

1 Supplementary materials for

2 **Metabolic engineering of a probiotic *Saccharomyces boulardii***

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7 (Running title: Metabolic engineering of *Saccharomyces boulardii*)

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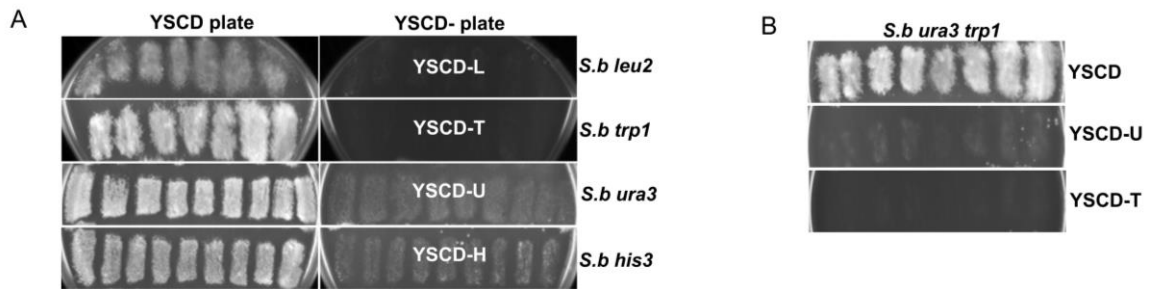
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24 **Fig. S1** The screen of auxotrophic markers inactivation. (A) The screen of single

25 deletion of each auxotrophic markers; (B) Identification of *ura3 trp1* double

26 inactivation using tandem guide RNA. *S. b* is short for *Saccharomyces boulardii*.

27 YSCD: yeast synthetic complete medium with 20 g/L glucose as a carbon source;

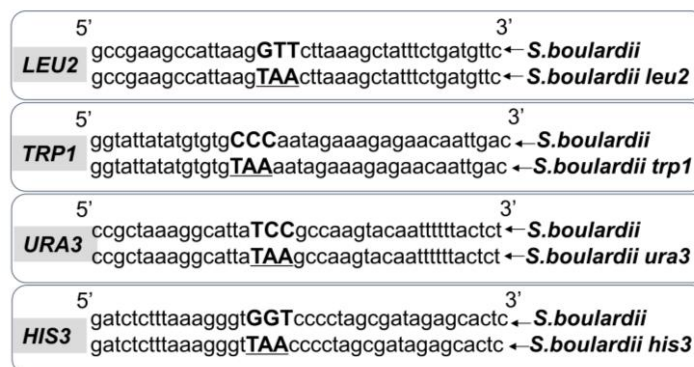
28 YSCD-Leu: YSCD medium minus leucine; YSCD-Trp: YSCD medium minus

29 tryptophan; YSCD-Ura: YSCD medium minus uracil; YSCD-His: YSCD medium

30 minus histidine.

31

32



33

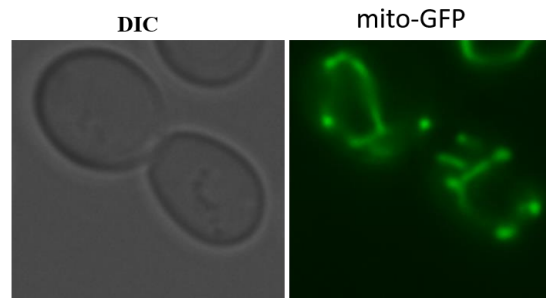
34 **Fig. S2** The sequencing results of four marker genes *LEU2*, *TRP1*, *URA3* and *HIS3*

35 indicated that the targeted nucleotides were replaced by the stop codon (TAA) as

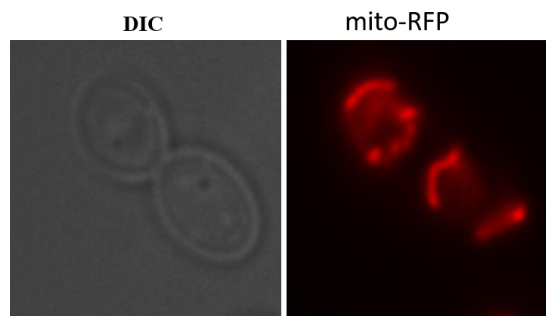
36 expected.

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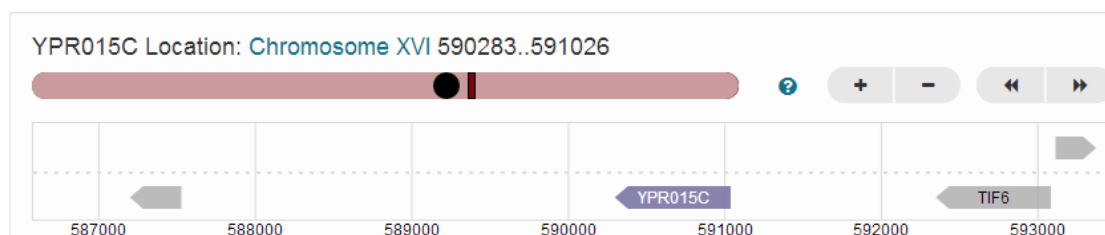
40 **Fig. S3.** Targeted localization of the green fluorescent protein (GFP) and red  
41 fluorescent protein (RFP) in mitochondria of *S. boulardii* expressing GFP and mRuby  
42 with a mitochondrial targeting sequence by introducing pVT100U-mtGFP and  
43 pVT100U-mt-mRuby plasmid, respectively. The left panel is differential interference  
44 contrast (DIC) images; right panel is GFP and RFP fluorescence.

45

#### 46 **Intergenic site CS8 for CRISPR-Cas9 based integration.**

47 The 2.7 kb non-coding region between YPR015C and a CDS of unknown function  
48 from chromosome XVI was chosen for further target gene insertion. The map and  
49 genome sequence from Yeastgenome.org are listed as follows:

50



51

<http://www.yeastgenome.org/locus/S000006219/overview>

52 **NNNN: CDS of functional gene of YPR015C**  
53 **NNNN: CDS of not essential protein, or protein with no function (or unknown**  
54 **function)**  
55 nnnn: Non-coding region  
56 nnnn: PAM sequence for CS8  
57 nnnn: Target sequence for guide RNA  
58 nnnn: Homologous region for integration  
59 nnnn: sequencing primer site

60  
61 **[CS8 region, Chromosome XVI]**

62 **.....TAGATCTTATACAAAAGCAACTGCGCGCTGTGGTAAAGCTACGGA**  
63 **AACAATGTCCTATCTGTGGGAAGGTTTGTTCGAGACCTTCAACACTGA**  
64 **GGACTCATTACTTAATACATACGGGAGACACACCTTCAATGTACTTG**  
65 **GGAGCATTGCAACAAATCTTCAATGTCAAGAGTAAACATGTTAAGGCA**  
66 **TTAAGAACCCATCAAAAAGAAAATAGCAAAGAAAAACATCAGTGA**aag  
67 cattgtttagaatatgttttcagtgtagtttgatgtaggtgtgacacttttaccagtaaaatgagtatagatatgtattagttc  
68 catataatattacatgtagccaacaatcaatcttactgacttccgattttgaaatgtgagaggattttgtcgaattataatttc  
69 tacaagaaattgttgcgaatgcatcagcattgttaacgggtctatactgccgcacgatacattttattctgtctagttcgtaa  
70 gacaaaggacctttcatttagtacaacgttccgacacggatgtcttgatgaatctctgtaccaggaattcaattacctgacta  
71 gtatgcagttcactatcaaaaaaaaaaagggttaaattggtataatcatggttaaagaacaaacagcctctttctctacaa  
72 aagtatgttaaattaggttagttcaaatgctgcacgtatcgcaacgttgttaatatataatgatccattggaacaatgaagt  
73 ttacagaagctcaaaataatgcaagaataattacgtcaagggagtcatacaaaactaagaaaacaaaggaattgactaag  
74 ttgagcgcgaatgatataatccatgcttgcattctgattcatacatatcgtttctgtcatttcaatggaagctttattatcaattacc  
75 acaaccttagtattgtcttatttggcctaaaggagttcatgctgtagtgatagtcacccatgaagtgattagtggaacat  
76 agaaggggataaatttctatcgatacaaaagaaaaaccaggattattgtttaccaccacatttcaactttcccgtgaat  
77 ttctactaccctcatttgaagaacgctttccactcttaggttggcgttgcattcttttccattcaatgcaccaagactta  
78 atttgttggttaaactcttatttctgtcagtttaaccaaaaaattacctacggtaattagtgaaggccaaaatctaattgtaca  
79 atgattcaatcattcttattgaggagaagctaccgagaagaaccctaccactggtacaacacaagggaacggctctcattg  
80 acctcgtgcataaccgggaaggtaacacatgaatgctgtgtttgaaatcatctccaatccaaactataggaatgaaaaat  
81 ttgggtacaatgtcttctacaatcgtattctgtcagcaatgttccaaggatgacagactcttctaagaaaagattca  
82 gttcaaaattfacataacagtgaaactatgcagaaaatttacttctagaccacgcatttaacaataatgtttcccgaacaata  
83 agtatttggcgtttaaagacgttatcagttattttagagaaggtgttaccattcgtgctcccatactaaaaccaaggggtaa  
84 taggaccacattcgttctgacagccaacaacgtgccactagcaggacagttattggaggttatattatgtaattattgttag  
85 ctgataaatgattacagaaagcgtgatgttactgggctgcttattcagcaatccccacgatatttacttcaagagaaag  
86 aatgtttgatgtaagtttatttgaacgtggaacttggcgtggggcatttatggctgctatcacacaaatcgcggagcagagta  
87 ccccgtgattataacacaactcactaaaaatcgtcaaaattgggggttacgggttagcgcggcagctcatcgagggaac  
88 agcacctagtgcacgtttaaattgatagatcttcagaacgaaatcaaaatcttcgcagtatcattacgtttcaagaaactaaagt  
89 gtttggagaaattaggaacgaaaaaagctactatagaatttagcctgtcttcaactgctgctcctcggcttttacttgccta  
90 agtatatgccgaatgggcgataagcgggagtgctggcagatctgtggtaaaaaaagatagcaataaaaaatgatgaatt  
91 taacagtagtcttgaattaaacaagtgatcttgcactgaatacttccatcaatgagtcgctaaaatgaaatggctgcacaat  
92 ctctccgcatactttaaaggcagcaatttagccagttgatccctacatttccacagaaacgaaaaagtgaatagttat  
93 tgcggccaatcgtaaagcttagcggccttagctttatgattgttgaagaaagtttgccttttactctccttcaggtgtca  
94 gtattaacaaacggcgttgaatgtttaaagttatttttattcatcaaatcgaacttgatactttctcgggtgatgctgctaattgatt  
95 ataccattaaggtgtcaccagtgtaaaaatgttcacggactgtgcaactacgtgtgaaggtaagttactcaacgcaaga

96 aacgtaacattttacaaatttagctgcagtactatgtttaagtaatccaaagggaactattgttatatcccagaattattaca  
97 ttcgtttcttagttcataaacaatgaatacctattgaatggatagaaattctgacttgattttacgagttattattgctgacattagtc  
98 caaagacatctcagttttgttcctctacaacccaatgaggaggctagccagggtgtcgccecaaaaaatagccaataaag  
99 cggcaactttctgt**ATGTCTTGCAATTTTTCCGCTGACTTGGGAGTAGAGTACA**  
100 **GCTGCGCGGAGTCGCGTATTACAAATCTTGTACTTTGCATTCTGTGCAT**  
101 **ACGTGAGGAAAAAGCAGCACCTGTTGTGAAAAAAGATAAATTTCTTTT**  
102 **TTTCATTTTTCTATCCTCAAAGGAAAATCTTTTCTGCGAAATTTCTAGC**  
103 **CGCTCTTCAGTTTCCGAAGTAAAGTCGCTGAGGGCGAAAAACAACTTT**  
104 **ATTTGCCACACGCTGTTATAGGCTTCCAGGGAGCAAAGCGCAATTG**  
105 **GGAATAAGGTTTCTCAATTGAATGGTTTGCTTCGCCTACAGCATTAAC**  
106 cagaaggaattgatctctgtatgtccgctaacagatctgtccattaattgtttattttcttcaggtaggctgagcccactttgt  
107 agtagctccctaccattactttggcctgtagttattggctttgatttttcttctatgacaatcccgttgagcaaccttgccaag  
108 ggctcttttgaaaaaaggcgtgggcaagatctctgtat.....

109

## 110 **Human lysozyme with chicken signal peptide.**

111 The gBlock sequence of human lysozyme with a chicken-lysozyme signal sequence

112 based on previous report (1, 2) is listed as follows:

113

114 NNNN: enzyme site for the confirmation of insertion of gBlock into plasmid

115 **nnnn: Chicken-lysozyme signal sequence**

116 **nnnn: Starting codon**

117 **nnnn: Stop codon**

118

119 GGTACC**atgaggtcttgtaaatcttggtgcttgcttctgccctggctgctctgggg**aaggtttcgaacgttgaat  
120 tggccagaactttgaagagattgggtatggacggtaccgtggtatctctttggtaactggatgtgtttggccaagtgggaat  
121 ctggttacaacactagagctactaactacaacgccggtgaccgttctactgactacggtatcttccaaattaactctagatact  
122 ggtgtaacgacggtaagactccagcgccgtaacgctgtcagttgtcttctgctttgttcaagacaacatcgctgac  
123 gccgttgctgtgtaagagagtcgtagagaccacaaggtatcagagcttgggtcgttggcgtaacagatgtcaaac  
124 agagacgtcagacaatacgttcaaggtgtggtgtc**taa**GTTCGAC

125

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