

## Supplementary Data

### Structural and biochemical analyses of the butanol dehydrogenase from *Fusobacterium nucleatum*

Xue Bai <sup>1,2,†</sup>, Jing Lan <sup>1,2,†</sup>, Shanru He <sup>1,2</sup>, Tingting Bu <sup>1,2</sup>, Jie Zhang <sup>1,2</sup>, Lulu Wang <sup>1,2,3</sup>, Xiaoling Jin <sup>1,2</sup>, Yuanchao Mao <sup>1,2</sup>, Wanting Guan <sup>1,2</sup>, Liying Zhang <sup>1,2</sup>, Ming Lu <sup>4</sup>, Hai-long Piao <sup>5</sup>, Inseong Jo <sup>6</sup>, Nam-Chul Ha <sup>7</sup>, Chunshan Quan <sup>1,2</sup>, Ki Hyun Nam <sup>8,9,\*</sup> and Yongbin Xu <sup>1,2,\*</sup>

<sup>1</sup> Department of Bioengineering, College of Life Science, Dalian Minzu University, Dalian 116600, Liaoning, China

<sup>2</sup> Key Laboratory of Biotechnology and Bioresources Utilization of Ministry of Education, College of Life Science, Dalian Minzu University, China

<sup>3</sup> School of Life Science and Biotechnology, Dalian University of Technology, No. 2 Linggong Road, Dalian 116024, Liaoning, China

<sup>4</sup> Shandong Provincial Key Laboratory of Energy Genetics, key Laboratory of Biofuel, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, Shandong, China

<sup>5</sup> CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, Liaoning, China

<sup>6</sup> Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea

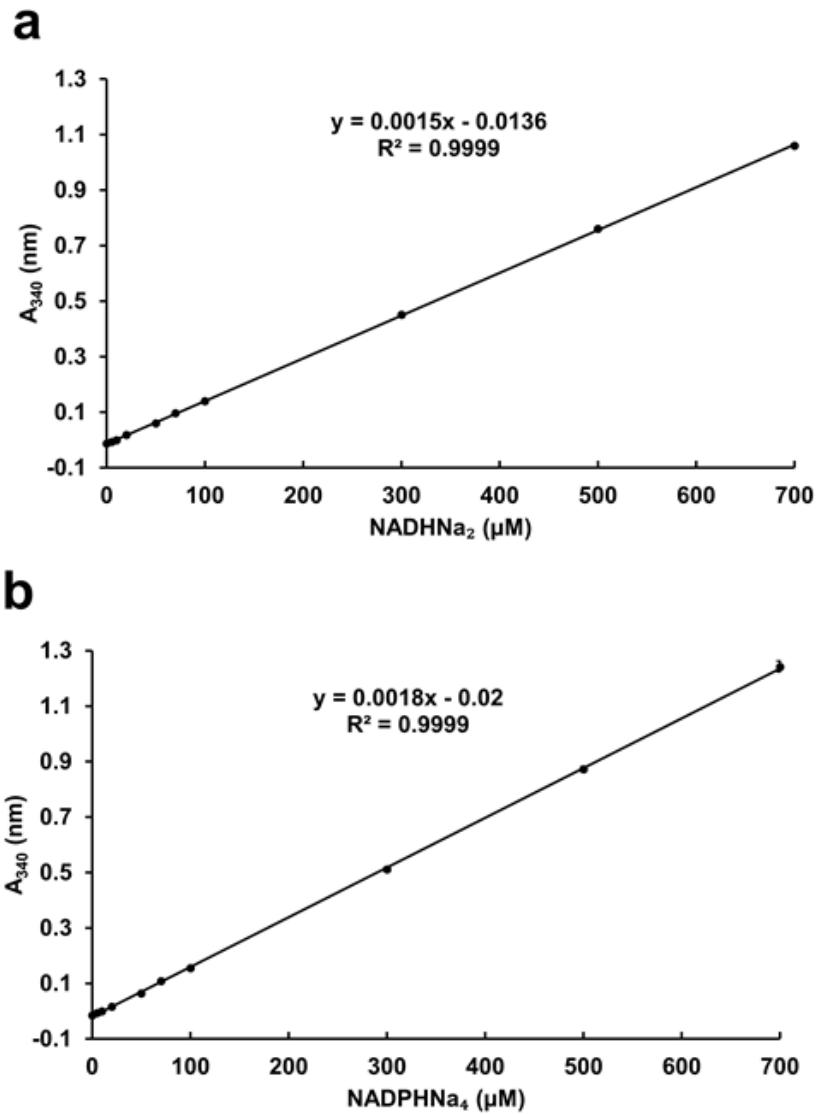
<sup>7</sup> Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Gwanak-gu, Seoul 00826, Republic of Korea

<sup>8</sup> Department of Life Science, Pohang University of Science and Technology, 35398 Pohang, Republic of Korea

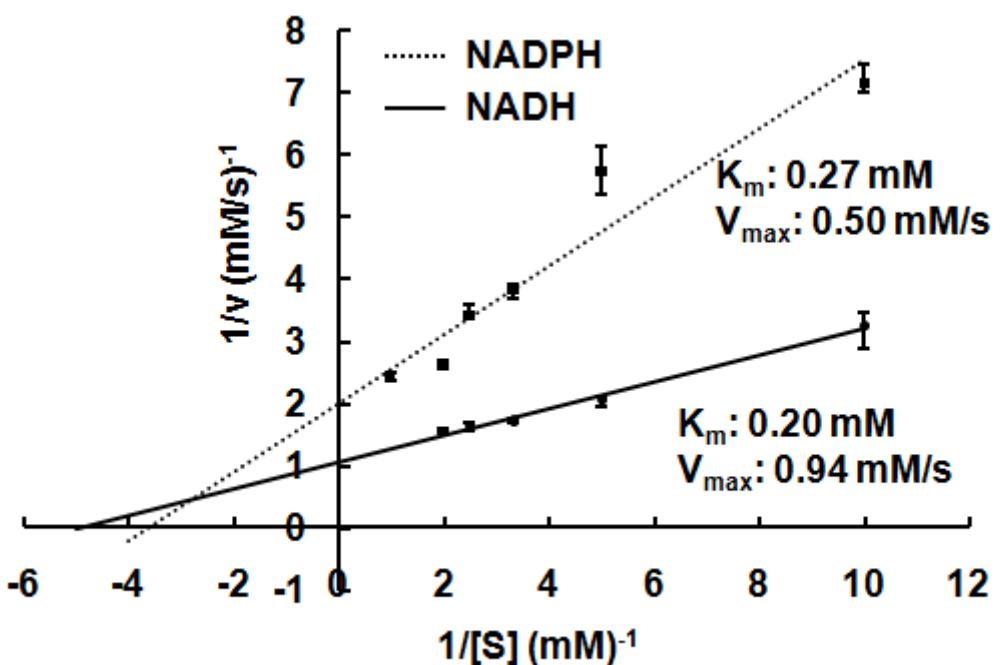
<sup>9</sup> POSTECH Biotech Center, Pohang University of Science and Technology, 35398 Pohang, Republic of Korea

\*Correspondence: structures@postech.ac.kr (K.H.N.); yongbinxu@dlnu.edu.cn (Y.X.)

† Both authors contributed equally to this work.



**Figure S1.** Calibration working curve constructed from the average slopes of (a) NADH, and (b) NADPH standards (0-700  $\mu\text{M}$ ), obtained from the three-fold assay repetition.



**Figure S2.** The double reciprocal plot was used to calculate  $V_{\max}$  and  $K_m$  of the enzyme in the presence of NADH and NADPH. Data represent average  $\pm$  SD of three different samples.

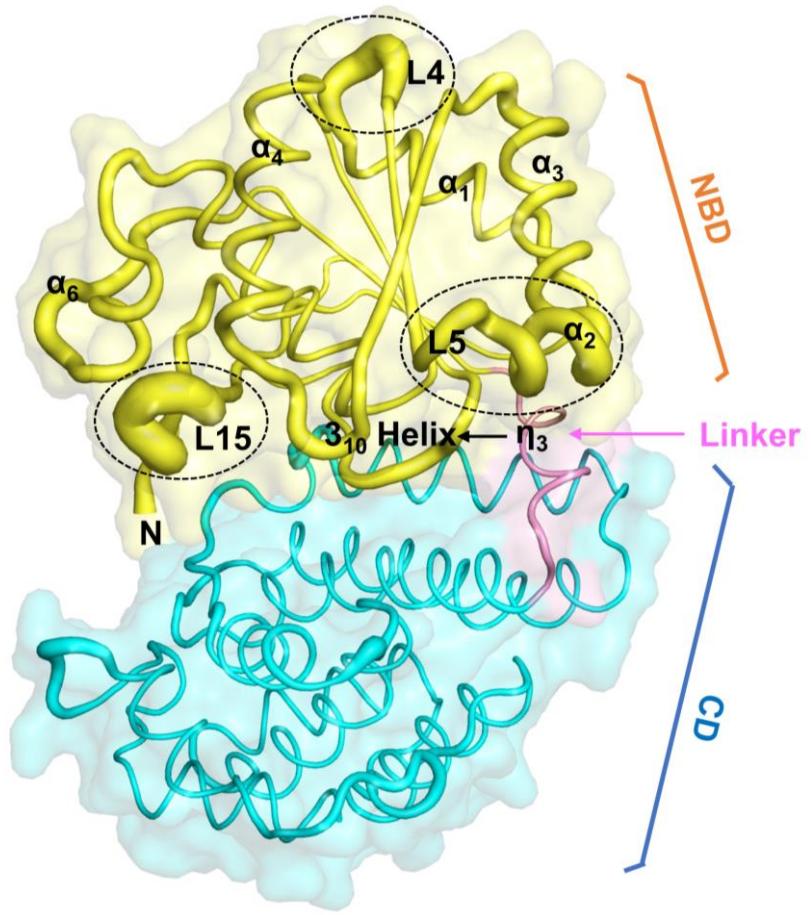
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FnYqdH	M	N	F	N	D	T	K	I	F	G	D
AfADH	M	N	F	I	F	K	S	G	T	K	I
CboADH	M	N	F	I	F	K	S	G	T	R	I
DrADH	M	N	F	I	F	E	N	P	T	K	I
EsADH	M	N	F	V	F	Q	N	P	T	K	I
CbaADH	M	I	N	F	T	E	Q	N	P	T	K
	Q	N	P	T	K	I	F	G	K	G	V
	Q	N	P	T	K	I	F	G	K	G	V
	Q	N	P	T	K	I	F	G	K	G	V
	Q	N	P	T	K	I	F	G	K	G	V
	Q	N	P	T	K	I	F	G	K	G	V

	110	120	130	140	150	160	170	180	190	200	
FnYqdH	G	G	S	V	I	D	A	K	I	A	M
AfADH	G	G	S	A	I	D	A	K	I	A	M
CboADH	G	G	S	A	I	D	A	K	I	A	M
DrADH	G	G	S	A	I	D	A	K	I	A	M
EsADH	G	G	S	V	I	D	A	K	I	A	M
CbaADH	G	G	S	V	I	D	A	K	I	A	M
	V	I	D	A	K	I	A	M	I	A	M
	V	I	D	A	K	I	A	M	I	A	M
	V	I	D	A	K	I	A	M	I	A	M
	V	I	D	A	K	I	A	M	I	A	M
	V	I	D	A	K	I	A	M	I	A	M

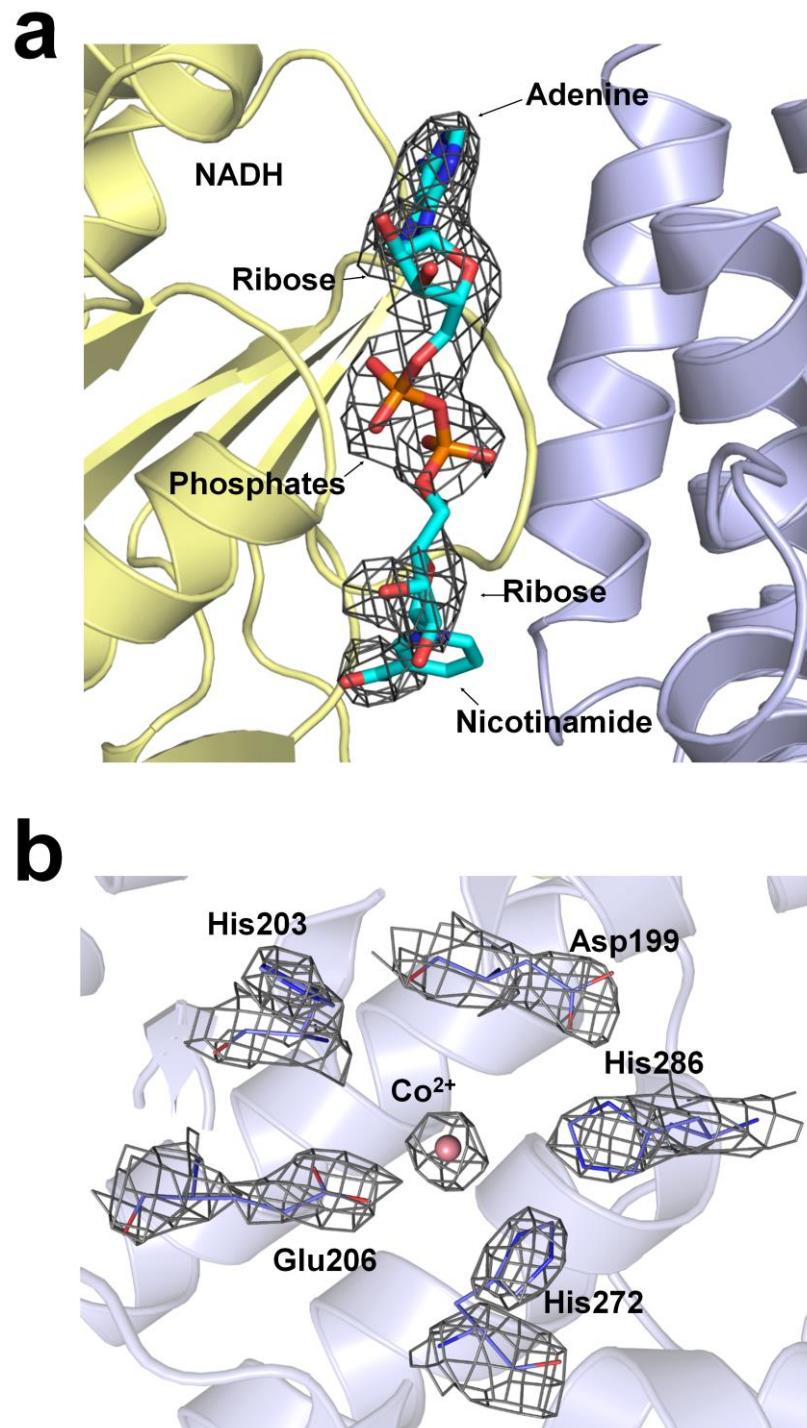
	210	220	230	240	250	260	270	280	290	300	
FnYqdH	L	S	H	M	E	R	Y	F	T	R	I
AfADH	L	S	H	M	E	R	Y	F	T	R	I
CboADH	L	S	H	M	E	R	Y	F	T	R	I
DrADH	L	S	H	M	E	R	Y	F	T	R	I
EsADH	L	S	H	M	E	R	Y	F	T	R	I
CbaADH	L	S	H	M	E	R	Y	F	T	R	I
	H	I	M	E	R	Y	F	T	R	I	M
	H	I	M	E	R	Y	F	T	R	I	M
	H	I	M	E	R	Y	F	T	R	I	M
	H	I	M	E	R	Y	F	T	R	I	M
	H	I	M	E	R	Y	F	T	R	I	M

	310	320	330	340	350	360	370	380														
FnYqdH	T	K	N	R	P	E	K	F	K	E	V	F	N	T	V	N	.	.	.	.	.	.
AfADH	V	K	D	E	M	R	F	V	Q	L	A	T	R	F	G	T	D	D	T	D	K	K
CboADH	V	K	E	N	M	R	F	V	Q	F	L	A	T	R	F	G	T	D	D	T	K	K
DrADH	V	K	H	D	I	N	F	V	Q	F	L	A	T	R	F	G	T	D	D	T	K	K
EsADH	V	K	H	D	P	N	F	V	Q	F	L	A	T	R	F	G	T	D	D	T	K	K
CbaADH	V	K	H	D	P	N	F	V	Q	F	L	A	T	R	F	G	T	D	D	T	K	K
	P	E	K	F	K	E	V	F	N	T	V	N	T	V	N	T	V	N	T	V	N	T
	P	E	K	F	K	E	V	F	N	T	V	N	T	V	N	T	V	N	T	V	N	T
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	P	E	K	F	K	E	V	F	N	T	V	N	T	V	N	T	V	N	T	V	N	T
	P	E	K	F	K	E	V	F	N	T	V	N	T	V	N	T	V	N	T	V	N	T

**Figure S3.** Sequence alignment of FnYqdH and homologous proteins. Sequence alignment of FnYqdH, AfADH (NCBI Reference Sequence: WP\_066501503.1), CboADH (GenBank: QGU94363.1), DrADH (NCBI Reference Sequence: WSLF01000004), EsADH (GenBank: NLK98329.1) and CbaADH (GenBank: NLY43117.1)



**Figure S4.** Analysis of the flexible region of FnYqdH. B-factor representation of FnYqdH; α2, L4, L5, and L15 show relatively high flexibility.



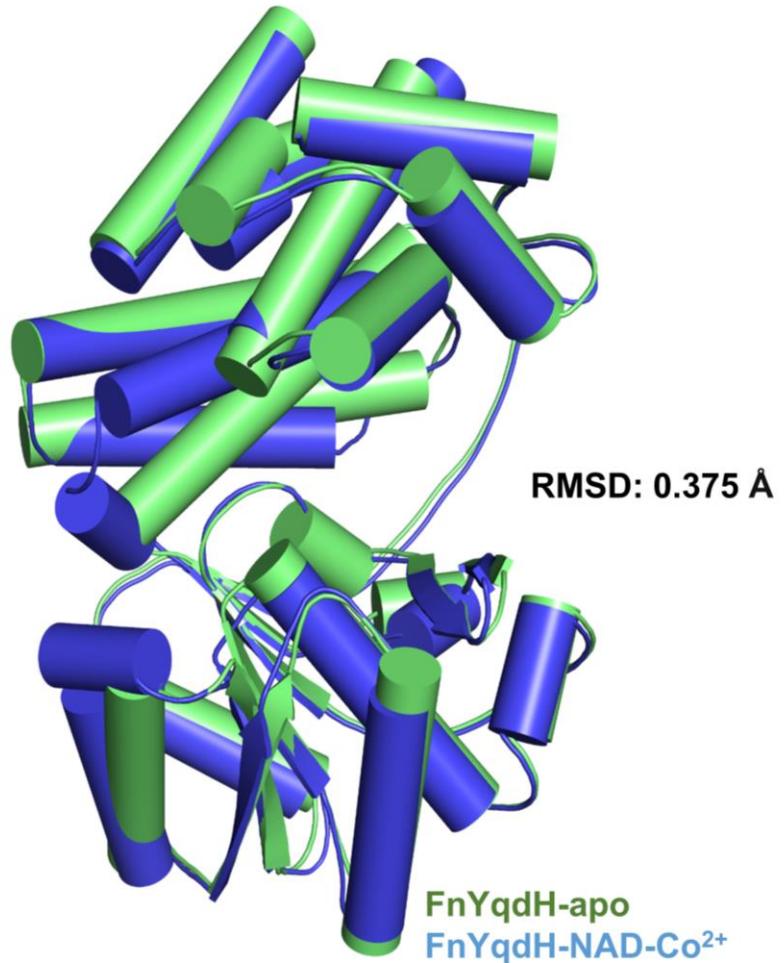
**Figure S5.** The electron density map of NADH and Co<sup>2+</sup>. The electron density map of (a) NADH and (b) Co<sup>2+</sup> are shown as grey mesh.

1	10	20	30	40	50	60	70	80	90	100
FnYqdH	M D N P N Y K N D T K I I F G K D N Y S E [I] G K N I K I P S K K T P [K] I L D H Y E A D E L I K K I C I Y E K V I S S Y K F D I E F I E I C G V V P N P R L S I V Y E G I K I C K K E N I T F I L A V									
CbBDHA	M K N F E F Y A P T R V I F G K D S E K Q I G T I I K .. N Q N C K K V L V H E G . G S S A K K S C I L D K I F E S Y K K S E I D Y V S I C G V V P N P R L S K V Y E G I N I L C K K E K V D F I L A V									
CaBDHA	M L G F D Y S I P T K V P F G K G K I D V I G E E I K .. K Y G S R V I L V Y G . G G S I K R N G I Y D R A T A I Y K C N N I A F Y E I S C G V S P N P R I T T V K K C I E I C R E N N V D L V L A I									
BmBDHA	M E N F T I W N P T K L I F G S N Q L E Q L K T E I P .. Q Y G K K V L L V Y G . G G S I K R N G L Y G R V V N Y E E I N P E I F E I S G V E P N P R I S T V R K G V E I C K R E G I E F I L A V									
ZmBDHA	M L N F D Y Y N E P T H I I V F G K G R I A Q L D T L L S .. . K D . A R V L U L Y G . . G S S A Q K I G T L D E V R K A E G D R . L T Y F E F G G I E P N P R S Y E T L M K A V E Q V R Q E K V D F I L A V									

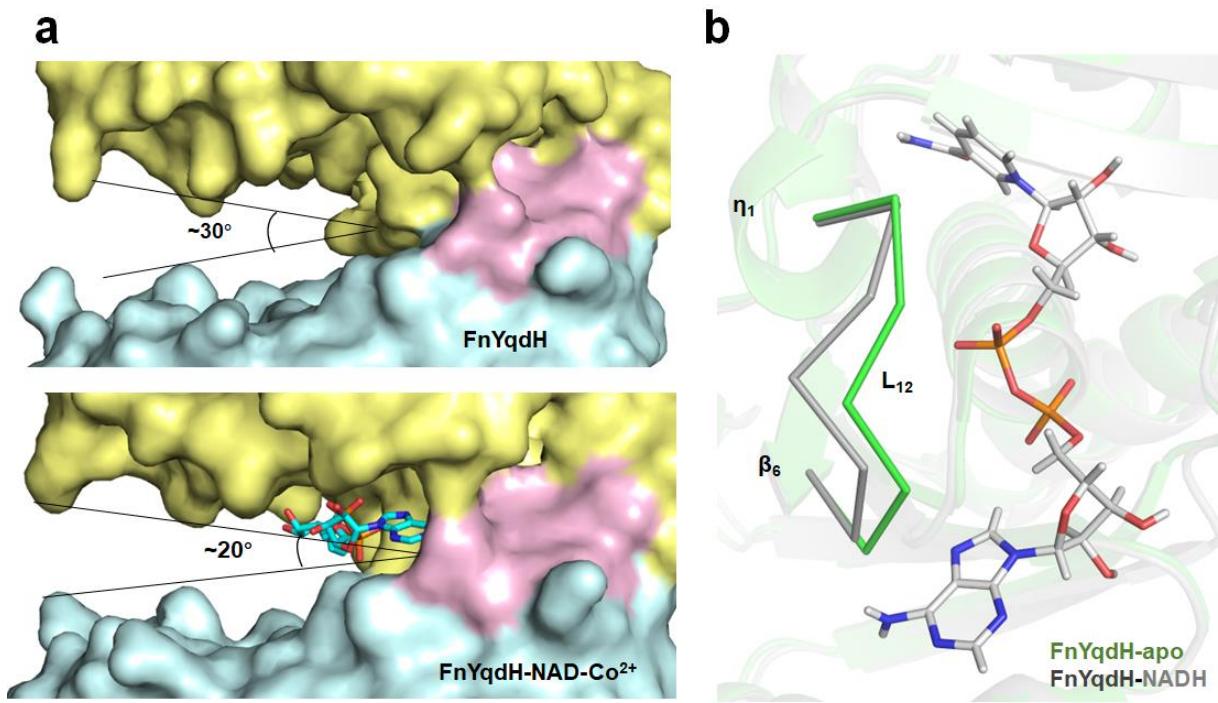
110	120	130	140	150	160	170	180	190
FnYqdH	G G S V I D S A K A I S L G A V D N G D V W D F F T A K R I . P Q D T L G I G V V L T I P G A G S E M S E S S I I T D E N K K Q K A V C D T E V N F P K F A I I N P E V C Y T I P D R L M R A G I V D							
CbBDHA	G G S V I D S A K A I G Y G M A N E C D V I D Y S R K V I I P T G C L P I G V V L T I A A A G S E M S N S V I T N E E G W I R G C N S E Y A R C K F A I M N P E L T Y T L P K Y Q T A G G A T D							
CaBDHA	G G S V I D C S K V I A A G V Y Y D G D T D M V K D P S K . I T K V L P I A S I L T I S A T G S E M D Q I A V I S N M E T N E K L G V G H D D M P E K F S V I L D P T Y I F T V P R N Q T A A G T A D							
BmBDHA	G G S V I D C T K A I A A G V K Y E G D P N D F V I K N A K . V K E A L P F G T V L T I A T G S E M N S G S V I T N W E L N E K R A W S S P A V P E K F S I L E P E N T Y T V P R D Q T V E G I V D							
ZmBDHA	G G S V I D G T K F V A A A V P Y E G E P P W E I L E T D G K K I K E A L P V G T V L T I P A T G S E M N R N S S V V I R K S I K S R G F H N D H V P E V F S I L L D P T K V Y T L P P R Q L A N G V V D							

200	210	220	230	240	250	260	270	280	290
FnYqdH	I I S H L M P Y F T K S I D T A L S D S I K P A T M K V I K Y G P L I L M K D R K N I A N Y C S Q I M W A A T M A H N G M I A C P R V A D P A S H R I E H E I S G I Y D I H G I G M A I I F P A W M K								
CbBDHA	I I M H T M P Y F T K E Q S M I L T D R I S P G L M R T V I H N V K I L M K N P K D I N A R A E V M W A A G L S H N I L T G C S S V C D P S C H O L E H E L C G M F D V I H G A C I A R V W G S W A R								
CaBDHA	I I M S H T F E S Y F S G V E G A Y V Q D G I A B A I L R T C I K Y G K I A M E K T D D I B A R A N L M W A S S L A I N G L L S L O K D R K P S C H P M E H E L S A Y Y D I T H G V G I A I L T P N W M E								
BmBDHA	I I M S H V F R Y F H P E E N T F P Q D R M P S S L I T V M E A A P K L I E N L E S B H R S T I L Y C G T M A L N G M L S M C F R G W P A T H K I F E H A V S A V Y D I F H G G G I A I L F P N W M K								
ZmBDHA	S E I H I T E Q Y L T Y P V D G M V Q D E F A E G L L R T L I K I G P E L L R D Q K N I D L A A N F M W T A I F L A N G L I G A C V P Q D W A T H M V G H E L T A A F G I D H G R T B A I I L P S L L Q								

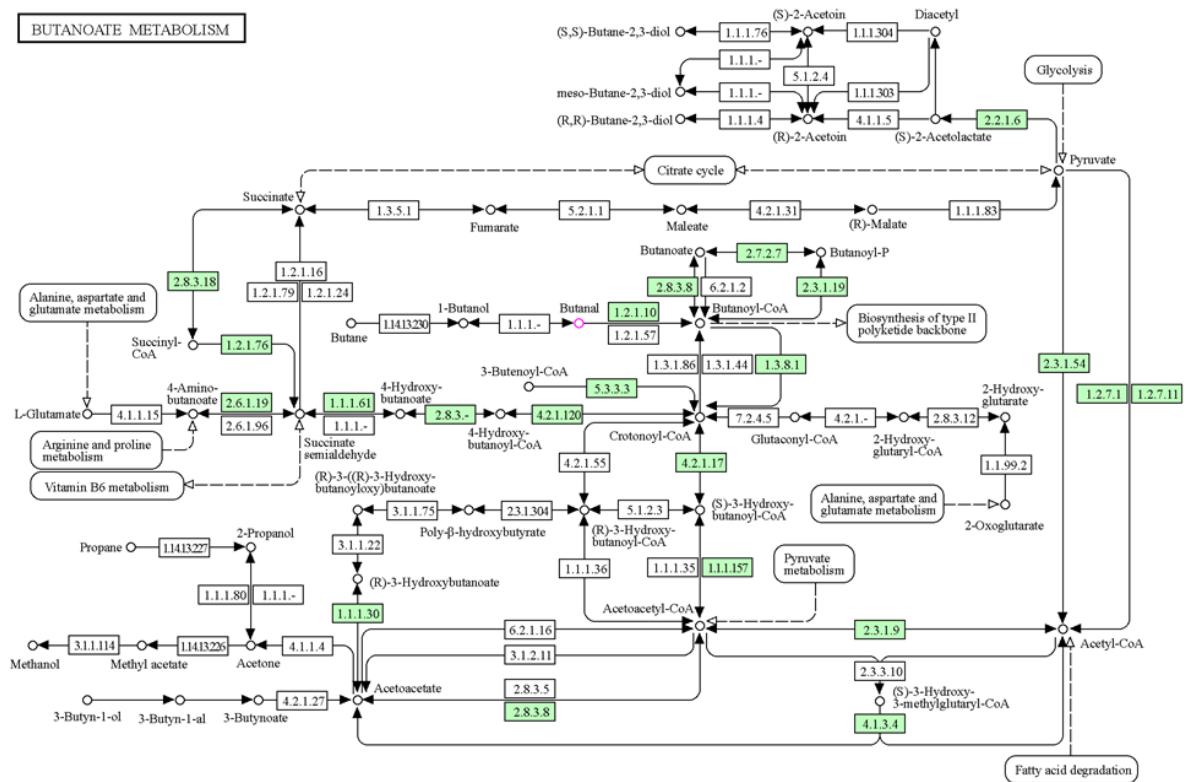
**Figure S6.** Sequence alignment of FnYqdH with BHD from *C. beijerinckii* (CbBDH, Accession No.: WP\_077844128.1), *C. acetobutylicum* (CaBDHA, WP\_010966572.1), *Bacillus megaterium* (BmBDHA, WP\_013085305), and *Zymomonas mobilis* (ZmBDHA, WP\_011241511.1).



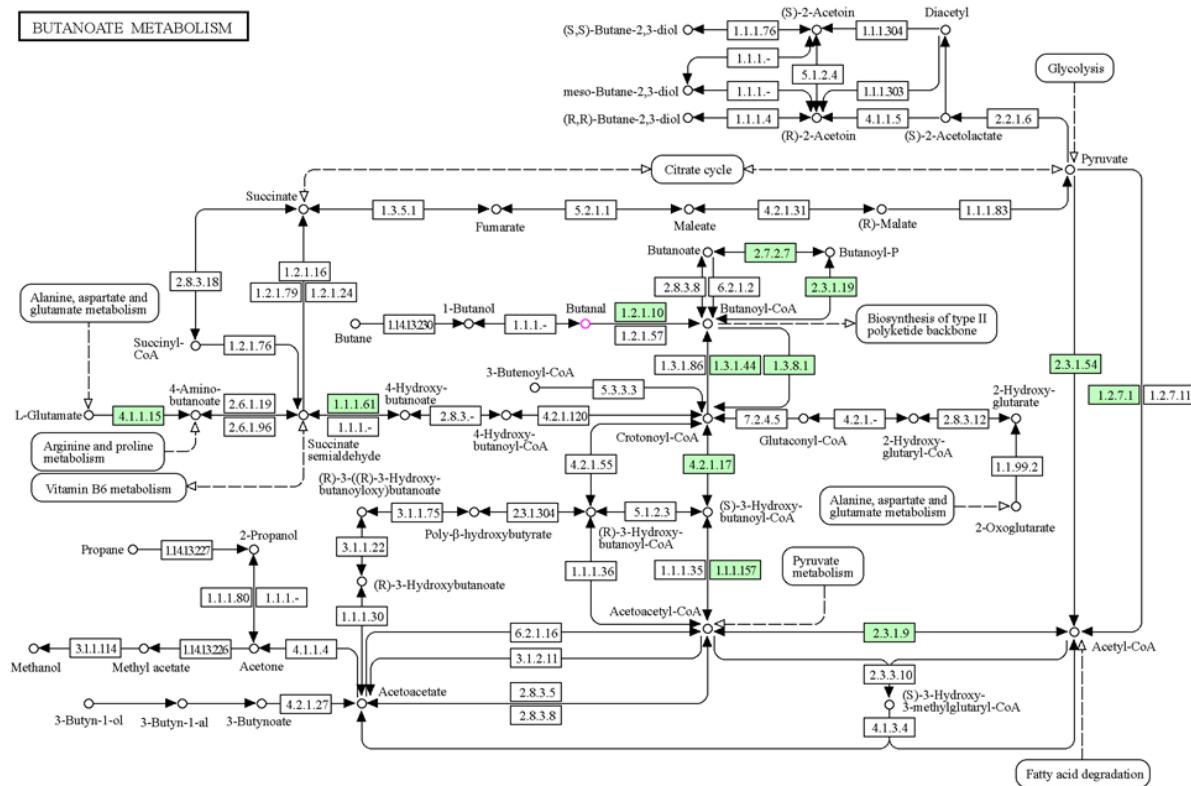
**Figure S7.** Overall fold of FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. Comparison of the structures of the FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. The crystal structures of the FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup> are colored green and blue, respectively.



**Figure S8.** Comparison of FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. (a) Surface comparison of FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. (b) Cartoon compilation of FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup>. FnYqdH-apo and FnYqdH-NAD-Co<sup>2+</sup> are colored green and grey, respectively.



**Figure S9.** Butanoate metabolism of *Clostridioides difficile*.



**Figure S10.** Butanoate metabolism of *Clostridium perfringens*.

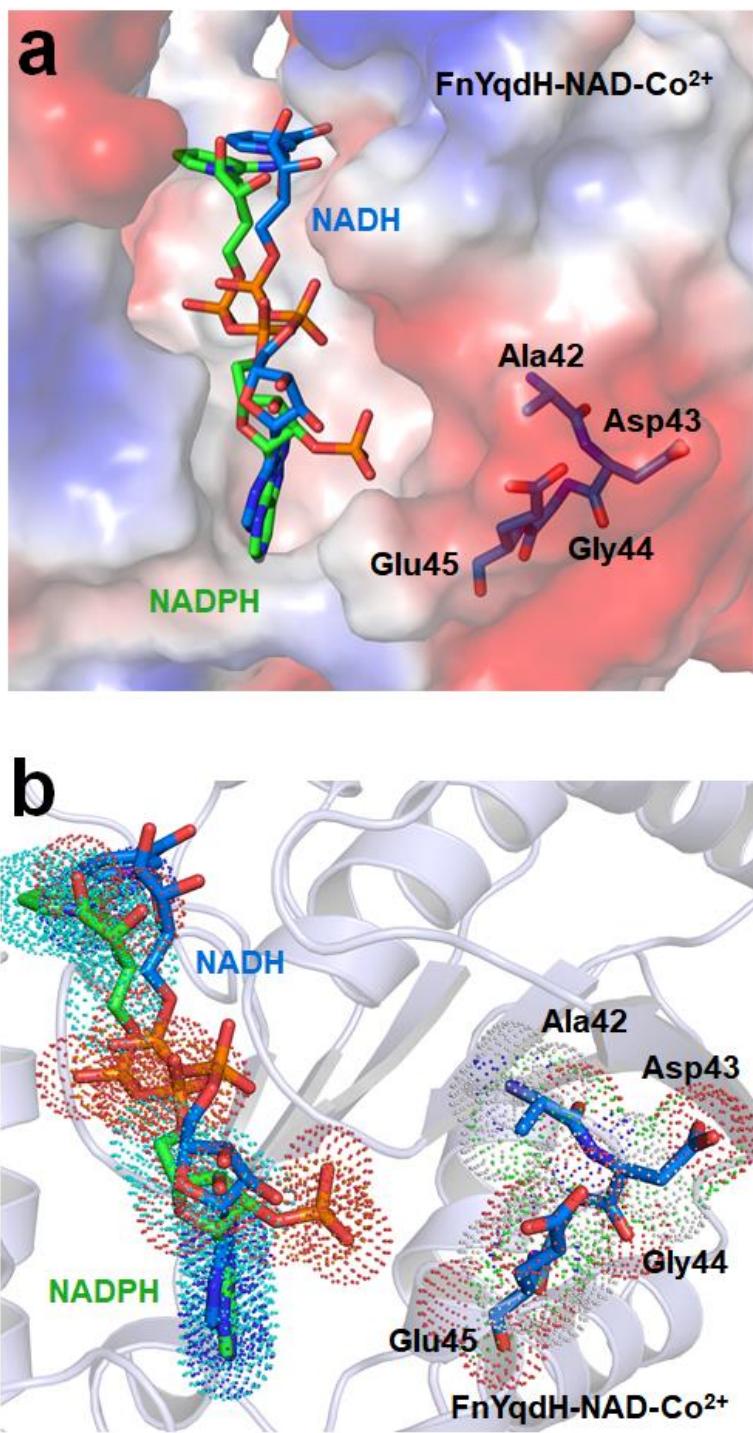
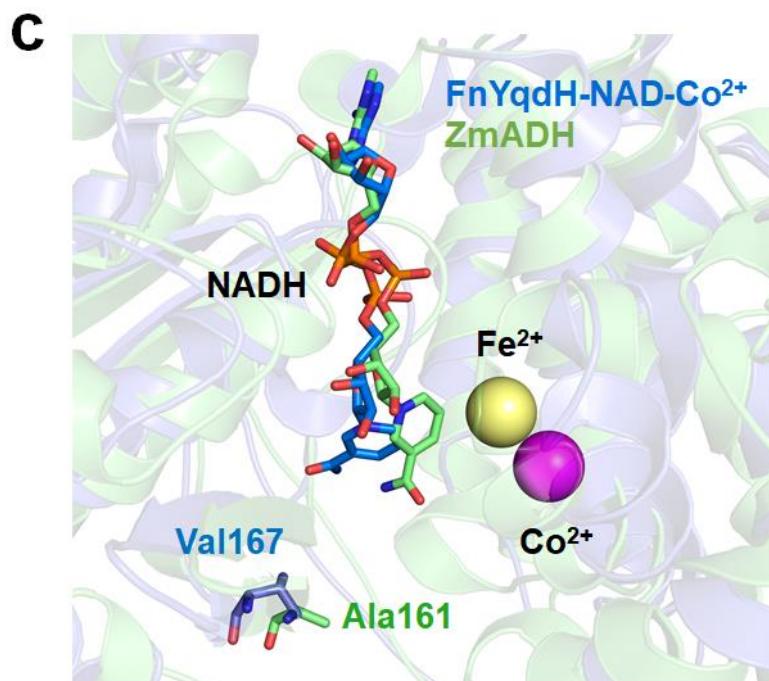
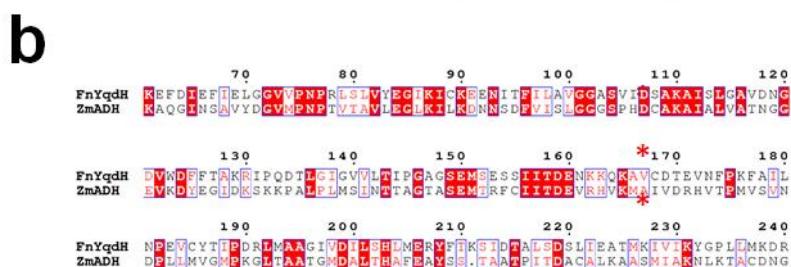
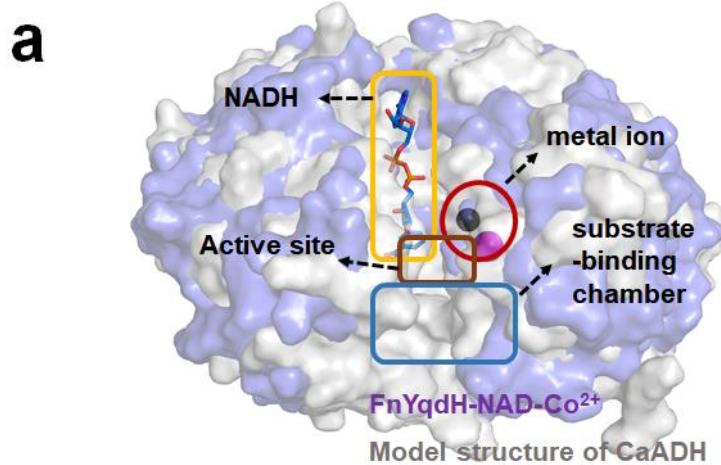


Figure S11. (a) The surface structure of the NAD(P)H-binding pocket of FnYqdH-NAD-Co<sup>2+</sup>, NADH is colored blue and NADPH is colored green. (b) Spatial distribution of NAD(P)H-binding pocket and NAD(P)H.



**Figure S12.** Comparison of FnYqdH-NAD-Co<sup>2+</sup> and other structures. (a) Schematic representation of FnYqdH-NAD-Co<sup>2+</sup> (colored blue) and CaADH (model structure, colored grey). (b) Partial sequence alignment of FnYqdH and ZmADH. The substrate-binding site of ZmBDH is indicated by a red \*. (c) Comparison of FnYqdH-NAD-Co<sup>2+</sup> (colored marine [blue]) and ZmADH (colored green).

**Table S1. Sequence of Primers.**

Primers	Sequence
F-FnYqdH-WT	GGGCC <u>ATGG</u> ATGGACAATTAAATTATAAAAATGATAC
R-FnYqdH-WT	GGG <u>CTCGAG</u> TTATTTGCTAAATTAAAGATA
F-FnYqdH-E41A	CTCTTGCA <u>TAT</u> <u>GCA</u> GCAGATGGAGAA
R-FnYqdH-E41A	TTCTCCATCTGC <u>TGC</u> ATAATGCAAGAG
F-FnYqdH-G44A	ATGAGGCAGAT <u>GCA</u> GAATTAATAA
R-FnYqdH-G44A	TTATTAA <u>TTCTG</u> CATCTGCCTCAT
F-FnYqdH-L46A	GCAGATGGAGAA <u>GCA</u> ATAAAAAAAACTT
R-FnYqdH-L46A	AAGTTTTTTAT <u>TGC</u> TTCTCCATCTGC
F-FnYqdH-S104A	GTTGGTGGAGCA <u>GCA</u> GTTATTGACTCA
R-FnYqdH-S104A	TGAGTCATAAAC <u>TGC</u> TGCTCCACCAAC
F-FnYqdH-D107A	GCAAGTGTATT <u>GCA</u> TCAGCTAAAGCA
R-FnYqdH-D107A	TGCTTAGCTGA <u>TGC</u> AATAACACTTGC
F-FnYqdH-T143A	GGTTGTTTA <u>GCA</u> ATTCCAGGAGCT
R-FnYqdH-T143A	AGCTCCTGGAAT <u>TGC</u> TAAAACAACC
F-FnYqdH-S152A	GGTTCTGAAATG <u>GCA</u> GAAAGCTCTATT
R-FnYqdH-S152A	AATAGAGCTTC <u>TGC</u> CATTCAGAACCC
F-FnYqdH-K165A	AATAAGAAACAA <u>GCA</u> GCTGTTGTGAT
R-FnYqdH-K165A	ATCACAAACAGC <u>TGC</u> TTGTTCTTATT
F-FnYqdH-T187A	GAAGTTGTTAT <u>GCA</u> ATTCCCTGATAGA
R-FnYqdH-T187A	TCTATCAGGAAT <u>TGC</u> ATAACAAACTTC
F-FnYqdH-D199A	GCTGGAATTGTA <u>GCA</u> ATTTTATCACAT
R-FnYqdH-D199A	ATGTGATAAAAT <u>TGC</u> TACAATTCCAGC
F-FnYqdH-H203A	GATATTATCA <u>GCA</u> TTAATGGAAAGA
R-FnYqdH-H203A	TCTTCCATTAA <u>TGC</u> TGATAAAATATC
F-FnYqdH-E206A	TCACATTAA <u>GCA</u> AGATATTTCACA
R-FnYqdH-E206A	TGTAAAATATCT <u>TGC</u> CATTAATGTGA
F-FnYqdH-H272A	GATTGGGCTTCC <u>GCA</u> AGAATTGAACAT
R-FnYqdH-H272A	ATGTTCAATTCT <u>TGC</u> GGAAGGCCAATC
F-FnYqdH-H286A	TATGATTGACT <u>GCA</u> GGTATTGGTATG
R-FnYqdH-H286A	CATACCAATAC <u>TGC</u> AGTCAAATCATA

The *N*col and *X*hol restriction sites are underlined. The mutant sites are marked red color with underlined.