Supplementary Table 1. List of PK genes.

Strain	GenBank ID	Group	Length (a.a.)	Identity (%)[a]	Activity against X5P	Activity against F6P	Reference
Bifidobacterium breve 203	GU936109	XFPK	825	–	Specific activity 29.0 (U/mg)	Specific activity 14.5 (U/mg)	This work
Bifidobacterium lactis	CAC29121	XFPK	825	94	V_{max}/K_m[b] 0.52 (U/mg/mM)	V_{max}/K_m[b] 0.6 (U/mg/mM)	(1)
Bifidobacterium animalis[c]	BAF37975	XFPK	825	95	–	–	(2)
Bifidobacterium longum[c]	AAN24771	XFPK	825	84	–	–	(3)
Bifidobacterium longum subsp. *infantis*[c]	ACJ52798	XFPK	825	83	–	–	(4)
Lactobacillus pentosus	CAC84393	XPK	788	46	Specific activity 4.5 (U/mg)	–	(5)
Lactobacillus paraplantarum	AAQ64626	XPK	788	46	–	V_{max}/K_m 98.3 (U/mg/mM)	(6)
Lactobachillus plantarum	CAD65631	XPK	803	47	V_{max}/K_m 1.14 (IU/mg/mM)	V_{max}/K_m 0.075 (IU/mg/mM)	(7)
Leuconostoc mesenteroides	AAV66077	XPK	813	42	–	Specific activity 0.3 (U/mg)	(8)

[a]Amino acid sequence idenitity with *Bifidobacterium breve* XFPK (*Bb*XFPK). The amino acid sequence identities between the XPK and XFPK groups are about 45%, but the identities within each group are more than 80%.

[b]Measured using native enzyme.

[c]Putative genes annotated by genome projects

Supplementary Table 2. The forward oligonucleotide primers used for construction of *Bb*XFPK mutants.

Name	Sequence of oligonucleotide primer
H64A	5'- ACCGTCTGGTCGGCGCCTGGGGCACCA -3'
H64N	5'- ACCGTCTGGTCGGCAACTGGGGCACCA -3'
H97A	5'- ATGGGCCCGGGCGCCGGCGGCCCGGCT -3'
H97N	5'- ATGGGCCCGGGCAACGGCGGCCCGGCT -3'
H142A	5'- GCGGCATCCCGTCGGCCTTCGCCCCGGAGA -3'
H142N	5'- GCGGCATCCCGTCGAACTTCGCCCCGGAGA -3'
H320A	5'- CTCCTGGCGTGCGGCCCAGGTCCCGCTGGCTT -3'
H320N	5'- CTCCTGGCGTGCGAACCAGGTCCCGCTGGCTT -3'
Q321A	5'- CTCCTGGCGTGCGCACGCCGTCCCGCTGGCTT -3'
S440A	5'- ACCGGACGAGACCGCTGCCAACCGCCTGAACG -3'
E479A	5'- ACCGAGCAGCTCTCCGCCCACCAGTGCGAGG -3'
Y501F	5'- ATCTGGAGCTCCTTCGAGTCCTTCGTCCA -3'
H548A	5'- GTGGCGTCAGGATGCCAACGGCTTCTCGCA -3'
N549A	5'- GGCGTCAGGATCACGCCGGCTTCTCGCACCA -3'
H553A	5'- AACGGCTTCTCGGCCCAGGACCCGGG -3'
H553N	5'- AACGGCTTCTCGAACCAGGACCCGGG -3'
K605A	5'- ATCTTCGCCGGCGCCCAGCCTGCTCCGA -3'

The mutated sequences are underlined

Supplementary Table 3. Data collection and refinement statistics of *Bb*XFPK mutants.

Data set	H64A	H142A	H320A	H553A
Data collection statistics				
Space group		*I422*		
Unit cell (Å)	a = 175.0	a = 174.6	a = 174.4	a = 175.1
	b = 175.0	b = 174.6	b = 174.4	b = 175.1
	c = 163.6	c = 163.6	c = 163.8	c = 164.0
Beam line	PF-BL5A-	PF-BL5A	PF-BL5A	PF-BL17A
Wavelength (Å)	1.00000	1.00000	1.00000	1.00000
Resolution (Å) [a]	50-1.90	50-2.10	50-2.10	50-2.10
	(1.93-1.90)	(2.14-2.10)	(2.14-2.10)	(2.14-2.10)
Total reflections	1,370,980	1,079,028	1,074,515	1,078,552
Unique reflections	99,057	73,408	73,346	74,103
Completeness (%)[a]	99.3 (100)	100 (100)	100 (100)	99.8 (99.9)
R_{merge} (%)[a]	5.8 (31.1)	7.2 (28.4)	7.1 (30.5)	7.7 (33.2)
$I/\sigma I$[a]	57.6 (8.8)	44.7 (9.7)	47.5 (10.1)	42.1 (6.9)
Redundancy[a]	13.9 (12.8)	14.7 (14.9)	14.7 (14.7)	14.6 (13.2)
Refinement statistics				
Resolution range (Å)	33.50-1.90	34.15-2.10	34.21-2.10	38.61-2.10
No. of reflections	92,268	69,663	69,576	70,211
R-factor/R_{free} (%)	16.3/20.6	15.8/19.6	15.0/19.4	15.8/19.1
r.m.s.d. from ideal				
Bond lengths (Å)	0.031	0.029	0.028	0.029
Bond angles (°)	2.221	2.139	2.031	1.962
Average B-factor (Å^2)				
Protein	26.1	22.8	24.2	29.1
Water	37.0	29.0	32.2	34.9
ThDP	22.0	19.6	19.1	26.1
Mg^{2+}	20.4	16.6	16.1	23.1
Ramachandran plot (%)				
Favoured	96.4	95.8	95.9	96.4
Allowed	3.6	4.1	4.1	3.6
Disallowed	0.0	0.1	0.0	0.0

[a]Values for highest resolution shell are given in parentheses.

Supplementary Figure Legends

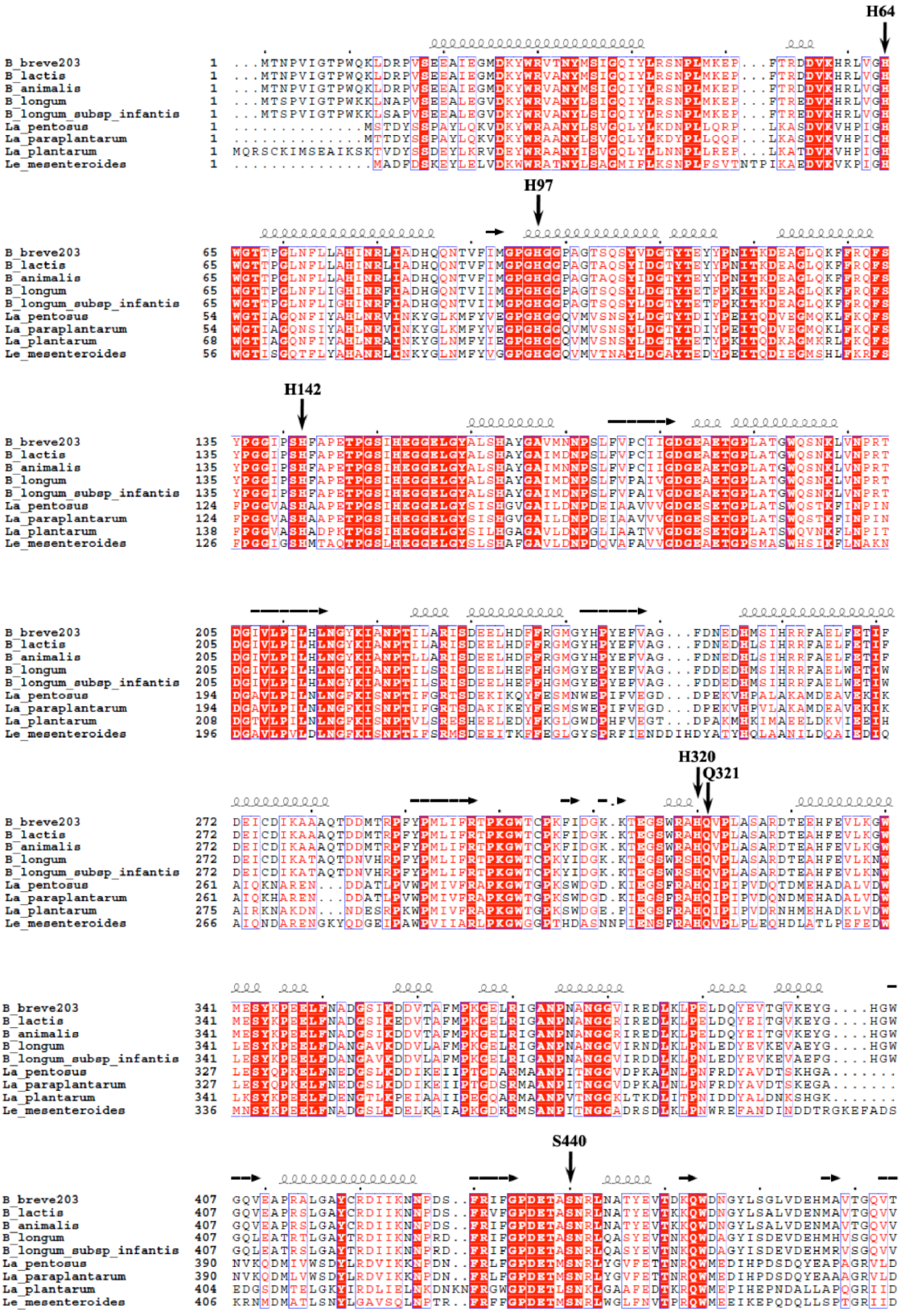
Supplementary Fig. 1. Amino acid sequence alimnment of PK. The sequence alignment was generated using ESPript (10). Residues important for catalysis and substrate binding characterized in this work are indicated by arrows.

Supplementary Fig. 2. Phylogenetic tree of PK. The tree was constructed from the alignment shown in Supplementary Fig. 1 by the neighbor-joining method.

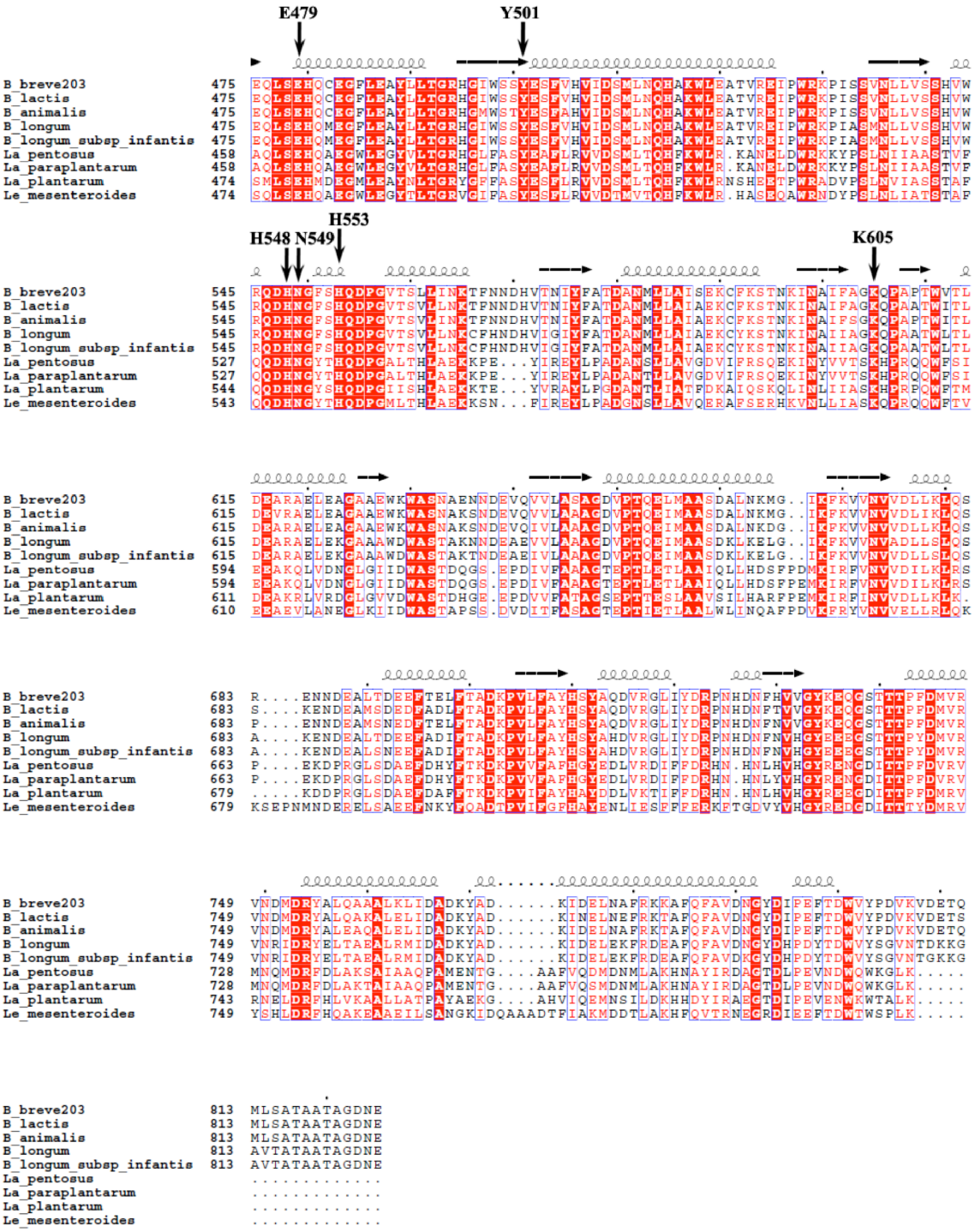
Supplementary Fig. 3. Stereoview of ThDP-binding manner in the active site of *Bb*XFPK. (A) Hydrophobic and hydrophilic interactions involved in the maintenance of V-conformation of ThDP. (B) The hexacoordinate geometry of Mg^{2+} ion and the interactions between diphosphate portion of ThDP and *Bb*XFPK.

Supplementary Fig. 4. $|F_o|-|F_c|$ omit electron density maps of the cofactors and their covalent adducts in the active site of *Bb*XFPK mutants. (A) Map of AcThDP (H142A) contoured at 3.5 σ. (B) Map of AcThDP (H320A) contoured at 3.5 σ. (C) Map of ThDP (H553A) contoured at 3.7 σ.

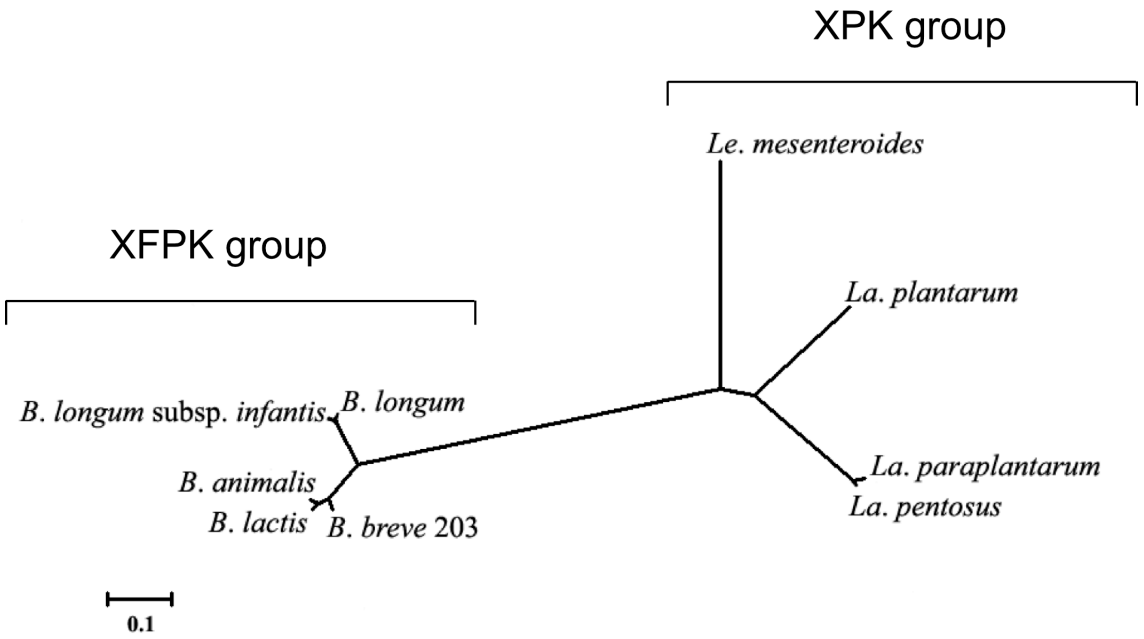
Supplementary Fig. 5. Stereoview of superimposition of the active site of wild-type (gray) and H64A mutant (colored) of *Bb*XFPK. Residues of H64A from PP domain of one subunit and Pyr domain of the other subunit are colored blue and yellow, respectively.



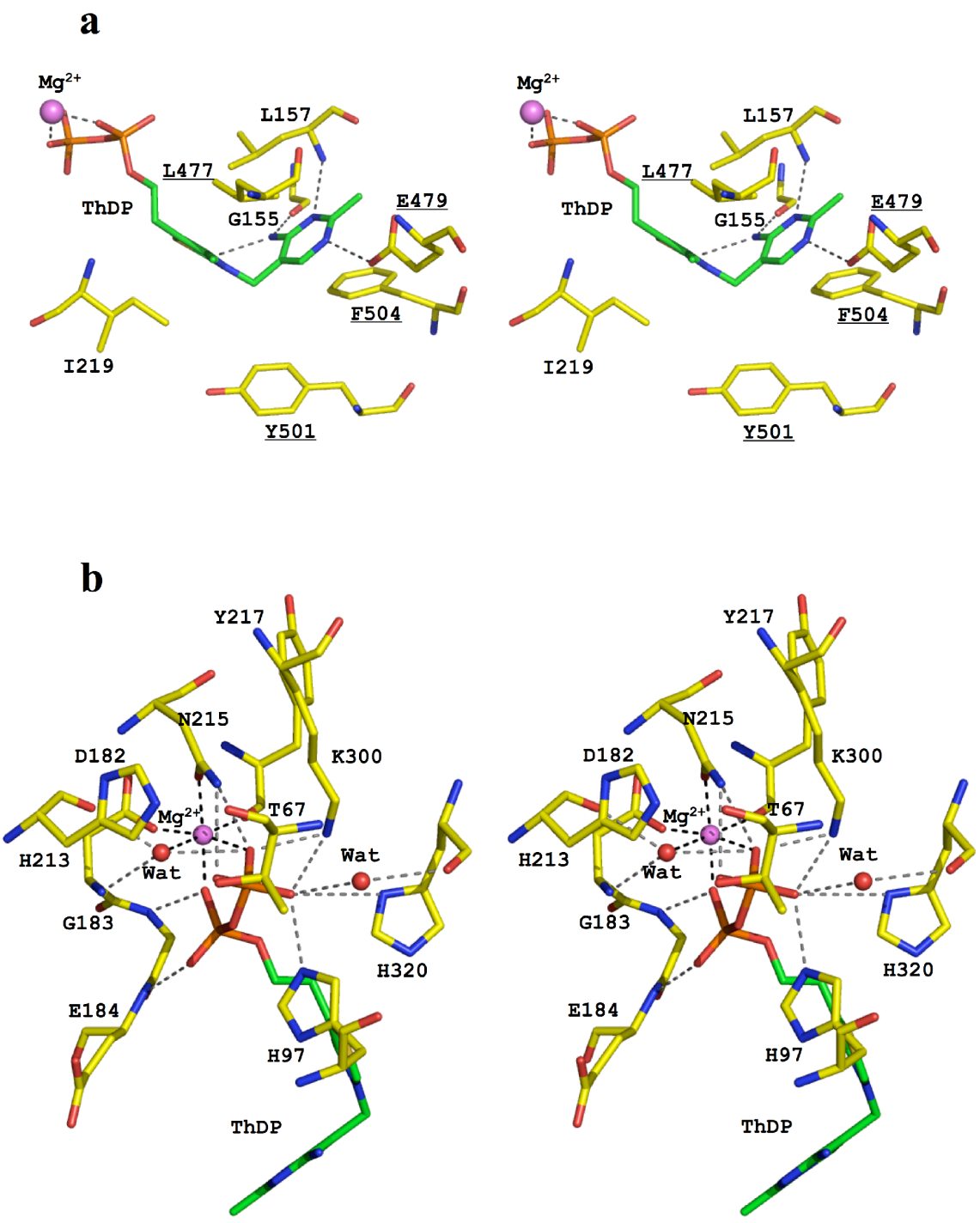
Suzuki et al., Supplementary Fig. 1



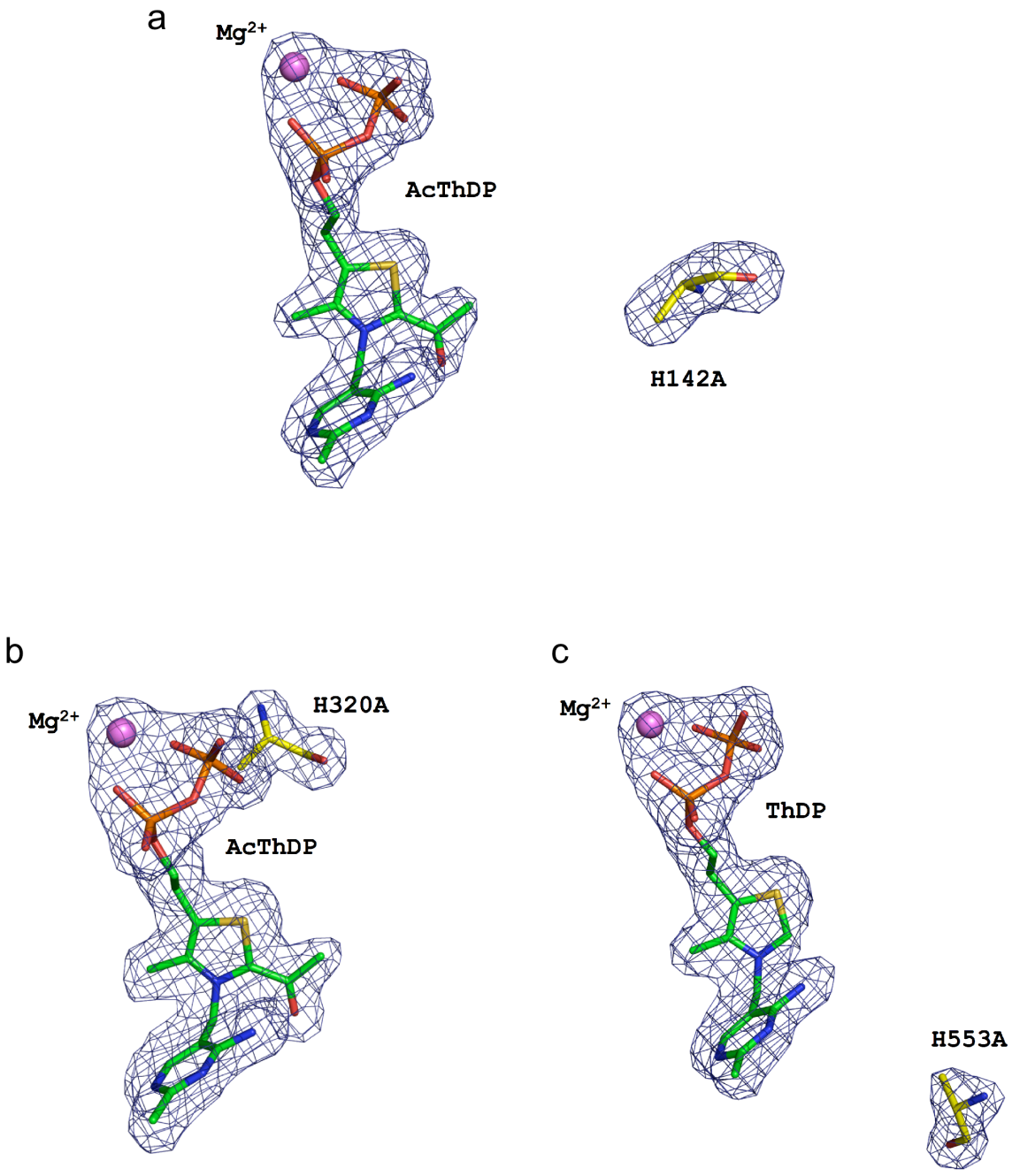
Suzuki et al., Supplementary Fig. 1, continued



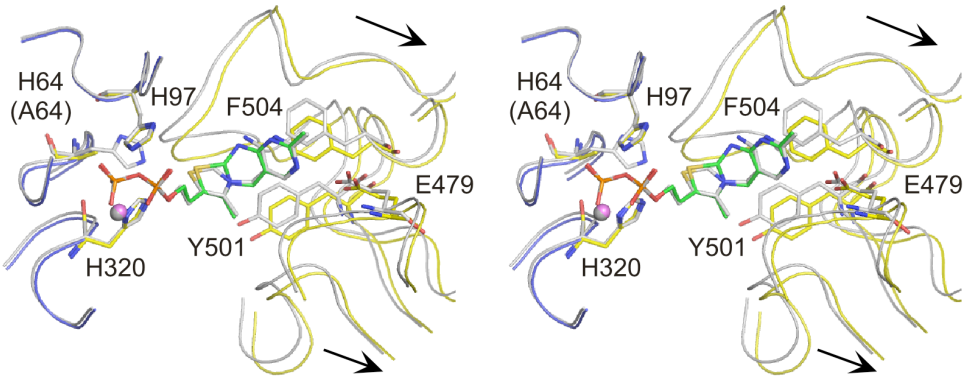
Suzuki et al., Supplementary Fig. 2



Suzuki et al., Supplementary Fig. 3



Suzuki et al., Supplementary Fig. 4



Suzuki et al.,
Supplementary Fig. 5

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