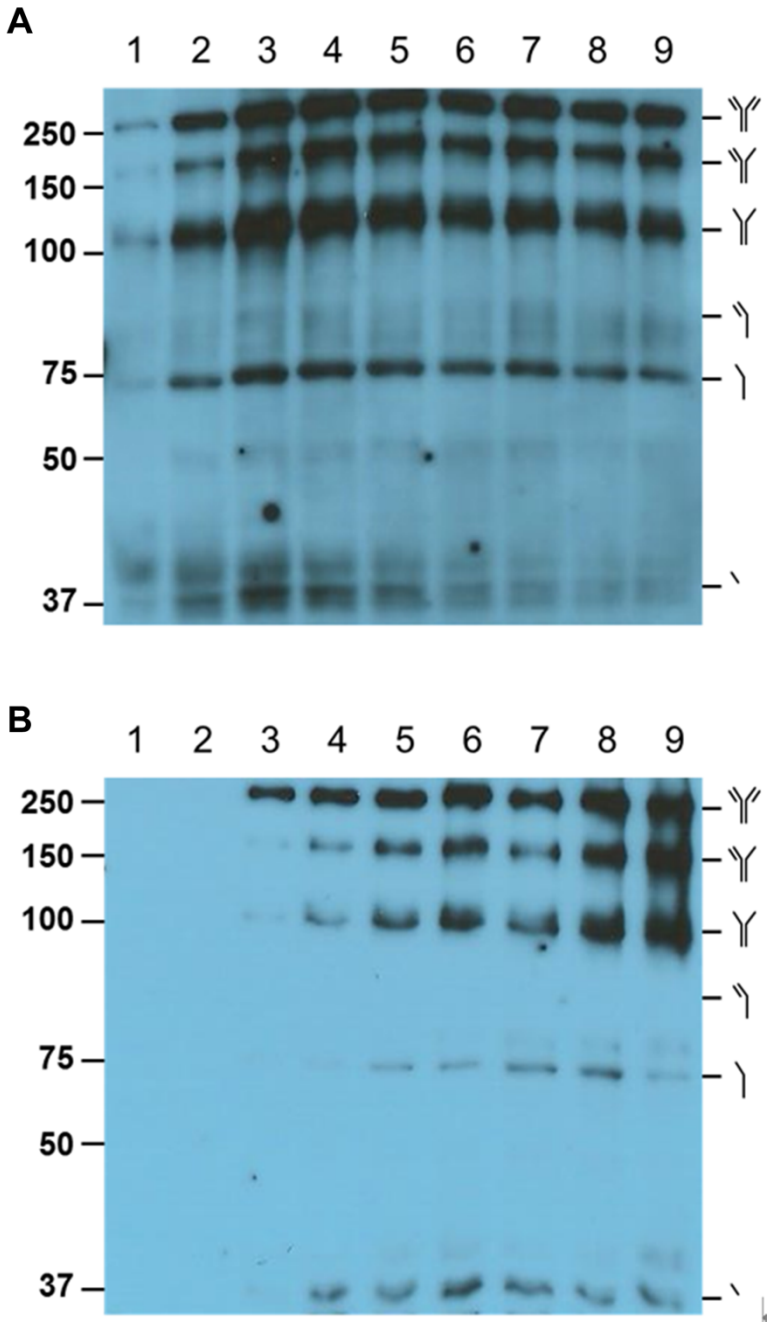

Figure S5. Western blot analysis after fed-batch cultivation for the production of IgG. (**A**) Periplasmic fraction. (**B**) Culture supernatant. Lane numbers represent each sampling point in the post-induction period. Stick figures indicate the predicted assembly of IgG.

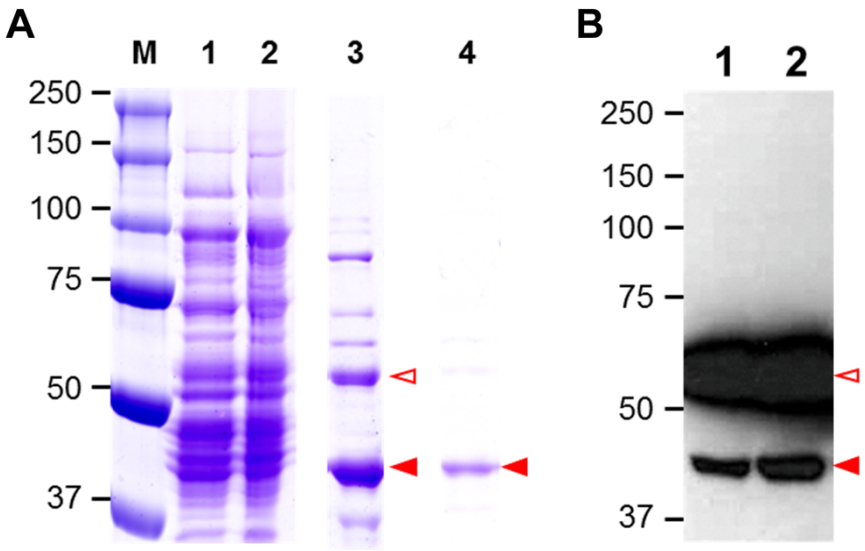

Figure S6. Expression and purification of G protein-coupled receptor mutant, NTR1 D03. (**A**) SDS-PAGE analysis after the purification of NTR1 D03. Lane 1, detergent-solubilized lysate; lane 2, unbound fraction of affinity chromatography; lane 3, eluents from Ni^{2+} affinity chromatography; lane 4, eluents from anion exchange chromatography. (**B**) Western blot analysis after flask cultivations for the expression of NTR1 D03. Lane 1, wild type *E. coli* MG1655 harboring pDrNTR1 and pBAD33*yidC*; lane 2, YJ002 harboring pDrNTR1 and pBAD33*yidC*. Open and closed arrowheads represent YidC and NTR1 D03, respectively.