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Supporting information for article:

Structure of d-alanine-d-alanine ligase from *Yersinia pestis*: nucleotide phosphate recognition by the serine loop

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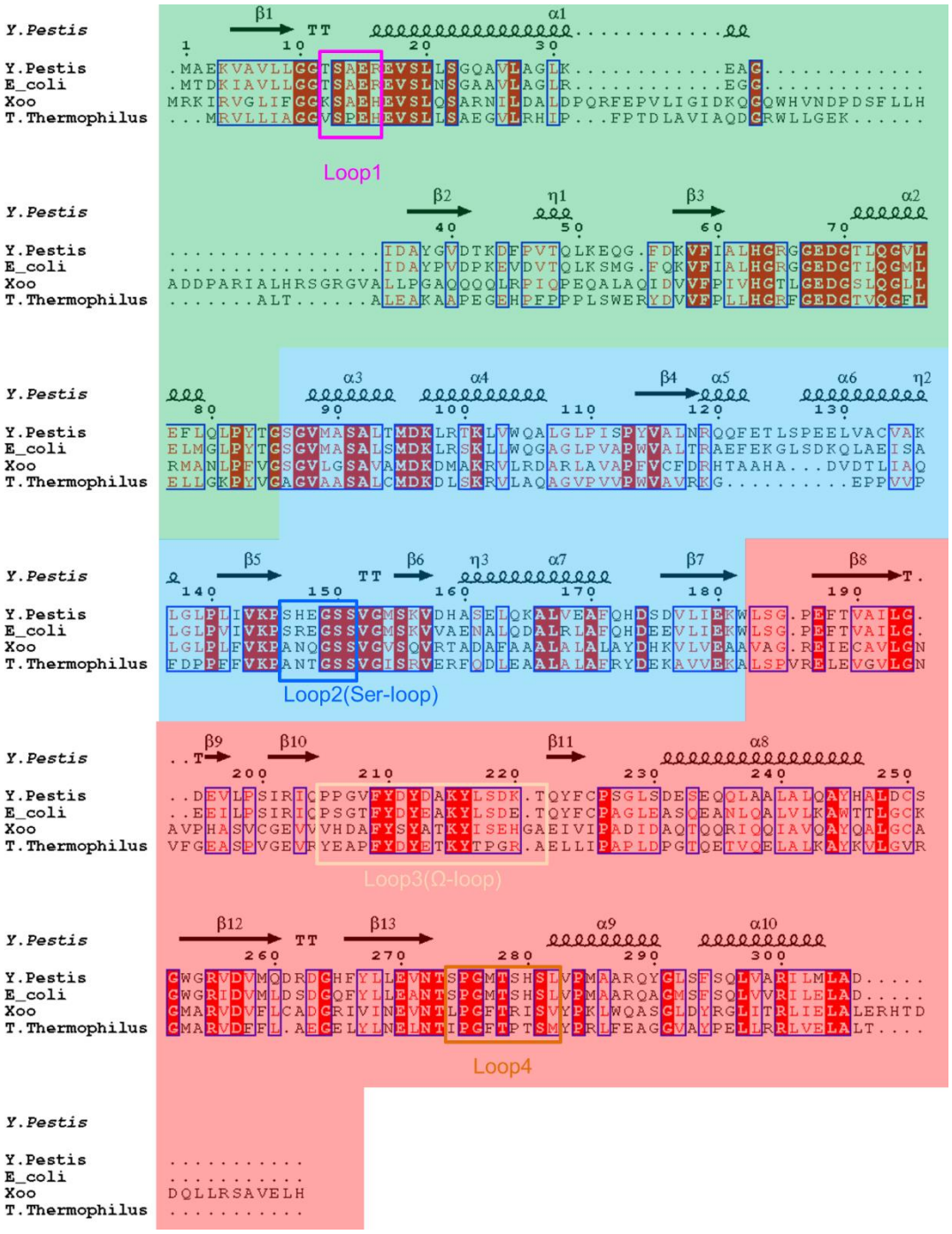

Figure S1 Sequence alignment of D-alanine-D-alanine ligases (DDLs) from *Yersinia pestis*, *Escherichia coli*, *Xanthomonas oryzae* pv. oryzae, and *Thermus thermophilus*. The N-terminal domain is green, the central domain is cyan, and the C-terminal domain is red. Loop 1, loop 2 (serine-loop), loop 3 (ω-loop), and loop 4 are purple, blue, salmon, and orange, respectively.

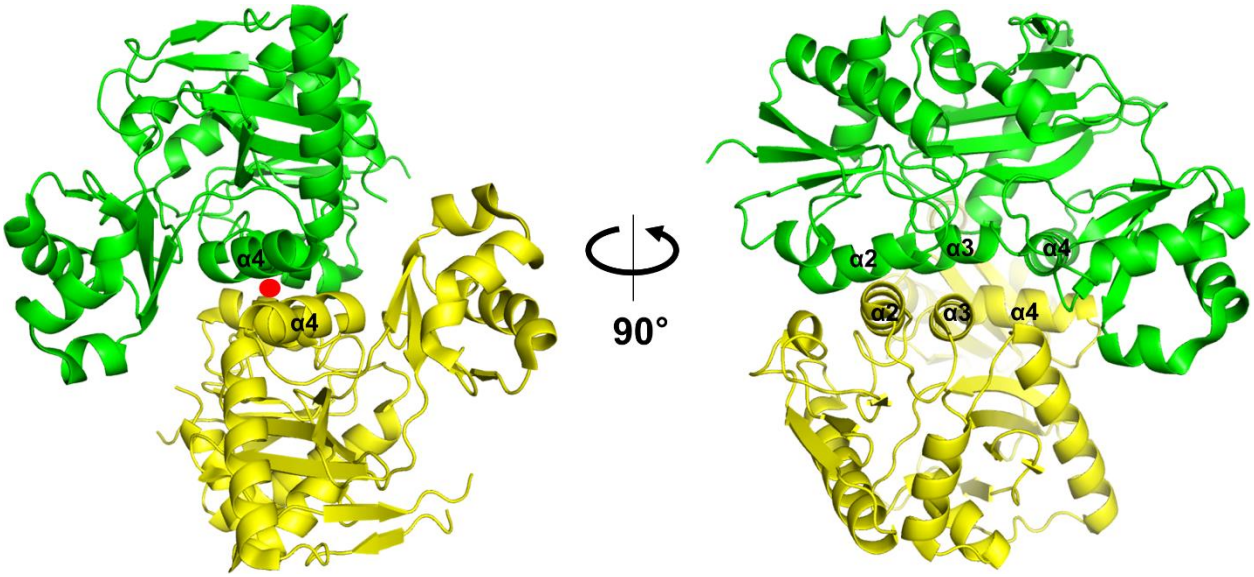

Figure S2 Dimer structure of YpDDL. Non-crystallographic two-fold symmetry is shown as a red ball. Three α-helices of α2, α3, and α4 which are mainly involved in dimerization are labelled.

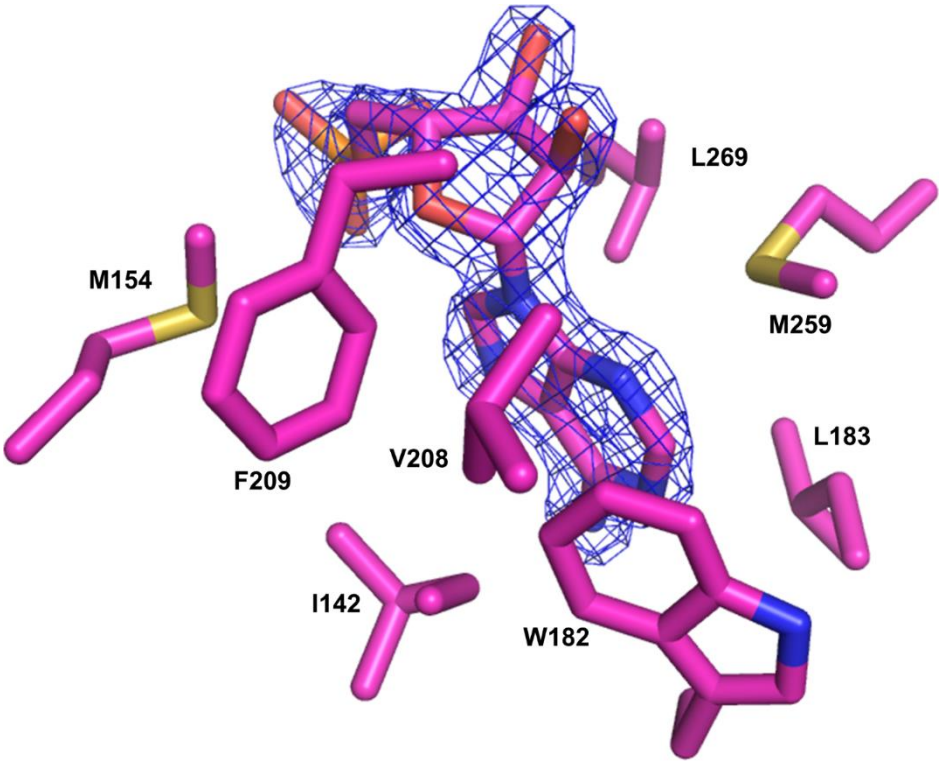

Figure S3 Hydrophobic adenine-binding pocket in AMP-bound YpDDL structure. The refined map (2Fo-Fc map contoured at 1.0 σ) of bound AMP is shown as a blue mesh.

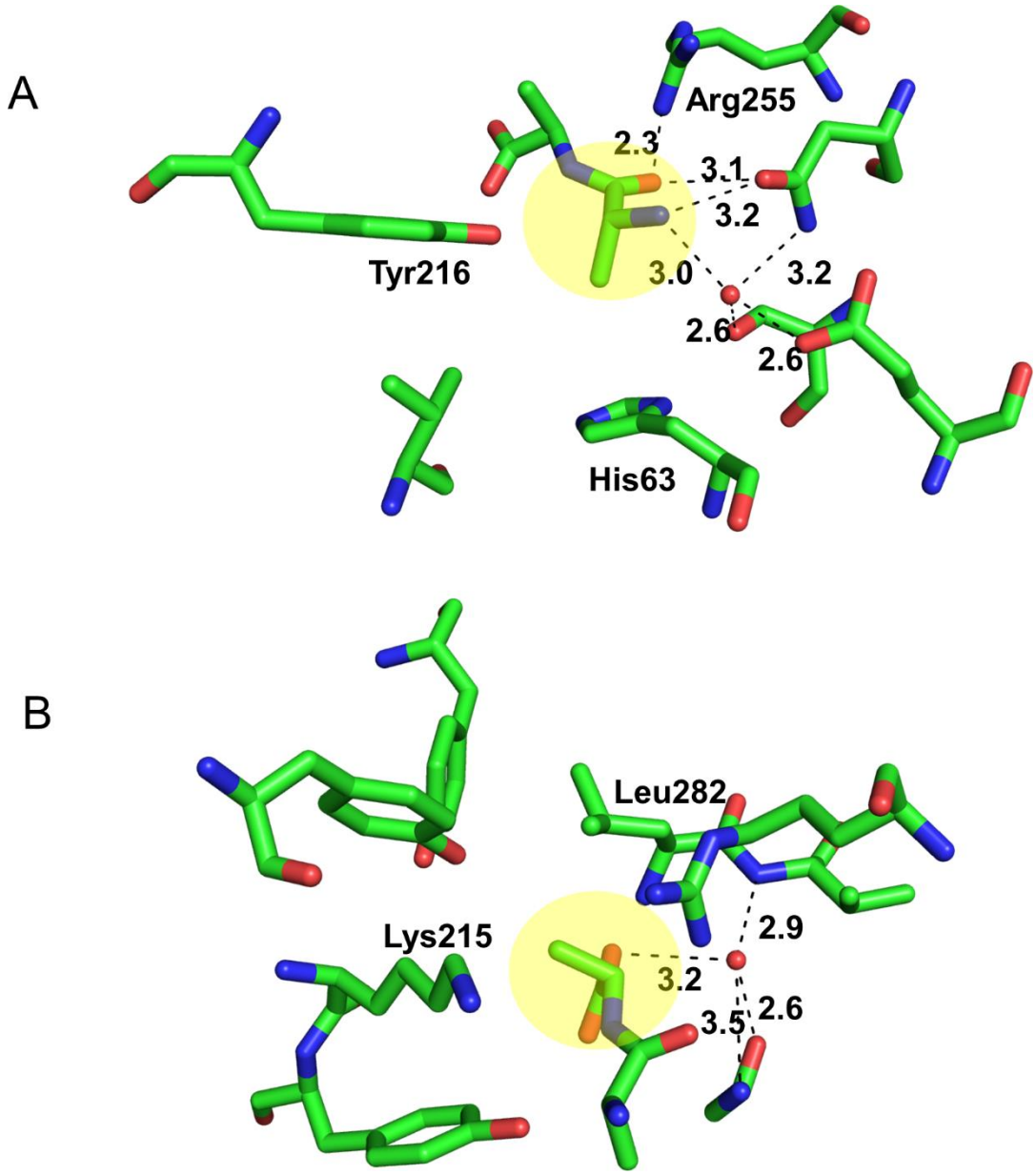

Figure S4 Structures of the D-alanine-binding sites. (a) Recognition of D-ala$_1$ in D-alanyl-D-alanine (D-ala-D-ala). D-Ala$_1$ is yellow. (b) Recognition of D-ala$_2$ in D-ala-D-ala. D-Ala$_2$ is yellow. Distance (Å) is labelled in dashed line.

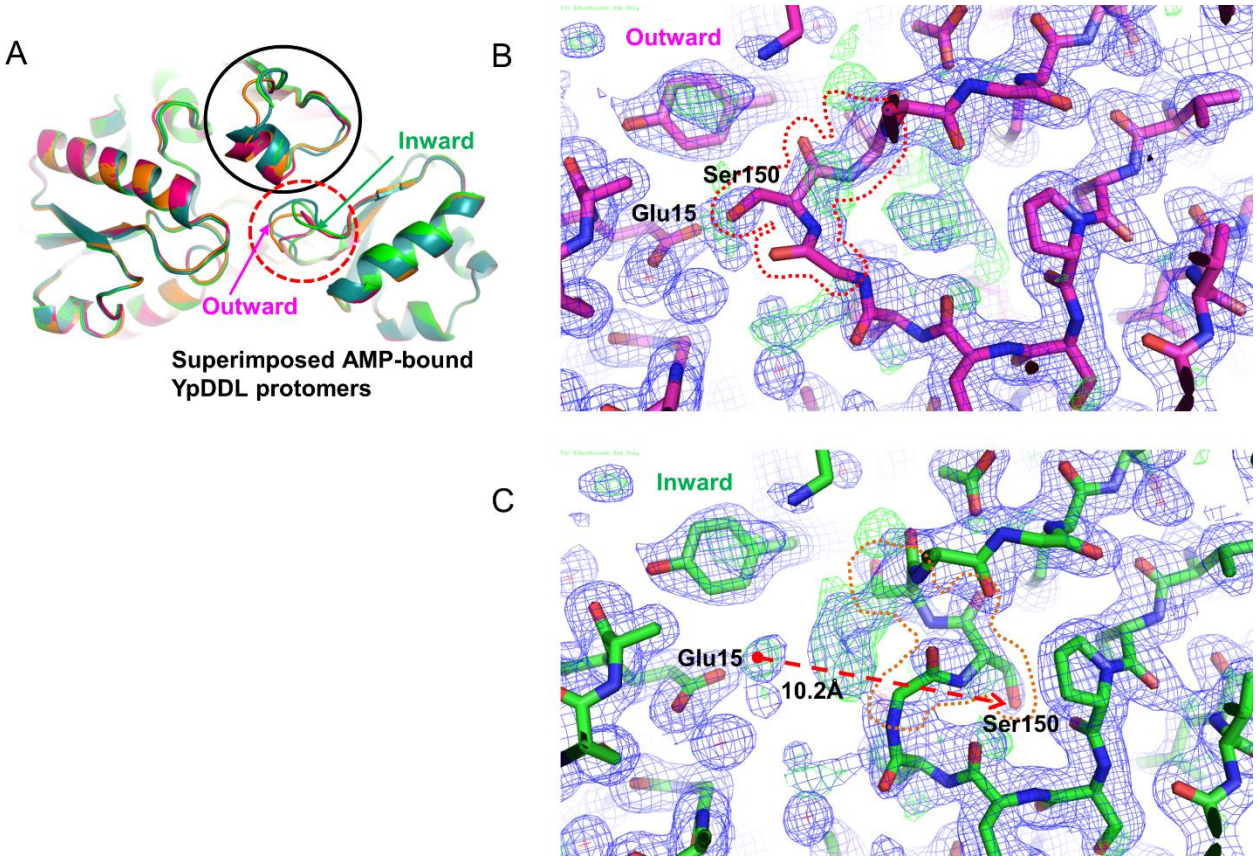

Figure S5 Outward and inward conformations of the ω-loop. (a) Four protomers of AMP-bound *Yersinia pestis* D-alanine-D-alanine ligase (YpDDL) structures were superimposed. The black circle represents the closed conformation of the ω-loop. The dashed red circle represents the flexible outward and inward serine-loops. (b) The outward serine-loop structure with an electron density map. The red dotted line shows the GSS motif in the serine-loop. The blue mesh shows a 2Fo-Fc map (contoured at 1.0 σ), and the green mesh shows Fo-Fc maps (contoured at 3.0 σ). (c) The inward serine-loop structure with an electron density map. The orange dotted line shows the GSS motif in the serine-loop. The red dashed line shows the movement of Ser150 from the outward conformation to inward conformation.

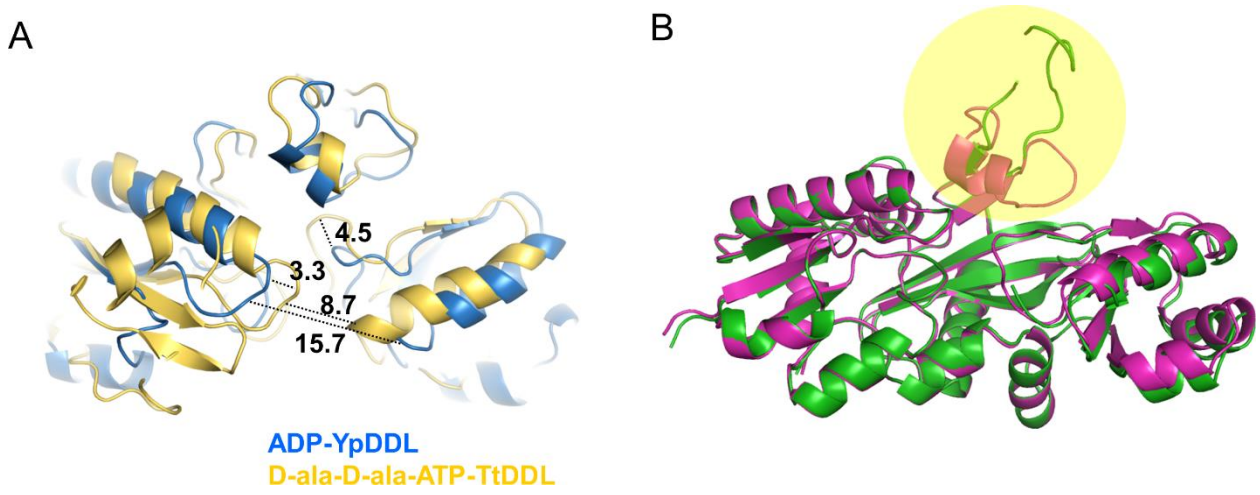

Figure S6 Interdomain and ω-loop conformation of YpDDL. (a) Comparison of interdomain conformations between *Yersinia pestis* D-alanine-D-alanine ligase (YpDDL) and *Thermus thermophilus* DDL (TtDDL). The ADP-bound YpDDL structure and D-alanyl-D-alanine (D-ala-D-ala)- and ATP-bound TtDDL structure are light blue and yellow, respectively. (b) Conformational changes of the ω-loop in YpDDL. Open and closed conformations of the ω-loop are yellow. Distance (Å) is labelled in dashed line.

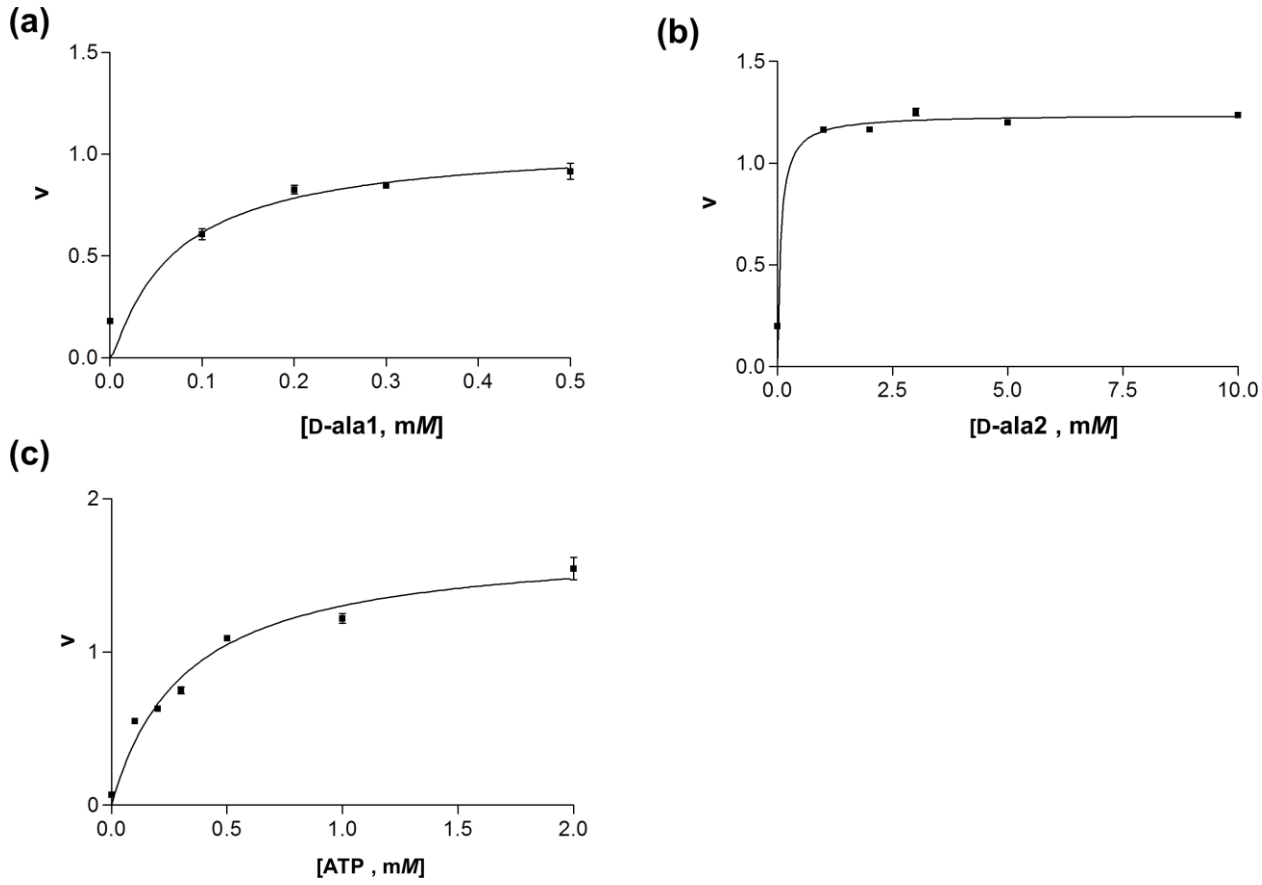

Figure S7 Enzyme kinetics of YpDDL for the first and second D-alanines and ATP. (a) Low concentration of D-alanine substrate-dependent initial velocity of YpDDL to determine K_{m1}. (b) High concentration of D-alanine substrate-dependent initial velocity of YpDDL to determine K_{m2}. (c) ATP substrate concentration-dependent initial velocity of YpDDL to determine $K_{m,ATP}$. Initial velocity is released Pi concentrations (m*M*) per min. Data were fitted with Prism by non-linear regression, and are means ± standard error from assays carried out in triplicate.

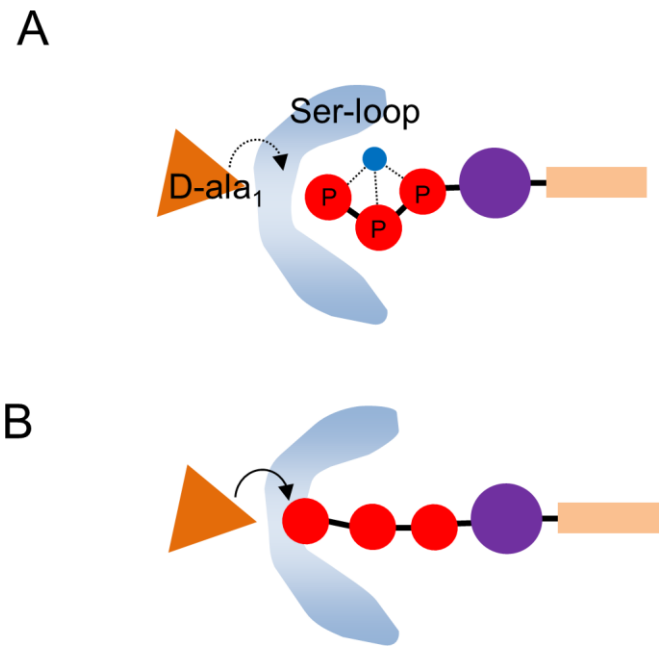

Figure S8 Recognition of nucleotide phosphates by the serine-loop with metal coordination. (a) The bent conformation of the 3 phosphates is stabilized by the hexacoordinated divalent metal ion and the serine-loop. (b) Loss of the metal coordination may be due to the conformational change of the serine-loop, which cannot hold the bent conformation of the 3 phosphates; which can stretch the 3 phosphates. The straight conformation of the 3 phosphates reduces the distance to D-ala$_1$; this change accelerates the nucleophilic attack by D-ala$_1$ on the γ-phosphate of ATP.