Supporting Information

Development of a potential protein display platform in *Corynebacterium glutamicum* using mycolic acid layer protein, NCgl1337, as an anchoring motif

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Supplementary Figures

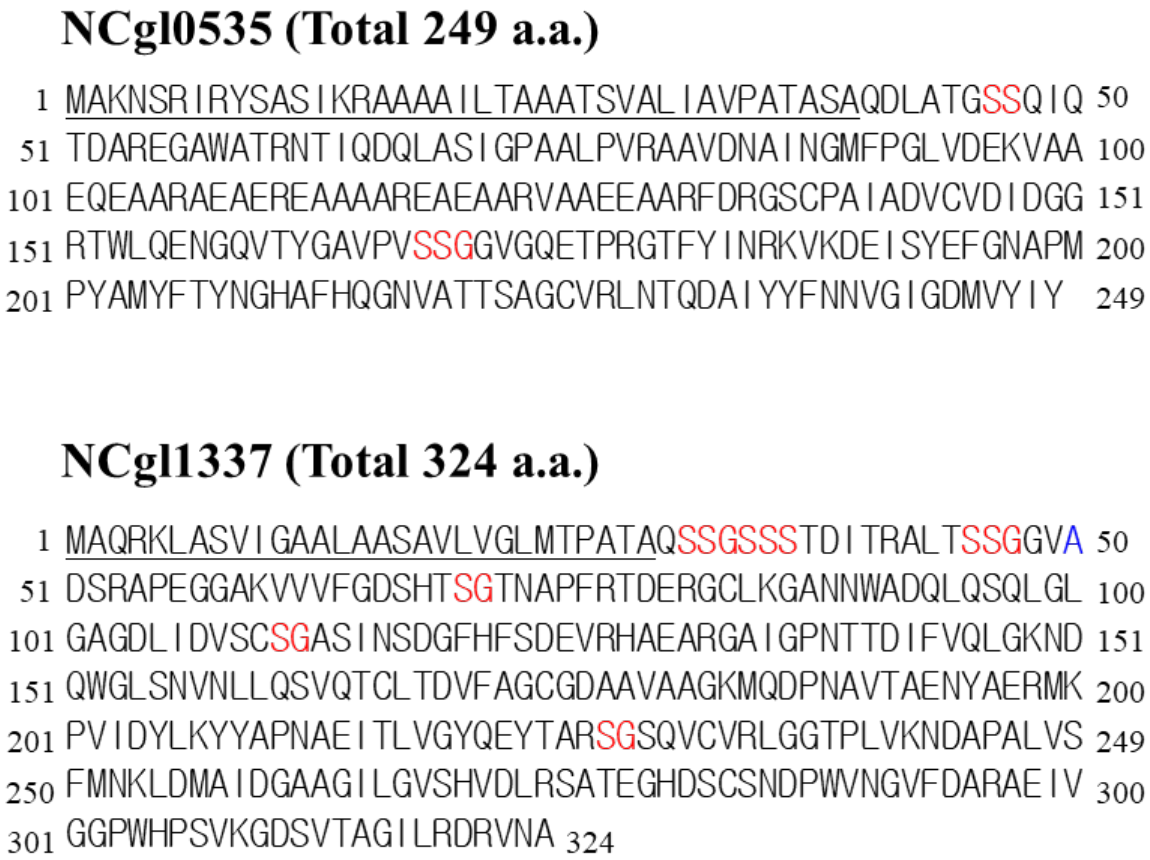

Figure S1. Amino acid sequences of two isolated proteins (NCgl0535 and NCgl1337). Underlined amino acids represents signal peptides. Red-color residues indicate putative *O*-mycoloylation sites (SS or SG). The blue-color Ala residue is the last amino acid in the short form of NCgl1337.

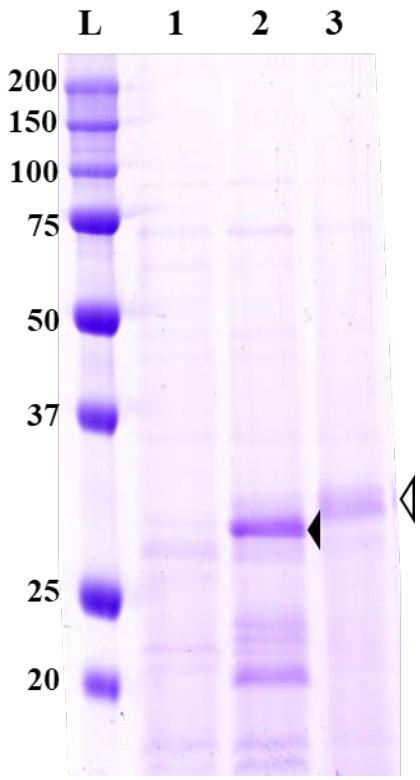

Figure S2. SDS-PAGE analysis of protein fraction in extracellular medium. Lane L represents molecular weight markers (kDa). Lanes 1 to 3 represent proteins samples in extracellular medium from cultivation with cells harboring pCES-RBS, pH36R-NCgl0535, and pH36R-NCgl1337, respectively. The closed and open arrow heads indicate the NCgl0535 and NCgl1337, respectively.

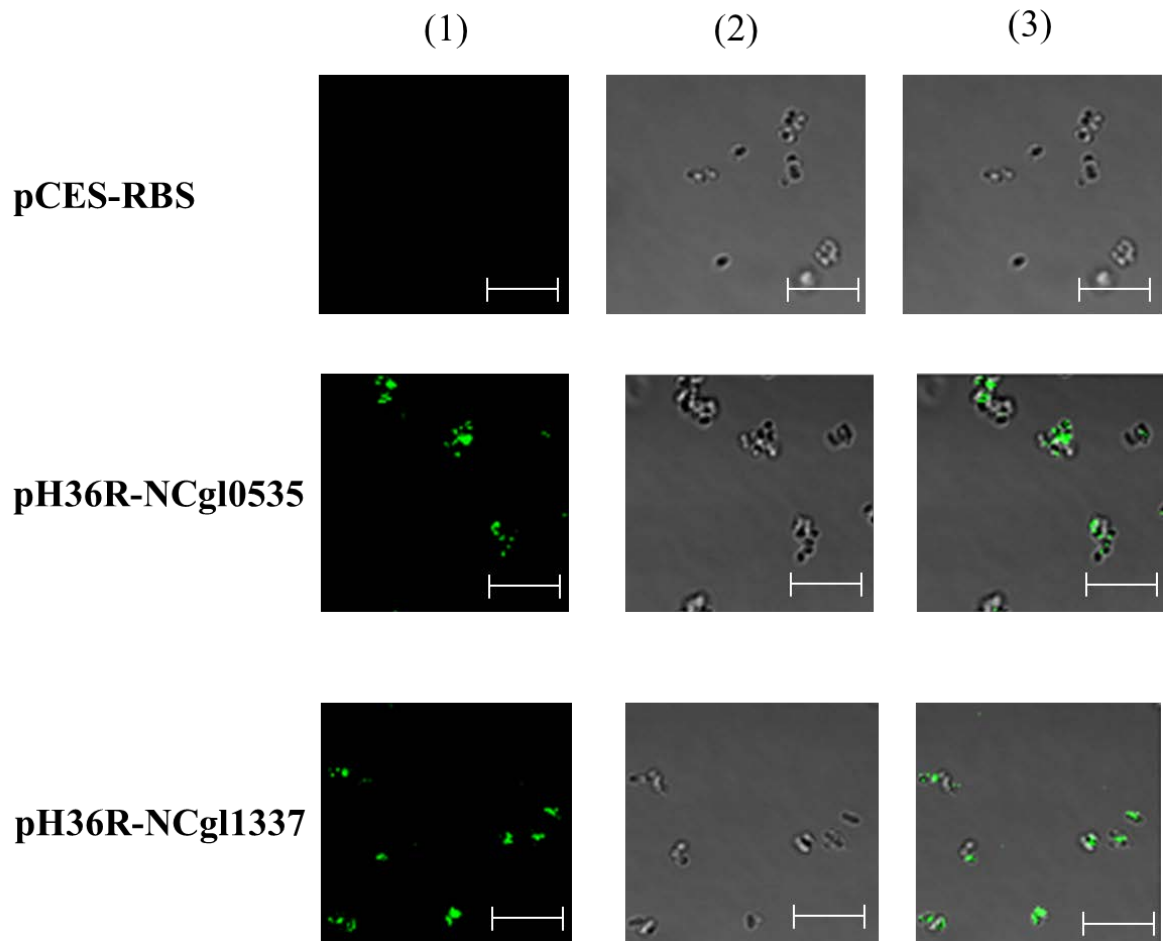

Figure S3. Confocal microscopy image of immunofluorescent labelled cells. (1), (2), and (3) represent the immunofluorescence image, Nomarski interference microscopy image, and merged image, respectively. Each scale bar represents 10 μm. M is mean value of FL1_Area.

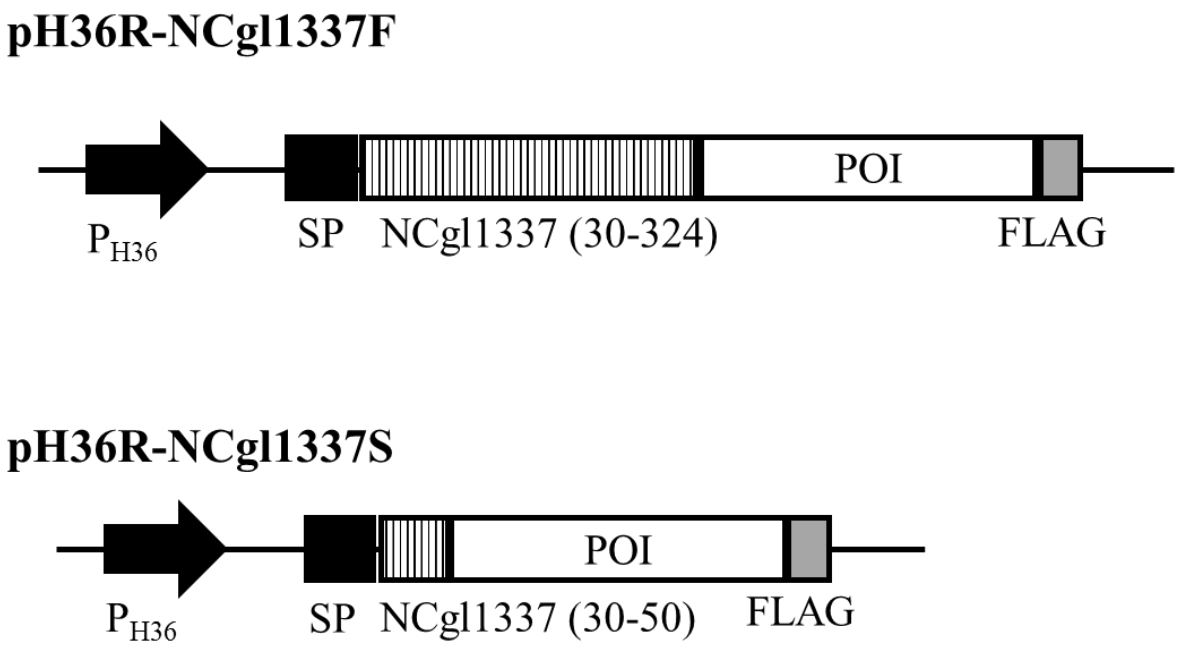

Figure S4. Schematic diagram of plasmids (pH36R-NCgl1337F and pH36R-NCgl1337S) carrying the full-length (1-324 a.a.) and short-length (1-50 a.a.) of NCgl1337 including its signal peptide (SP, 29 a.a.), respectively. P_{H36} indicates a strong and constitutive promoter. FLAG tag was fused to C-terminus of each POI (protein of interest).

Table S1. List of mycolic acid layer proteins in *C. glutamicum*.

Locus tag	Gene annotation
NCgl0336	Trehalose mycoloyltransferase cMytC
NCgl0987	Trehalose mycoloyltransferase cMytD
NCgl2101	Trehalose mycoloyltransferase cMytF
NCgl2777	Trehalose mycoloyltransferase cMytA
NCgl2779	Trehalose mycoloyltransferase cMytB
Cg3008	PorA
NCgl0933	PorB
NCgl0329	Putative ABC-type cobalamin/Fe3+-siderophore transport system, periplasmic component
NCgl0776	Putative ABC-type cobalamin/Fe3+-siderophore transport system, periplasmic component
NCgl0776	Putative secreted trypsin-like serine protease
NCgl1480	Putative cell wall-associated hydrolase
NCgl2375	Putative protein ABC-type transporter, periplasmic component
NCgl0381	Hypothetical protein
NCgl0513	Hypothetical protein
NCgl0535	Hypothetical protein
NCgl1337	Hypothetical protein
NCgl2430	Hypothetical protein
NCgl2789	Hypothetical protein