

1 **Supplementary Information**

2

3 **AraBAD Based Toolkit for Gene Expression and Metabolic Robustness**  
4 **Improvement in *Synechococcus elongatus***

5

6 **Yi-Qi Cao<sup>1</sup>, Qian Li<sup>1</sup>, Peng-Fei Xia<sup>1</sup>, Liu-Jing Wei<sup>2</sup>, Ning Guo<sup>1</sup>, Jian-Wei Li<sup>1</sup>,**  
7 **Shu-Guang Wang<sup>1,\*</sup>**

8

9 <sup>1</sup>School of Environmental Science and Engineering, Shandong University, 27 Shanda  
10 Nanlu, Jinan 250100, China

11 <sup>2</sup>State Key Laboratory of Bioreactor Engineering, East China University of Science and  
12 Technology, 130 Meilong Road, Shanghai 200237, China

13 \*Correspondence and materials should be addressed to S.-G.W. (email: wsg@sdu.edu.cn)

14

15 **Figure legends**

16 **Figure S1.** Fluorescence of eGFP and mtGFP led by P<sub>BAD</sub> promoter in *S. elongatus* after  
17 inducing with 2 g/L *L*-arabinose for 2 days.

18 **Figure S2.** Standardized fluorescent intensity (RFU/OD<sub>600</sub>) of *E. coli* BW25113, YQe1  
19 (P<sub>BAD</sub>) and YQe2 (P<sub>Ttc</sub>) under non-induced condition. An asterisk (\*) represents a  
20 statistical difference ( $p < 0.05$ ).

21 **Figure S3.** Fluorescence images of *S. elongatus* strains. (A) YQs1 and (B) YQs2 after  
22 inducing with 20 g/L *L*-arabinose and 5 mM IPTG for 2 days, respectively.

23 **Figure S4.** Growth curve of *S. elongatus* in continuous light condition with or without 2  
24 g/L *L*-arabinose for 10 days.

25

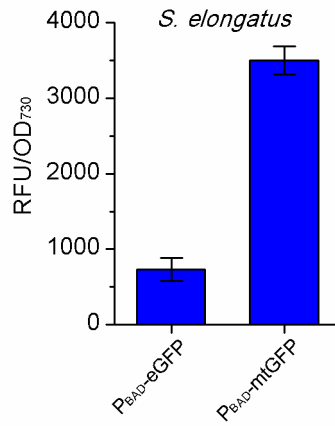
26 **Table legends**

27 **Table S1.** Plasmids used in this study

28 **Table S2.** Primers used in this study

29

30 **Figures**

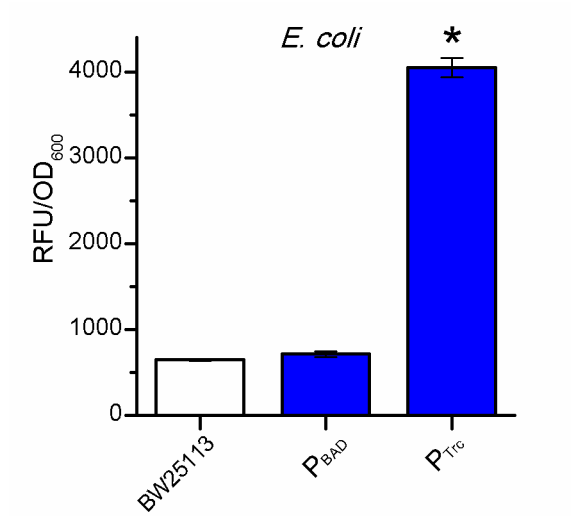


31

32 **Figure S1.** Fluorescence of eGFP and mtGFP led by P<sub>BAD</sub> promoter in *S. elongatus* after

33 inducing with 2 g/L *L*-arabinose for 2 days.

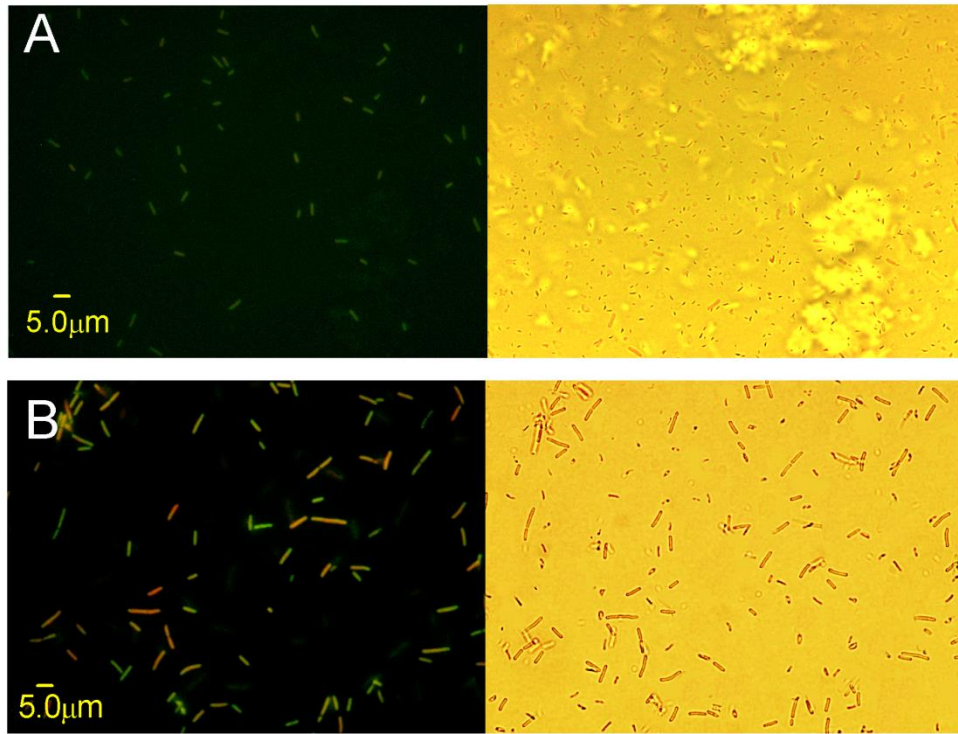
34



35

36 **Figure S2.** Standardized fluorescent intensity (RFU/OD<sub>600</sub>) of *E. coli* BW25113, YQe1  
37 (P<sub>BAD</sub>) and YQe2 (P<sub>Tre</sub>) under non-induced condition. An asterisk (\*) represents a  
38 statistical difference ( $p < 0.05$ ).

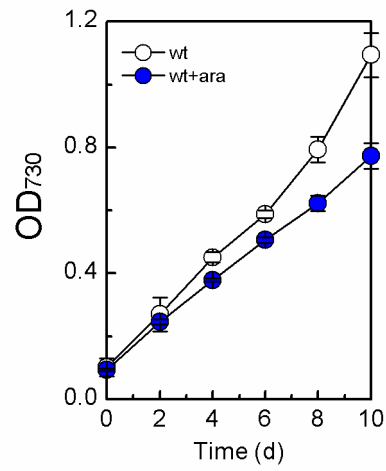
39



40

41 **Figure S3.** Fluorescence images of *S. elongatus* strains. (A) YQs1 and (B) YQs2 after  
42 inducing with 20 g/L *L*-arabinose and 5 mM IPTG for 2 days, respectively.

43



44

45 **Figure S4.** Growth curve of *S. elongatus* in continuous light condition with or without 2

46 g/L *L*-arabinose for 10 days.

47

48 **Tables**49 **Table S1.** Plasmids used in this study.

<b>Plasmids</b>	<b>Genotypes</b>	<b>Sources</b>
pAM2991	NSI targeting vector; $P_{\text{Trc}}$ ; $\text{Sp}^{\text{R}}$	Addgene #40248
pAM1573	NSII targeting vector; $\text{Cm}^{\text{R}}$	Addgene #40239
pcrRNA.ind	<i>araC</i> - $P_{\text{BAD}}$ ; $\text{Amp}^{\text{R}}$	Addgene #61284
pcrRNA.con	$P_{\text{J23119}}$ ; $\text{Amp}^{\text{R}}$	Addgene #61285
pYQ1	pAM2991, but $P_{\text{Trc}}$ : <i>araE</i>	This study
pYQ2	pAM2991, but $P_{\text{Trc}}$ : <i>mtgfp</i>	This study
pYQ3	pAM2991, but $P_{\text{Trc}}$ : <i>araE</i> - <i>mtgfp</i>	This study
pYQ4	pcrRNA.ind, but $P_{\text{BAD}}$ : <i>mtgfp</i>	This study
pYQ5	pAM2991, but $P_{\text{BAD}}$ : <i>mtgfp</i>	This study
pYQ6	pYQ1 containing $P_{\text{BAD}}$ - <i>mtgfp</i>	This study
pYQ7	pcrRNA.con, but $P_{\text{J23119}}$ : <i>araA-araB-araD</i>	This study
pYQ8	pAM1573, but $P_{\text{J23119}}$ : <i>araA-araB-araD</i>	This study

50

51

52 **Table S2.** Primers used in this study.

Primers	Sequences (5'-3')
Q1	ccggaattcatggttactatcaatac ggaatctg
Q2	ccggaattctcagacgccgatatttctcaa
Q3	ccggaattcatggttactatcaatac ggaatctgct
Q4	gtgaatagttcctcgccttttgacatgac gcc gatatttctcaactt
Q5	aagttgagaaatcgcgc gtc atgtcaaaa ggc ga ggaactatt
Q6	ccggaattcttacttgataa ttc atccatgccca
Q7	ccggaattcatgtcaaaa ggc ga ggaactatt
Q8	cccaagcttttacttgataa ttc atccatgccca
Q9	aaggaaaaaagcggccgcttatgacaactgac ggc tacatca
Q10	aaggaaaaaagcggccgcaaaa ggc catcc gtca ggat
Q14	catggcgcgaaggc atattac gggca gtaaacgca gaa gc ggtc tgataaacagaatt
Q15	ccacacttcataattatcaaaaatcgtcat gtaccgagctcgaattc gctagc gctagca
Q16	tgctagc gctagc gaattc gagc tc ggtac atgac gat tttt gataa tta tgaagt gggttt gtcatt ggca gc
Q17	gccaaaatc gaggccaattgcaatc gccat tta gc gac gaaaccc gtaatac acttc gttccagc gca g
Q40	ccgctc gaggctcat gttt gac agc tta tcatc gat
Q41	tgctctagaaaaaggccatcc gtca ggat
Q50	ccggaattcatgtcaaaa ggc ga ggaactatt
Q51	cgcggatccttacttgataa ttc atccatgccca