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Supporting information for article:

Divalent metal ion-based catalytic mechanism of the Nudix
hydrolase Orf153 (YmfB) from *Escherichia coli*

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Supporting Information**Table S1** Sequences of oligonucleotide primers used for construction of YmfB mutants.

Primer	Sequence 5' --- 3'
E23Q_F	TAGTCGTTGAACAGACGATTAATGGT
E23Q_R	ACCATTAATCGTCTGTTCAACGACTA
H37A_F	CAACCTGCCGGGGCCCTGGAAGCCG
H37A_R	CGGCTTCCAGGGCCCCGGCAGGTTG
E51Q_F	CCGCCGCCCGTCAGCTATGGGAAG
E51Q_R	CTTCCCATAGCTGACGGGCGGCGG
E54Q_F	GTGAGCTGTGGCAGGAAACCGGCATC
E54Q_R	GATGCCGGTTTCCTGCCACAGCTCAC
E55Q_F	GCTGTGGGAACAGACCGGCATCAGC
E55Q_R	GCTGATGCCGGTCTGTTCCACAGC
D98N_F	GCAGCCTCATAACAGCGATATCG
D98N_R	CGATATCGCTGTTATGAGGCTGC
D100N_F	CTCATGACAGCAATATCGACTGC
D100N_R	GCAGTCGATATTGCTGTCATGAG
R119A_F	CGTCAAATCTTGCGTCGCCGCTGG
R119A_R	CCAGCGGCGACGCAAGATTTGACG

Table S2 R^2 values for the least-squares adjustment of k_{cat} and K_M for GDP and GTP.

	GDP	GTP
WT	0.9920	0.9808
E23Q	0.9321	0.9681
H37A	0.9665	0.9881
E51Q	N/A	N/A
E54Q	0.9389	0.9214
E55Q	N/A	N/A
D98N	0.9523	0.9645
D100N	0.9808	0.9930
R119A	0.9825	0.9968

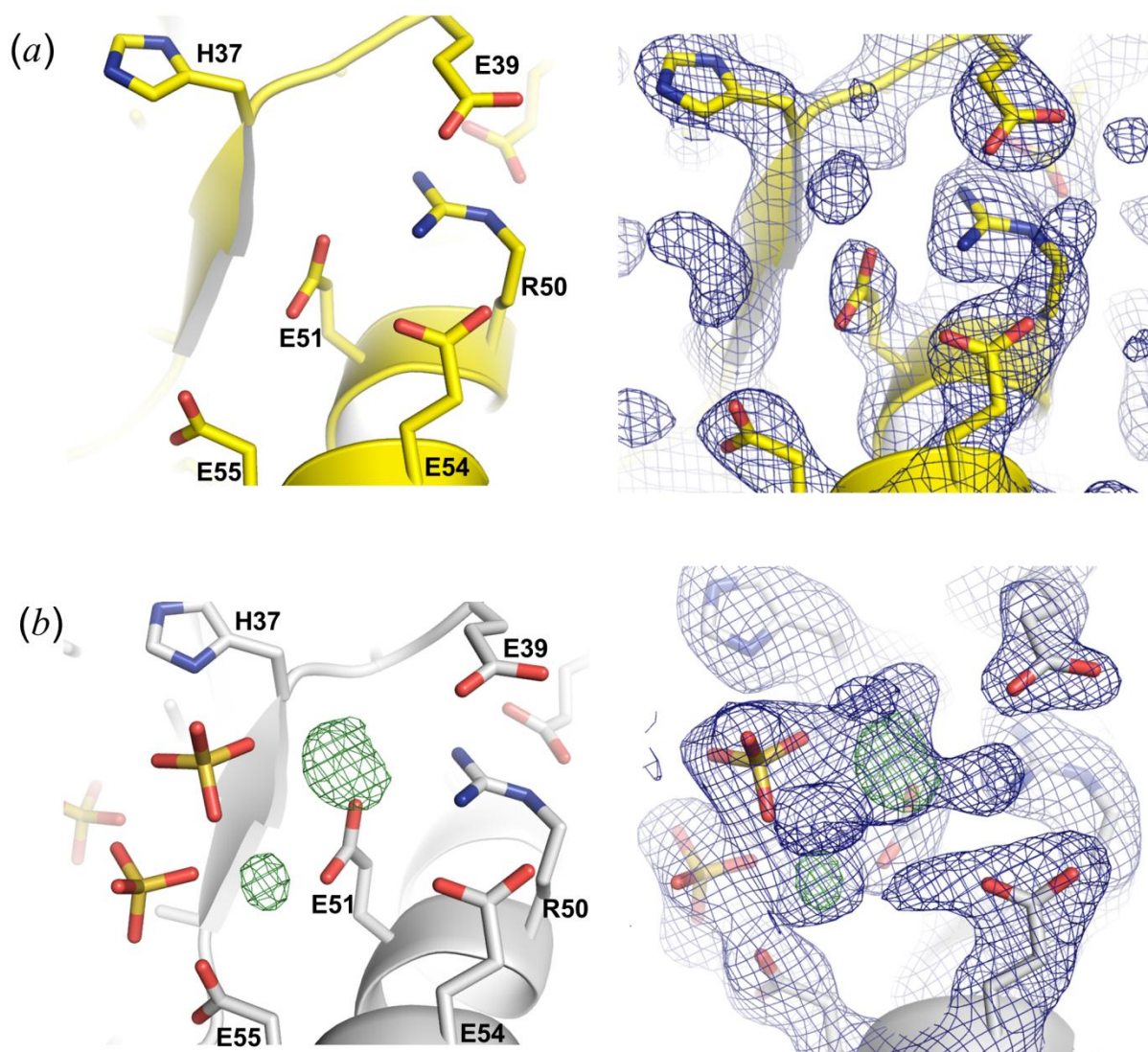


Figure S1 Omit maps of metal and water molecules in the active site of apo-YmfB and metal-bound YmfB structures. (a) The active site of apo-YmfB structure did not show the positive density in Fo-Fc map (contoured at 4.0σ) (left). (b) The metal-bound YmfB structure showed two positive densities (green) for metal ions in Fo-Fc map (contoured at 4.0σ) (left). The right figures showed both 2Fo-Fc maps (contoured at 1.0σ , blue) and Fo-Fc maps (contoured at 4.0σ , green) without metal and water molecules.

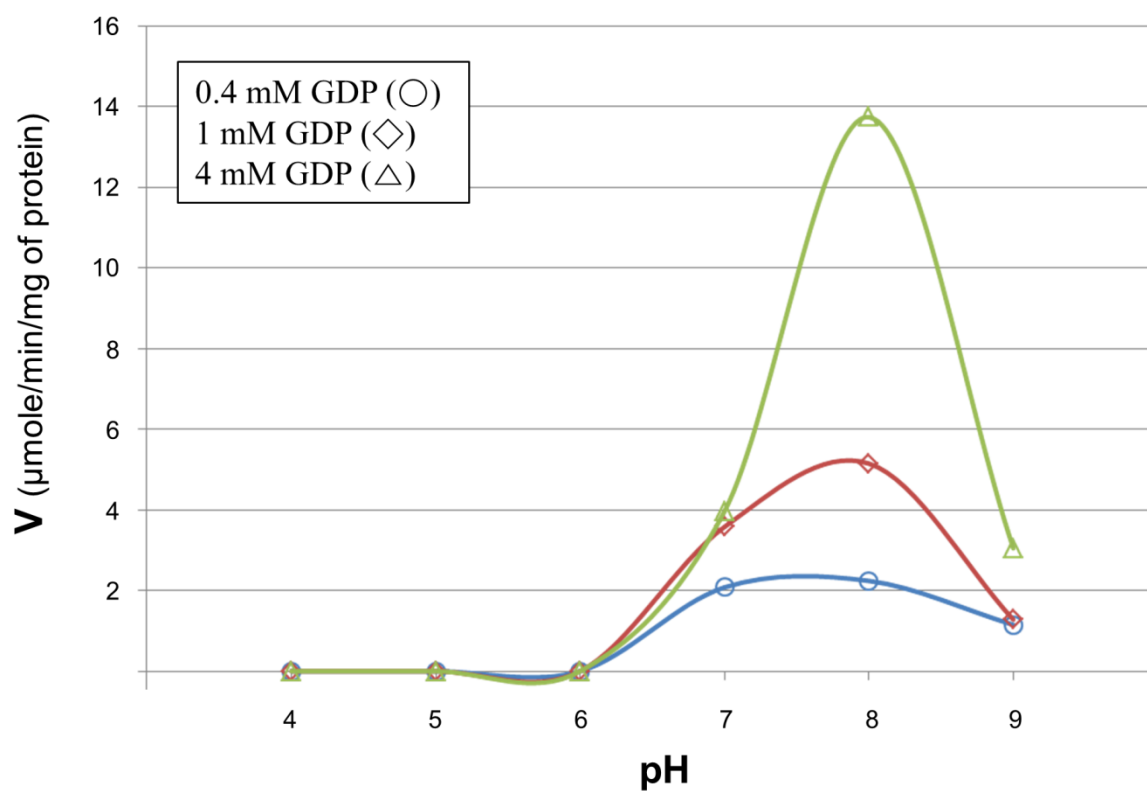


Figure S2 The pH dependence of the YmfB catalytic activity. Enzymatic assays were carried out with 0.4 mM GDP (○), 1 mM GDP (◇), and 4 mM GDP (△) at various pHs.