

Supplemental Figures (S1–S7) and Table S1

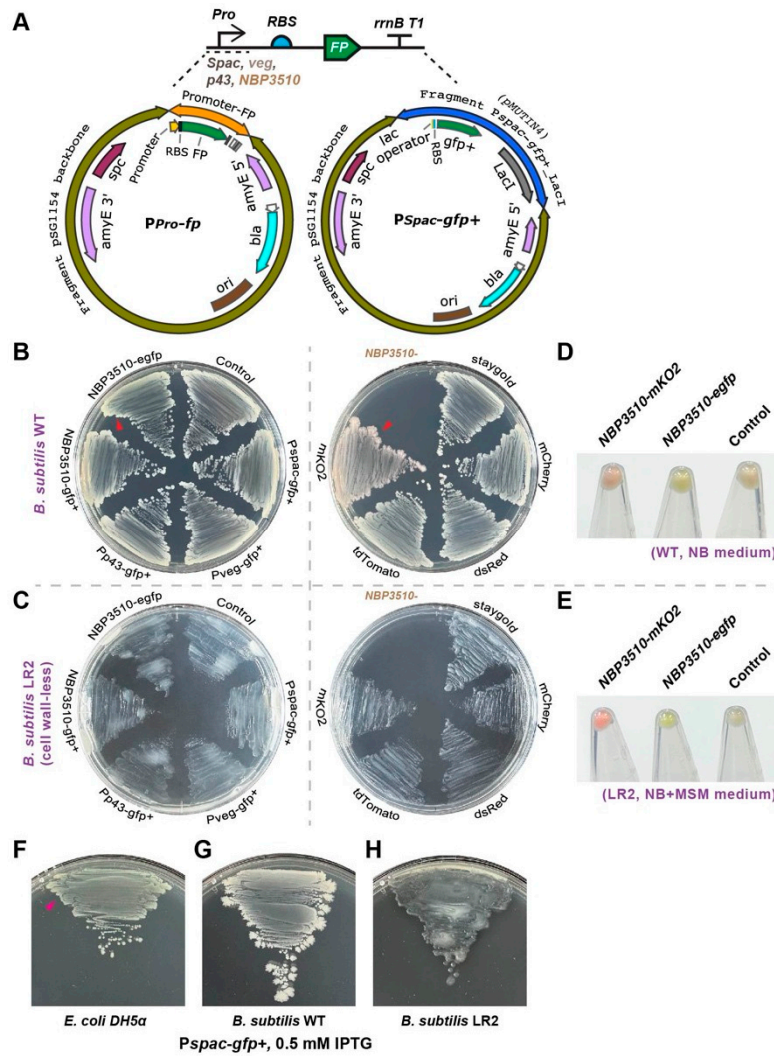


Figure S1. Plasmid construction and direct observation of expressed FPs in *B. subtilis* WT and L-form LR2. (A) Construction of the fluorescent protein (FP) expressing vectors (*PPro-fp* and *PSpac-gfp+*) under various constitutive promoters (*Pro*: *Pveg*, *P43*, and *NBP3510*) and IPTG-inducible promoter *Spac*. (B) All generated FP-expressing strains of *B. subtilis* WT (B) and LR2 (C) were grown on NA plate or NA/MSM plate, respectively. The red arrow represents apparent red or green colony in the *NBP3510-egfp* or *NBP3510-mKO2* WT strain. Cell pellets from indicated strains cultured in liquid medium were also collected for comparisons (D&E). Comparison of different fluorescent strains with *PSpac-gfp+* in the presence of IPTG (F–H). (F) *E. coli* DH5α strain containing *PSpac-gfp+* was grown on LB plate with 0.5mM IPTG, the red arrowhead indicates the apparent green color. *B. subtilis* WT (G) and LR2 (H) strain containing *PSpac-gfp+* was grown on NA or NA/MSM plate with 0.5 mM IPTG, respectively.

Pspac: *ttgtgagcggataacaat**taagcttaaggaggt*
Pveg: *aattttgtcaaaaataattttattgacaacgtcttattataacggtgatataatttaaa*
*ttttattttgacaaaaatggcgtcgtttgtacaataaatgt**tactagagaaaggtggtgaa*
Pp43: *attttacatttttagaatggcgtgaaaaaaacgcgcgattatgtaaaaatataa*
tactagagaaaggtggtgaa
NBP3510: *acttctcaaaatcccatttaaaaaatttttttaaaaaaatatttgacatttttaa*
ataaagcgtttataatataatgtagaacaacaaaggaggagattttgtttgacaaa
*gtagaacgt**gattaactaataaggaggacaaac*

Promoter sequence
RBS sequence

Figure S2. The sequence of different promoters used in this study. The promoter and RBS sequences were marked with underline and italics, respectively.

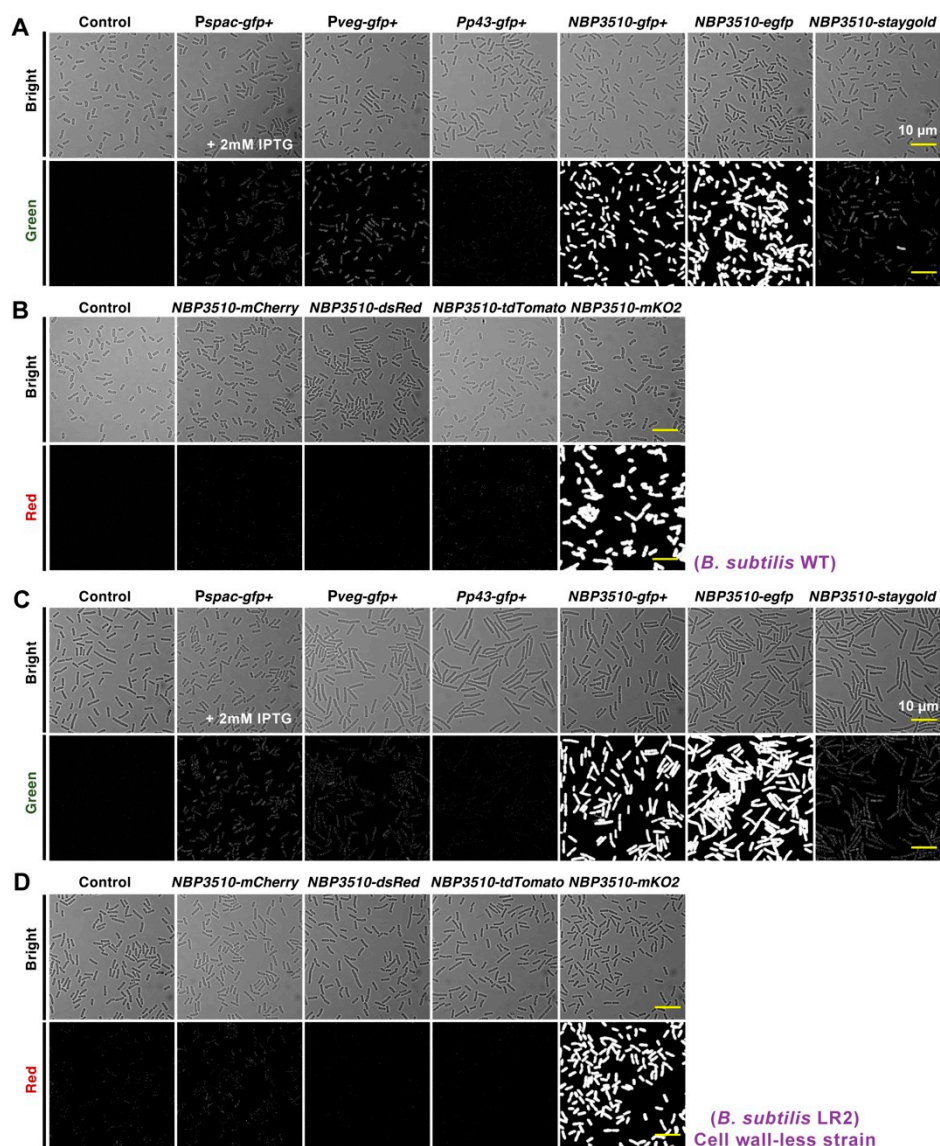


Figure S3. Microscope images of all constructed fluorescent strains. Images of tested green (A) and red (B) fluorescent strains in *B. subtilis* WT. Images of all tested green (C) and red (D) fluorescent strains in *B. subtilis* LR2. All bacteria were grown to late logarithmic phase in C minimal medium (CMM). Scale bar = 10 μ m.

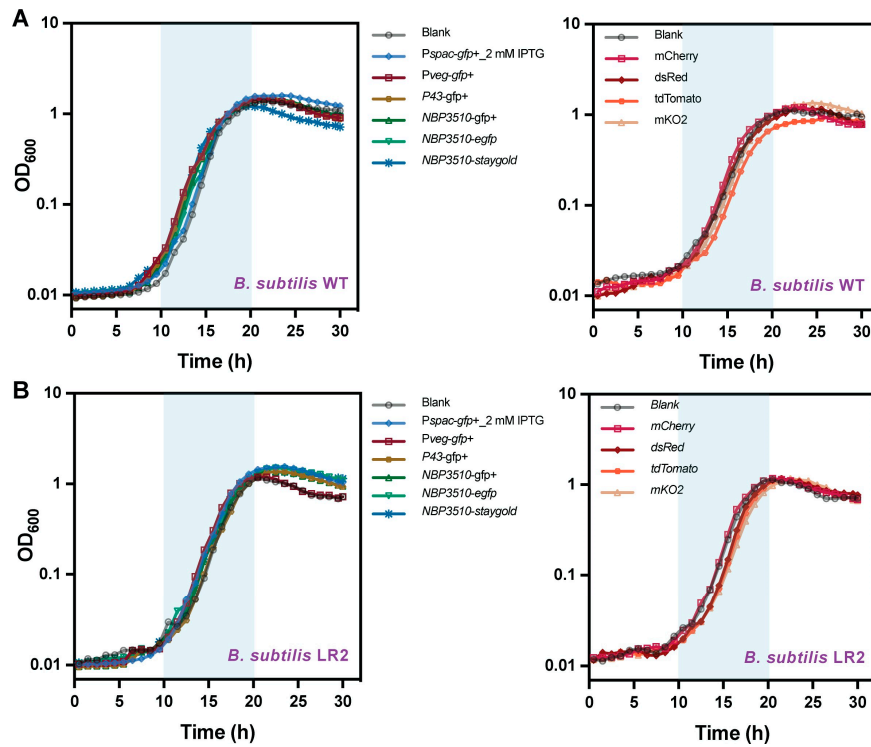


Figure S4. Growth curve of all strains used in this study. The growth curve (OD₆₀₀) for all stains expressing green (left panel) or red (right panel) fluorescent proteins in *B. subtilis* WT (A) and L-form LR2 (B) were shown.

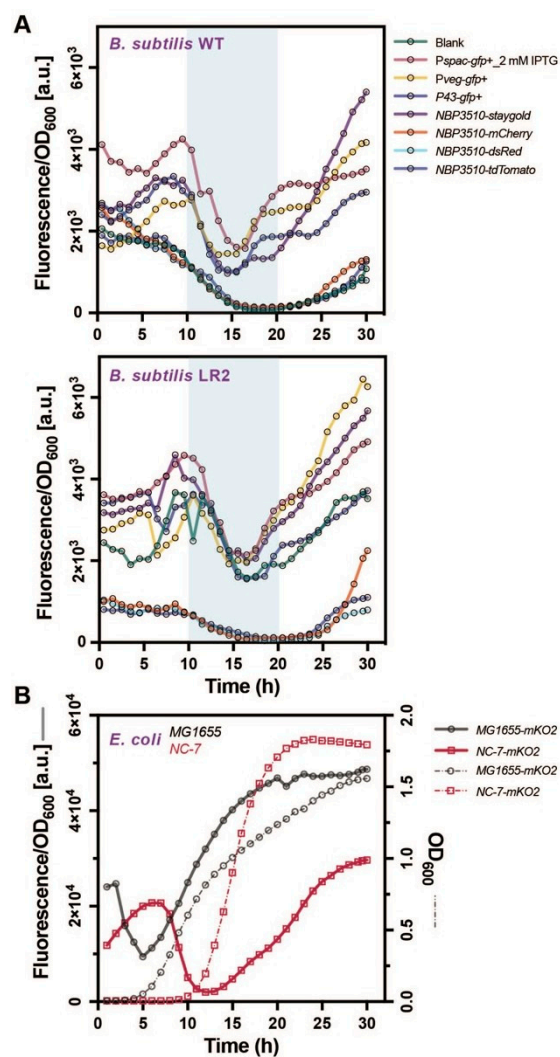


Figure S5. The expressions of fluorescent proteins in *B. subtilis* WT, L-form LR2, *E. coli* MG1655 and L-form NC-7. (A) The fluorescent expression level and pattern measured by the microplate reader in *B. subtilis* WT (upper panel) and L-form LR2 (lower panel). (B) The fluorescent expression level and growth pattern in *E. coli* MG1655 and L-form NC-7.

Table S1 List of primers used in this study

Name	Sequence (5' to 3')
<i>Pspac</i> F1	gtgatgataagtgggaaggac
<i>Pspac</i> R1	gctttcgtatgtagcatcac
<i>Promoter</i> F1	gactatattcaaaggtgaaagcactcg
<i>Promoter</i> R1	gtacctctagatgcttcaccacct
<i>Promoter</i> F2	gtttgtcgggaacgctctctac
<i>Promoter</i> R2	cgcgattccaatgaggttaagag
<i>gfp</i> + R1	ccgtatgtagcatcaccttcacc
<i>egfp</i> R1	ccgtatgttgcacaccttcac
<i>staygold</i> R1	ccatgacatcggtagttttccac
<i>mCherry</i> R1	cttgagccgtacatgaactgag
<i>dsRed</i> R1	ggaaggacagcttcattgtagtcg
<i>tdTomato</i> R1	catgttgtgtcctcggaggag
<i>mKO2</i> R1	cgcgtagtgtcatctcttgatg
<i>gfp</i> + F2	ccgttcaactagcagaccattatc
<i>egfp</i> F2	cggttcaactagcagaccattatc
<i>staygold</i> F2	gatgtcccatatcactggattcg
<i>mCherry</i> F2	ctgaggtcaagaccacctacaagg
<i>dsRed</i> F2	caaccactacgtggactccaag
<i>tdTomato</i> F2	ctcctccgaggacaacaacatg
<i>mKO2</i> F2	gattcttgaaatgccaggagacc
<i>transform</i> F3	cgcgaattatcaagctctgagc
<i>transform</i> R3	cgaggggaagcggttcacagtttc
<i>transform</i> F4	catcattaatcatccttcagg
<i>transform</i> R4	gctgaccgttagcgtttaagtac
<i>HM-pUC19</i> F	gtgcccttaaacgccctgtcagaccaagttaactcat
<i>HM-pUC19</i> R	gagaagtgcattgctccagtcgggaaacctgtcgtg
<i>HM-Ppro-mKO2</i> -F	ccgactggaagcatgcacttctcaaagatcca
<i>HM-Ppro-mKO2</i> -R	gtgtgacgttctgcggccgctctagaaccggaac
<i>HM-pETcoco-1</i> -F	ctagagcggccgcagaacgtcacaccgtcagcag
<i>HM-pETcoco-1</i> -R	cttggtctgacaggcggtttaagggcaccaataactgcc
<i>Fragment 3+1</i> F1	ctggcgattcaggttcattcatgc
<i>Fragment 3+1</i> R1	gaagaacagtatgtgtatctgcgctc
<i>Fragment 1+2</i> F2	gcgttatccctgattctgtgg
<i>Fragment 1+2</i> R2	ctaagtcaaacgcgaaaggcattg
<i>Fragment 2+3</i> F3	ggtcgacgggtatcgataagcttg
<i>Fragment 2+3</i> R3	cttacgtgccgatcaacgtctc

