(Supplemental Material)

Supplemental Material Figure legends

Fig. S1. The relative fluorescence of the linker region-deletion mutants. The red fluorescence of displayed mRFP1 was measured as described in the method. The deletion mutant (missing 24 ~ 948 region) showed only 33 % of the fluorescence of the other mutants having predicted linker regions. This result showed that the 785 ~ 948 region plays an important role for efficient display of heterologous proteins.

Fig. S2. The overview of construction of pATLIC vector (A). The passenger domain was deleted by PCR using the primers containing TEV protease recognition and SmaI restriction site sequences. Amplified PCR products were treated by T4 DNA polymerase without dNTPs for annealing of single-stranded ends. A typical ligation independent cloning (LIC) procedure for display of proteins of interest (POI) using pATLIC vector was shown in (B).

Fig. S3. The protease accessiblity test for surface-displayed or periplasmic mRFP1. For periplamic mRFP1, we expressed mRFP1 in pATLIC having an identical signal sequence but lacking the translocator domain by incorporating a stop codon followed by the mRFP1 sequence (pATLIC*-mRFP1).We observed the change of relative fluorescence of whole-cells after trypsin digestion as described in the method. The periplasmic mRFP1 was not susceptible to trypsin treatment but surface-displayed mRFP1 was degraded by trypsin. The fluorescence of untreated (□) and treated (■) cells are presented in the figure.

24

25 Fig. S4. (A) Optimal inducer concentration for the maximum surface display and its

26 effect on the cell growth. Effects of inducer (L-(+)-arabinose) concentration on surface

27 display of Aga16B. The amount of reducing sugars produced at the range of 0 ~ 0.2% of

28 inducer. (B) Growth of host cells (*E. coli* BW25113) displaying various proteins was

29 measured by OD_{600}. *E. coli* BW25113 harboring pATLIC-*aga50A* (●), pATLIC-*aga16B*

30 (♦), pATLIC-*aga86C* (▲), pATLIC-*aga50D* (■) and pATLIC-aga86E (X) were grown

31 for 24 h at 16˚C. An asterisk (*) indicates the inducing time point with L-(+)-arabinose.

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33

34 Fig. S5. The neoagarobiose hydrolase (NABH) activity in the culture supernatant of

35 NABH displayed cells. Before NABH displayed cells were treated by TEV protease, the

36 culture supernatant of the cells was concentrated, incubated in the reaction mixture and

37 analyzed by TLC. Lane 1, L-(+)-arabinose (L-Ara); lane 2, D-galactose (D-Gal); lane 3,

38 3,6-anhydro-L-galactose (L-AHG); lane 4, neoagarobiose (DP2); lane 5, the culture

39 supernatant

40

41 Fig. S6. The red fluorescence unit (RFU) curve of mRFP1. The correlation between the

42 red fluorescence unit and amount of mRFP1 was calculated from the curve.

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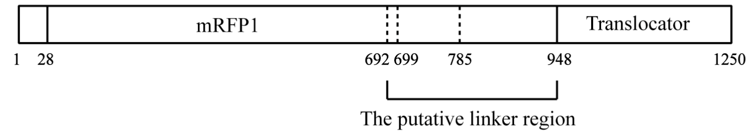

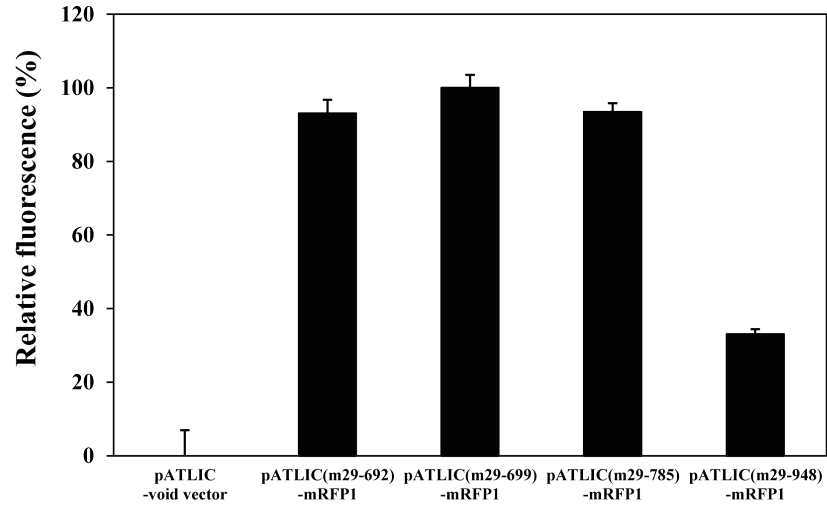

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46 (Fig. S1)

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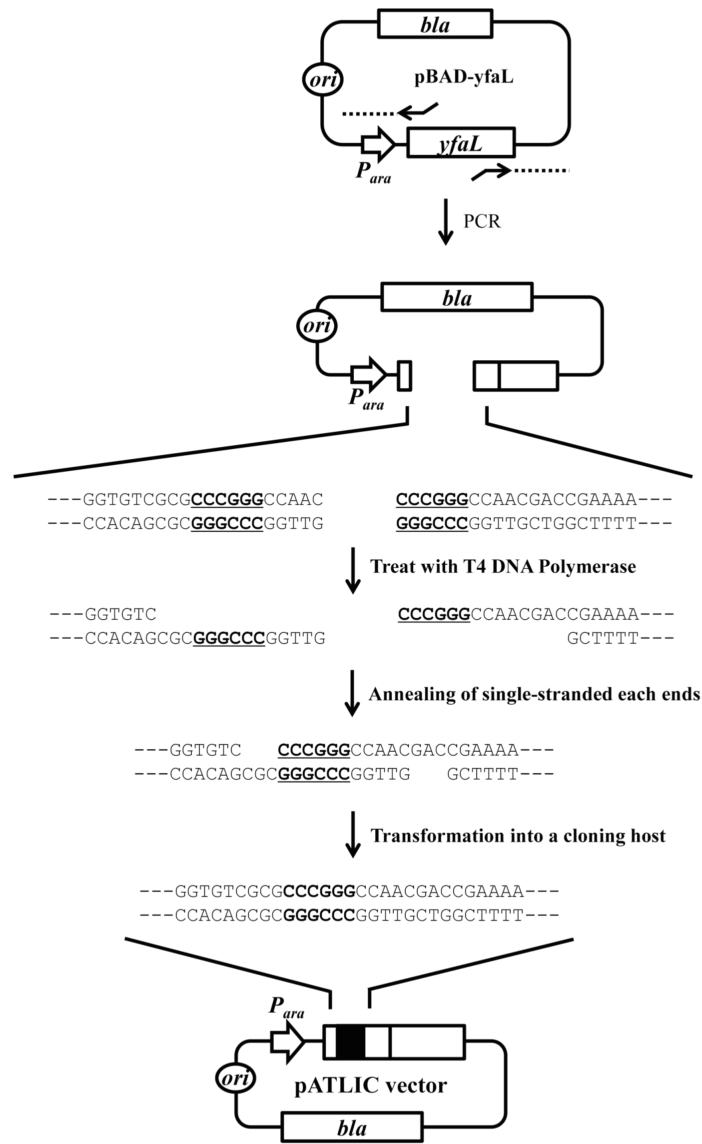

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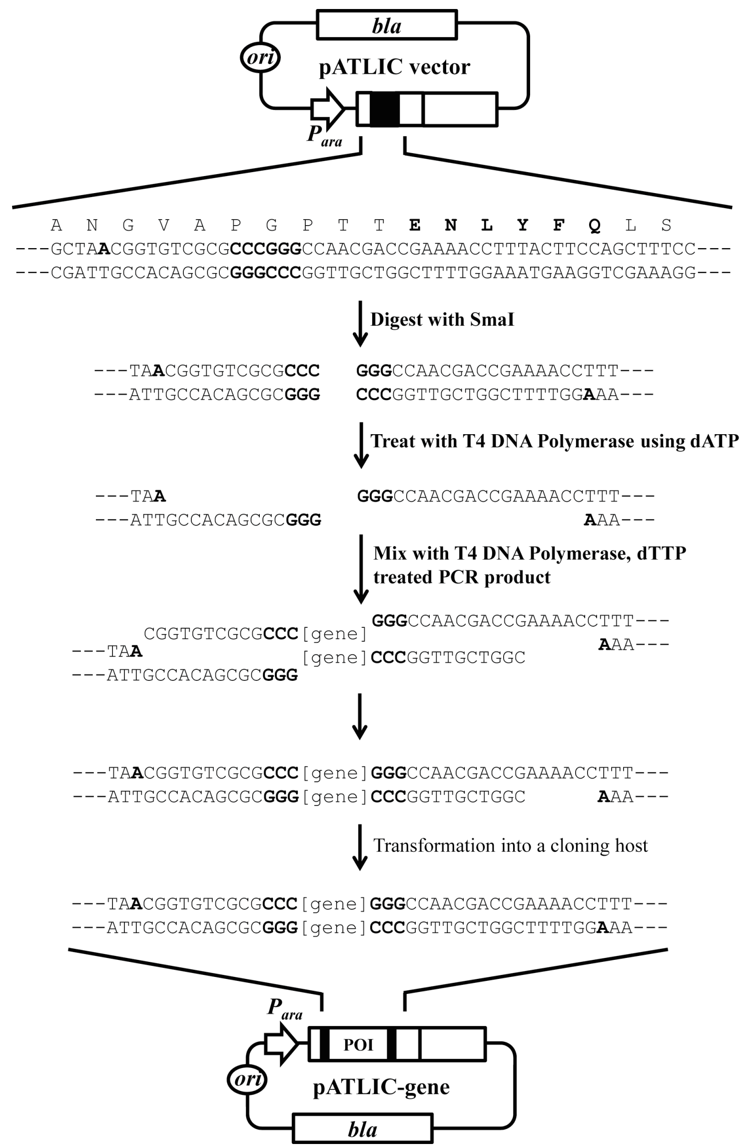
50 (Fig. S2A)



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52 (Fig. S2B)

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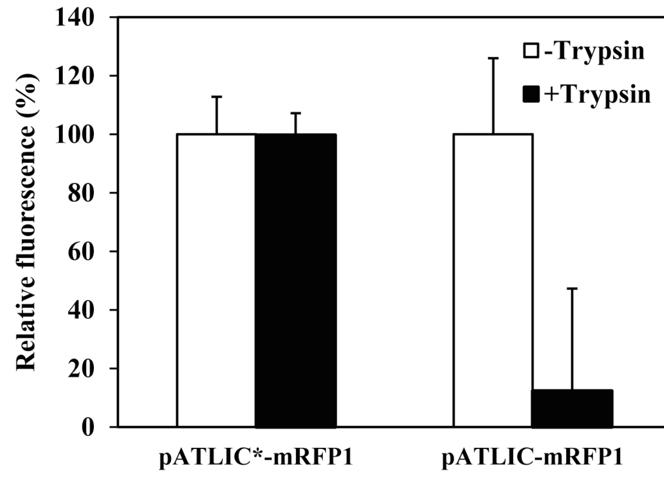

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55 (Fig. S3)

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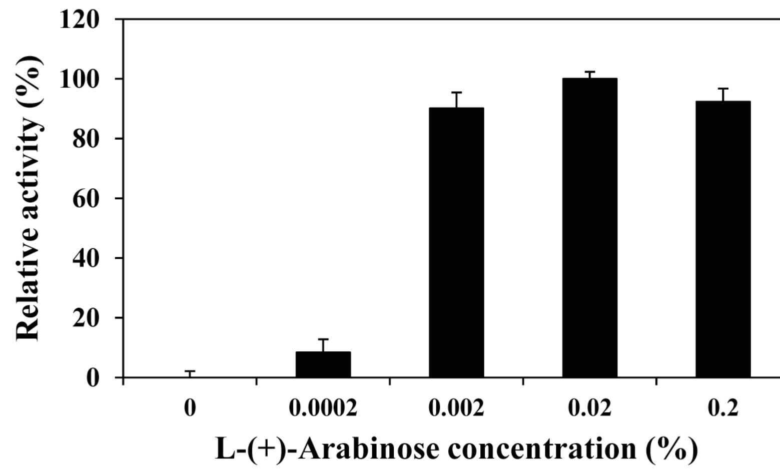

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58 (Fig. S4A)

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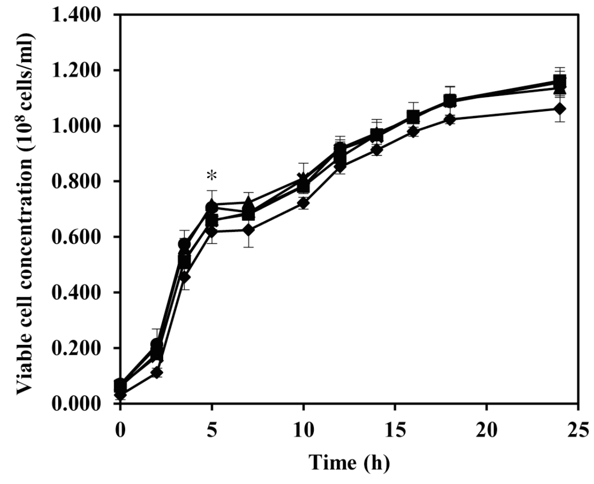

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61 (Fig. S4B)

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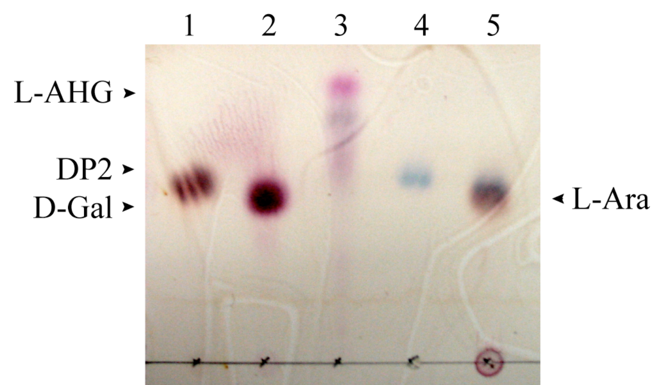

64 (Fig. S5)

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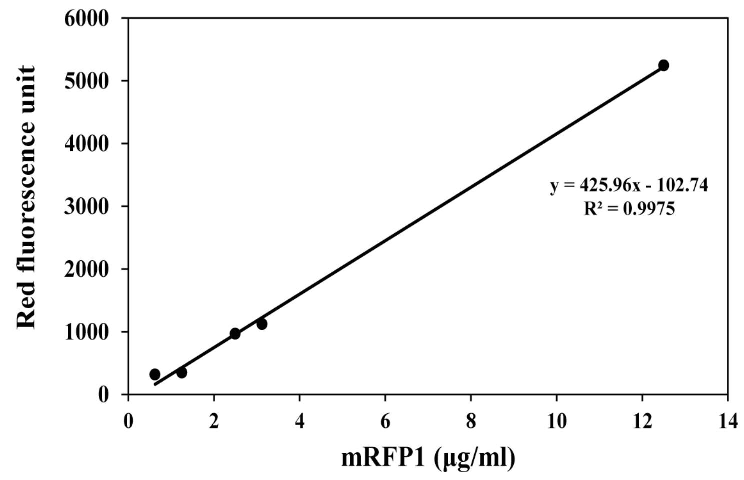

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67 (Fig. S6)