Conversion of a soluble protein into a potent chaperone *in vivo*

Supplementary Information

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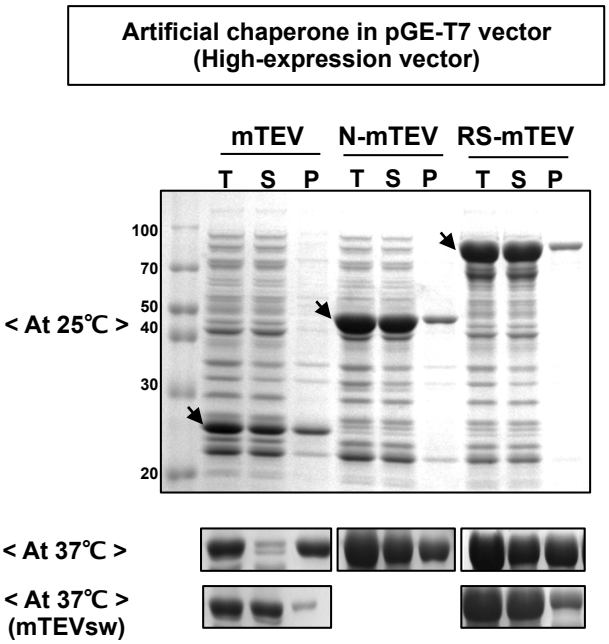
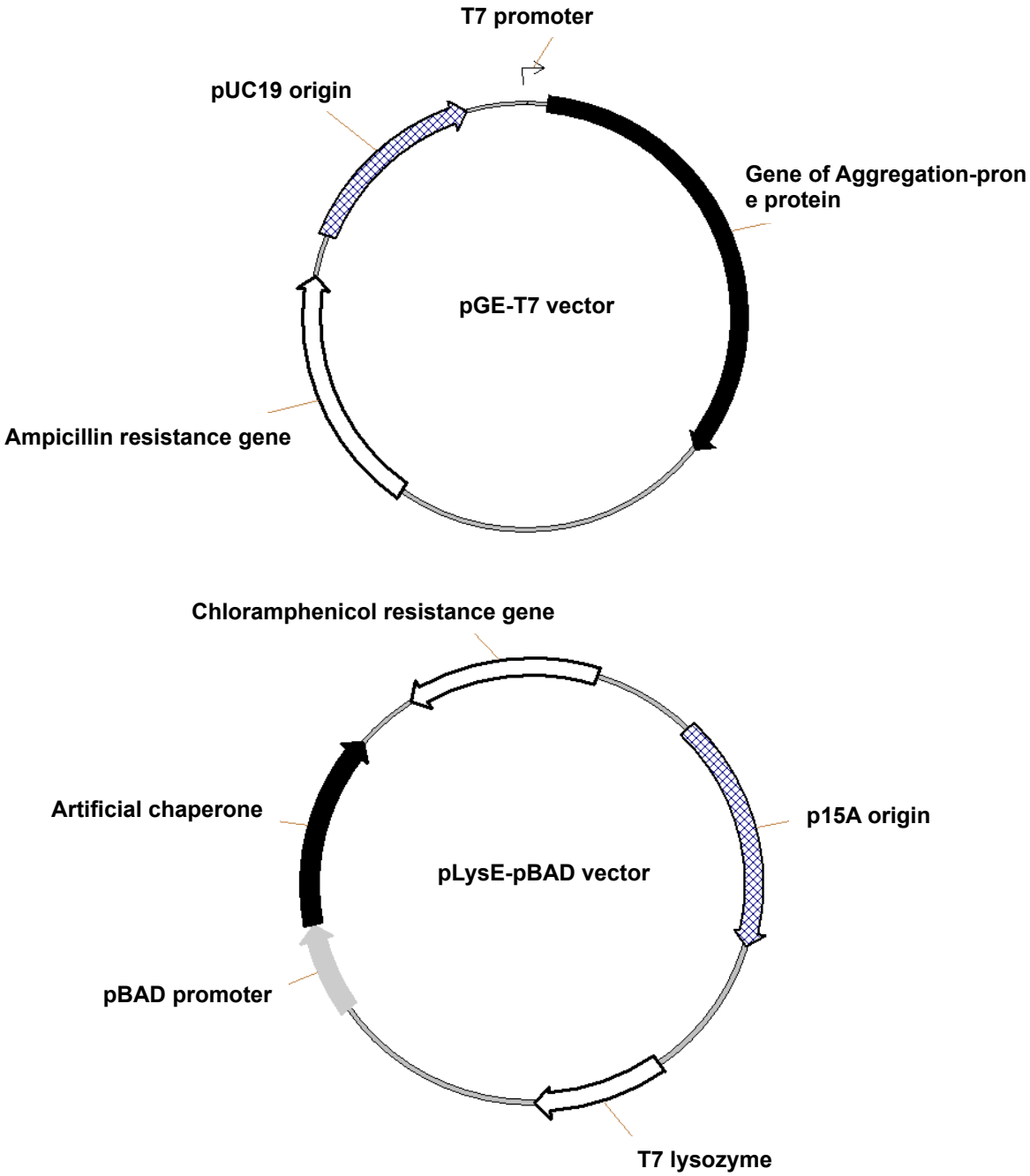
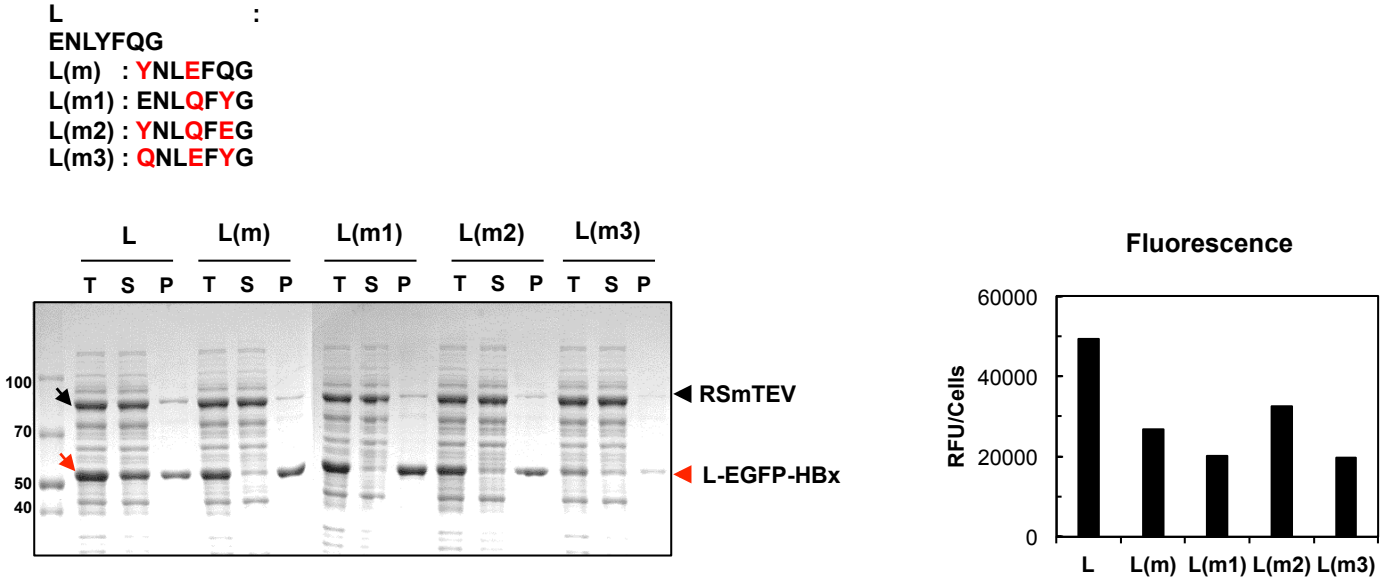


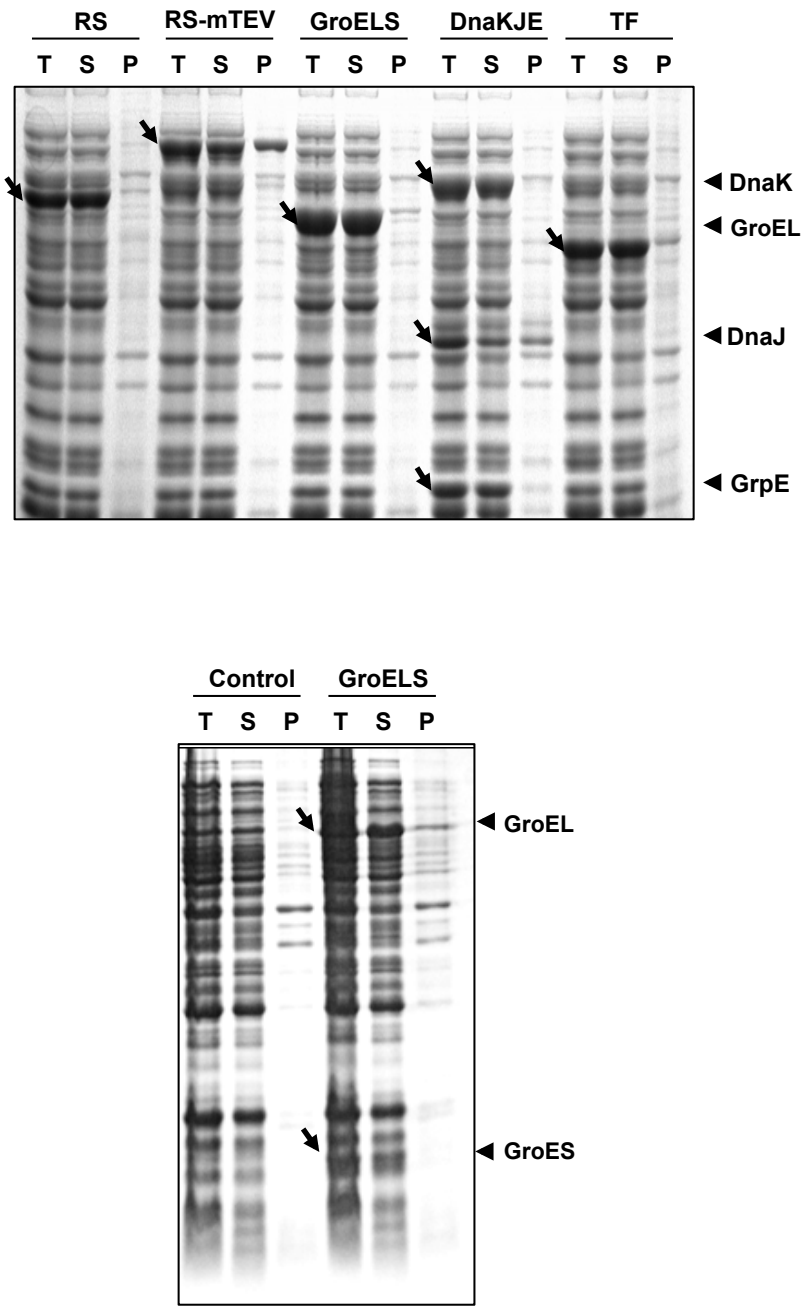
Supplementary Figure S1. Expression of mTEV and its derivatives in *E. coli.* mTEV, N-mTEV, and RS-mTEV were expressed in *E. coli* at 25 °C and 37 °C. N and RS represent the N-terminal domain of *E. coli* LysRS and the whole LysRS, respectively. More soluble mTEV variant (mTEVsw) and its derivative (RS-mTEVsw) were also expressed in *E. coli* at 37 °C. The solubilities of the three mTEV variants expressed at 25 °C were similar, whereas that of mTEV decreased at 37 °C. mTEVsw alone was highly soluble even at 37 °C.



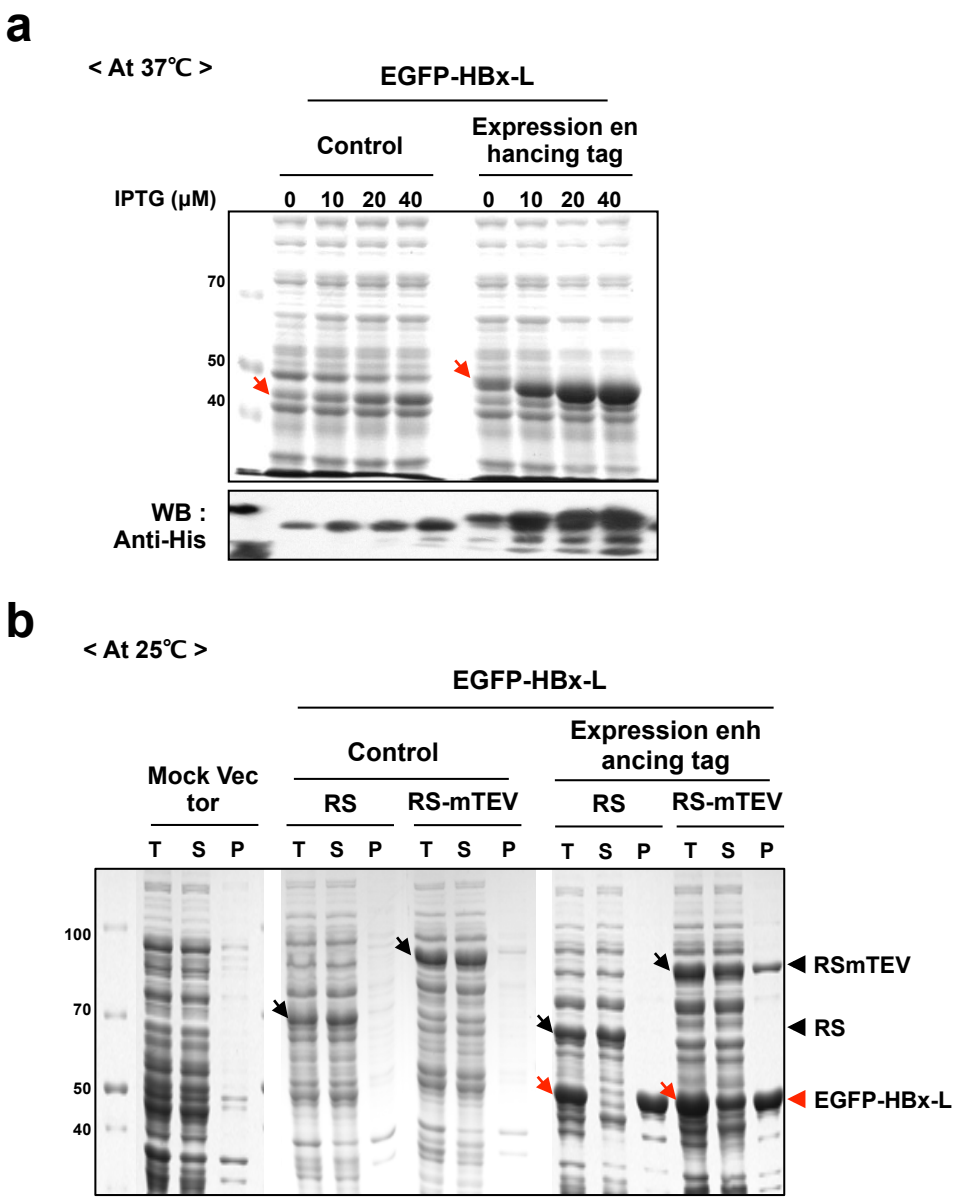
Supplementary Figure S2. Diagram of co-expression vectors used for aggregation-prone proteins and artificial chaperones. pGET7 vector used for the expression of aggregation-prone substrate proteins harbours an ampicillin-resistance gene and a pUC19 origin of replication. Protein expression under control of T7 promoter was induced by IPTG. pLysEpBAD used for chaperone expression carries a chloramphenicol-resistance gene and a p15A origin of replication. Expression of artificial chaperones under the control of the pBAD promoter was triggered by L-arabinose.



Supplementary Figure S3. **Mutation of the "L" tag in L-EGFP-HBx alters interaction with RS-mTEV.** A mutation was introduced in the conserved residues of the "L" tag, and the resultin g mutant variants [L(m), L(m1), L(m2), and L(m3)] were attached to the N-terminus of EGFP-H Bx, respectively. These proteins were co-expressed with RS-mTEV in *E. coli*. Mutated sequence s in the "L" tag are indicated in red. Expressed proteins were analysed using SDS-PAGE and ver ified by measuring fluorescence (histogram, right).



Supplementary Figure S4. Confirmation of the expression of RS, RS-mTEV, and molecular chaperones. RS, RS-mTEV, GroELS, DnaKJE, and TF were expressed in *E. coli*, and their expression was analysed using SDS-PAGE. Each target band is indicated by an arrow. In the case of GroELS, the SDS-PAGE result (down) was added to clearly see the expression of GroES, a relatively small sized protein, which was not shown in the upper SDS-PAGE result obtained after a long running time for a better resolution.



Supplementary Figure S5. **The N-terminal MSEQ tag increases the expression of the substrat e proteins of RS-mTEV.** (**a**) EGFP-HBx-L in the presence or absence of this tag was expressed in *E. coli* at 37 °C and induced at various IPTG concentrations (0–40 μM). Total lysates of each sampl e were analysed using SDS-PAGE and western blot. Red arrows indicate EGFP-HBx-L expression. (**b**) EGFP-HBx-L in the presence or absence of the tag was expressed in *E. coli* at 25 °C (induced b y 100 μM IPTG) along with RS or RS-mTEV co-expression, followed by SDS-PAGE analysis.