

Suppl. Material

Journal: Applied Microbiology and Biotechnology

Dual engineered bacteria improve inflammatory bowel disease in mice

Yong-Qi Wu^{a, #}, Zhen-Ping Zou^{a, #}, Ying Zhou^{a *}, and Bang-Ce Ye^{a, b *}

^a Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

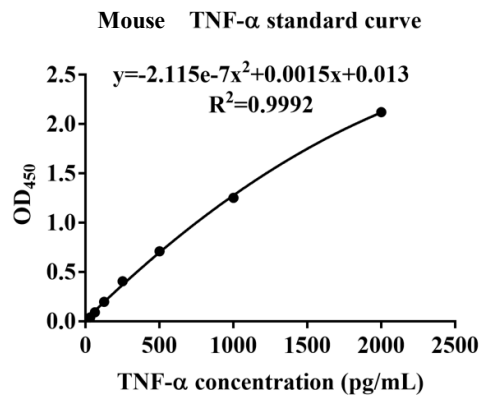
^b Institute of Engineering Biology and Health, Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, China

[#] Y.-Q. W. and Z.-P. Z. contributed equally to this paper

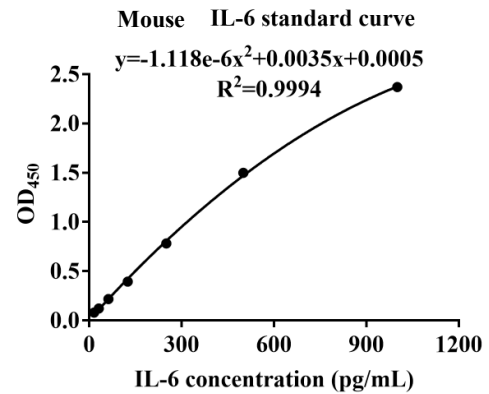
^{} Email: zhouying@ecust.edu.cn (Y.Z.); bcyeye@ecust.edu.cn (B.-C. Y.)*

Suppl. Figures

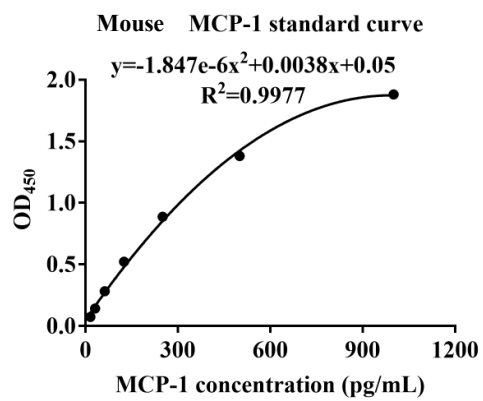
A



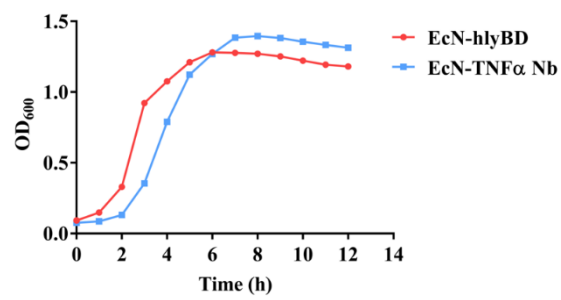
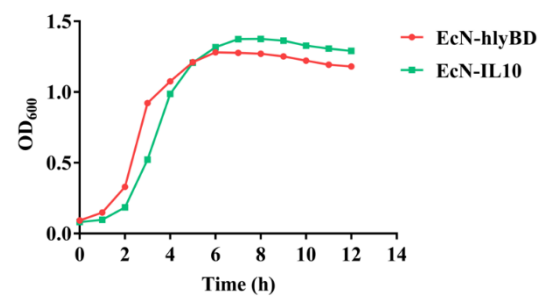
B



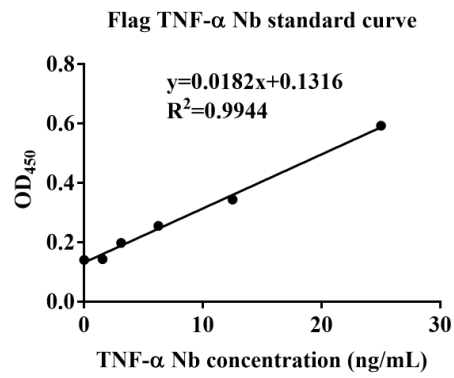
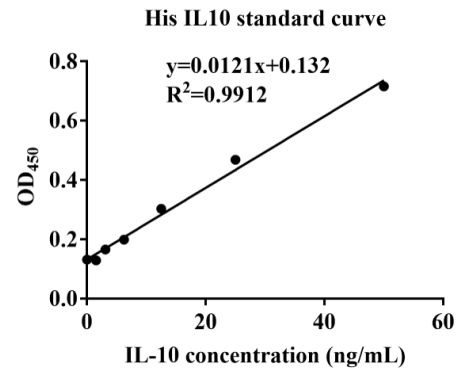
C



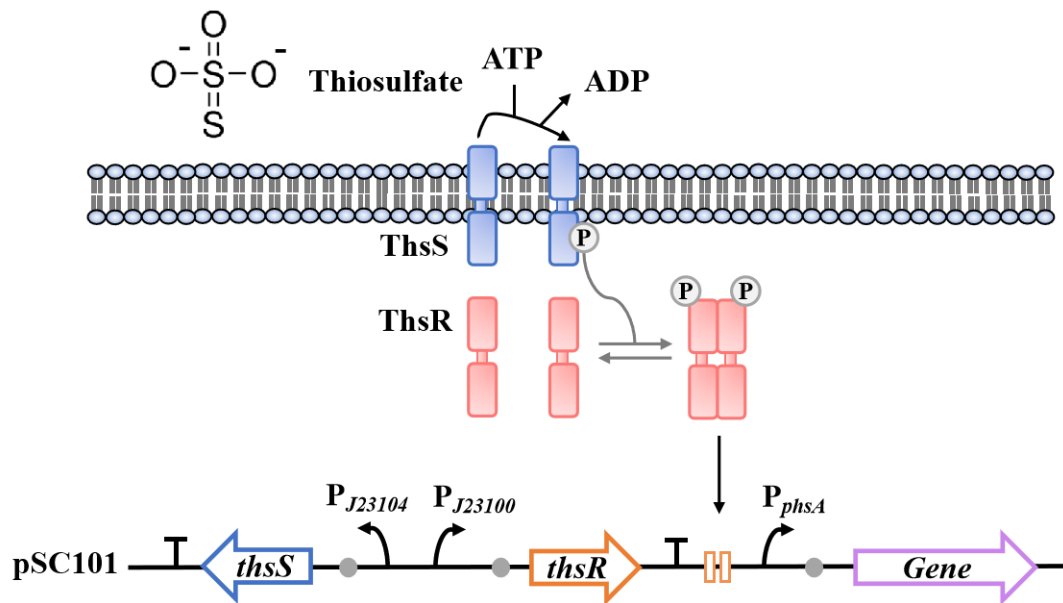
Supplemental Fig. S1 Mouse TNF- α standard curve (A); Mouse IL-6 standard curve (B); Mouse MCP-1 standard curve (C).

A**B**

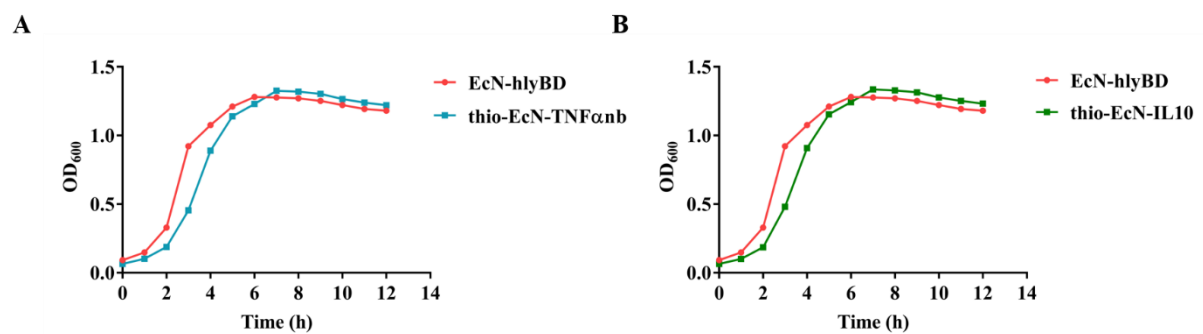
Supplemental Fig. S2 Growth curve of constitutive engineered bacteria EcN-TNF- α Nb (A) and EcN-IL10 (B).

A**B**

Supplemental Fig. S3 Standard curve of anti-TNF- α nanobody (A) and IL-10 (B).



Supplemental Fig. S4 Schematic diagram of the thiosulfate two-component system (Daeffler et al. 2017)



Supplemental Fig. S5 Growth curve of inducible engineered bacteria thio-EcN-TNF- α Nb (A) and thio-EcN-IL10 (B).

Suppl. Tables

Supplemental Table S1 Strains and plasmids used in this work.

Strain/Plasmid	References/Sources
<i>Escherichia coli</i> DH5 α	TransGen Biotech, Beijing, China
<i>Escherichia coli</i> BL21(DE3)	TransGen Biotech, Beijing, China
pET28a-TNF α Nb-BL21	This study
pET28a-IL10-BL21	This study
<i>Escherichia coli</i> Nissle 1917 (EcN)	DSMZ Cat# DSM6601
EcN-hlyBD	(Zou et al. 2023)
EcN-mcherry	This study
pWT-TNF α Nb	This study
EcN-TNF α Nb	This study
pWT-IL10	This study
EcN-IL10	This study
thio-EcN-TNF α Nb	This study
thio-EcN-IL10	This study

Supplemental Table S2 Overview of all sequences of genetic parts used in this work.

Gene/encoded protein	Nucleotide sequences	Amino acid sequences
Anti-TNF- α antibody	CAGGTGCAGCTGCAAGATAGCGGTGGTGGTCTGGTTCAGG CAGGTGGTAGTCTGCGCCTGAGTTGCGCAGCCAGTGGCGG TACATTTAGTAGCATTATTATGGCCTGGTTCCGTCAGGCACC GGGCAAAGAACGTGAATTTGTGGGCGCAGTGAGCTGGAGT GGTGGCACCACCGTTTATGCAGATAGTGTCTGGGTGCTTT TGAAATTAGCCGCGATAGTGCCCGTAAAAGTGTGTATCTGC AAATGAATAGCCTGAAACCGGAAGATACCGCCGTTTATTATT GCGCAGCCCGCCCGTATCAGAAATATAATTGGGCCAGCGC AAGCTATAATGTGTGGGGCCAGGGCACCCAGGTGACCGTT AGTAGT	QVQLQDSGGGLVQAGGSLRLSCAASGGTFSSII MAWFRQAPGKEREFVGAVSWSGGTTVYADSVL GRFEISRDSARKSVYLMNSLKPEDTAVYYCAAR PYQKYNWASASYNVWGQGTQVTVSS
IL-10	AGCCGTGGCCAGTATAGTCGTGAAGATAATAATTGCACCCA CTTTCCGGTGGGTCAGAGCCACATGCTGCTGGAAGTGC GC ACCGCATTTAGCCAGGTGAAAACCTTTTTTTCAGACCAAAGAT CAGCTGGATAACATTCTGCTGACCGATAGTCTGATGCAGGA TTTTAAAGGTTACCTGGGCTGCCAGGCACTGAGTGAAATGA TTCAGTTTTATCTGGTGGAGGTGATGCCGCAGGCAGAAAAA CATGGCCCGGAAATTAAGAACATCTGAATAGTCTGGGCGA AAAAGTGAAGAACCTGCGCATGCGCCTGCGTCGCTGTCATC GTTTTCTGCCGTGTGAAAATAAAAGCAAAGCCGTTGAACAG GTTAAGAGCGATTTTAATAAGCTGCAGGATCAGGGTGTTTAT	MSRGQYSREDNNCTHFPVGQSHMLLELR TAFS QVKTFQTKDQLDNILLTDSLMDQDFKGYLG CQAL SEMIQFYLVEVMPQAEKHGPEIKEHLNSLGEK LK TLRMRLRRCHRFLPCENKSKAVEQVKSDFNK LQ DQGVYKAMNEFDIFINCIEAYMMIKMKS

mcherry	AAAGCCATGAATGAATTTGACATCTTCATCAACTGCATCGAA GCCTATATGATGATTAAGATGAAGTCC ATGGTGAGCAAGGGGCGAGGAGGATAACATGGCCATCATCA AGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGT GAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGG CCGCCCCCTACGAGGGCAGCCAGACCGCCAAGCTGAAGGTG ACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGT CCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCAC CCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGA GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGC GGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACG GCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTC CCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCT GGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCG CCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGA CGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAG GCCAAGAAGCCCGTGACGCTGCCCCGGCGCCTACAACGTCA ACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACC ATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCA CCGGCGGCATGGACGAGCTGTACAAG TTAGCCTATGGAAGTCAGGATAATCTTAATCCATTAATTAAT GAAATCAGCAAAATCATTTTCAGCTGCAGGTAATTTTGATGTT AAAGAGGAAAGAGCTGCAGCTTCTTTATTGCAGTTGTCCGG TAATGCCAGTGATTTTTTCATATGGACGGAACCTCAATAACTTT GACAGCATCAGCA	MVSKEEDNMAIIKEFMRFKVHMEGSVNGHEFEI EGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILS PQFMYGSKAYVKHPADIPDYLKLSFPEGFKWER VMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTN FPSDGPVMQKKTMGWEASSERMYPEDGALKGE IKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYN VNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYK
hlyA secretory peptide	TTAGCCTATGGAAGTCAGGATAATCTTAATCCATTAATTAAT GAAATCAGCAAAATCATTTTCAGCTGCAGGTAATTTTGATGTT AAAGAGGAAAGAGCTGCAGCTTCTTTATTGCAGTTGTCCGG TAATGCCAGTGATTTTTTCATATGGACGGAACCTCAATAACTTT GACAGCATCAGCA	LAYGSQDNLNPLINEISKIISAAGNFDVKEERAAAS LLQLSGNASDFSYGRNSITLTASA

References

- Daeffler KN, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, Britton RA, Tabor JJ (2017) Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation. *Mol Syst Biol* 13: 923. <https://doi.org/10.15252/msb.20167416>
- Zou ZP, Du Y, Fang TT, Zhou Y, Ye BC (2023) Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice. *Cell Host Microbe* 31: 199-212. <https://doi.org/10.1016/j.chom.2022.12.004>