Supplemental Information

O$_2$-inducible H$_2$O$_2$-forming NADPH oxidase is responsible for the hyper O$_2$ sensitivity of *Bifidobacterium longum* subsp. *infantis*

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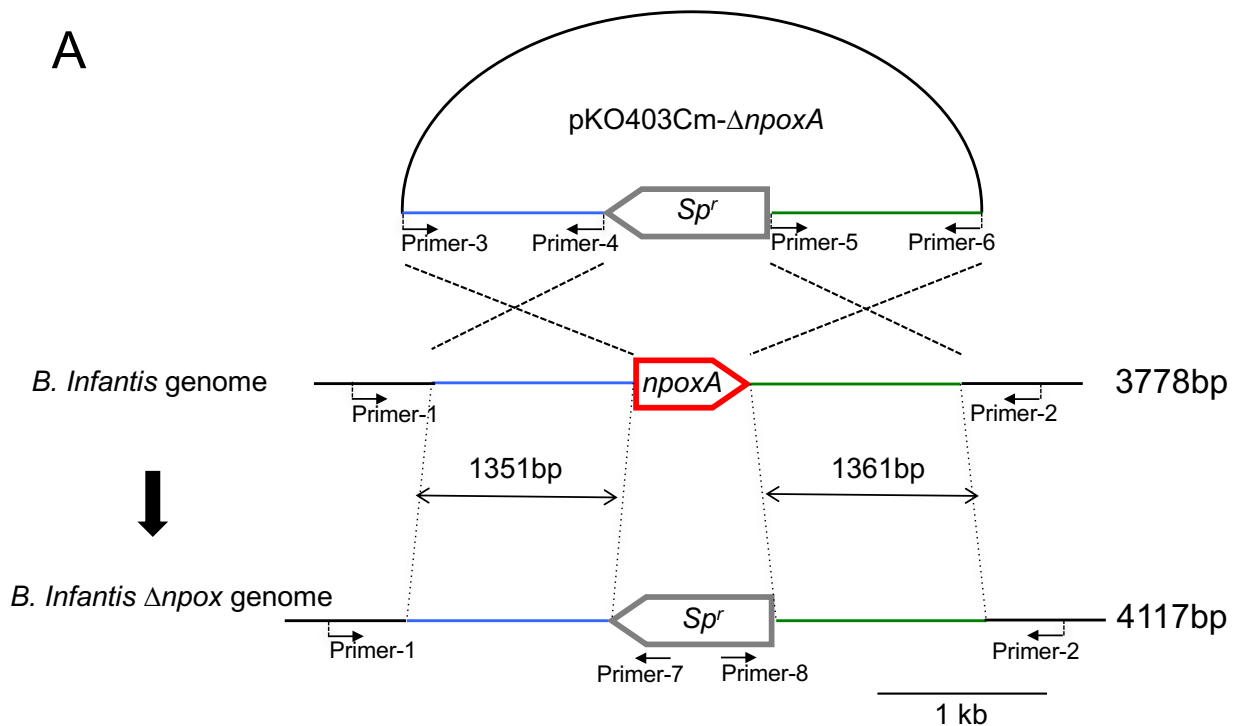[2]Department of Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan
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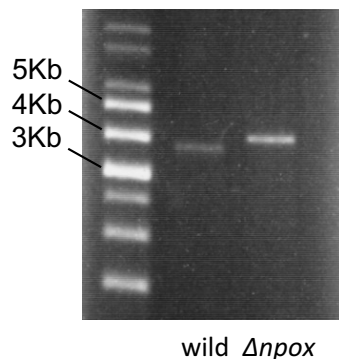
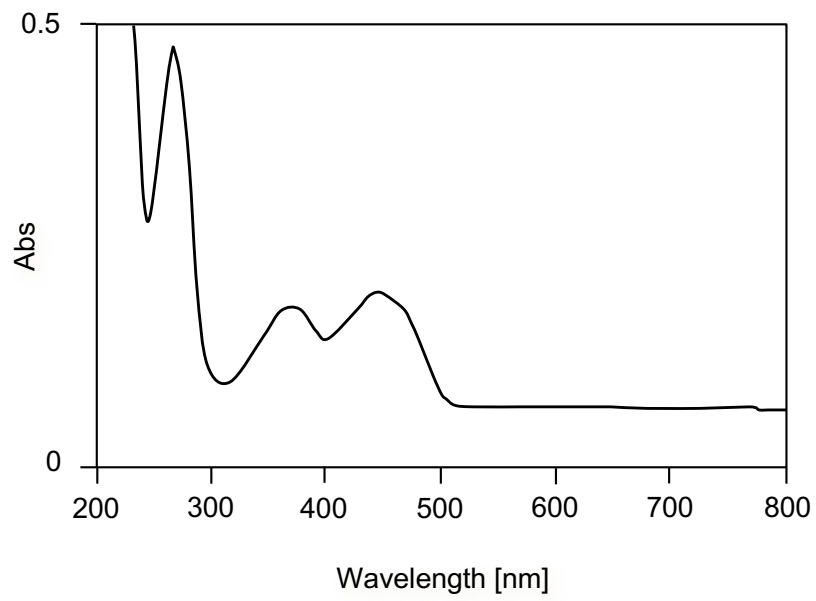*Correspondence: Shinji Kawasaki, kawashin@nodai.ac.jp, Tel&Fax+81-3-5477-2764

A

pKO403Cm-ΔnpoxA

Sp^r

Primer-3   Primer-4   Primer-5   Primer-6

B. Infantis genome

Primer-1   npoxA   Primer-2   3778bp

1351bp   1361bp

B. Infantis Δnpox genome

Primer-1   Sp^r Primer-2   4117bp

Primer-7   Primer-8

1 kb

B

Primer-1	CAG GCC AGC GTA ATC ACA G
Primer-2	GTG CCG GAC AAC GAA GTC AA
Primer-3	acaGTTCGATGACGTTATCTCCCT
Primer-4	aagGGCTTTATTGTCGCACGG
Primer-5	gaaTGAGACCAGGACCAACGA
Primer-6	ctaGAGCGCATCGATTTGTTTCCA
Primer-7	AGC AGT TCG TAG TTA TCT TGG AG
Primer-8	GTT ATT TAT AGA TTT CAT TGG CTT

C

5Kb
4Kb
3Kb

wild Δnpox
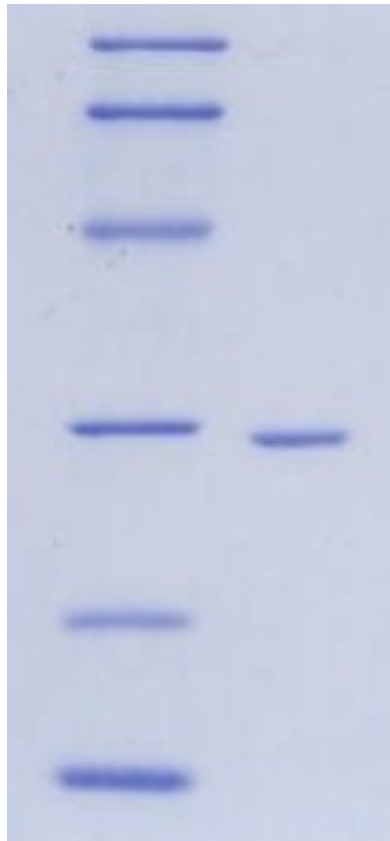
Supplementary Figure S1
Procedure for *B. infantis npox* gene disruption. (A) A plasmid pKO403Cm-ΔnpoxA for the knockout of the *B. infantis npox* gene was constructed. (B) Primers used for the gene knockout experiments. Small letters are the 5' overhang for the Golden Gate cloning experiment. (C) The deletion of *npoxA* was confirmed by PCR using Primer-1 and Primer-2. The PCR products were sequenced to confirm the precise disruption.

Supplementary Figure S2
Absorption spectrum of the *B. infantis* NPOX protein. The absorption (Abs) spectrum of the purified enzyme was determined in 50 mM sodium phosphate, pH 7.0.

Supplementary Figure S3
Original SDS-PAGE image of the purified *B. infantis* NPOX used to generate Figure 2B.